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Short Communication

Effect of PVP on the electroosmotic mobility of wet-etched glass microchannels

We present an experimental study on the effect of polymer PVP on EOF mobility of microchannels wet etched into optical white soda lime glass, also known as Crown glass. We performed experiments to evaluate the effect of PVP concentration and pH on EOF mobility. We used on-chip capillary zone electrophoresis and a neutral fluorescent dye as a passive marker to quantify the electroosmotic flow. We performed experiments under controlled conditions by varying pH from 5.2 and 10.3 and concentration of PVP from 0 to 2.0% w/w at constant ionic strength (30 mM). Our experiments show that PVP at concentrations of 1.0% or above very effectively suppress EOF at low pH (6.6). At high pH of 10.3, PVP has a much weaker suppressing effect on EOF and increasing its concentration above about 0.5% showed negligible effect on EOF mobility. Finally, we briefly discuss the effects of pH on using PVP as an adsorbed coating. Our experiments provide useful guidelines on choosing correct pH and concentration of PVP for effective EOF suppression in glass channels.

PVP is used in many applications to suppress protein adsorption [1], extract analytes [2], or separate biomolecules [3]. PVP is also used as a simultaneous dynamic coating and sieving matrix for DNA separations [4, 5]. Most relevant here, PVP is used to control and suppress EOF in electrophoresis-based techniques, including CZE [6,7], and ITP [8,9]. Improved coating capacity has been reported for PVP used as an outer layer wall coating for EOF suppression [10]. For example, a glass surface can be silanized and PVP subsequently adsorbed as an upper polymer layer by the hydrogen abstraction method [11]. Further, PVP has been used as a dynamic coating for composite glass and PDMS [12] or glass-on-silicon (substrate) chips [13] for PCR systems.

We here focus on using PVP as a dynamic surface passivation additive. Dynamic coating (including the additive in the buffer of interest) is simple and cost-effective; thus attractive across a broad range of applications. Further, aqueous PVP solutions have relatively low viscosity [14] and, therefore, facilitating buffer loading into capillaries and on-chip. To our knowledge, the only study to systematically evalu-

ate PVP as a dynamic coating for EOF suppression was that of Kaneta et al. [6] who employed it for EOF suppression in a standard CZE experiment in silica capillaries. Kaneta et al. [6] reported a tenfold reduction of EOF at 1.0% concentration of PVP compared to untreated silica capillary. They varied PVP concentration, PVP molecular weight, and pH, and studied EOF mobility in the presence of SDS. Despite its popularity and common use as an EOF suppressant in on-chip capillary electrophoresis, we know of no systematic experimental study on the effects of PVP concentration and pH on the glass surfaces of microchannels. Here, we report EOF mobility measurements taken in microchannels etched in white soda lime at varying PVP concentrations and pH values. We used a neutral marker [15] (rhodamine B (RB) fluorescent dye) and straightforward electrokinetic injection experiments.

We here only summarize our materials and methods while details can be found elsewhere in Milanova et al. [7]. We performed standard on-chip CZE experiments and used a neutral dye RB (Sigma-Aldrich, St. Louis, MO, USA) at 200 μM to quantify EOF mobility. We explored the 16 background electrolyte chemistries listed in Table 1. We prepared buffer solutions of glycine (chemistries 1–4), tricine (5–8), MES (9–12), and acetic acid (13–16) titrated with sodium hydroxide (NaOH) to measured pH values of 10.3, 8.5, 6.6, and 5.2, respectively. We explored PVP (MW = 1 000 000, Polysciences Inc., Warrington, PA, USA) concentrations of 0, 0.5, 1.0, and 2.0% w/w of water. We here refer to PVP concentration using the usual

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Abbreviation: RB, rhodamine B

Table 1. Details of background electrolyte buffer composition in our electroosmotic mobility study

	Measured, predicted pH	PVP (MW = 1 000 000) [w/w]	C (acid) [mM][a]	C (base) [mM]	I [mM]
1–4	10.3, 10.11	0, 0.5, 1.0, 2.0%	Glycine (+1, 39.5, 2.32), (−1, 37.4, 9.78) 40	NaOH 30	30
5–8	8.5, 8.49	0, 0.5, 1.0, 2.0%	Tricine (−1, 26.6, 8.5) 40	NaOH 30	30
9–12	6.6, 6.43	0, 0.5, 1.0, 2.0%	MES (−1, 26.8, 6.13) 40	NaOH 30	30
13–16	5.2, 5.09	0, 0.5, 1.0, 2.0%	Acetic acid (−1, 42.4, 4.756) 40	NaOH 30	30

a) In parenthesis, we list, respectively, species valence, absolute mobility as factors of 10^{-9} $m^2V^{-1}s^{-1}$, and acid dissociation constants (pK_a).

designation as a percentage concentration by weight (PVP density is 1.2 g/cm³, so 1.0% PVP corresponds to 12 mg/mL at 25°C). We used a Corning Pinnacle 542 pH/conductivity meter (Nova Analytics, Woburn, MA, USA) for pH measurements. Predicted pH values were from the Peakmaster tool [16] and often differed by approximately 0.1–0.2 pH units from measured values, possibly due to the effect of ionic strength on acid dissociation constants [17] and experimental error.

Details of our optical and experimental setup are provided in Milanova et al. [7], and depicted here in Fig. 1. Briefly, we performed measurements on an inverted epifluorescent microscope (IX70, Olympus, Hauppauge, NY, USA) equipped with a mercury lamp, a U-MWIBA filter cube from Olympus

Figure 1. The experimental apparatus for capillary zone electrophoresis includes microfluidic chip, epifluorescence microscope, CCD camera, high-voltage switching system, 1.2 kV DC power supply, and a data acquisition system. We used a 10× objective (numerical aperture of 0.4) for all experiments. We used exposure times between 50 and 100 ms, depending on fluorescence signal strength. The chip was a cross-type Caliper NS 95 with 12-μm etch depth and 10-μm mask width in the separation channel. Precise measurements of channel center contour lengths of various regions are: 5.0 mm (I), 16.3 mm (II), 8.4 mm (III – the separation channel), 16.1 mm (IV), and 4.3 mm (V). We used rhodamine B as a neutral dye loaded into the north reservoir (N). The inset table summarizes an empirically optimized voltage scheme for sample stream pinching and injection. Our main voltage (from W to E) was 1.2 kV, yielding an electric field of 29.4 V/cm in the separation channel, oriented left to right.

(460–490 nm excitation, 515 nm emission) and a 10× (NA of 0.4) UPlanApo objective. We imaged a region only 1.5 mm into the separation channel (∼1/10th of the separation channel length) as longer lengths results in overly weak signals at conditions of strongly suppressed EOF (migration times were as long as order 10^3 s). We analyzed images to quantify the migration time, t_{EOF}, for the maxima of the neutral dye peak. We quantified the local electric field, E, (∼29.4 V/cm) in the separation channel by treating electrolyte filled channels as a network of resistors to compute the relation between channel geometries, applied potentials, and electric fields. For more details, see the Supporting Information of [7].

For uniform conductivity buffers with negligible pressure disturbances, EOF mobility μ_{EOF} can be expressed simply in terms of the EOF velocity V_{EOF} as:

$$\mu_{EOF} = \frac{\nu_{EOF}}{E} = \frac{L}{Et_{EOF}} \tag{1}$$

where E is the electric field, L is the travel distance (here $L = 1.5$ mm), and t_{EOF} is the migration time. Our white soda lime glass microchips had a negative surface at all conditions, resulting in positive EOF in the direction of electric field.

The essence of the passive, neutral marker method is that the neutral fluorescent marker has negligible electrophoretic mobility and does not interact with the background electrolyte or channel walls. We observed no evidence (e.g. peak tailing, background fluorescence) of wall adsorption. Cationic RB fits these criteria as it is suitably soluble and has a reported pK_a value of 3.22 [18], and is approximately neutral for our pH range of interest (5.2<pH<10.3).

We described our injection protocol in the Supporting Information of [7]. Briefly, we used a "pinching" step to inject a finite amount of sample in the separation channel, and application of electric field in the separation channel included a "retraction" step to quickly interrupt sample flow into the channel. We show a chip schematic and summarize the two-stage voltage control scheme in Fig. 1. Our chip was an off-the-shelf optical white soda lime glass microfluidic chip (model NS-95 from Caliper Life Sciences, Mountain View, CA, USA), composed primarily of SiO_2 (69.5%), K_2O (8.3%), Na_2O (8.1%), CaO (7.1%), and several other minor oxide additives (McReynolds, R.J., Caliper Life Sciences, personal communication, June 19, 2012). Before introducing
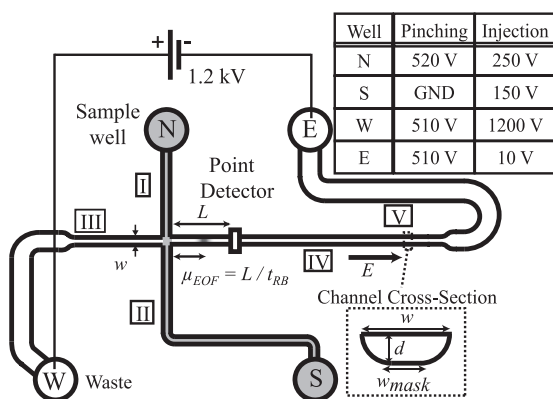
each new background electrolyte chemistry, we flushed the channels with 40 µL of 0.5 M NaOH for 10 min by applying vacuum to well labeled *S* in Fig. 1, followed by deionized water for 5 min, then 100 mM HCl for 3 min, and then deionized water again for 3 min. Between repeats of the same buffer chemistries, we only rinsed with deionized water.

We note the ambient temperature for our experiments varied between 21 and 23°C. We report the observed experimental uncertainties from the mean for five realizations (using 95% confidence interval and the Student *t*-distribution). Uncertainties in the mean were about 2.9% for the 2.0% PVP and 7.0% for 0% PVP data. We verified that Joule heating effects were negligible (see Supporting Information of [7]).

We measured the EOF in the presence of PVP for 16 background electrolyte chemistries. We explored PVP concentrations ranging from 0 to 2.0% w/w, keeping the background electrolyte ionic strength fixed at 30 mM. We note that RB visibly precipitates in background electrolytes with less than about 30 mM ionic strength [7]. Figure 2 shows measured electroosmotic mobility on a logarithmic scale versus concentration, for measured pH values of 5.2, 6.6, 8.5, and 10.3. At low pH, we observed a considerable reduction (110-fold drop to a value of 0.44×10^{-9} m^{2}V^{-1}s^{-1}) relative to the maximum value at pH 10.3 and 0% PVP. However, we observed comparatively weaker effective EOF suppression at higher pH values. For example, adding 2.0% PVP concentration at pH 10.3 causes a slight drop in EOF mobility from 48.2
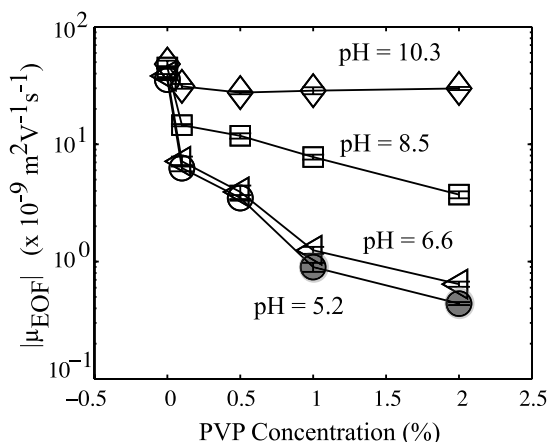

Figure 2. Here we present electroosmotic mobility as deduced from motion of the neutral dye rhodamine B. We explored pH values of 5.2 (○), 6.6 (◁), 8.5 (□), and 10.3 (◇) and PVP concentrations ranging from 0 to 2.0% w/w. We placed the detector at $L = 1.5$ mm (see Fig. 1) in the separation channel. Data show EOF mobility decreases with increasing polymer concentration and decreasing pH. EOF mobility at pH 5.2 with 2.0% PVP is 0.44×10^{-9} m^{2}V^{-1}s^{-1}, more than 100-fold lower than the comparison case of equal pH but no polymer. We note that at pH of 5.2, RB is 1% ionized and has an effective mobility in the order of 0.1×10^{-9} m^{2}V^{-1}s^{-1}. The electroosmotic mobility and the electrophoretic mobility of rhodamine B at pH 5.2 and PVP concentrations of 1.0 and 2.0% are on the same order, and so the EOF mobility data at these conditions is correct only within an order of magnitude.

$\times 10^{-9}$ to 29.9×10^{-9} m^{2}V^{-1}s^{-1}. In contrast to this, the case of adding 2% PVP at pH 5.2 results in a drop from 35.5×10^{-9} to 0.44×10^{-9} m^{2}V^{-1}s^{-1}. Rhodamine B is a weakly basic dye with a p$K_{a,+1}$ of 3.2 [18,19]. Therefore, at pH values of 6.6, 8.5, and 10.3, RB is less than 0.1% ionized and its electrophoretic mobility is negligible compared to the measured EOF mobilities at these pH values. At pH 5.2, RB is 1% ionized, and has an effective mobility in the order of 0.1×10^{-9} m^{2}V^{-1}s^{-1}. This mobility magnitude is negligible compared to the measured EOF mobilities at pH 5.2 for PVP concentrations equal to 1.0% and higher. For the lowest EOF mobility data at pH 5.2 and PVP concentrations of 1.0 and 2.0%, our reported values of EOF mobility may therefore be biased above the actual EOF mobility value due to the (cationic) electrophoretic mobility of RB. We therefore highlight these two data points in Fig. 2 using filled circles and caution that the corresponding values are correct only within an order of magnitude.

For all pH values and concentrations of PVP used in the current experiments, we observed no evidence of interaction of rhodamine B with channel walls. For example, we observed negligible increases in background fluorescence. Further, the rhodamine B peaks in electropherograms were symmetric. In the Supporting Information Fig. S1, we present 16 representative electropherograms corresponding to each experimental condition shown in Fig. 2.

Our data provide guidelines for designing electrophoresis experiments requiring precise control of EOF. Overall, we found that the largest reduction in EOF occurs when PVP concentration is varied from 0 to 1.0%. Above 1.0% PVP, there is far less decrease in EOF mobility. For pH 10.3, EOF mobility drops less than a factor of 2 between 0 and 0.1% and levels off above PVP concentrations of about 0.1%. The data of Fig. 2 for pH values lower than 10.3 show more significant decrease of electroosmotic mobility with increasing PVP concentration. Interestingly, in the lower pH range (of 6.6 and below), the EOF mobility drops by about fivefold for PVP concentration of 0–0.1% and an additional 20-fold for 0.1–2.0%.

The observed strong dependence of suppression capacity of PVP on pH is very consistent with previous reports. For example, Kaneta et al. [6], Yu et al. [20], and Wang et al. [21] each reported strong pH-dependence of the degree of EOF mobility suppression in fused silica channels using dynamic coatings in CZE experiments. These studies used PVP (MW = 40 000, 360 000, and 1 000 000), poly(*N*-isopropylacrylamide) (PNIPAM), and P(VP-co-DMAEMA) copolymer coatings, respectively. A few of their observations are worth noting. Yu et al. [20] reported a 30-fold suppression of EOF at pH 5.84 versus a tenfold suppression at pH 7.43. They attributed this to hydrogen bonding between the silanol groups of the silica surface and the oxygen atoms of the carbonyl groups of PNIPAM. Both Kaneta et al. [6] and Wang et al. [21] showed that their coatings were stable only between pH of about 6 and 8; whereas we observe efficient and stable suppression at pH as low as 5.2. Further, Kaneta et al. [6] reported only a tenfold reduction in EOF by PVP, whereas our optical white soda lime glass data show roughly 100-fold reduction. All

three studies [6, 20, 21] reported a gradual reduction of EOF (and/or associated surface charge) with increasing polymer concentration, similar to our data for pH 8.5 and below. Wang et al. [21] noted a slight leveling off of EOF mobility above PVP concentrations of about approximately 1.25%, while Kaneta et al. [6] observed near uniform, monotonic EOF reduction at PVP concentrations larger than 1.0%.

PVP as a dynamic surface coating for EOF reduction is attractive as it is a low viscosity polymer, which interacts with glass surfaces noncovalently. We have presented experimental data of electroosmotic mobility in the presence of a dynamic suppressing polymer PVP (MW = 1 000 000) on channels wet etched into optical white soda lime glass. We performed on-chip CZE experiments at pH values of 5.2, 6.6, 8.5, and 10.3; and polymer concentrations between 0 and 2.0%. We used the fluorescent dye rhodamine B as a neutral marker. PVP is most effective for pH 5.2 and 6.6, moderately effective at pH 8.5, and least effective at pH 10.3. For example, at pH 5.2, 2.0% w/w PVP yields a 100-fold reduction in EOF mobility, but an equal concentration of PVP at pH 10.3 results in an EOF reduction of less than about twofold. We believe these data are useful and provide guidance in designing capillary electrophoresis experiments.

The authors have declared no conflict of interest.

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