

Original

## Studies on Polyester Chips with Modified Inner Surface for Open-Channel Electrochromatography

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### Abstract

Modification of polyester microchip inner surface was carried out through incorporation of functional moieties into polyester matrix during chip fabrication procedure. Olefin alcohols with different alkyl chain length were chosen to serve as stationary phases for open-channel electrochromatography. The modified polyester microchannels exhibited a high-resolution efficiency. The 10-undecene-1-ol modified polyester chip was evaluated in terms of its mass loadability, effect of chain length of olefin alcohols, effect of the content of 10-undecene-1-ol, effect of buffer pH, effect of methanol content in running buffer, and reproducibility. The study on van Deemter plot revealed the factors resulting in band broadening in 10-undecene-1-ol modified polyester chips. The considerable large retention of samples on 10-undecene-1-ol modified polyester channels showed that 10-undecene-1-ol plays a significant role in separation. The migration order of Rhodamine B could be regulated by the content of 10-undecene-1-ol in the polyester matrix. The separation of laser dyes was based on the hydrophobic-hydrophobic interaction, while static interaction between charged components and negatively charged channel walls also cannot be excluded.

**Keywords:** microchip, electrophoresis, open-channel chromatography

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### Introduction

With the emerge of microfluidic devices [1], interest in microchip electrochromatography (EC) is growing up rapidly due to its capability of expanding the range of compounds that can be separated and analyzed on microchips. This area is still considered to be unexplored since so far only few publications are related to on-chip separation by EC [2-4].

The technical difficulties encountered in preparing frits might still be a barrier for the widespread application of packed column EC on chips. Hjertén and co-workers have proved that by using in situ polymerized continuous bed in the chip channels could eliminate such problem [2]. In that case, however, the complicated manipulation in both preparations of continuous polymer bed and later sample injection as well as separation appeared to be another problem, thereby, hindering its further development. In view of the facts mentioned above, open-tubular techniques that utilize capillary wall as separation matrix in capillary EC (CEC) would be

much more suitable for performing EC on chips owing to its obvious advantages over monolithic columns — much simpler instrumentation, easier preparation and manipulation.

Open-tubular EC has been extensively discussed for several years [5-11]. Theories and some column techniques that have been developed and being used in open-tubular EC might be acceptable in EC on chips. The first operation of EC on chips could be traced back to 1994 reported by Jacobson [3]. In their report, the surface of the channel was chemically modified with octadecylsilane to serve as a reversed phase, on which the separation of three neutral dyes was attained. The significant contributions to total plate height of the chip systems were discussed in detail. The same group later reported solvent-programmed microchip EC [4]. The channel was prepared through static coating. The column preparation techniques employed on chips are still chemically bonding through -Si-O-Si-C, or coating. These techniques are always associated with short sample retention time due to low phase ratio,

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low column efficiency, and poor pH resistibility, hampering the applications of EC to chips. Relatively small channels (usually with the channel height in the vicinity of 5–10  $\mu\text{m}$ ) will arise other problems, such as low mass loadability, difficulties in column preparation. To find an alternative approach and solve these problems out is becoming more and more important.

From the practical point of view, polymer materials may be a promising separation matrix capable of offering sufficient solute retention, good column stability and relatively high phase ratio except the disadvantage of low column efficiency resulted from the slow diffusion of the solutes in the stationary phase [12–16]. Recently, we developed a method for the fabrication of polyester microchips [17], through which functional groups are added into pre-polymers and then undergone polymerization. In this case, any kind of moieties, severed as stationary phase, can be incorporated into the polymer matrix during the chip fabrication procedure, which is similar with the sol–gel technology for the preparation of stationary phases [8]. By this method, modification of the inner surface of channels can be easily carried out.

Stationary phases modified with diol–group are favorable for proteins and dipeptides analysis due to their hydrophilic surface compared with conventional  $\text{C}_{18}$  and  $\text{C}_8$  stationary phases in high performance liquid chromatography (HPLC) [9]. In this study, modified polyester channels with olefin alcohols having different chain lengths were prepared and their chromatographic separation performances were investigated. Microchips modified with 10–undecene–1–ol were evaluated in terms of mass loadability, chain length of the modifiers, the content of 10–undecene–1–ol, buffer pH and methanol content in background electrolyte, channel length, as well as reproducibility. Band broadening in 10–undecene–1–ol modified polyester channels was also discussed.

## Experimental Section

**Chemicals.** For chip fabrication, DUFR–2506 (p)–L mask blank coated with a thin layer of 50 nm Cr/Cr<sub>2</sub>O<sub>3</sub> was received from Ul-coat (Tokyo, Japan). Positive photo–resist PMER P–RZ 300 and developer PMER P–1S were obtained from Tokyo Oka Kogyo (Tokyo, Japan). Unsaturated linear polyester for curing (Clear polyester<sup>®</sup>, two–liquid type) was obtained from Epoc (Osaka, Japan). 4–pentene–1–ol, 7–octene–1–ol and 10–undecene–1–ol were purchased from Tokyo Kasei Kogyo Co. Ltd. (Tokyo, Japan).

For chromatographic evaluation, Sulforhodamine B (SRB) and Sulforhodamine 101 (SR 101) used as test samples were obtained from Kanto Chemicals (Tokyo, Japan). Rhodamine B (RB) was obtained from Tokyo Kasei Kogyo Co. Ltd. (Tokyo, Japan). Borate buffer with the concentration of 25 mM at pH 9.6 is used as background electrolyte. To avoid sample stacking, SRB, RB, and SR 101 were dissolved in 50 mM borate buffer at pH 9.6 to give

the concentration of  $3 \times 10^{-5}\text{M}$ ,  $1 \times 10^{-5}\text{M}$  and  $1 \times 10^{-5}\text{M}$ , respectively. Boric acid–citric acid–sodium phosphate with the constant ion strength of 30 mM is chosen as a buffer system for the study of pH effect. Other chemicals are of analytical grade. Peak identification was accomplished by injection of a mixture of samples spiked with the corresponding compound.

**Apparatus.** The total analytical system used in this study is the same as described previously [17]. Briefly, it consists of a 1 mW of He/Ne laser (543 nm, Model LHGP–0051, Japan laser Co., Shinjuku, Tokyo, Japan) as a light source, an optical fiber (core 400  $\mu\text{m}$ , Polymicro Technologies, Phoenix, Arizona, USA) for collecting fluorescence, a mechanical chopper ( $f = 825\text{ Hz}$ , NF Circuit Design Block, Yokohama, Japan), a photomultiplier tube (Hamamatsu Photonics R 535, Hamamatsu, Japan), a lock–in amplifier (LI 574, NF Circuit Design Block, Tokyo, Japan) and an integrator (Chromatopac CR–4 A, Shimadzu, Kyoto, Japan).

A serpentine channel design with four reservoirs shown in Figure 1 was used in this study. Pinched injection and three–way separation modes were employed according to Jacobson's scheme [18]. Three high voltage supplies were applied to four reservoirs through a home–made changeover. The determination of electroosmotic flow (EOF) was accomplished through current monitoring method [19]. The magnitude of EO mobility was calculated by the following equation [17],

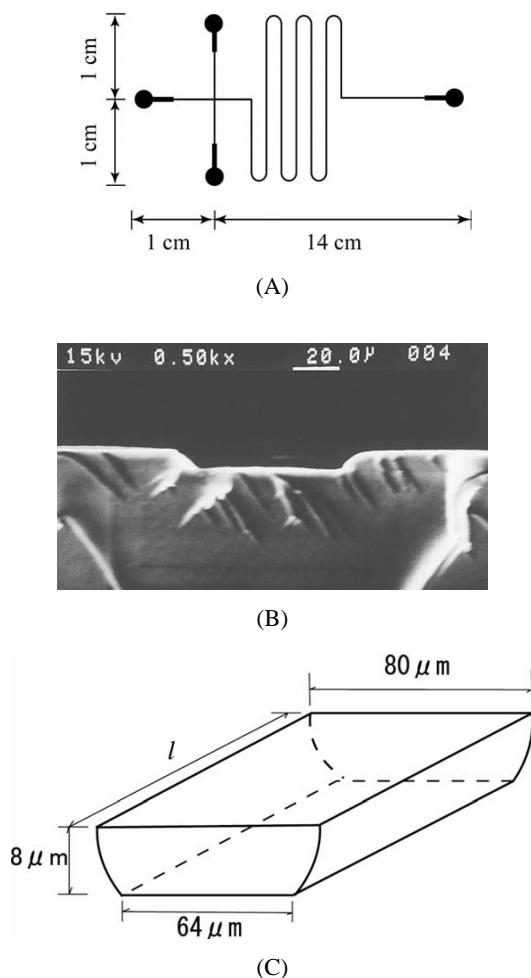
$$\mu_{EO} = \frac{E_2 l_1 + E_1 l_2}{E_1 E_2 t} \quad (1)$$

where  $\mu_{EO}$  is the electroosmotic mobility;  $E_1$  and  $E_2$  are the electrical field strengths in the channel from buffer reservoir to intersection point, and separation channel, respectively;  $l_1$  and  $l_2$  are the lengths of the two corresponding channels; and 't' is the duration for the electric current of the whole channel to reach stable.

To perform conventional capillary electrophoresis (CE) separation, a laboratory–made CE setup consisting of a high voltage power supply (Matsusada, Precision Inc., Kusatsu, Shiga, Japan), a CE–971 E intelligent UV–Vis detector (Jasco, Tokyo, Japan), an uncoated fused silica capillary (No. 1010–31142, 50  $\mu\text{m}$ –i.d., GL Science, Tokyo, Japan) and a C–R4A Chromatopac integrator (Shimadzu, Kyoto, Japan) was used. The UV detection at the cathode end was performed at 280 nm. Acetone was used as neutral marker for the determination of EOF.

All separations were carried out at the electric field strength of 200 V/cm, otherwise stated.

**Fabrication of master channel.** The fabrication of master channel followed the procedure reported previously [17]. Briefly, a serpentine channel pattern shown in Figure 1 (A) was transferred onto a Cr–coated glass plate with a thin layer of positive photoresist by photolithography. After removing the Cr layer of the glass plate,



**Figure 1.** Serpentine channel pattern (A) and scanning electronic microscopic image of the cross section of polyester channel (B). (C): Channel height, 8  $\mu\text{m}$ ; Upper width, 80  $\mu\text{m}$ ; and Bottom width, 64  $\mu\text{m}$ .

the positive relief channel on the glass plate was obtained through wet-chemical etching in 1 M  $\text{NH}_4\text{F}$  / HF solution at room temperature.

**Preparation of olefin alcohol modified polyester chips.** Fourteen grams of clear polyester was premixed with 1.4 ml of 10-undecene-1-ol, and then into this mixture was added 80  $\mu\text{l}$  of curing agent to initiate cross linking. After curing at room temperature for about 45 min., the two polyester plates were peeled off from the corresponding glass plates respectively and then brought into contact. Channel sealing was accomplished through further curing between the two polyester plates for another 12 hours. After complete curing for 24 hours, chips are ready for use.

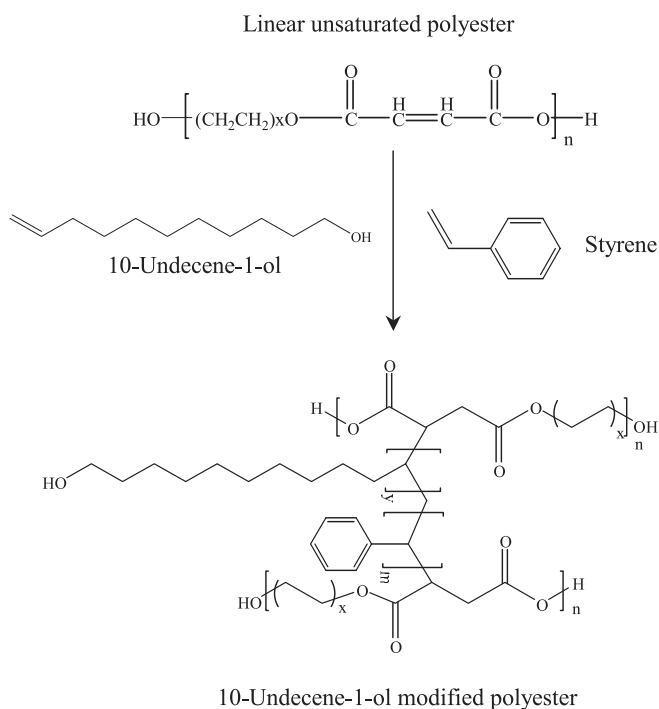
For the fabrication of 4-pentene-1-ol and 7-octene-1-ol modified polyester chips, 0.69 ml 4-pentene-1-ol and 1 ml 7-octene-1-ol were added into the clear polyester instead of 10-undecene-1-ol, respectively. Other steps were the same.

**Channel structure.** Scanning electron microscopic (SEM) image and channel dimensions are shown in Figure 1 (B). Due to isotropic etching, polyester channel is in trapezoidal shape (cross section shown in figure 1 (C)). The upper width of the channel is  $\sim 80 \mu\text{m}$ , the bottom width is  $\sim 64 \mu\text{m}$ , the channel width at the half height is  $\sim 72 \mu\text{m}$ , and the height is  $\sim 8 \mu\text{m}$ . The aspect ratio of the channel is approximately 9. The surface-to-volume ratio of the channel used in this study is approximately  $0.282 \text{ m}^{-1}$ .

## Results and Discussion

### Modification procedure:

The exact structure of linear unsaturated polyester is not available due to enterprise secrets. Generally, raw materials used for synthesizing unsaturated polyester includes anhydrous maleic acid or fumaric acid, phthalic acid, glycols, vinyl monomers and some other additives. The main structure of the linear unsaturated polyester is shown in Figure 2. Polyester used in this work is the mixture of unsaturated polyester, styrene and some other additives according to the data sheet presented by the producer. Since the content of styrene has been determined to be approximately 35% [20], the amount of olefin alcohol added into the clear polyester can be adjusted by controlling the molar ratio of enol-to-styrene. The possible modified polyester network with 10-undecene-1-ol is also illustrated in Figure 2.



**Figure 2.** Schematic diagram of modification procedure of native polyester with 10-undecene-1-ol.

### Preparation of modified polyester chips:

Peeling time is a crucial parameter to accomplish a successful fabrication [17]. It is easy to control the time in the case of native polyester especially when the curing temperature is controlled, since the time duration between gelling and complete curing is considerably long. While the addition of other monomers for crosslinking would change the curing time and usually make it shorter, so that it is difficult to know the exact time for peeling. Most experiments are, therefore, based on trial and error when choosing different monomer additives. In the cases of 4-pentene-1-ol, 7-octene-1-ol and 10-undecene-1-ol, the right peeling time was found to be 40 minutes for 14 grams of unsaturated polyester with 80  $\mu$ l of curing agent (the maximum molar ratio of enol-to-styrene is 0.143).

To our experience, the maximum content that can be added for modifying 14 g of native polyester, in the case of 10-undecene-1-ol, is 1.4 ml, which in the molar ratio of enol-to-styrene is 0.143. As for 4-pentene-1-ol, the maximum ratio can reach up to 0.333. In that case, however, the possibility of failure is considerably high.

### Hydrophilicity of the modified polyester channels:

As we previously reported [17], the native polyester channels have a relatively hydrophobic inner surface. It is difficult to wet the channel surface with aqueous buffer solution without containing any organic solvents. The results from the measurement of contact angle showed that the addition of olefin alcohol greatly improved the channel hydrophilicity, ca. 10 ° changes in the case of 10-unde-

cene-1-ol. It was found that there are no obvious changes in the contact angles of the channels modified with 4-pentene-1-ol. The reason can be attributed to the longer alkyl chain of 10-undecene-1-ol, the hydroxyl groups of which can stretch out of the benzene rings of the styrene molecules on the channel surface, thus, exhibiting a distinct effect on the channel hydrophilicity. The easy access of aqueous solution into the 10-undecene-1-ol modified channels also proved the remarkable influence of the chain length. For 4-pentene-1-ol and 7-octene-1-ol with the same enol-to-styrene ratio as 10-undecene-1-ol, bubbles are formed when filling the channels with aqueous electrolytes.

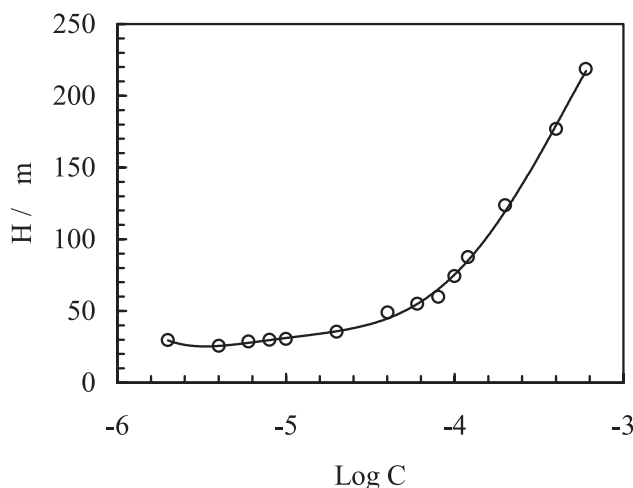
### Mass loadability.

In EC, for charged samples, solute retention can be obtained with the aids of differences in their inherent electrophoretic mobilities, as in CE, and the interaction with the stationary phases, as in HPLC. In order to determine the chromatographic retention factors of the test solutes on the inner surface of the polyester microchannels modified with 10-undecene-ol, the separation was performed using both conventional fused silica capillaries and modified polyester chips under the same conditions. The corresponding data are listed in Table 1. It was found that the EO mobility is a little bit smaller than that in the fused silica capillary. The retention factors of SRB and SR 101 on the modified channel surface, induced by purely chromatographic process, were 0.101 and 0.267, respectively, corresponding to a low phase ratio. Though with such low solute retention, the modified channels still exhibited a good resolution for the test samples, due to high column efficiency.

**Table 1.** Comparison of modified polyester chip with fused silica capillary.

		Fused silica capillary		Modified polyester chip
obs ( $\text{cm}^2\text{s}^{-1}\text{V}^{-1}$ )	SRB	5.084	$10^{-4}$	3.926
	SR101	5.001	$10^{-4}$	3.347
eff ( $\text{cm}^2\text{s}^{-1}\text{V}^{-1}$ )	SRB	-1.358	$10^{-4}$	-1.755
	SR101	-1.441	$10^{-4}$	-2.334
eo ( $\text{cm}^2\text{s}^{-1}\text{V}^{-1}$ )		6.442	$10^{-4}$	5.681
$k_{\text{obs}}$	SRB	0.267		0.447
	SR101	0.288		0.697
k	SRB	-		0.101
	SR101	-		0.267
Rs	0.61	1		4.687

**Table 1.** Running buffer, 25 mM  $\text{H}_3\text{BO}_3$ -NaCl / NaOH pH 9.6; Electric field strength, 200 V/cm; (a) fused silica capillary (I. D.50  $\mu\text{m}$ , O. D.375  $\mu\text{m}$ ); (b) 10-undecene-1-ol modified polyester chip, enol-to-styrene ratio, 0.143; Other conditions see experimental section.



**Figure 3.** Effect of sample concentration on plate height for 10-undecene-1-ol modified polyester chip (enol-to-styrene ratio, 0.143). Conditions as in Table 1.

Relatively low phase ratio would inevitably arise another problem, low mass loadability. Column overload is one of the practical problems that readily happen in open tubular chromatography by injecting larger sample amount, which results in wide, distorted bands, reduced retention times, and consequently poorer resolution [21]. The mass loadability of the modified polyester chips was assessed by injecting different concentrations of the most retained solute, SR 101, into the channel cross section. Figure 3 illustrated the relationship between plate height and sample concentration for the channels modified with 10-undecene-1-ol. As can be seen in Figure 3, the height equivalent to a theoretical plate started to increase at a concentration of  $2 \times 10^{-5} \text{M}$ , which demonstrates overloading.

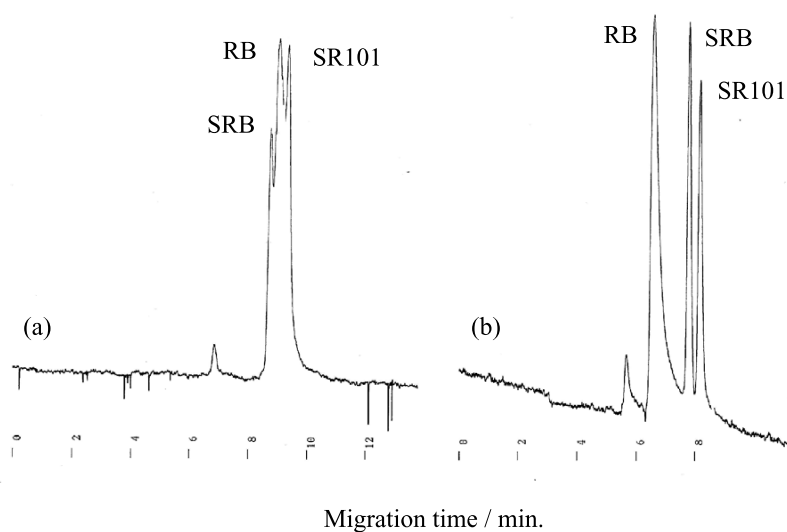
#### Effect of chain length.

Olefin alcohols used in this work are the group of enols hav-

ing a hydroxyl group at one end of alkyl chain and a double bond at the other end. The influence of the chain length on resolution was investigated using 4-pentene-1-ol, 7-octene-1-ol and 10-undecene-1-ol modified polyester channels. As a comparison, the separation was also carried out under the same conditions in the fused silica capillary where the separation is only resulted from difference in electrophoretic mobilities. The effect of chain length on the resolution and EO mobilities in a native polyester chip, 4-pentene-1-ol, 7-octene-1-ol or 10-undecene-1-ol modified polyester chips are presented in Table 2. Since the channels modified with 4-pentene-1-ol as well as 7-octene-1-ol have less hydrophilicity than that with 10-undecene-1-ol, as discussed above, aqueous solution gave unstable electric current in these channels when high voltages were applied. Consequently a buffer containing 20% (v/v) content of methanol instead of aqueous electrolytes was used for chip evaluation. The separation tended to become poorer with increase of the alkyl chain length, and the best resolution was obtained in the native polyester channels. While 4-pentene-1-ol modified channels offered sufficient retention for SRB and SR 101 ( $k_{SRB} = 0.248$ , and  $k_{SR101} = 0.494$ , induced by purely chromatographic process). It is reasonable that poor separation was due to the changes from hydrophobic channel surface to hydrophilic one, and the partition coefficient becomes smaller. Interestingly, the EO mobilities in the three modified channels exhibited a decreasing tendency with increase in the carbon number of the alkyl chains. This reason is not clear due to complicated polymer network. In the buffer solution with 20% (v/v) methanol, the channel surface modified with 10-undecene-1-ol has much more similarity with the inner surface of the fused silica capillary, since almost no retention was found for SRB and SR 101. The separation was induced only by the different electrophoretic mobilities.

**Table 2.** Comparison data. 4-P-1-ol, and 10-U-1-ol are 4-pentene-1-ol, 7-octene-1-ol and 10-undecene-1-ol, respectively.

	Fused silica capillary	Polyester microchip			
		Native	4-P-1-ol	7-O-1-ol	10-U-1-ol
$\mu_{SRB}^{obs}$ ( $\text{cm}^2 \text{s}^{-1} \text{V}^{-1}$ )	$2.766 \cdot 10^{-4}$	$2.472 \cdot 10^{-4}$	$2.804 \cdot 10^{-4}$	$2.287 \cdot 10^{-4}$	$1.944 \cdot 10^{-4}$
$\mu_{SR101}^{obs}$ ( $\text{cm}^2 \text{s}^{-1} \text{V}^{-1}$ )	$2.741 \cdot 10^{-4}$	$2.130 \cdot 10^{-4}$	$2.325 \cdot 10^{-4}$	$2.155 \cdot 10^{-4}$	$1.911 \cdot 10^{-4}$
$\mu_{eo}$ ( $\text{cm}^2 \text{s}^{-1} \text{V}^{-1}$ )	$3.752 \cdot 10^{-4}$	$3.602 \cdot 10^{-4}$	$4.485 \cdot 10^{-4}$	$3.306 \cdot 10^{-4}$	$2.922 \cdot 10^{-4}$
$k'_{SRB}$	-	0.058	0.248	0.015	0
$k'_{SR101}$	-	0.216	0.494	0.065	0.003
<b>Rs</b>	0.488	4.5431	3.5698	1.401	0.544



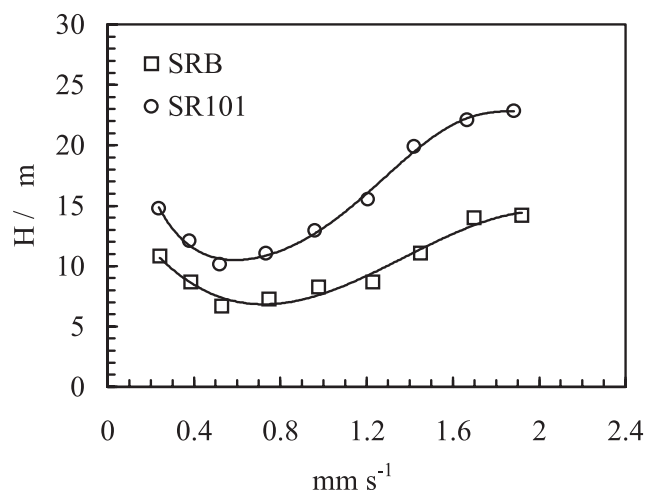
**Figure 4.** Dependence of migration order of SRB, RB and SR 101 on the content of 10-undecene-1-ol. (a) enol-to-styrene ratio, 0.067; (b) enol-to-styrene ratio, 0.091. Other conditions as in Table 1.

#### Effect of 10-undecene-1-ol content.

The hydrophilicity of the polyester channels also can be controlled by the amount of 10-undecene-1-ol added, consequently affecting separation. Figure 4 shows the electropherograms of three laser dyes, SRB, RB and SR 101 using modified polyester chips with different content of 10-undecene-1-ol. RB has the similar chemical structure to SRB. In the buffer solution over pH 9, RB is considered to be neutral, while SRB is negatively charged. Theoretically speaking, in the case of the fused silica capillary, neutral RB should be eluted faster than negatively charged SRB. As discussed above, relatively high hydrophilic channel surface would behave much more like the fused silica capillary. This is proved by the electropherogram shown in Figure 4 b, where the modified polyester chip had the enol-to-styrene ratio of 0.091, and a rather hydrophilic channel surface. The reversed migration order of SRB and RB on the modified channels with the enol-to-styrene ratio of 0.067 revealed that hydrophobic interaction gave the dominant contribution to the solute retention, which is further proved by the separation of SRB, SR 101 and RB on the hydrophobic channels modified with 4-pentene-1-ol. RB was found to be the most retained solute in this case.

#### Relationship between plate height and migration velocity.

It is very important to well understand significant factors, which contribute to band broadening for a specific separation system. Figure 5 shows the relationship between linear velocity and plate heights. The linear velocity is adjusted by varying the electric field strength. Consistent with the instances reported [3,4], there is

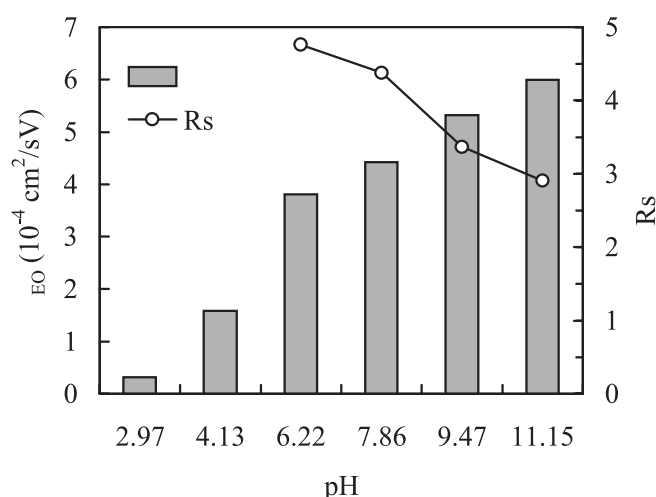


**Figure 5.** Dependence of plate height equivalent to a theoretical plate number on the migration velocity. Conditions as in Table 1.

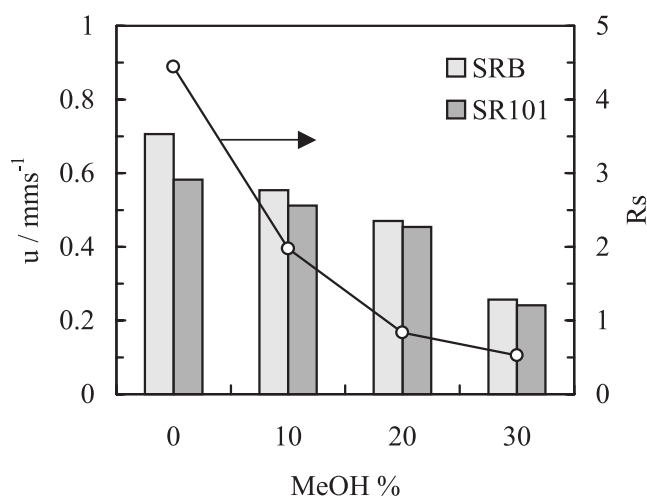
an optimal linear velocity at which the plate height has a minimum value, i.e., the highest column efficiency. As can be seen, the curve in Figure 5 has the same tendency as van Deemter type plot. A loss in column efficiency happened with increasing the velocity of the solute, which suggests dominant contribution to plate height at higher velocity is from axial diffusion, that is, mass transfer for mobile phase.

**Effect of pH and methanol content:** We have already discussed about the effect of buffer pH on the magnitude of EO mobility in 10-undecene-1-ol modified channels [17]. It was demonstrated

that there were no remarkable changes in EO mobility when pH was above 8, due to the complete dissociation (>90%) of carboxylic group on the channel surface [17]. It however should be noted that the enol-to-styrene ratio, in that case, was approximately 0.1, indicating a less hydrophilic surface than the channels with the enol-to-styrene ratio of 0.143 used in this section. The studies on the effect of chain length on EO mobilities also suggest that the channel hydrophilicity would affect the magnitude of the EO mobilities. Therefore, in this case, the influence of pH on EO mobilities was investigated again. The results are plotted in Figure 6. It can be seen that the magnitude of EO mobilities are a little bit larger than that in polyester channels with the enol-to-styrene ratio of 0.1. This probably results from the adsorption of other ions, which affect zeta potential. This further indicates that hydrophilic channel



**Figure 6.** Effect of pH on the migration velocity of SRB and SR 101 as well as  $R_s$ . Conditions as in Table 1.



**Figure 7.** Effect of methanol content in the running buffer on the migration velocity of SRB and SR 101 as well as  $R_s$ . Conditions as in Table 1.

surface is much more favorable for the adsorption of ions from the background electrolyte. Since the EO mobility was not large enough to elute the negatively charged solutes out of the cathode end below pH 6, the effect of pH on  $R_s$  could not be observed within this pH range. It was noted that a loss in  $R_s$  happened when the velocity of EOF is larger.

Apart from the buffer pH that would affect the velocity of EOF, consequently, organic modifier in buffer solution should also be discussed. However, in EC, the effects of organic modifiers on the EOF velocity as well as on the partition equilibrium between the solutes and the stationary phase, are much more complicated. The plot in Figure 7 shows that the separation was lost seriously when the content of methanol reached to 30% (v/v).

#### Effect of channel length.

The effect of channel length on separation is listed in Table 3. For the test mixture of SRB and SR 101, base line separation, corresponding to  $R_s = 1.5$ , was obtained at the channel length of >3 cm. Generally, the theoretical plate number for a specific solute would not be affected by column length. The noticeable deviation in  $N$  at the channel length of 1 cm might be from the detection system, which was not optimized in our work.

**Reproducibility:** The reproducibility was investigated in terms of the migration times of SRB and SR 101 in the case of 10-undecene-1-ol modified polyester channels. RSD% ( $n=8$ ) from run-to-run experiments is 3.16 for SRB, and 3.47 for SR 101, respectively; RSD% ( $n=5$ ) from chip-to-chip experiments is 3.18 for SRB, and 3.56 for SR 101, respectively, showing a good reproducibility. Note that the buffers used in this work have high pH value of 9.6, which suggests that the channel surface is stable enough to tolerate high basic buffer conditions.

#### Conclusions

Conclusively, the method developed in our work is a promising approach for the operation of open-channel EC on chips. The modified channels exhibited good separation performance. The reproducibility experiments showed that the modified channel surface was tolerable to strong basic buffer conditions. Through controlling the content of 10-undecene-1-ol, the reversed migration order of SRB and RB was accomplished, suggesting tunable retention behavior of the modified channels for specific solutes. Not only olefin alcohol, other functional moieties, such as chiral groups, could be useful for the surface modification, through which chiral EC on chip might become possible.

#### Acknowledgments

Support for this work by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Cul-

ture, Japan and grant from Japan Society for the Promotion of Science are gratefully acknowledged.

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