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Stacking open-capillary electroosmotic pumps in series to boost the pumping pressure to drive high-performance liquid chromatographic separations

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ABSTRACT

Numerous micropumps have been developed, but few of them can produce adequate flow rate and pressure for high-performance liquid chromatography (HPLC) applications. We have recently developed an innovative hybrid electroosmotic pump (EOP) to solve this problem. The basic unit of a hybrid pump consists of a +EOP (the pumping element is positively charged) and a −EOP (the pumping element is negatively charged). The outlet of the +EOP is then joined with the inlet of the −EOP, forming a basic pump unit, while the anode of a positive high voltage (HV) power supply is placed at the joint. The inlet and outlet of this pump unit are electrically grounded. With this configuration, we can stack many of such pump units in series to boost the pumping power. In this work, we describe in details how an open-capillary hybrid EOP is constructed and characterize this pump systematically. We also show that a hybrid EOP with ten serially stacked pump units can deliver a maximum pressure of 21.5 MPa (~3100 psi). We further demonstrate the feasibility of using this hybrid EOP to drive eluents for HPLC separations of proteins and peptides.

1. Introduction

High-pressure liquid chromatography (HPLC) is arguably the most commonly utilized separation technique in analytical laboratories, due to its robustness, reproducibility, and flexibility. Since the pioneering work of Horvath and co-workers [1,2] in liquid chromatography (LC), there has been a continuous interest in performing LC separations in ever smaller scales. A miniaturized HPLC offers several significant benefits such as reduced sample and reagent consumption, diminished waste generation, shrunk system size, and improved performance. The portability of a miniaturized HPLC is particularly important for point-of-care measurements and remote Chemical and Biological Warfare Agent detections. One of the major challenges toward downsizing an HPLC is the lack of a high pressure micropump capable of generating adequate flow rate and pressure to drive HPLC separations.

A variety of micropumps have been developed since the early 1980s [3], and several articles have reviewed the progress in this area [4–8]. Few of these pumps have been used for HPLC applications. Electroosmotic pump (EOP) is one of the most promising candidates that has shown potential for practical HPLC separations [7–12]. EOPs have several inherent advantages over other types of micropumps. For example, EOPs are capable of generating pulse-free flows, the flow magnitude and direction of an EOP are convenient to control, the pump can be fabricated using standard microfabrication technologies (and thus it is readily integratable with lab-on-chip devices), and EOPs have no moving parts.

The development of EOPs can be traced back to the early 1970s when Pretorius et al. [13] used electroosmotic flow (EOF) from a 5-cm-long and 1-mm-i.d. (inner diameter) glass columns packed with 1–20 μm silica particles to drive liquids for chromatographic separations. Theeuwes [14] miniaturized and applied EOPs in controlled drug delivery systems. More recently, Paul and Rakestraw [9] used 1–3 μm silica bead packed capillaries to generate pressures of up to ~5000 psi. Zeng et al. [15] used a polymer frit-confined bed of silica particles to generate a maximum pressure of ~30 psi and a maximum flow rate of 0.8 mL/min. Chen et al. [16,17] used parallel columns packed with 2–3 μm silica beads to generate pressures of ~2000 psi and flow rates of a few μL/min. Polymer- and silica-based monolithic materials have also been used in EOPs [18,19]. While these pumps worked well, electrical connections were troublesome [16,17,20].

Open capillary EOPs were developed in the early 1990s [21–24] for microflow analysis. Moderate flow rates and pressures were generated for capillary-based flow injection and sequential

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injection analyses. Lazar and Karger [25] reported a multiple open-channel EOP to deliver peptide samples for electrospray ionization-mass spectrometric detection. Pu and Liu [26] integrated an EOP with an ion-exchange membrane electric field decoupler and an off-chip selection valve for enzyme assay. More recently, Byun et al. [27] developed an EO pumped nanopipetter capable of accurately picking up and delivering pL–μL aliquots. Open channel EO pumping was also used in other applications [28–30]. The problem associated with these pumps is their limited pumping pressure (usually less than 100 psi).

Takamura et al. [31] developed a so-called cascade EOP, which seemed to be a solution to the above problem. A cascade EOP is basically a group of EOPs connected in series. Because a high voltage (HV) was applied to the inlet, and a ground potential (GND) was applied to the outlet of each EOP, if one simply connected the outlet of one EOP to the inlet of a second EOP, the HV on the second EOP would be joined directly to the GND of the first EOP, causing a short circuit. To solve this problem, Takamura et al. [31] used a fat connecting channel to "isolate" the HV and the GND. This connecting channel needed to be fat in order to reduce its resistance against the pump flow. The dilemma was: the fat channel could not "isolate" the HV and the GND effectively, and a lot of current of the HV supply was drained through this capillary.

We have recently disclosed an innovative hybrid EOP capable of addressing this issue [32]. In this report, we describe the detailed procedure of how to fabricate such an EOP, characterize its performances and demonstrate its feasibility for HPLC separations.

2. Experimental

2.1. Chemicals and materials

Methacryloyloxypropyl-trimethoxysilane (MPTS, also named 3-(trimethoxysilyl) propyl methacrylate) (98%) was purchased from Acros (Fairlawn, NJ). [2-(Methacryloyloxy)ethyl]-trimethylammonium chloride (META, 75 wt% in water) was obtained from Sigma–Aldrich (St. Louis, MO). *p*-Styrenesulfonic acid sodium salt (pSSA) was supplied by TCI (Tokyo, Japan). Acrylamide, [*N*,*N*′-methylene bisacrylamide] (Bis), *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED), and ammonium persulfate (APS) were purchased from Bio-Rad Laboratories (Hercules, CA). Stearyl methacrylate (SMA, techn.) and 2,2′-azobisisobutyronitrile (AIBN, 98%) were obtained from Aldrich (Steinheim, Germany). Ethylene glycol dimethacrylate (EDMA, 98%) was purchased from Alfa Aesar (Ward Hill, MA). Cyclohexanol was obtained from J.T. Backer (Phillipsburg, NJ). 1,4-Butanediol (99%) was supplied by Emerald BioSystems (Bainbridge Island, WA). HPLC peptide standard mixture H2016 was supplied by Sigma–Aldrich. Other chemicals were obtained from Fisher Scientific International Inc. Ultrapure water purified by a NANO Pure Infinity Ultrapure water system (Barnstead, Newton, WA) was used for solution preparations. Fused silica capillaries were purchased from Polymicro Technologies (Phoenix, AZ).

2.2. Preparation of pump capillaries

Capillaries with both positively and negatively charged walls were used to assemble our EOPs. Briefly, fused-silica capillaries (1-m length × 5-μm i.d. × 150-μm o.d.) were washed with 1.0 M NaOH for 1 h, DI water for 40 min and acetonitrile for 40 min, and then dried with helium. These capillaries were flushed with a solution containing 10% (v/v) 3-(trimethoxysilyl)propyl methacrylate and 0.2% acetic acid in acetonitrile for 2 h, rinsed with acetonitrile for 30 min, and then dried with helium. To prepare capillaries with positively charged walls, the above pretreated capillaries were flushed with a degassed solution containing 2 mL of 1.50% (w/w) META, 5 μL of 10% (w/w) APS and 0.5 μL TEMED at 4 °C for 30 min, and then rinsed with DI water. To prepare capillaries with negatively charged walls, the above solution was substituted with 2 mL of 0.20 M pSSA and 0.004% Bis, 10 μL of 10% (w/w) APS and 1 μL TEMED and the flushing time was increased from 30 min to 35 min.

2.3. Measurement of electroosmotic mobility

Electroosmotic mobilities were measured to examine the coating quality and uniformity. A capillary electrophoresis (CE) setup with a UV absorbance detector was employed for these tests. A 100-cm-long coated capillary was cut evenly into 4 segments. An optical window was created approximately in the middle of each segment by removing the polyimide coating with a razor blade, so the effective capillary length was ~12.5 cm. After the capillary was installed in the UV absorbance detector, it was flushed with 0.1% trifluoroacetic acid (TFA). The solution-entrance end of the capillary was placed in a solution containing 2% (v/v) dimethyl sulfoxide (DMSO) and 0.1% TFA, while the solution-exit end was placed in a solution containing 0.1% TFA. An electric field (E) was then applied across the capillary, and the migration time (t) of DMSO was recorded. The electroosmotic mobility (μ_{eo}) was computed as $\mu_{eo} = 12.5\ \text{cm}/(t \times E)$.

2.4. EOP construction

Fig. 1a presents a schematic diagram of a basic pump unit. The unit consists of a +EOP and a −EOP. The +EOP was constructed using capillaries with positively charged walls, while the −EOP was constructed using capillaries with negatively charged walls. Consequently, the EOF inside the +EOP went from cathode to anode and its direction was reversed inside the −EOP. With the connection of the +EOP outlet to the −EOP inlet, the EOF flowed from the +EOP to the −EOP smoothly as the +HV was on. The electric–fluidic (E–F) interface in Fig. 1a is used to facilitate the application of an external potential to the fluidic system. Fig. 1b exhibits a detailed configuration of the E–F interface. It was made from a micro Tee, with two of its leads being connected fluidic-ly to pump or connection capillaries and the third lead connected electrically to a capillary filled with immobilized polyacrylamide gel. The gel-based interface was prepared following the protocols reported in Ref. [27], and it could sustain a pressure of 6000 psi without any problem. The pump capillaries were first bundled inside a PEEK tubing. To secure the capillaries in the PEEK tubing and prevent liquid-leaking, some epoxy adhesive was applied to the outside of the pump capillaries and the inside of the PEEK tubing. The free-end of the gel-filled capillary was inserted into a buffer solution where an external HV was applied. The gel served as a salt bridge that allowed the continuity of electricity but inhibited liquid from flowing across it. As a voltage was applied to the buffer solution, the electric potential extended to the solution inside the Tee. Electrolysis occurred and the electrolysis-induced bubbles formed only in the solution inside the buffer container, not inside the fluidic system. Referring back to Fig. 1a, both the inlet and the outlet of this pump unit were at the same electric potential (ground), which allowed us to conveniently connect many of these EOP units in series.

2.5. Measurement of the maximum flow rate and maximum pumping pressure of an EOP

An empty capillary with 50 or 100 μm i.d. was connected by a union to the outlet of an EOP. As the EOP was on, the speed (υ) of the meniscus inside the empty capillary was monitored and measured

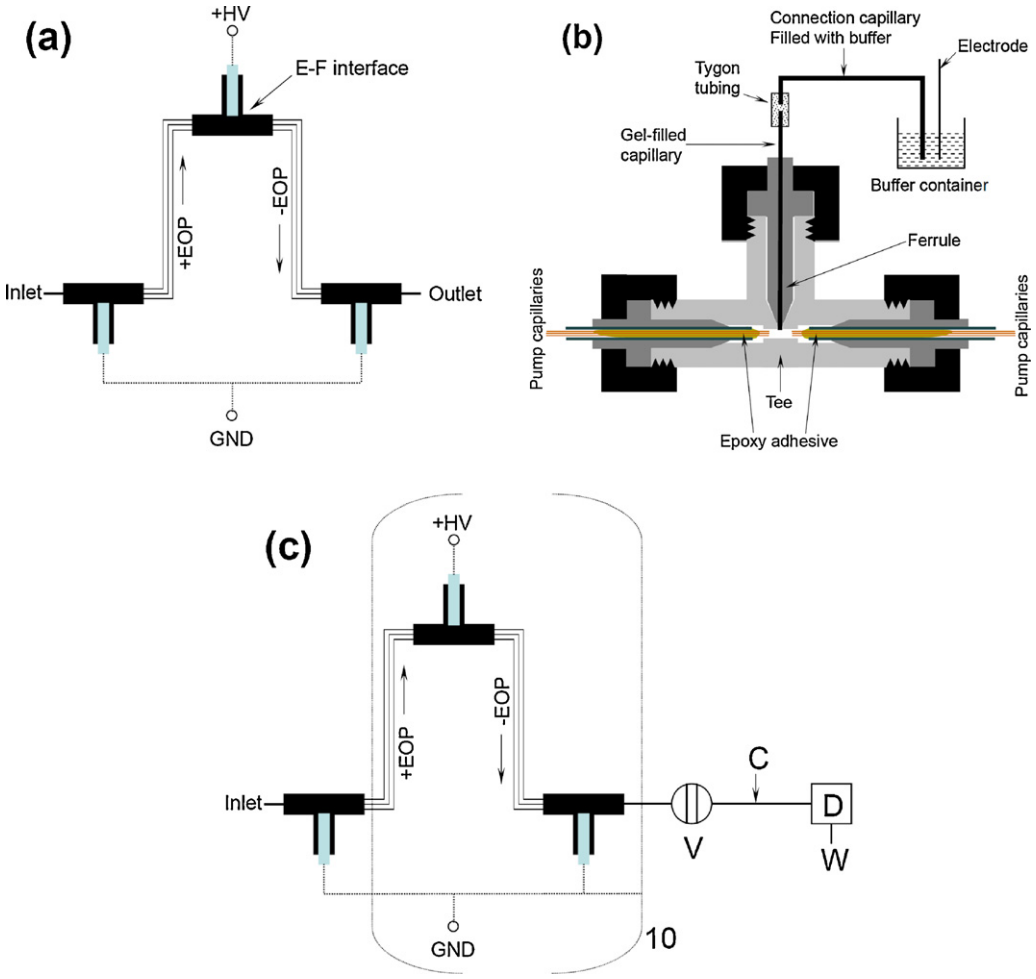

Fig. 1. Schematic configuration of a hybrid EOP. (a) Basic unit of a hybrid EOP. Each unit consists of 52 +EOP and 52 −EOP capillaries. The solid-lines indicate the fluidic pathways and the dashed lines indicate the electricity pathways. HV, high voltage; and GND, ground. (b) A detailed configuration of the E–F interface in (a). (c) An HPLC system with a ten-unit hybrid EOP. The parenthesis indicates ten duplicate basic pump units connected in series. V, a 4-nL injection valve; C, a monolith capillary column; D, a UV absorbance detector with a wavelength set at 210 nm; and W, waste (effluent).

using a microscope. The maximum flow rate (which equals to Q_{EO}) of the EOP was calculated by,

$$Q_{EO} = \frac{\pi d^2 \upsilon}{4} \quad (1)$$

where d is the i.d. of the empty capillary.

The maximum pumping pressure of an EOP was measured as described previously [33]. Briefly, one end of a capillary (25-cm length × 50-μm i.d. × 360-μm o.d.) was blocked by some epoxy glue. (Note: it is important to ensure that some of the glue gets inside the capillary tip.) The other end of the capillary was connected to the EOP outlet via a union, with an air plug being trapped inside the capillary. The length of the air plug was recorded as L as the pump was off (at ambient pressure). Then, the pump was turned on and ran continually until the air plug could not be compressed any longer. The length of the air plug was recorded as L'. The maximum pumping pressure (Δp) of the EOP was calculated by,

$$\Delta p = \left(\frac{L}{L'} - 1\right) \times 14.5(\text{psi}) \quad (2)$$

2.6. Preparation of monolith capillary

Monolith columns were used in this work for protein and peptide separations. These columns were prepared similarly following a published procedure [34]. Briefly, the inner wall of a fused silica capillary (40-cm length × 75-μm i.d. × 360-μm o.d.) was cleaned by flowing 1.0 M NaOH for 1 h, water for 30 min, 1.0 M HCl for 1 h, water for 30 min, and acetonitrile for 30 min through the capillary. The capillary was then flushed with 10% 3-(trimethoxysilyl)propyl methacrylate in acetonitrile (containing 0.2% acetic acid) for 4 h, rinsed with acetonitrile for 20 min, and dried with nitrogen. A solution was prepared by mixing 0.45 g SMA, 0.30 g EDMA, 0.962 g cyclohexanol, 0.788 g 1,4-butanediol and 7.5 mg AIBN. After this mixture was sonicated to dissolve all chemicals and purged with helium for ~10 min, it was introduced into the above treated capillary via a Hamilton syringe until 30 cm of the capillary was filled. After both ends of the capillary were sealed with rubber stoppers, the capillary was submerged in a 60 °C water bath for 20 h to allow the monolith to form. The monolith was then washed with methanol and water using a HPLC pump. A 1–2 mm detection window was created right outside the monolith by removing the polyimide coating with a razor blade. Excess monolith was cut off to leave an effective column (monolith) length of 20 cm.

2.7. An HPLC system with serially stacked EOPs

Fig. 1c presents an HPLC system with ten EOP units connected in series. The outlet of this serially stacked pump was linked with a 4-nL injection valve (Valco Instruments Co. Inc., Houston, TX) and

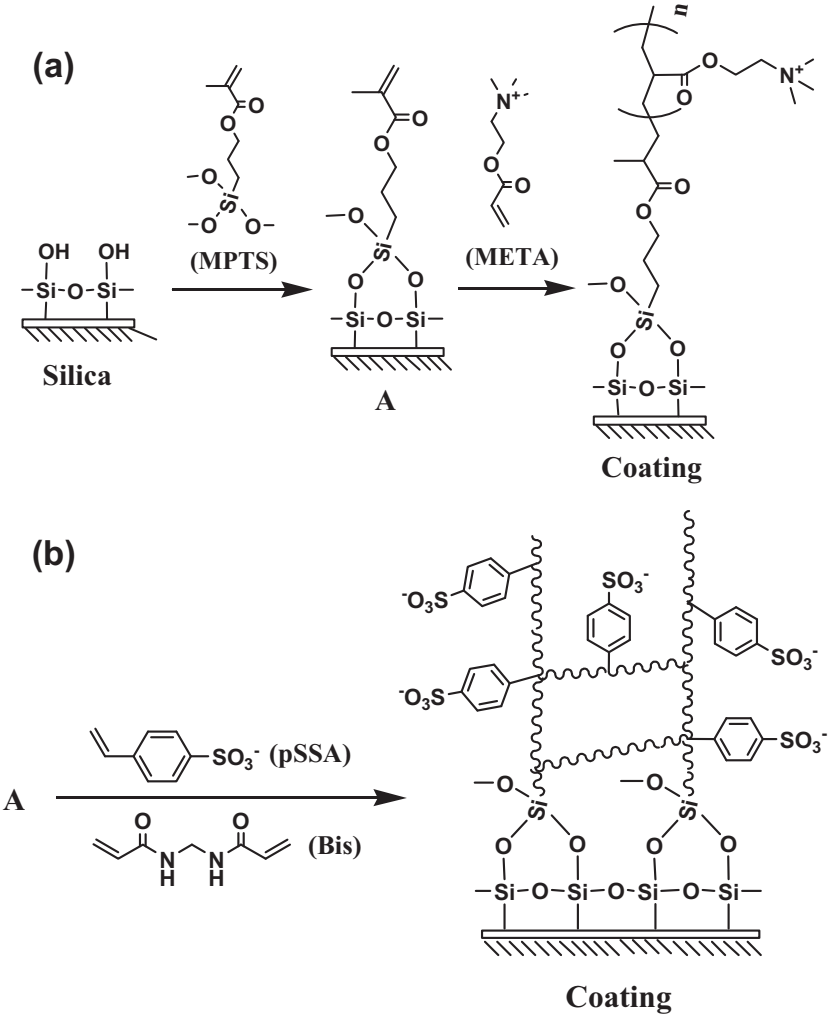

Fig. 2. Chemistry schemes for capillary wall coatings. (a) Production of positive coating. (b) Production of negative coating.

then a monolith column. The separated peptides and proteins were monitored using a Linear UVIS 200 absorbance detector at 215 nm. The absorbance signal was acquired using a NI multifunctional card DAQCard-6062E (National Instruments, Austin, TX), and the data was processed with an in-house written Labview program.

3. Results and discussion

3.1. Derivatization of capillary inner surfaces

Our intention of this project was to develop a serially stacked EOP to boost its pumping power (pressure and flow rate) for HPLC separations of proteins and peptides. Since these separations are usually carried out using eluents containing 0.1% trifluoroacetic acid, we had to derivatize the inner walls of the pump capillaries so that these capillaries could produce significant EOF under this acidic condition.

Fig. 2 presents two chemistry schemes we used to derivatize our capillaries. These chemistries were selected because we frequently coat capillaries with cross-linked polyacrylamide [35] for DNA and protein separations and are familiar with these processes. The capillaries we used before had inner diameters of $\geq$50 μm and lengths of $\leq$60 cm. In this work, we utilized 1-m-long and 5-μm-i.d. pump capillaries for constructing our EOPs. One noticeable difference was the enhanced flow resistance of the 5-μm-i.d. capillary. When we flushed a solution through this capillary, it took several minutes for the solution to go from end to end. Because derivatization reactions were carried out by flushing reactant solutions through a capillary, the reaction conditions at one end of the capillary could be different from those at the other end of the capillary. To examine whether these differences yielded non-uniform coatings, we cut the coated capillary evenly into 4 segments and measured the EOF of each segment. We noticed that positive coatings were pretty uniform. For the negative coatings, however, the EOF was lower near the solution-entrance end than that near the solution-exit end of the capillary. At this stage, we do not understand completely why the EOF changed. It was likely caused by the varying reaction times.

We also observed that the stability of the negative coatings improved dramatically with the addition of a small amount of cross-linker. Without the cross-linker, the EOF declined rapidly (to <20% of its original value in 10 days) under the test conditions. To maximize the EOF and improve the coating stability, we also optimized the monomer concentration and polymerization time. For negative coatings, the optimized monomer concentration was [pSSA] = 0.20 M, and the polymerization time was ~35 min. For the positive coatings, the optimized monomer concentration was [META] = 1.5%, and the polymerization time was ~30 min. Under these optimized conditions, the electroosmotic mobility, $|\mu_{eo}|$, could often be close to or greater than 4×10^{-4} cm^2 V^{-1} s^{-1}.

Fig. 3 presents the results of a stability test. The test started right after the capillaries were prepared. The test was performed by flushing the capillaries (n = 5) with an acidic eluent (0.10% TFA in

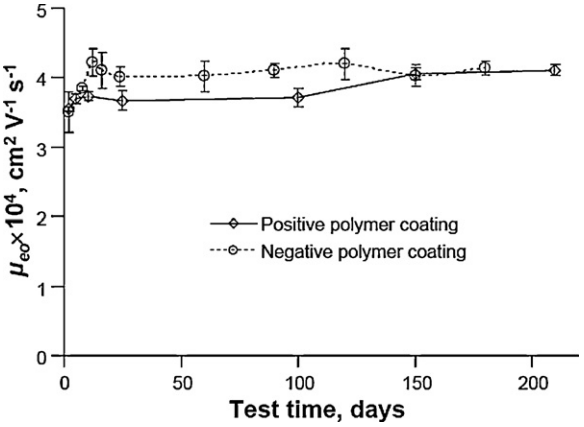

Fig. 3. Coating stability. The capillaries were normally filled with an aqueous solution containing 0.1% TFA and 15.9% ACN.

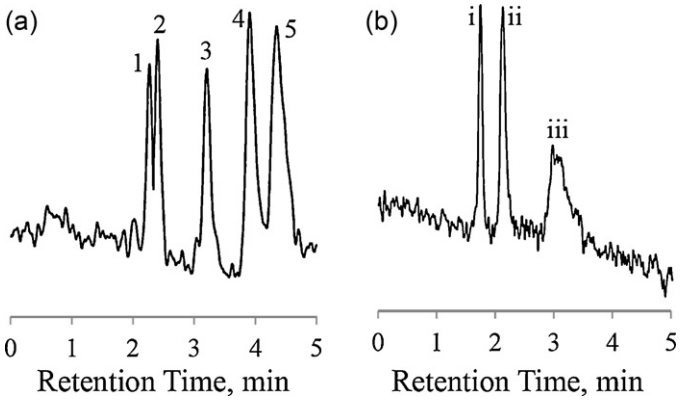

Fig. 4. Typical chromatograms for peptide and protein separations. The separations were performed using a ten-unit hybrid EOP and a poly(SMA-EDMA) monolithic capillary column. The effective column length was 20 cm. The total voltage applied on the EOP was 20 kV. The eluent contained 0.1% TFA and 15.9% ACN in water. Under these conditions, the pump flow rate was 58 nL/min and pumping pressure was 1.8 MPa. The wavelength of UV detector was set 210 nm. (a) The peptide sample contained 0.040 mg/mL Gly-Tyr, 0.040 mg/mL Val-Tyr-Val, 0.040 mg/mL Met enkephalin, 0.080 mg/mL Leu enkephalin, and 0.080 mg/mL Angiotension II. The mixture was dissolved in 0.10% TFA. (b) The protein sample contained 0.50 mg/mL Ribonuclease A, 0.80 mg/mL Insulin, and 1.4 mg/mL Cytochrome C in 0.10% TFA.

15.9% acetonitrile and 84.1% water), measuring the electroosmotic mobilities of the capillaries as described in Section 2, and storing the capillaries with the eluent filled inside. The results seemed "unbelievable", compared to the notorious EOF fluctuations in CE. One explanation is that we used the same pump solution all the time, which prevented the inner walls of the pump capillaries from contaminations. It should be pointed out that these coatings were not stable under basic conditions.

3.2. Construction of serially stacked EOP

In order for the EOP to produce adequate pump pressure, we need to use narrow and long pump capillaries. We tried to use smaller i.d. (e.g., 2 μm) capillaries initially but ran into problems with wall coating. We ended up with 5-μm-i.d. and 1-m-long pump capillaries. Using the above established coating conditions, we prepared both positively and negatively coated capillaries and assembled +EOPs and −EOPs using these capillaries. Fifty two parallel pump capillaries (1-m-long and 5-μm-i.d.) were utilized to build our pumps. The 1-m-long capillaries were used so that we could apply 20–30 kV across these capillaries without producing a lot of current to protect the gel-filled capillary (see Fig. 1b). If a high current passes through a gel-filled capillary constantly, bubbles will form inside the capillary to break the electric continuity. Referring back to Fig. 1a, there were 104 capillaries (52 positively coated and 52 negatively coated) in that basic hybrid EOP unit. The pump solution was 0.10% TFA in 15.9% acetonitrile and 84.1% water, and this solution was also the eluent for our HPLC separations. The composition of the eluent was optimized to ensure baseline resolutions for isocratic separations of the selected proteins and peptides.

After the +EOP and −EOP were assembled, we measured their maximum flow rates (the flow rates at a zero backpressure) and maximum pumping pressures (the backpressures at a zero flow rate) when different external voltages (from 5 kV to 40 kV) were applied. The flow rate and pumping pressure increased linearly with the external voltage applied (r^2 = 0.996–0.998). The good linear coefficients indicated that Joule heating was effectively dissipated as 40 kV was applied. Although we applied 40 kV to examine the linear range of the maximum flow rate and pressure, we normally did not apply voltages beyond 25 kV. [Extra cautions must be taken when high voltages are applied.] We then combined the +EOP and −EOP into a basic EOP unit (see Fig. 1a) and measured its maximum flow rate and pumping pressure again. As expected, the maximum pumping pressure of the hybrid EOP was the sum of the pumping pressures of the +EOP and −EOP, while the maximum flow rate remained approximately the same. Such a pump unit could work reliably when an external voltage of 20 kV was applied, producing a maximum flow rate of ~90 nL per minute and a maximum pumping pressure of ~2.5 MPa.

An outstanding feature of this EOP is that we can conveniently connect many of these basic pump units in series, because the inlet and outlet of this pump unit are at the same potential (e.g., GND in Fig. 1a). An excellent outcome of this serial-stacking is an enhanced pumping pressure. Very good linear relationship (r^2 = 0.999) was obtained between the maximum pumping pressure of this EOP and the number of pump units. As a result, a ten-unit serially stacked EOP produced a maximum pressure of 21.5 MPa (~3100 psi) as an external voltage of 20 kV was applied. This pump drew a current of ~140 μA and ~3 W power under these conditions. Compact HV systems capable of supplying 400 μA at 25 kV are commercially available (EMCO, Sutter Creek, CA), and these HV systems allow us to construct 28-unit EOP without problems.

3.3. Application of serially stacked EOP

To demonstrate the feasibility of using this pump to drive HPLC separations, we constructed a monolithic-capillary-based HPLC system as presented in Fig. 1c. Fig. 4 presents typical chromatograms for isocratic separations of peptide and protein. The analytes were baseline-resolved in about 5 min. The reproducibility tests showed a relative standard deviation of ≤5% (n = 5) for these separations.

The pump rate and pressure were fairly sensitive to eluent composition changes. As the acetonitrile concentration increased, the electroosmotic mobility decreased from 4.6×10^4 cm^2 V^{-1} s^{-1} (at 0.1% TFA and 0% acetonitrile in water) to 4.0×10^4 cm^2 V^{-1} s^{-1} (at 0.1% TFA and 20% acetonitrile in water), and to 3.6×10^4 cm^2 V^{-1} s^{-1} (at 0.1% TFA and 50% acetonitrile in water). While this was not a problem for isocratic elutions, it would be problematic if a gradient eluent needs to be pumped.

4. Conclusions

We have developed an innovative hybrid EOP that can be connected in series to boost pumping pressure, and pumping pressures of more than 3000 psi have been achieved. Theoretically, we can always enhance the pumping pressure by adding more hybrid EOPs in the series, as long as the high voltage power supply can provide

the necessary voltage and current. We have also demonstrated the feasibility of using this serially stacked EOP to directly drive eluents for HPLC separations of proteins and peptides. However, the pump rate and pressure were sensitive to eluent composition changes, which made the pump unreliable to drive gradient elutions. We are building alternative EOPs with gradient-elution capability, and the results will be published elsewhere.

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