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Fabrication of polyester microchannels and their applications to capillary electrophoresis

Wei Xu, Katsumi Uchiyama, Takuya Shimosaka, Toshiyuki Hobo*

Department of Applied Chemistry, Graduate School of Engineering, Tokyo Metropolitan University, 1-1 Minami-ohsawa, Hachioji, Tokyo 192-0397, Japan

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Abstract

Inexpensive and disposable polyester microchips were fabricated through photolithographic and wet-chemical etching procedure, followed by replication using an imprinting method at room temperature. Laboratory-scale laser-induced fluorescence equipment was employed as a detection system. The generation of electroosmotic flow (EOF) on the polyester channels was discussed in this paper. Surfactants in the running buffer had a significant effect on the EOF depending on their types. The ζ potential of the electric double layer formed by adsorbing sodium lauryl sulfate molecules on the wall of polyester channels seemed to be constant within the buffer pH investigated. EOF could also be suppressed to zero by adding polyoxyethylene 23 lauryl ether into the running buffer. The separation of two laser dyes was obtained using polyester chips through both micellar electrokinetic chromatography and capillary zone electrophoresis. The polyester channels modified with 10-undecen-1-ol exhibited a dramatically high-separation efficiency compared with the conventional fused-silica capillary tubes.

Keywords: Microchannels; Chip technology; Electroosmotic flow; Undecenol; Polyester

1. Introduction

Miniaturized microfluidic devices have been being a charming topic as a hybrid of photolithographic technologies and capillary electrophoresis (CE). Since the first glass chip was used in capillary electrophoresis [1], miniaturization of CE instruments has undergone rapid growth, in which silica and quartz are often used as device compartments. More recently, researchers are putting their attentions on disposable polymer chips. However, usage of synthetic polymer materials for capillary electrophoresis instruments is actually originated from isotachophoresis. After Mikkers et al. [2] introduced polymer tubes to capillary electrophoresis, application of polymer materials for device compartments were discussed by several groups [3–10], and polymer chips are developed. The reason why researchers are interested in polymer, especially plastics chips, can be explained from following aspects: (1) Easy fabrication: compared with silica or quartz chips, the fabrication of polymer chips is much simpler [11,12]. (2) Cost-effectiveness and disposability: once a master template is produced, a lot of same-size channels can be replicated. This method has been used for the fabrication of most of the polymer

*Corresponding author. Fax: +81-426-77-2821.
E-mail address: hobo-toshiyuki@c.metro-u.ac.jp (T. Hobo).

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microfluidic devices [13–15]; moreover, since the plastics themselves are very cheap, the polymer chips could also be disposable. (3) Easy modifiability of channel inner surface. It is easier to give modification sites in polymer chips than in silica or quartz by incorporating functional monomers although it is yet to be reported. In the case of silica chips Jacobson et al. had reported open-channel electrochromatography [16].

Methods such as UV-laser photoablation [17], injection molding [13], and wire imprinting [11], have been employed and discussed for the fabrication of polymer microfluidic devices by several groups. The materials used include polycarbonate [17], polystyrene [17,18], poly(ethylene terephthalate) [17], cellulose acetate [17], acrylic [13,18], poly(methyl methacrylate) (PMMA) [11,12], poly(dimethylsiloxane) (PDMS) [14,15,19], co-polyester [12,18] and styrol [20]. We noticed that in almost all cases the channel sealing technique is problematic. Usually base and cover plates are bonded by heating, using adhesives, or chemical bonding. They, however, are very difficult to accomplish a completely sealed channel without failure.

In this paper, we employed unsaturated polyester for the fabrication of chips. An imprinting method at room temperature used for PDMS chip preparation [11,12] was applied. The procedure includes fabrication of glass template with positive relief channels, replication of channels to polyester through curing and then channel sealing. One of the advantages of our procedure is that channel sealing can be easily manipulated through further curing by bringing two polyester plates that are procured for ca. 30 min into contact at room temperature. The polyester chip fabrication procedure in this work is time and cost effective, and can be carried out in any laboratories without the need of special instruments. Also, in this paper, we will discuss the generation of electroosmotic flow (EOF) in the polyester channels. The effect of surfactants in running buffer on the magnitudes of EOF was revealed. This is the first report regarding the polyester channels with chemically modified inner surface in capillary electrophoresis and the effect of buffer pH on EOF was investigated. The performance of polyester chips was evaluated by the separation of two laser dyes.

2. Experimental

2.1. Chemicals

A mask blank coated with a thin layer of 50 nm Cr/Cr_2O_3 (DUFR-2506 (p)-L, Ulcoat, Japan) was used as a mask plate. Positive photo resist PMER P-RZ300 and developer PMER P-1S are purchased from Tokyo Oka Kogyo (Tokyo, Japan). Unsaturated polyester for curing (Clear Polyester, two-liquid type) is used as received from Epoc (Osaka, Japan). Sulforhodamine B and Sulforhodamine 101 (SRB and SR101) as samples are obtained from Kanto Chemical (Tokyo, Japan). The sample solution is prepared by mixing the same amount of SRB and SR101 in 50 m*M* borate buffer solution. The borate buffer solution was also used as the background electrolyte. A constant ionic strength of 60 m*M* was adjusted by adding NaCl into the background buffer solution [21]. Other chemicals are of analytical grade. Peak identification was made by injecting a mixture of samples spiked with the corresponding compound.

2.2. Apparatus

2.2.1. Analysis system

The system employed in this work is shown in Fig. 1. A He–Ne laser (1 mW, LHGP-0051, PMS Electro-Optics, Japan) as a light source was used to generate an excitation light of 543 nm which was

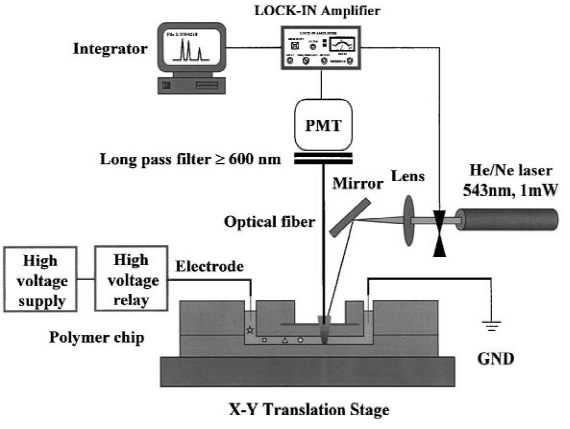

Fig. 1. Schematic diagram of the analysis system.

modulated by a light chopper (f=825 Hz, NF Circuit Design Block, Japan), focused by a lens (f=200), and then reflected by a mirror onto the detection window of polyester microchip, giving a laser spot of 1 mm in diameter. The fluorescence is collected by an optical fiber (core 400 μm, Polymicro Technologies, USA), filtered through two long pass glass filters ($\lambda \geq 600$ nm, O-58, Irie, Tokyo, Japan) and transformed into an electric signal by a photomultiplier tube (Hamawatsu Photonics R535, Japan), then amplified by a LOCK-IN amplifier (LI574, NF Circuit Design Block, Japan). The electropherogram is obtained through an integrator (Shimadzu Chromatopac CR-4A; Shimadzu, Tokyo, Japan). The detection system in this study is not optimized.

Three high-voltage supplies were used in this work. A laboratory-made changeover switch was employed to manually change injection mode to separation mode and vice versa. Samples were electrokinetically introduced into the channel intersection by applying −300 V to sample waste reservoir with the other three reservoirs electrically grounded. According to Jacobson et al., sample leakage from side channels and separation channel could be avoided by pinched injection method [22]. Electrophoresis separation was carried out at the electrical field of 209 V/cm in a separation channel by applying −7.0 kV to the waste reservoir, while the voltages at sample and sample waste reservoirs were kept at −300 V, respectively.

The magnitude of electroosmotic mobility was determined by current monitoring method [23]. A chart recorder for the monitoring of current was connected in parallel to the high-voltage supply circuit through an electric resistance of 1 kΩ. After the channel was filled with a buffer solution 1 [10 m*M* borate containing 12 m*M* sodium lauryl sulfate (SDS) at pH 8.1] and a constant electrical current of 4 μA was obtained, the buffer solution 1 in the buffer, sample and sample waste reservoirs were removed and changed to buffer solution 2 (20 m*M* borate containing 12 m*M* SDS at pH 8.1). The buffer waste reservoir was concurrently refilled with buffer solution 1 again to avoid the hydrodynamic gravity effect [18]. The separation mode was started by the change-over switch. Thus, EOF drove buffer solution 2 to fill the whole channel and finally gave a constant electrical current of 7 μA. The time needed to obtain a constant current is considered to be the EOF rate migration time. Since the electrical field strengths of the four channels in this case were different, the time (t_1) it took buffer 2 to move from the buffer reservoir to the intersection point was different from the time (t_2) to move from the intersection point to the buffer waste reservoir. The migration times t_1 and t_2 fit the following equations:

$$\mu_{\text{eof}} E_1 = \frac{l_1}{t_1} \tag{1}$$

$$\mu_{\text{eof}} E_2 = \frac{l_2}{t_2} \tag{2}$$

$$t = t_1 + t_2 \tag{3}$$

Combining Eqs. (1), (2) and (3) gives Eq. (4) to calculate the magnitude of electroosmotic mobility:

$$\mu_{\text{eof}} = \frac{E_2 l_1 + E_1 l_2}{E_1 E_2 t} \tag{4}$$

where μ_{eof} is the electroosmotic mobility; E_1 and E_2 are the strengths of the electrical fields across the channels from the buffer reservoir to the intersection point and the separation channel, respectively; l_1 and l_2 are the lengths of the two corresponding channels; and t is the time to fill the whole channel with buffer solution 2. In our case, E_1, E_2, l_1, and l_2 are 256.6 V/cm, 209.0 V/cm, 1 cm and 32.2 cm, respectively. Then Eq. (4) can be written as:

$$\mu_{\text{eof}} = \frac{0.158}{t} \tag{5}$$

2.2.2. Traditional CE setup

A laboratory-made CE setup consisted of a high voltage power supply (Matsusada, Precision Inc., Japan), a CE-971E intelligent UV–Vis detector (Jasco, Japan), a C-R4A chromatopac integrator (Shimadzu) and an uncoated fused-silica capillary (I.D. 0.050×O.D. 0.375 mm) from GL Science, Tokyo, Japan. The total length of the fused-silica capillary is 50 cm and the effective length is 29 cm. The UV detection at the cathode end was performed at 215 nm. The fused-silica capillary was pretreated by flushing with 1 *M* sodium hydroxide solution for

5 min, followed by water for another 30 min. Samples are introduced into the capillary by gravity injection method (10 cm for 5 s).

Other instruments used in this work include scanning electron microscope (JSM-6100, Jeol Personal Spec., Japan) and size-exclusion chromatographic (SEC) columns of Shodex GPC KF-802 (Showa Denko, Japan) and JAIGEL 3H-AF (Japan Analytical Industry, Japan).

2.3. Chip fabrication

The polyester microchip was prepared through a photolithographic and a wet-chemical etching procedure followed by replication, which is improved from the previous report [24].

2.3.1. Fabrication of master glass channel template

A mask glass plate was coated with a thin layer of positive photo resist PMER P-RZ300 film by a spin coater (K-359 S-1, Kyowariken, Japan) at 600 rev./min for 2 min and baked in an oven at 90°C for another 15 min. A serpentine channel design shown in Fig. 2a drawn by using the software Adobe Illustrator 8.0 (Adobe Systems, Japan), and printed out on a positive film (Kodak, Japan) was transferred onto the mask plate by exposing it to a long-wavelength ultraviolet lamp (FLB-15, Toshiba, Japan) for 2 min, followed by developing in PMER P-1S for 3 min. The mask plate with channel design was further baked at 90°C for 15 min. The Cr/Cr_2O_3 layer was then removed with the mixture of 17% (w/v) aqueous cerium(IV) diammonium nitrate solution–perchloric acid (95:5, v/v) to define the channel on the mask plate. After rinsing in 2 *M* nitric acid for 5 min, a positive relief channel was obtained by etching the glass in 1 *M* ammonium fluoride–1 *M* hydrofluoric acid solution at room temperature for 12 min to give the height of the relief channel of about 15 μm. After silanization in trimethylchlorosilane–toluene (5:95, v/v) for 24 h at room temperature, the master glass template was kept in a clean box before use.

2.3.2. Chip replication

Chip fabrication consists of two concurrent procedures as shown in Fig. 2b–2d. Four silicone tubes

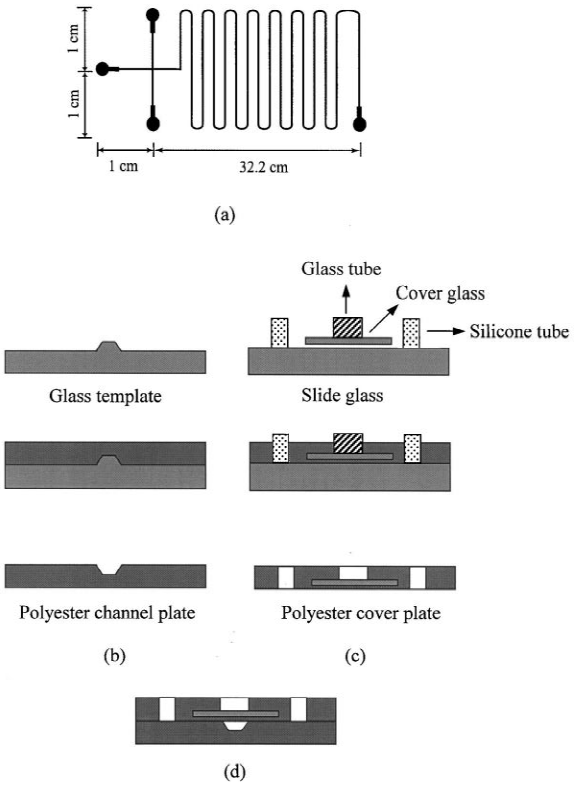

Fig. 2. Chip fabrication procedure. (a) Design of channel network used to prepare polyester chip; (b) channel replication; (c) fabrication of polyester cover plate with a detection window and four buffer reservoirs; (d) channel sealing by bringing the cover plate onto the channel plate for further polymerization.

were placed on a glass plate using water-soluble glue to define four buffer reservoirs. A cover glass was hung over on a *Z*-translation stage 1 mm above the glass plate to serve as a detection window. Fourteen grams of clear polyester was mixed with four drops of curing agent, half of which was poured onto the glass plate with positive relief channel to duplicate the channels, while the other half of which was used to make a cover plate with four reservoirs and a detection window, which was later used to seal the channels. After curing at room temperature for 45 min, the two polyester plates were peeled off from the glass plates and the channel was sealed by putting them together for further crosslinking. The polyester chip was kept in a clean box for use after 2-day complete curing at room temperature.

The same procedure is used for the fabrication of

10-undecen-1-ol modified polyester chips. Twelve grams of native clear polyester was first mixed with 0.72 g of 10-undecen-1-ol and then four drops of curing agent were added to initiate the crosslinking. Channel sealing was performed after curing at room temperature for 60 min.

3. Results and discussion

3.1. Chip fabrication

Fig. 3 shows scanning electron microscopic (SEM) images of the intersection of polyester chip and its master glass template as well as a photograph of the polyester chip prepared in this work. The four small holes in Fig. 3c serve as buffer and sample reservoirs, while the large hole serves as a detection window. High voltage is applied through four platinum electrodes slotted in the four small reservoirs. The volumes of the four reservoirs were calculated to be ca. 50 μl based on the size of the silicone tubes placed to define the reservoirs while curing.

Compared with PDMS and other plastic chips, the polyester chip prepared in this study has a considerably rough outer surface, which is resulted from the evaporation of some molecules from its outer surface when exposing to the atmosphere during curing. To avoid Rayleigh scattering, a cover glass

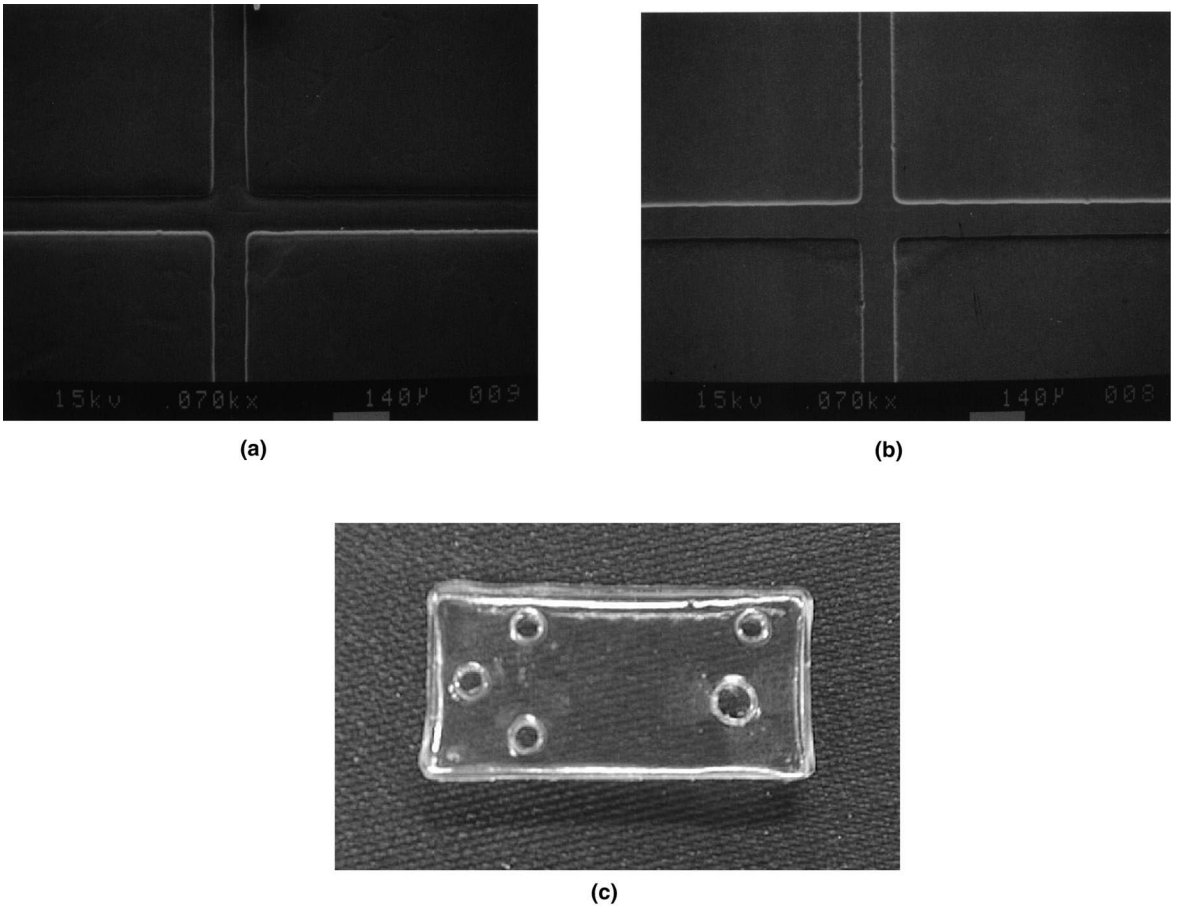

Fig. 3. Microscopic images of channels and a photograph of polyester chip. (a) Intersection of positive relief channel on glass plate (glass template); (b) intersection of channel on polyester plate; (c) photograph of polyester chip prepared and used in this work. Channel width, ~100 μm; channel depth, ~15 μm; chip dimensions, 6.2×3.1×0.8 cm.

was placed into the polyester cover plate to serve as a detection window. The distance from the channel to the cover glass remained 1 mm for every chip made in this experiment, which was adjusted by a *Z*-translation stage. The relationship between the distance and the detection sensitivity was not evaluated in this study because it is not our major aim.

The time to peel the polyester plates from their corresponding glass template is very important for preparing chips without failure. If the curing time is too long, the polyester plates will become hard, which is unfavorable for peeling and also will make the channel sealing much more difficult. Thus, the buffer leakage from between the two polyester plates will happen easily. On the other hand, if the curing time is too short, the channels are easily clogged through further curing for channel sealing. Then the chips have to be discarded. In this study, the right time for peeling was found to be 45 min when curing at room temperature. Controlling the curing temperature is very important for fixing the peeling time. In addition, silanization of glass template will also aid the peeling [14].

Since the microchannels were completely sealed by further curing between the two polyester plates at room temperature, we do not have to use any other sealing methods ever discussed in both preparation of polymer and glass chips, which makes the whole fabrication procedure easy to be carried out in any laboratories.

Microscopic uniformity of the channel proves that the polyester channel can be easily replicated from the master plate. Unfortunately, we could not measure the shrinking degree of the channel structure after its complete curing, although the SEM pictures did not show obvious dimensional changes between the polyester channel and its glass master.

3.2. Chip properties

Unlike elastic PDMS chip, the polyester chip prepared by our procedure is as rigid and hard as any other plastics such as PMMA [13,14]. The introduction of buffer solution into microchannel is easily done using only a simple syringe instead of any complicated mechanical pumps. This may be considered to be one of the advantages of choosing unsaturated polyester as chip compartment. Moreover, the polyester chip has a considerable thermal stability. It can tolerate temperatures up to 120°C without changing its channel structure. Like other plastics, the polyester chip also has very good transparency. The only disadvantage is its optical quality. In its UV–Visible spectrum the absorption appears up to the wavelength of 370 nm, which limits the detection light source in the range of 400–700 nm, resulting in a limitation of the amount of samples to be detected. Therefore, no polymer chip is comparable to silica chip in terms of optical quality.

Since Joule heat is an important parameter affecting capillary electrophoretic column performance, the capability to dissipate Joule heat is a basis for selecting a new material [25]. Although the coefficient of heat conductivity of the polyester used in these experiments was not determined, the Ohm's plot showed a linear relationship between the electrical current and the voltage applied up to 16 kV. A positive deviation appeared at the voltage of 17 kV, indicating insufficient power dissipation. However, the heat dissipation was below 1 W/min within the range of voltages investigated (0–22 kV), which suggests that the capability of polyester to dissipate Joule heat is efficient. Thus, the contribution of Joule heat to the height equivalent to a theoretical plate can be neglected [22,25,26]. The electrical current became zero when the applied voltage is over 22 kV.

3.3. Inner surface of the polyester channels

Since the unsaturated polyester for curing consists of styrene monomer, linear unsaturated polyester with average molecular mass of about 2300 (determined by SEC) and very tiny amount of additives such as silicone dioxide and methylstyrene, a hydrophobic inner surface is expected after curing. In fact, the bubble formation due to the difficulties in wetting the polyester channels by aqueous solution is easily found. We found that the presence of SDS in the buffer can facilitate the introduction of the buffer solution into the hydrophobic polyester channels. Here SDS, reducing the surface tension of aqueous solution, makes it easy to penetrate into the hydrophobic channel [15]. The difficulty in filling aqueous buffer solution into the hydrophobic polyester channels, however, prevented us from performing capil-

lary zone electrophoretic separation. Thus, micellar electrokinetic chromatography (MEKC) is carried out in the case of native polyester chips.

3.4. Electroosmotic flow

EOF is a wall-generated phenomenon that plays an important role in separation. Zeta potential (ζ) of electrical double layer on the inner surface is directly determined by the surface charge density. Since EOF is one of the most important parameters directly connected with a successful separation, its determination and optimization become inevitable.

EOF has been fully discussed in the case of fused-silica capillary tube. Also, the generation of EOF in the silica or glass chip is due to the dissociation of silanol groups on the channel surface at pH≥2 as well as the adsorption of OH^- [27]. The measurement of EOF in the silica or glass chip has been made by indirect fluorometry using water as an unretained neutral marker [20]. More recently, current monitoring method has been employed to measure the magnitude of EOF in polymer channels [15,18,23,28]. Locascio et al. measured electroosmotic mobilities in acrylic, polystyrene, and polyester channels. The lowest EOF rate was found in polystyrene sheets while the highest was found in the polyester sheets which exhibited an EOF similar to a fused-silica capillary [18]. In this study however, due to the complicated polymer components, the factor responsible for the generation of EOF could not be ascertained. Nevertheless, in our previous report, the separation of SRB and SR101 under MEKC condition shows that the direction of EOF in the polyester chip was toward the cathode. The inner surface of the polyester channel might be negatively charged when using 10 m*M* borate as running buffer containing 12 m*M* SDS at pH 8.1 [29].

We have reported MEKC separation of two laser dyes, SRB and SR101, using polyester chip and its comparison with traditional silica capillary tube [29]. Since the comparison was made under the same condition, it is possible to predict the magnitude of EOF in the polyester channels, as reported by Duffy et al. [15]. In the case of MEKC, the observed electrophoretic mobility of the analyte can be expressed as follows:

$$\vec{\mu}_{\mathrm{obv}} = \vec{\mu}_{\mathrm{eof}} + \vec{\mu}_{\mathrm{eff}} \quad (6)$$

where $\vec{\mu}_{\mathrm{ob}\upsilon}$ is the observed electrophoretic mobility of the analyte; $\vec{\mu}_{\mathrm{eof}}$ is the electroosmotic mobility; and $\vec{\mu}_{\mathrm{eff}}$, the effective mobility of the analyte which is determined by the charge, mass and shape of the analyte molecules as well as its partition between the free buffer solution and micelle phase [30]. The analyte will have the same μ_{eff} values in both polyester chip and fused-silica capillary tube provided that other conditions in both cases are the same. Therefore, the magnitude of electroosmotic mobility in polyester chip can be predicted by Eq. (7):

$$\vec{\mu}_{\mathrm{eof}}^{\mathrm{Chip}} = \vec{\mu}_{\mathrm{obv}}^{\mathrm{Chip}} + \vec{\mu}_{\mathrm{eof}}^{\mathrm{Tube}} - \vec{\mu}_{\mathrm{obv}}^{\mathrm{Tube}} \quad (7)$$

The EOF in the fused-silica capillary tube was determined to be 6.961×10^{-4} $cm^2/V\,s$ using toluene as a neutral marker. Electropherograms gave the values of observed mobilities of 5.199×10^{-4} $cm^2/V\,s$ and 3.455×10^{-4} $cm^2/V\,s$ in the chip and fused-silica tube, respectively. Substituting values of $\vec{\mu}_{\mathrm{ob}\upsilon}^{\mathrm{Chip}}$, $\vec{\mu}_{\mathrm{eof}}^{\mathrm{Tube}}$, and $\vec{\mu}_{\mathrm{ob}\upsilon}^{\mathrm{Tube}}$ into Eq. (7) yields the predicted value of EOF in the polyester channels as 8.705×10^{-4} $cm^2/V\,s$. We also measured the electroosmotic mobility using current monitoring methods to be 8.090×10^{-4} $cm^2/V\,s$ ($n=3$), a little bit smaller than the calculated value.

It can be seen that the electroosmotic mobility in the polyester channel is greater than that in the fused-silica tube, suggesting a much greater surface charge density. This makes us think about what functions incorporated in the polyester contribute to form an electrical double layer on the inner surface of channels and drive the bulk buffer solution toward the cathode. It is very likely that the carboxyl group on the channel surface is mainly responsible for EOF formation, while some potential-determining ions (OH^-) adsorbed on the wall also cannot be excluded [8]. As discussed for fused-silica capillaries, carboxyl groups should also be deprotonated as buffer pH increases, resulting in an increase in charge density on the inner wall, therefore a dramatic increase of EOF was observed [27]. We investigated the effect of buffer pH on the migration time of SRB and SR101 in the presence of 12 m*M* SDS. The results shown in Fig. 4 indicated that there were no obvious changes

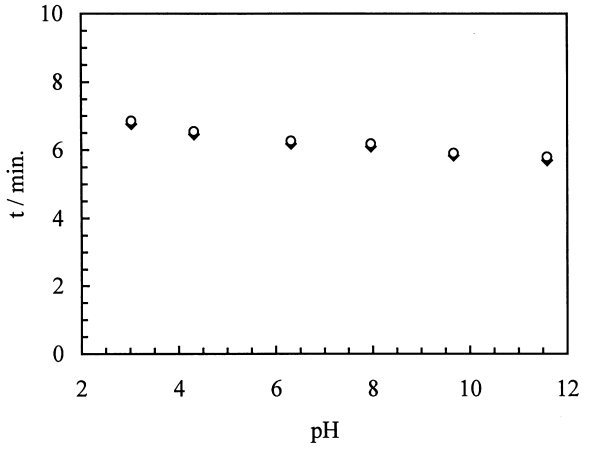

Fig. 4. Effect of buffer pH on the migration time of SRB and SR101. Running buffer, boric acid-citric acid-sodium phosphate at the constant ion strength of 30 m*M* in the presence of 12 m*M* SDS. ♦, SRB and ○, SR101.

in migration time as buffer pH increased up to 11.6. It was also noted that even at very low buffer pH of 3.0, EOF was observed, which was not the case in the fused-silica capillary tubes. Fig. 5 shows the electropherogram of SRB and 101 separation at pH 3.0 in the presence of 12 m*M* SDS. It seems that surface charge density in this case does not change with the change of buffer pH, which is not consistent with what Roberts et al. have reported [17]. To prove

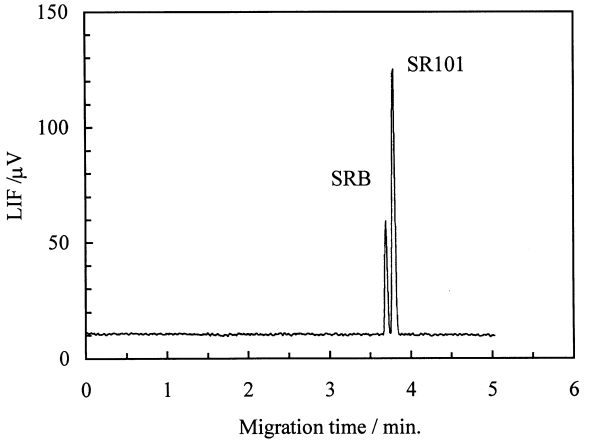

Fig. 5. Electropherograms of separation of two laser dyes by native polyester channels under MEKC conditions at low buffer pH. Running buffer, 20 m*M* borate-sodium phosphate containing 12 m*M* SDS at pH 3.0; other conditions see Experimental section.

this, the effect of buffer pH on the electroosmotic mobilities in the native polyester channels was investigated. Fig. 6 shows the plot of the magnitude of electroosmotic mobilities vs. buffer pH. It can be seen that electroosmotic mobilities in the native polyester channels do not exhibit obvious changes with buffer pH. The electroosmotic mobility even exhibited quite a large value of 7.303×10^{-4} cm^2/V s at pH 3.0, which has not been observed in the cases of both glass or polymer channels and tubes; As shown in Fig. 6a, electroosmotic mobilities in fused-

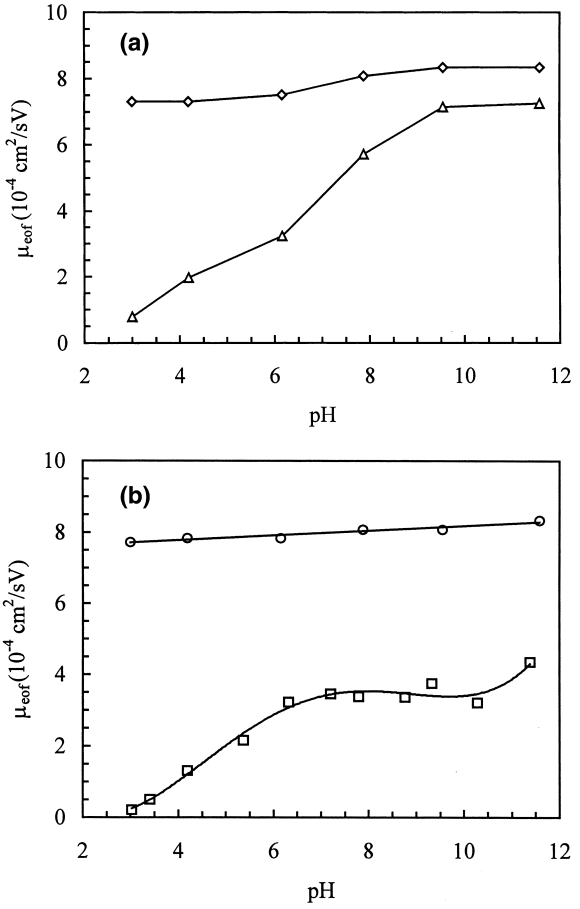

Fig. 6. Effect of buffer pH on electroosmotic mobilities. (a) Running buffer, boric acid–citric acid–sodium phosphate at the constant ion strength of 30 m*M* in the presence of 12 m*M* SDS; ◇, native polyester chip; △, fused-silica capillary; (b) running buffer, boric acid–citric acid–sodium phosphate at the constant ion strength of 30 m*M*. ○, in the presence of 12 m*M* SDS, and □, in the absence of SDS. Chip, 10-undecen-1-ol modified polyester chip.

silica capillary tubes increased from 7.94×10^{-5} $cm^2/V\ s$ at pH 3.0 to 7.15×10^{-4} $cm^2/V\ s$ at pH 9.55 and then the curve remained flat. Considering this phenomenon and the ease of filling buffer solution into the hydrophobic channels in the presence of SDS as previously mentioned, it may be assumed that SDS molecules which are adsorbed onto the inner surface due to the hydrophobic–hydrophobic interaction hide the intrinsic acidic functional groups on the polyester channels, forming a new dynamic double layer which makes the surface charge density remains constant even when the buffer pH values change. If this assumption holds, it is expected that EOF can be suppressed by adding non-ionic surfactant into the running buffer. The magnitude of EOF in the presence of polyoxyethylene 23 lauryl ether (Brij 35) instead of SDS at concentration of 20 mg/ml was measured. As expected, we did not observe any changes of electric current within the pH range investigated from 3 to 11.6, which means EOF, in this case, was almost zero. This proved that the previous assumption is true, which is also consistent with the case of isotachophoresis where EOF is suppressed using cellulose derivatives in organic synthetic materials instrumentation [8].

As discussed by Wang et al. [28], the concentration of surfactant in the running buffer may have some effects on electroosmotic mobilities; In their case, with the increase of the surfactant concentration, the electroosmotic mobilities decreased rapidly. At higher concentration, the direction of the electroosmotic mobilities was reversed. In our case, the increase of the electroosmotic mobilites would be expected with the increase of the concentration of SDS in the running buffer [31]. However, the magnitude of electroosmotic mobility in the presence of SDS at the concentration of 1 m*M* gave the same value as that at SDS concentration of 12 m*M* in the running buffer. It suggests that the permanent electric double layer has been formed at the concentration of 1 mM; thus, the surfactant concentrations did not have any effect on EOF. Therefore, extremely low concentrations of the surfactants are necessary for investigation. Unfortunately, this experiment could not be carried out due to the difficulties of filling the channels with the buffer solution at very low surfactants concentrations.

As mentioned above, SDS molecules not only involve in a separation behaving as a pesdo-stationary phase by forming micelles in buffer solution, but also contribute themselves to the formation of dynamic electric double layer on the surface of the polyester channels. At least, they reduce surface tension, and make the buffer solution accessible into the hydrophobic channels. Based on this point, channel surface modification is feasible either by coating or chemically bonding.

3.5. Polyester microchannels with modified inner surface

In the case of unsaturated polyester, the inner surface of the channels can be modified by adding functional compounds with double bonds into native unsaturated polyester before curing. In this work, polyester chip modified with 10-undecen-1-ol was prepared. It was found that the addition of alcohol improved the hydrophilic properties of the inner surface. It can be used for CZE separation and investigation the real effect of buffer pH on the ζ potentials in the polyester channels. The effect of buffer pH on electroosmotic mobilities in the modified polyester channels is shown in Fig. 6b. A curve similar to a titration curve was obtained as previously obtained with polymer tubes [8]. The inflection point of the curve appeared around the pH value of 4.6, corresponding to the pK_a value of carboxylic acids. The carboxylic groups incorporated in the polyester might partly contribute to surface charge which directly determines the ζ potentials on the polyester channels. When pH is over 10, another increase in electroosmotic mobilites was observed, which might be originated from some other potential-determining ions adsorbed onto the channel surface. In addition, the plot of the effect of buffer pH in the presence of 12 m*M* SDS on electroosmotic mobilities in the modified channels exhibited the same tendency as in the case of native polyester channels (Fig. 6b). It suggests that the adsorption also happened in the modified channels, which further proved our previous assumption.

The separation of two laser dyes was obtained by modified polyester channels using 50 m*M* borate solution as running buffer at pH 9.6 (Fig. 7). As can be seen, the polyester channels (Fig. 7a) exhibited

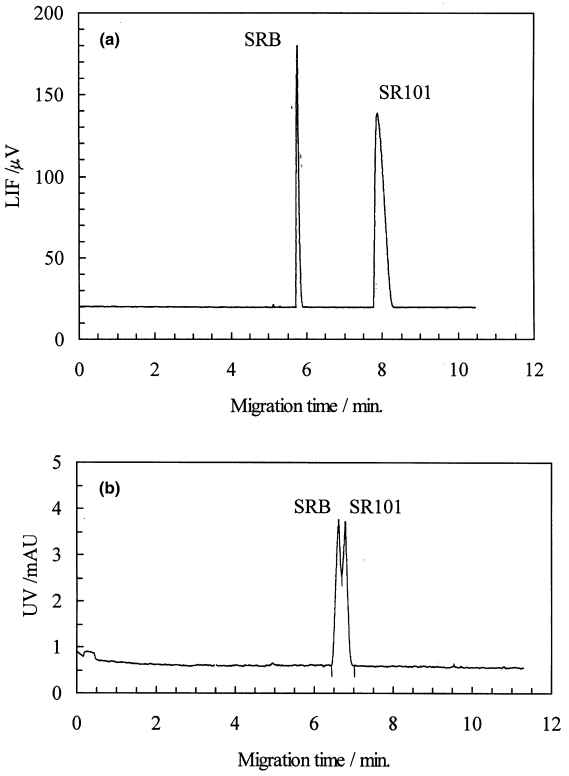

Fig. 7. Electropherograms of separation of two laser dyes using 50 m*M* borate as running buffer at pH 9.6. (a) 10-Undecen-1-ol modified polyester channels (6%, w/w); and (b) fused-silica capillary tube. Other conditions see Experimental section.

more than 10 times higher resolution efficiency compared to the conventional fused-silica capillary tubes (Fig. 7b). 10-Undecen-1-ol modified polyester channels were compared with the conventional fused-silica capillary tubes in Table 1. The electroosmotic mobility in polyester channels was found to be similar with that in the fused-silica capillaries, which is considered to be a crucial parameter that determines a separation. Thus, the high resolution obtained by modified polyester was assumed to result from the interaction between sample molecules and functional groups on the inner surface of the channels. It is very likely that benzene rings and undecanol groups, which are stretching out from the channel surface toward the buffer solution, are responsible for the interaction. If this assumption is true, open-channel chromatography in polymer chips will become possible. In addition, it was noticed in Fig. 7a that the bandwidth of SR101 is broader than that of SRB, corresponding to eight times lower separation efficiency. This may be resulting from the stronger interaction between SR101 molecules and the functional groups (1-undecanol) on the inner surface of the channels as well as the low diffusion speed of SR101 molecules. This part of the work is now under investigation.

4. Conclusion

Combined with our low-cost and easy-performed fabrication procedure, unsaturated polyester can be considered to be a promising alternative material for plastic microchip. The polyester chips prepared in this work possess considerable thermal and mechanical stability as well as relative solvent resistance. The binary buffer system mixed with organic solvents, such as methanol, can be used. To our experience, the maximum mixable volume content of methanol in the buffer solution could be 50%. Furthermore, the polyester chips can be used repeatedly for more than 1 month with due care, although they can be disposed after use for once or several times.

Even at low buffer pH, zeta potential of the double layer formed by the adsorption of SDS molecules on

Table 1
Comparison of modified polyester chip with fused-silica tube

	Modified polyester chip	Fused silica tube
Electroosmotic mobility ($cm^2\ V^{-1}\ s^{-1}$)	5.918×10^{-4} ($n=3$)	5.442×10^{-4} ($n=3$)
Eff. EP mobility ($cm^2\ V^{-1}\ s^{-1}$)	-2.012×10^{-4} (B)	-1.591×10^{-4} (B)
	-3.097×10^{-4} (101)	-1.687×10^{-4} (101)
Peak efficiency (m^{-1})	7.935×10^{4} (B)	1.908×10^{4} (B)
	1.161×10^{4} (101)	4.004×10^{4} (101)
Resolution (R_s)	6.204	0.585

the channel surface remain almost the same as that at high buffer pH. It provides a very special condition for the separation of those compounds that are not stable or tend to deposit at higher pH. It can be carried out without slowing down the separation speed. Alternatively, the presence of non-ionic surfactants can suppress EOF to be almost zero, through which isoelectric focusing on chip can be easily performed. Also, modification of the inner surface of the polyester channels through chemical bonding will make the separation of a broad range of compounds possible by open channel electrochromatography on polymer chips.

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