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Flow manipulation for sweeping with a cationic surfactant in microchip capillary electrophoresis

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Abstract

Flow manipulation in sweeping microchip capillary electrophoresis (CE) is complicated by the free liquid communication between channels at the intersection, especially when the electroosmotic flows are mismatched in the main channel. Sweeping in traditional CE with cationic micelles is an effective way to concentrate anionic analytes. However, it is a challenge to transfer this method onto microchip CE because the dynamic coating process on capillary walls by cationic surfactants is interrupted when the sample solution free of surfactants is introduced into the microchip channels. This situation presents a difficulty in the sample loading, injection and dispensing processes. By adding surfactant at a concentration around the critical micelle concentration and by properly designing the voltage configuration, the flows in a microchip were effectively manipulated and this sweeping method was successfully moved to microchip CE using tetradecyltrimethylammonium bromide (TTAB). The sweeping effect of cationic surfactant in the sample solution was discussed theoretically and studied experimentally in traditional CE. The flows in a microchip were monitored with fluorescence imaging, and the injection and sweeping processes were studied by locating the detection point along the separation channel. A detection enhancement of up to 500-fold was achieved for 5-carboxyfluorescein.

Keywords

Sweeping; Microchip; Capillary electrophoresis; Cationic surfactant; Flow control

1. Introduction

As a typical representative of micro total analysis systems, microchip capillary electrophoresis (CE) is presently one of the most promising analytical methods due to its high separation power and the possibility of using plastic as the next generation of material for its reduced cost and ease of fabrication [1,2]. However, detection sensitivity is a problem due to the small injection volume and the short detection pathlength in optical detection, although more sensitive detection schemes such as laser-induced fluorescence have been commonly used [3]. Online sample preconcentration is a convenient alternative to overcome this barrier. Sweeping [4,5]

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and stacking [6,7] are the common approaches used for online sample preconcentration. Sweeping is superior to sample stacking because it is suitable for both neutral and charged solutes by choosing appropriate surfactants. Sweeping in traditional CE has been widely investigated and applied in pharmaceuticals and biologicals [8–11]. Detection enhancements from 100- to million-fold were achieved with the common surfactant, sodium dodecyl sulfate (SDS) [12,13].

The usual condition of sweeping requires that the separation buffer contain micelles while the sample is free of surfactant. By picking and accumulating, the analytes in the sample plug are piled up and then separated through micellar electrokinetic chromatography [12]. A key factor determining the final preconcentration extent is the partition coefficient, k , defined as the ratio of analyte numbers in the micellar and the water phases, which indicates the strength of interaction between micelles and analyte as well as the hydrophobicity or hydrophilicity of the analytes. For neutral analytes, both anionic and cationic surfactants usually work well in sweeping, while for charged analytes, the oppositely charged surfactants have stronger interaction with analytes and to produce better preconcentration by sweeping [14,15]. Thus, cationic micelle is potentially a superior choice for sweeping anionic analytes. Sweeping in traditional CE is usually conducted at low pH values to make the electroosmotic flow (EOF) negligible [12], and the sample solution usually is introduced into the capillary using hydrodynamic approaches. When the separation buffer produces a significant EOF, an electrokinetic injection scheme can be used for electrokinetic stacking injection (EKSI) [4, 16,17], resulting in a larger injection volume and greater enhancement.

Sweeping has been transferred onto microchip CE with electrokinetic injection [3,15,17] because of the difficulty in performing hydrodynamic injection in microchips owing to the complexity of branched channels interconnected at the cross section. Two ways, dynamic [18] and gated injection [19] modes, can be used to introduce a large sample plug into the separation channel for the subsequent sweeping. The gated injection is a better choice that can keep the sample solution free of contamination by the pushback of the separation buffer during the dispensing/separation step. Under an applied voltage, the negatively charged SDS micelles move against the EOF [15] in a negatively charged channel. Similarly, cationic micelles migrate in the opposite direction of the EOF reversed relative to the normal direction by the adsorption of the cationic surfactant on the negatively charged walls [20]. Therefore, the final migration direction of micelles is determined by the relative magnitudes of the bulk flow and the electrophoresis. However, it is difficult to maintain the bulk flow velocity when the sample solution free of the cationic surfactant is introduced into the channels, because the dynamic coating process is interrupted by the fresh sample solution, resulting in a decrease in the local EOF and correspondingly the bulk flow. Moreover, the sample solution should be electrokinetically pumped through the intersection of the microchip for preparation of injection, so the fresh sample solution washes away the cationic surfactant adsorbed on the channel walls, resulting in reduced or reversed EOF relative to the initial situation. Therefore, the dynamic coating process imposes difficulty in performing sweeping in microchip CE using cationic surfactants. One possible way to solve this problem is to modify the channel walls with positively charged components, such as non-bonded temporary coating [21–23] or a bonded permanent coating [24]. Another strategy is to mix the cationic surfactant with the sample solution to maintain the dynamic coating process on the channel walls.

In this paper, sweeping with a cationic surfactant, tetradecyltrimethylammonium bromide (TTAB), was performed in microchip CE. The flows were effectively manipulated by adding the surfactant to the sample solution and by applying a suitable voltage program. The effect of the surfactant in the sample solution was analyzed theoretically and studied experimentally through traditional CE with the model analyte: fluorescein disodium salt (FL). A four-step procedure with the modified gated injection was designed for the sweeping microchip CE, and

the preconcentration method was evaluated by determining the detection enhancements for 5-carboxyfluorescein (5-FAM) and FL.

2. Experimental

2.1. Instrumentation

Traditional CE system—The traditional CE experiments were performed with a P/ACE MDQ instrument from Beckman Coulter (Fullerton, CA, USA). Fused silica capillaries with 50 μm I.D. and 375 μm O.D. were purchased from Polymicro Technologies (Phoenix, AZ, USA). The total length of the capillary was 40.2 cm and the effective length was 30.0 cm from the inlet to the detection point. The excitation wavelength from an argon ion laser was 488 nm and emission was collected at 520 nm. The capillary was thermostatted at 25 °C.

Microchip CE system—The microchip CE system was custom-built and has been described previously [25]. Briefly, a standard single-cross glass microchip (Micralyne, Edmonton, AB, Canada) was used. The voltage program was applied to reservoirs with platinum electrodes using a high-voltage power supply (Micralyne, Alberta, Canada) controlled by a Labview program (National Instrument Corporation, Austin, TX, USA). The light source was a 150-watt xenon lamp supplying a filtered excitation beam (480 ± 20 nm) which is focused on the microchip channels using a 20x objective of an inverted microscope (Nikon Eclipse TE300, Nikon Corp., Melville, NY, USA). The fluorescence was collected by the same objective and was passed on to a photomultiplier tube (PMT, Hamamatsu R3896, Bridgewater, NJ, USA) after being filtered (535 ± 25 nm). The current signal from the PMT was preamplified by a preamplifier (Model SR570, Stanford Research Systems). Data were recorded at 20 Hz controlled by Turbochrome software (PE Nelson, San Jose, CA, USA). In addition, a video recording system including a CCD-100 camera (Dage-MTI, Michigan City, IN, USA), a VCR recorder and monitor (Model BWMC, Javelin Systems, Torrance, CA, USA) was coupled to the bottom of the microscope and was used to record and qualitatively observe the flows in the microchip channels.

2.2. Chemicals and reagents

TTAB, 1.0 M NaOH solutions and acetonitrile were purchased from Sigma-Aldrich (St. Louis, MO, USA). Monobasic and dibasic sodium phosphate was purchased from Fisher Scientific (Fair Lawn, NJ, USA). 5-FAM was obtained from Aldrich Chemical Company (Milwaukee, WI, USA). FL was obtained from ICN Biomedicals (Aurora, OH, USA). Water was purified with a Milli-Q system (Millipore, Bedford, MA, USA). All chemicals and reagents were used without further treatment.

The stock solutions of TTAB and phosphate buffer were prepared in purified water at concentrations of 200 mM, respectively. The pH 8.0 phosphate stock solutions (200 mM) were prepared by mixing monobasic and dibasic phosphate at the volume ratio of 1/9. Stock solutions of FL and 5-FAM were prepared in 30% methanol (v/v) at concentrations of 1.0 and 0.44 mM, respectively. For sample preparation, the solutes were diluted to 500.0 nM or 200.0 nM with purified water. All the solutions loaded into the capillaries and the microchip channels were degassed and filtered through 0.44- μm syringe filters purchased from Gelman Laboratory, Pall Corp. (Ann Arbor, MI, USA).

2.3. General experimental conditions

Traditional CE—New capillaries were sequentially conditioned with 1 M NaOH, deionized water and separation buffer for 10 min each under a pressure of 30 psi. Before performing a separation, the capillary was reconditioned sequentially with 1.0 M NaOH, water and separation buffer for 2 min at a pressure of 40 psi. Sample solution was introduced into the

capillary by a pressure of 2 psi for 1.0 min. The separation was performed by applying a voltage (20.1 kV) across the capillary at the reversed polarity.

Microchip CE—The new microchips were sequentially conditioned with 1.0 M NaOH, deionized water and separation buffer for 10 min each under a vacuum applied at the sample waste (SW) reservoir. Before a new sample loading, the microchip was reconditioned with 1.0 M NaOH, deionized water and separation buffer for 5 min, respectively. After the NaOH wash, all the reservoirs were flushed several times with deionized water. Different volumes of separation buffer were loaded into the buffer reservoir (BR, 50 μ L), BW (50 μ L), the SW (30 μ L). The sample reservoir (SR) was washed several times with deionized water and then was filled with sample solution (50 μ L). The Labview and Turbochrome programs were manually switched on simultaneously. All microchip CE experiments were performed at ambient temperature. All the data were processed with Turbochrome software version 4.0.

Buffer conductivities were measured and adjusted with an ExStik EC400 Conductivity/TDS/Salinity meter manufactured by Extech Instruments (Waltham, MA, USA). The video was recorded on a video tape, and the flow pictures were selected according to the progress of flows.

3. Results and discussion

3.1. Problems in sweeping microchip CE with cationic surfactants

Cationic surfactants are a promising choice for online sample preconcentration of anionic analytes with sweeping considering the electrostatic interaction enhancing the partition of analytes into the cationic micelles [14,26,27]. The adsorption of cationic surfactants in the separation buffer makes the silica channel walls positively charged in terms of the dynamic coating process [20], resulting in reversed EOF when a voltage is applied across the channel. When the surfactant-containing buffer in the capillary is replaced by a buffer devoid of cationic surfactants, the dynamic coating process will be interrupted and the positive charge on the walls will be reduced, resulting in a decrease in the local EOF [4], and even finally the capillary walls return to the inherent condition with the original negative charge when contacting the nonsurfactant buffer for enough time. This situation complicates the flows in the microchip channel and presents a challenge to the flow control in sweeping microchip CE using cationic surfactant as shown in Figure 1. During the loading process, the migrating micelles against the EOF sweep the analytes back to the SR (Figure 1a), which prevents the sample flow from reaching the injection intersection. In the sample injection process (Figure 1b), the sweeping front still stays in the injection channel although some analyte enters the separation channel. Moreover, sample leaking occurs during the dispensing step due to the sweeping effect in the side channel (Figure 1c).

To maintain the dynamic coating process and the local EOF of the sample plug, one straightforward way is to add an amount of surfactant to the sample solution. However, the surfactant in the sample zone probably affects the sweeping enhancement of analyte. This effect is studied theoretically and experimentally in traditional CE in the following section.

3.2. Interaction between analytes and surfactants/micelles

Quirino and Terabe [28] have studied theoretically and verified experimentally that the swept zone is determined by the retention factor (k) of the analyte in the micellar phase. For both neutral and charged analytes, the length of the swept zone (l_s) can be determined by the same equation [28] when the sample solution is free of surfactant.

$$l_s = \frac{l_{inj}}{1+k} \quad (1)$$

where l_{inj} is the originally injected sample plug length before sweeping. When surfactant is added to the sample solution, the surfactant molecules possibly form ion pairs with analytes, because surfactants, such as SDS and TTAB, are good ion-pairing reagents. Anionic SDS molecules can form strong ion pairs with cationic solutes [29], and even with neutral analytes [30]. The interaction between analytes and surfactant monomers can be very strong and should not be ignored, since ion pairing will affect the effective mobility of the analytes [31,32].

For a system containing analyte and surfactant at a concentration below the CMC, the interactions between analytes and monomers include the first- and second-order, as well as higher-order ion pairs. For simplicity, only the first-order ion pairs are considered here. The effective electrophoretic velocity ($V_{A,eff}^m$) of the analyte molecule under the ion-pairing condition is the average of the free and paired analytes weighted by their individual fraction of existence modes as shown in Equation 2.

$$V_{A,eff}^m = \frac{V_A + k_1 [m^+] V_{Am}}{1 + k_1 [m^+]} \quad (2)$$

where V_A and V_{Am} are the electrophoretic velocities of the free analytes and the ion-pairs, respectively, k_1 is the ion-pair formation constant, and $[m^+]$ is surfactant concentration. Similarly, when the surfactant concentration reaches the CMC, the effective electrophoretic velocity correspondingly is determined by Equation 3 with the surfactant concentration replaced by the CMC.

$$V_{A,eff}^{CMC} = \frac{V_A + k_1 (CMC) V_{Am}}{1 + k_1 (CMC)} \quad (3)$$

Thus, the swept zone (l_s) can be derived to Equation 4.

$$l_s = \frac{l_{inj}}{1+k} \times \frac{V_{mc} - V_{A,eff}^{CMC}}{V_{mc} - V_{A,eff}^m} \quad (4)$$

Where V_{mc} is the electrophoretic velocity of the micelle. When the surfactant concentration is above the CMC, retention factor k , according to its definition, is the ratio of the number of analyte molecules in the micellar phase to that in the aqueous phase,

$$k = \frac{[Amc]}{[A] + [Am]} \quad (5)$$

where Amc refers to the analyte-micelle entity. According to Equation 4, there are several cases, which are discussed below.

Case 1. There is no ion-pairing effect, i.e. $k_1 = 0$, then $V_{A,eff}^{CMC} = V_{A,eff}^m = V_A$, and the swept zone width is determined by Equation 1. In this case, the existence of surfactant in the sample solution below the CMC will not affect the sweeping results.

Case 2. Ion-pairing effect exists, i.e. $k_1 \neq 0$. Thus, l_s will change with the increase of surfactant concentration in the sample solution. When there is no surfactant in the sample solution ($[m^+] = 0$), then $V_{A,eff}^m = V_A$, and the swept zone (l_s^0) is determined by Equation 6.

$$l_s^0 = \frac{l_{inj}}{1+k} \times \frac{V_{mc} - V_{A,eff}^{CMC}}{V_{mc} - V_A} \quad (6)$$

This is the usual result under the common sweeping condition with the sample solution devoid of surfactant. Considering the diffusion effect, the swept zone width determined experimentally should be larger than that obtained theoretically according to Equation 1. However, in the studies by Quirino and Terabe [28], the experimental results are much smaller than the theoretical ones. According to Equation 6, this difference may be due to the ion-pairing effect besides the experimental errors. When $[m^+] = \text{CMC}$, $V_{A,eff}^{CMC} = V_{A,eff}^m$, the swept zone (l_s^{CMC}) is determined by Equation 1. When the surfactant concentration in the sample solution is between 0 and CMC, the absolute magnitude of $V_{A,eff}^m$ decreases with the increase of $[m^+]$, and the swept zone (l_s) should accordingly become wider and finally reach the maximum l_s^{CMC} when $[m^+] = \text{CMC}$. However, experimental results as shown in Figure 2 do not demonstrate the expected trend. The peak height (or half-peak width) decreased, and then increased with the increase of the surfactant concentration in sample solution, and reached the maximum height at the CMC. This unconventionality possibly is the result of the enhancement (increase of k) of the interaction between analyte and micelles due to the existence of the ion-pairing effect in the sample solution similar to the situation studied by Kim et al. [33].

When the surfactant concentration in the sample solution is above the CMC, the interaction of analytes with micelles will further alter the mobility of the analyte. Then the swept plug length is determined by Equation 7.

$$l_s^{>CMC} = l_{inj} \left(\frac{1+k_s}{1+k} \right) \quad (7)$$

where k_s is the retention factor when the surfactant above the CMC is in the sample solution. The retention factor is a linear function of the surfactant concentration when the micellar concentrations are low [30,34].

$$k = P_{mw} \tilde{v} (C_T - \text{CMC}) \quad (8)$$

where P_{mw} is the partition coefficient of a solute between the micelles and the water, $\tilde{v}$ is the molar volume of the micelles, and C_T is the total concentration of the surfactant. For a given surfactant concentration above the CMC in the separation buffer, the swept zone can be controlled by adjusting the surfactant concentration in the sample solution according to Equations 7 and 8. By substituting Equation 8 into Equation 7, the swept zone can be expressed as Equation 9.

$$\frac{l_s}{l_{inj}} = mC_T + b \quad (9)$$

where

$$m = \frac{P_{mw} \tilde{v}}{k+1} \quad (10)$$

and

$$b = \frac{1}{1+k} - \frac{P_{mw} \tilde{v} CMC}{1+k} \quad (11)$$

and C_T is the total surfactant concentration in the sample solution, and l_{inj} can be regarded as the peak width at the half peak height when the sample solution has the same surfactant concentration as the separation buffer. From the combination of Equations 10 and 11, CMC can be determined by the following relationship

$$CMC = \frac{1}{m(k+1)} - \frac{b}{m} \quad (12)$$

Generally, the surfactant concentration (C_T) in the separation buffer is less than 0.1 M, and the model analyte can be selected so that its retention factor in the surfactant of interest is much larger than unity, i.e. $k \gg 1$. Therefore, the first term on the right side of Equation 12 is negligible when m is not very small, and the CMC is determined by the ratio of the slope and the intercept expressed in Equation 13.

$$CMC = -\frac{b}{m} \quad (13)$$

A series of sweeping experiments for FL was performed with TTAB and DTAB to test the linear relationship (Equation 4) between the relative width of the swept zone and the surfactant concentration in the sample solution using the same running buffer with a fixed surfactant concentration. The peak width at its half height was obtained directly from the electropherograms. The migration velocity was adjusted using another short injected sample plug. As expected according to Equation 9, the swept zone width (l_s) is linearly related to the TTAB concentration (C_T) in the sample solution with the least-square regression equation of $l_s = 0.127C_T - 0.563$ and the R^2 value of 0.993. A similar relationship was also obtained for DTAB. According to Equation 13, the CMCs were obtained at 4.4 mM and 14.2 mM for TTAB and DTAB, respectively. The reported CMCs in similar conditions are 1.6 mM [31] and 11.3 mM [35] for TTAB and DTAB, respectively. Although this method is not reliable for determining CMCs of surfactants due to the possible stacking effect owing to ionic transport number mismatch during sweeping [15], the experimental results demonstrate consistency with the theoretical analysis.

In summary, the surfactant in the sample solution affects the sweeping results. The best sweeping can be obtained with TTAB in the sample solution at the concentration of the CMC instead of being free of surfactant. The cationic surfactant at the CMC can maintain a steady reversed EOF [20]. Therefore, TTAB at the CMC was added to the sample solution to manipulate flows in sweeping microchip CE using TTAB.

3.3. Optimization of voltage program in microchip CE

The gated injection method [15,36,37] was used to introduce a sample plug into the separation channel for the subsequent sweeping and separation. In microchips for CE, one or more side channels are usually connected with the main channel for serial injections and separations, and they are in free communication with the main channel. Therefore, an appropriate voltage configuration needs to be optimized for the BR and SW reservoirs to prevent leaking. Especially, sweeping microchip CE involves a high concentration of TTAB in the channels. During the sample injection process, the leaking of TTAB into the sample plug may induce a pre-sweeping effect or deteriorate the subsequent sweeping [15]. As can be seen from the flow image of Figure 3a, the leaking is significant when the BR and SW are floated while the injection voltage is applied between SR and BW, which resulted in weak sweeping and produced a wide peak as shown in Figure 3a. On the other hand, when a voltage is applied to BR, the sample solution flows to the BR and is swept by the micelles in the BR channel. Part of the swept zone in the BR channel is pushed into the separation channel during the dispensing step as shown in Figure 3b. This part of the sample mixed with micelles cannot be further swept and concentrated, which results in tailing of peaks when the injection is conducted for a relatively long time (Figure 3b). As pointed out by Liu et al. [15], this part of the sample possibly merges with the other concentrated zone due to ionic transport number mismatch during sample sweeping. However, for a large sample plug, the merging effect may not be completed before detection. To solve this problem, an additional step termed 'flushing' was inserted, in which SR and BW are floated and a voltage is applied across the BR and SW. This step pushes the concentrated sample in the SR channel to the SW. Furthermore, the gated injection generally is conducted with a voltage at the SR to maintain the flowing of sample solution to SW during the dispensing step. The standing application of the voltage to SR possibly accelerates the sample depletion [38,39] and induces bias of the sample solution [37]. In the gated injection method, the SR was floated to reduce the voltage effect on the sample solution during the dispensing/separation process while the leaking from the SR channel was pushed to the SW by the incoming flow.

According to the above discussion, a four-step procedure was designed as shown in Figure 4 and the corresponding voltage configuration for each step is shown underneath. The sample loading step pumps the sample solution through the intersection until it reaches a steady state. The injection step introduces a sample plug into the separation channel in terms of stacking injection [16]. The flushing step is to clear the swept sample zone in the SR channel. The final step includes the subsequent sweeping and separation. The time duration for each step can be selected experimentally. Especially for the flushing step, a shorter time can reduce the diffusion of the swept zone in the separation channel, so that a high voltage is applied to shorten the duration.

3.4. Detection enhancement and sample plug length

An advantage of CE using microchips instead of traditional capillaries is the random selection of the detection point along the separation channel. The injection and the sweeping processes were monitored by moving the detection point to the proper locations. Figure 5a shows that the fluorescence response increased with the detection points going farther from the intersection. The introduction of sample solution into the separation channel was carried out by using the electrokinetic stacking injection (EKSI) [4], in which the injection and the

sweeping were simultaneously proceeding. It takes time for the sweeping front to reach the detection point, and the analyte concentration in the sweeping front is time-dependent, which is reflected in the increase of fluorescence signal with detection locations. A longer injection time surely can introduce more sample solution for sweeping; however, the maximum length of the sample plug is limited by the total length of the separation channel and the retention factor of the analyte. A longer separation channel tolerates a larger injection volume and the stronger interactions between analytes and micelles produce higher concentration enhancements. A compromise should be optimized between the injection volume and the resolution for a given microchip and the analytes of interest. At the detection location of 6.00 cm from the intersection, 60.0-s injection plus 10-s flushing produced optimal preconcentration and resolution for the model analytes, 5-FAM and FL (Figure 5b(ii)), under the voltage configuration in Figure 4. The detection enhancements relative to the normal injection (0.20 s) with BR floated and without the flushing step (Figure 5b(i)) are 340- and 250-fold for 5-FAM and FL, respectively. 5-FAM has -3 charges while FL has -2 under the buffer condition. Therefore, the stronger electrostatic interaction between micelles and analytes favors the retention factor and concentration of 5-FAM. 100-s injection performed for 5-FAM produced a detection enhancement of 500-fold.

The injection plug length was estimated by monitoring the sample plug front during the injection step. The detection point was positioned at 1.00 cm from the intersection, and the fluorescence response showed the approaching of the sample plug front. With this method, the plug injection velocity was determined as 0.024 cm/s. Accordingly, 40-, 60- and 100-s injection times introduced sample plugs of 0.97, 1.46 and 2.43 cm, respectively. The real sample plug should include the swept length during the EKSI, which depends on the electrophoretic velocity of the micelle.

4. Conclusions

These experiments demonstrate that the presence of surfactant at a proper concentration on the one hand maintains the local EOF and on the other hand enlarges the sweeping concentration enhancement. The flows in the microchip channels are well controlled by the combination of TTAB (at the CMC) in the sample solution and with properly designing the voltage configuration. Although additional steps in microchip CE may make the experimental procedure laborious, it is really crucial for the right control of the injections and separations. The modified gated injection can be considered for real analyses when sample solutions are sensitive to the application of voltages. These results can provide meaningful insight for sweeping in microchip CE.

Acknowledgments

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Figure 1.

Flow images in the sweeping microchip CE with sample solution free of TTAB. Images were recorded with 200 μ M 5-FAM in the phosphate buffer. Arrows indicate the direction of the bulk flow.

Figure 2.

Concentration effect of TTAB in the sample solutions. Separation buffer comprised 40.0 mM phosphate, 60.0 mM TTAB and 10% acetonitrile (pH 8.0). Injection was carried out by a pressure of 1.0 psi for 60 s. FL 2.0 nM was prepared in phosphate buffer (pH 8.0) with various concentrations of TTAB.

Figure 3.

(a) Leaking of separation buffer during sample injection with BR and SW floated. (b) Dispensing without the flushing step. Both used 5 nM 5-FAM prepared in phosphate buffer with 1.5 mM TTAB added. Separation buffer comprised 20.0 mM phosphate and 40.0 mM TTAB (pH 9.0) Arrows indicate bulk flows.

Figure 4.

4-Step procedure. (a) Sample loading; (b) Electrokinetic stacking injection (EKSI); (c) Flushing; (d) Dispensing/separation. SR = Sample Reservoir; SW = Sample Waste reservoir; BR = Buffer Reservoir; BW = Buffer Waste reservoir.

Figure 5.

(a) Peak sizes versus detection locations along the separation channel: (i) 2.0 cm; (ii) 3.0 cm; (iii) 4.0 cm; and (iv) 5.0 cm. Injection was performed for 40 s; flushing for 6 s. Sample and separation buffer are the same as in Figure 3. (b) Electropherograms of normal injection and sweeping. (i) Normal injection; sample (500 nM 5-FAM and FL, respectively) was prepared in the separation buffer; injection (0.20 s) was performed with SR floated and without the flushing step. (ii) Sweeping: injection for 60 s, flushing for 10 s. The sample contained 5-FAM and FL at 5.0 nM each, and the separation buffer is the same as in Figure 3.