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Study of an electroosmotic pump for liquid delivery and its application in capillary column liquid chromatography

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Abstract

A packed-bed electroosmotic pump (EOP) was constructed and evaluated. The EOP consisted of three capillary columns packed in parallel, a gas-releasing device, Pt electrodes and a high-voltage power supply. The EOP could generate output pressure above 5.0 MPa and constant flow rate in the range of nl/min to a few μl/min for pure water, pure methanol, 2 mM potassium dihydrogenphosphate buffer, the buffer–methanol mixture and the pure water–methanol mixture at applied potentials less than 20 kV. The composition of solvent before/after pumping was quantitatively determined by using a gas chromatograph equipped with both flame ionization detector and thermal conductivity detector. It was found that there were no apparent changes in composition and relative concentrations after pumping process for a methanol–ethanol–acetonitrile mixture and a methanol–water mixture. Theoretical aspect of the EOP was discussed in detail. An capillary HPLC system consisting of the EOP, an injection valve, a 15 cm × 320 μm i.d., 5 μm Spherigel C_{18} stainless steel analytical column, and an on-column UV detector was connected to evaluate the performance of the EOP. A comparative study was also carried out with a mechanical capillary HPLC pump on the same system. The results demonstrated that the reproducibility of flow rate and the pulsation-free flow property of the EOP are superior to that of mechanical pump in capillary HPLC application.

Keywords: Electroosmotic pump; Pumps; Instrumentation

1. Introduction

Miniaturized liquid delivery devices used in microfluidic systems have been studied extensively in recent years [1–8]. For packed capillary high-performance liquid chromatography (μ-HPLC), the operating pressure normally exceeds 5 MPa at flow rate of 0.5–5 μl/min. Although there are very reliable commercial mechanical pumps used in such a low flow range, the costs of these are very high and some of them often suffer from unstable liquid delivery because of the leakage both from the check valves and dynamic sealing of piston at pressures above 5 MPa. Most of the micropumps studied so far are for low-pressure purposes except certain type of electroosmotic pump [9–13] and a thermal expansion pump [14]. The state of the art of the thermal pump is for interrupted operation rather than continuously pumping. The open-channel electroosmotic pumps (EOPs) are widely used in capillary electrophoresis and in lab-on-a-chip system, and a novel multiple open-channel electroosmotic pumping system for microfluidic sample handling has been reported recently by Lizar and Karger [15]. The disadvantages of the open-channel pump are the unstable flow rate and low pumping output pressure, in most cases lower than 100 cm hydraulic pressure. On the other hand, the high-pressure packed channel EOPs based on electroosmosis principle have been investigated by several authors in recent years [16,9–13]. Gan, et al. [16] reported an EOP pump, which could generate about 1 ml/min flow rate and 0.15 MPa pressure for water at applied voltage of 500 V, used in a flow injection analysis system; Paul et al. [10] reported a packed capillary EOP, which could generate about 0.04 μl/min flow rate and 1 MPa pressure for water buffer at applied voltage of 1.5 kV; Zeng et al. [12] described an EOP, which could generate flow rate of 3.6 μl/min and pressures in excess of 2 MPa for deionized water at 2 kV. Guan et al. utilized 25 cm × 0.32 mm i.d. columns packed with 5 μm irregular silica in their EOP, which could generate pressures up to 20 MPa and flow rate about 1.6 μl/min for water buffer at applied voltage of 28 kV

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[17]. The EOP shows potential applications not only in low-pressure micro-fluidic systems, but also in high-pressure packed capillary liquid chromatography (μ-HPLC).

The long-term stability of pumping rate, the pressure can be reached at a given output flow rate, and the properties of mobile phases can be delivered by EOP are the three key factors for practical application. The diaphragm-isolated chamber technique used in some EOPs has the advantage of isolating mobile phase to be delivered with the dielectrical fluid inside the pumping system, allowing delivery of almost all mobile phase regardless the properties of the solvents; while the disadvantage of the technique is the syringe-like function of the diaphragm chamber in which check valves has to be employed to control the suction-output action. The leakage of the check valves can be hardly avoided or neglected, especially for microliter liquid delivery.

The direct pumping mode in EOP has all the advantages of the pump mentioned above, but is limited to use only buffers in most literatures. The composition of mobile phases used in μ-HPLC contains organic solvents and water mixture in most applications, and even pure organic solvent occasionally. It is very important to be able to pump such mobile phases at pressure above 5 MPa.

The maximum flow rate at pressure above 5 MPa is another limiting factor of EOP in practical application. The output flow rate of an EOP is dependent on the output pressure, and is reaching maximum value at pressure equal to 0. It is more practical to characterize the EOP with flow rate at a given output pressure rather than the maximum values of them. Typical μ-HPLC needs at least 1.5 μl/min flow rate at a pressure of 5 MPa, when a 25 cm × 250 μm i.d. packed capillary column is used for separation.

Previous study showed that the flow rate of different solvents was not only dependent on the applied voltage and the characteristics of EOP, but also dependent upon the properties of the solvent [9–16]. It had never been approved by experiment about the possible molecular discrimination effect of an EOP for a mixture of organic solvents or a water–organic solvent mixture commonly used in reversed-phase HPLC. It would be questionable to be used in μ-HPLC if the EOP had the problem of molecular discrimination.

The objective of this paper is to study an new type (EOP), including its theoretical aspect, and evaluate the property of liquid delivery for pure water, buffer solution, pure methanol, methanol–water mixture and methanol–ethanol–acetonitrile mixture.

A comparative study was carried out between the EOP and a mechanical pump in μ-HPLC to evaluate the applicability of the EOP in μ-HPLC.

2. Theory

The electroosmosis is generally attributed to the formation of an electric double layer at the interface between a solid and a liquid containing ions or polar solvents. The surface of the fine silica particles packed in the microchannel, filled with a buffer solution of pH more than 3, or polar organic solvents such as methyl alcohol, becomes negatively charged as a result of the deprotonation of acidic silanol groups. To provide overall charge neutrality, the fluid adjacent to the surface of the fine grains becomes positively charged so that an electric double layer is formed. When an external dc electric field was applied to the two end of the system the liquid would move to form the electroosmotic flow (EOF), and the direction of the EOF is from positive to negative.

In the packed channel, the interstitial spaces between the particles act like multiple flow passages in parallel. We can model the structure of packed channel as an array of N capillaries with inner radius equal to the average pore radius of particles, a and with the length of the tortuous flow path through the pores, L_e. The total behavior of EOF in the packed channel can be estimated by using the behavior of EOF in a single capillary as discussed below. The net electroosmotic velocity, $v_z(x)$, of a liquid in a capillary of radius, a, is the difference of the electroosmotic flow velocity under a potential gradient, $E_z = V/L_e$, and the counter flow inside the channel at a pressure gradient, $P_z = \Delta P/L_e$ [18].

$$v_z(x) = -\frac{\varepsilon\zeta E_z}{\eta}\left[1 - \frac{\zeta(\kappa x)}{\psi}\right] - \frac{P_z a^2}{4\eta}\left[1 - \left(\frac{x}{a}\right)^2\right] \quad (1)$$

where the minus sign in the first term of Eq. (1) means that when the zeta potentia ζ is negative, the direction of electroosmotic velocity is the same as that of electric field E; ε is the dielectric constant, η the viscosity of the liquid, P_z the pressure gradient along the flow direction z, E_z the electrical field, κ is the reciprocal of the double-layer thickness, ψ the wall potential in capillary, and ζ the zeta potential at a point distance x from the axis. The nondimensional potential distribution associated with electric double layer ions, ζ/ψ can be calculated by solving the Poisson–Boltzmann equation for a cylindrical geometry. In order to simplify the analysis, we make the Debye–Huckel approximation that the potential energy associated with each double layer ion is small compared with the kinetic energy of the ions [19]. For Debye–Huckel charge layers, the potential is given below [18]:

$$\zeta(\kappa x) = \psi\frac{I_0(\kappa x)}{I_0(\kappa a)} \quad (2)$$

where I_0 is the zero-order modified Bessel function of the first kind. Integrating Eq. (1) for $v_z(x)$ over a cross-section πx^2 gives the volume flow rate in a single capillary:

$$q = \int_0^a v_z(x)\,\mathrm{d}(\pi x^2) = -\frac{\pi a^2\varepsilon\zeta E_z}{\eta}\left[1 - \frac{2I_1(\kappa a)}{\kappa a I_0(\kappa a)}\right] - \frac{\pi a^4 P_z}{8\eta} \quad (3)$$

where I_1 is the first-order modified Bessel function of the first kind. The flow rate of the porous medium, Q, which is calculated for N of these capillaries, is:

$$Q = Nq = -\frac{N\pi a^2 \varepsilon \zeta E_z}{\eta}\left[1 - \frac{2I_1(\kappa a)}{\kappa a I_0(\kappa a)}\right] - \frac{N\pi a^4 P_z}{8\eta} \quad (4)$$

In order to convert the dimensions of the actual tortuous-path pore channels to the physical dimensions of the packed channel, two parameters are defined [12]. First, tortuosity is defined as $\tau = L_e/L$, where L_e is the average length of migration along the pore path and L the physical length of the packed channel [20]. The second parameter is porosity, defined as $p = V_{dry}/V_{wet}$, where V_{dry} and V_{wet} are the void and total volumes of the packing materials, respectively. The effective cross-sectional area of the porous medium is then approximated as $A_e = N\pi a^2 = (p/\tau)A$, where A is the cross-sectional area of the packed channel. The total flow rate of the entire porous medium is then:

$$Q = -\frac{pA\varepsilon\zeta V}{\tau^2 \eta L}\left[1 - \frac{2I_1(\kappa a)}{\kappa a I_0(\kappa a)}\right] - \frac{pA\Delta P a^2}{8\tau^2 \eta L} \quad (5)$$

where ΔP is the pressure difference along the length of the capillary.

When the counter flow rate inside the channel eventually counterbalances the electroosmotic flow, i.e., the net flow rate is zero, the maximum pressure ΔP_m generated across the porous structure is obtained from Eq. (5).

$$\Delta P_m = -\frac{8\varepsilon\zeta EL}{a^2}\left[1 - \frac{2I_1(\kappa a)}{\kappa a I_0(\kappa a)}\right] \quad (6)$$

The maximum flow rate Q_m of the entire porous medium under the condition of no counter pressure is:

$$Q_m = -\frac{pA\varepsilon\zeta E}{\tau^2 \eta}\left[1 - \frac{2I_1(\kappa a)}{\kappa a I_0(\kappa a)}\right] \quad (7)$$

Substituting Eqs. (6) and (7) into (5), a relationship between the flow rate and the pressure of an EOP is obtained:

$$\Delta P = -\frac{\Delta P_m}{Q_m} Q + \Delta P_m \quad (8)$$

For a given length of a packed channel, a given particle material and working fluid, where ε, ζ, L, a^2, τ^2, p, A, and η are all fixed values, Eqs. (6) and (8) indicate that both the pressure and the flow rate are proportional to the electric field E (applied voltage), and the pressure is proportional to the length of the packed channel under ideal condition. Eq. (5) shows that the flow rate is proportional to the cross-sectional area of the packed channel. The maximum flow rate Q_m is independent of the length of the packed channel (contrary to Ref. [12]), but the Q is related indirectly with L when the load of the EOP is taken into account. It was proved by our experiments described elsewhere [21].

3. Experimental

3.1. Electroosmotic pump

The EOP system designed in this study was a one-stage, triple channel system where three packed columns were used as electroosmosis channels and were connected in parallel in order to increase the flow rate capacity while maintain the efficiency of heat dissipation of the columns. The system is outlined in Fig. 1A. A gas-releasing device was added at the outlet of the EOP in order to expel the gases produced by electro-chemical reaction. Cylindrical shaped Pt electrodes were placed at both ends of the packed columns (see Fig. 1A).

The preparation of the packed capillary columns of EOP was one of the key steps for construction of the EOP. The packing equipment consisted of a high-pressure pump (Dalian Institute of Chemical Physics, Dalian, China) and a 20 cm × 1 mm i.d. stainless-steel slurry reservoir. About 0.3 g of porous silica spheres (average particle size 2 μm, Dalian Institute of Chemical Physics) were mixed with 1 ml chloroform. Then the solution was sonicated by an ultrasonic bath (Shanghai Barnson, China) for 3 min to disperse the packing particles. The slurry was then transferred into the packing reservoir connected to a column blank. The unoccupied volume of reservoir was filled with chloroform before connecting it to the packing pump. The packing pressure was increased at 5 MPa/s to 20 MPa and kept for pumping till about 100-fold of column volumes of pure chloroform was flown through the column, the pump was switched off. When the pressure returned to zero, the column was equilibrated with methanol for 1 h, then with pure water for another 2 h before use [13,21]. Three columns of 25 cm × 530 μm i.d. were prepared, and were used in the EOP. Alternatively, the columns can also be prepared by dry-packing method [22], and then conditioned by methanol water (70:30, v/v) for 1 h, pure water for another 2 h before

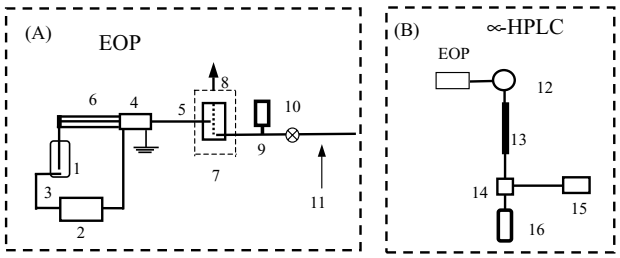

Fig. 1. Schematic diagram of the one-stage EOP and the μ-HPLC system. (A) The EOP system: 1, solvent reservoir, covered with an insulating sheath; 2, high-voltage direct current source module; 3, Pt wire; 4, hollow electrode (grounded); 5, capillary conduit; 6, packed columns, three packed columns connected in parallel; 7, gas releasing device (8, representation of gas leaving direction); 9, liquid pressure sensor; 10, open/close valve; 11, measurement point of flow rate. (B) The μ-HPLC system: 12, a four-port injection valve; 13, analytical capillary HPLC column; 14, an on-column UV-Vis detector; 15, chromatographic data station; 16, waste liquid bottle.

use. The packing process must be carried out in a ventilating cabinet because of the hazardous property of chloroform.

The packed columns of the EOP were filled with liquid being pumped either by capillary suction or another pump until all the packing was wet. When a high voltage was applied, the liquid moved from the solvent reservoir to the output end of the pump by EOF effect. The outlet of the pump was connected with a pressure sensor and an open-close valve as shown in Fig. 1. When the valve was closed (no net flow), the pressure was built up until it was counterbalanced by the pressure-driven flow, a internal flow from the outlet end back to the reservoir within the column, and an maximum hydraulic static pressure ΔP_m was established. The pressure value was read from the display board of the liquid pressure sensor (model: MSP-300-600-B-5-W-1, Measurement Specialties, Valley Forge, PA, USA).

The maximum flow rate Q_m was measured by a set of volume-micrometer attached to the outlet of the pump when pressure $\Delta P = 0$. The volume-micrometers were made of micro liter glass syringes (Shanghai Anting, China). Before each measurement, the meter was connected to a vacuum line to dry-out the residual liquid. A stopwatch was used to record the time lap of a given volume of liquid that entered into the metering range of the meter.

When a given length of a packed capillary HPLC column was connected to the outlet of the EOP, a pressure ΔP was established because of the resistance of the load column to the flow of mobile phase delivered by the EOP. The pressure values were red from the display board of the pressure sensor, and the flow rates were measured by the same method described above. By using deferent length of load columns, we measured a series of ΔP and Q values of the EOP at a given applied voltage. For each experiment, the ΔP and Q values were measured four times under the same conditions. All experiments were performed at room temperature.

3.2. μ-HPLC system

The system consisted of a four-port injection valve with a internal loop of 200 nl (VICI, Switzerland), a laboratory, 15 cm × 320 μm i.d. 5 μm Spherigel C_{18} stainless steel analytical column and a Jasco 1575 on-column UV-Vis detector (Jasco, Japan). The system was schematically shown in Fig. 1B.

The mobile phase used was a methanol–water mixture containing no salt or other additives.

A Jasco PU-1580 intelligent HPLC pump with minimum flow of 1 μl/min was used for a comparative study.

3.3. Chemicals

Bidistilled pure water was prepared in our laboratory starting from pure water (Wahaha Hangzhou, China); analytical grade potassium dihydrogenphosphate was from Shenyang Chemical Reagents Factory (Shenyang, China) and was prepared to a concentration of 2 mM buffer in bidistilled water; chromatographic grade methanol, ethanol and acetonitrile were from Tedia Company, USA; the mixture of methanol with the buffer (50:50, v/v), the mixture of water with methanol (50:50, v/v), and the mixture of methanol, ethanol and acetonitrile (1:1:1, v/v/v) were prepared. They were used as the mobile phase to evaluate the performance of the EOP. All mixtures were sonicated for about 5 min before use.

Samples containing thiourea (0.1 mM), benzene (0.3 mM), toluene (0.2 mM), naphthalene (0.2 mM), biphenyl (0.2 mM), phenanthrene (0.2 mM) and anthracene (0.3 mM) (Shenyang Chemical Reagents Factory, analytical grade) were prepared in mobile phase.

The concentration of the mobile phase before/after pumped may change because of the electro-driven principle. To clarify the suspicion, a Perkin-Elmer LX-Auto-System gas chromatograph equipped with both flame ionization detection (FID) and thermal conductivity detection (TCD) systems was used to measure the composition of the solvents. A 30 m × 0.53 mm i.d. 0.6 μm SE-54 capillary column (KeFen, Dalian Institute of Chemical Physics) together with FID was used to measure the mobile phase of methanol ethanol acetonitrile. While the mobile phase containing water and methanol was measured on a column of 1 m × 2 mm i.d. packed with GDX401 porous polymer material (KeFen) using TCD. Hydrogen was used as carrier gas. The injection volume was 0.1 μl. Four repeated measurements were carried out for each sample.

4. Results and discussion

4.1. The maximum pressure and flow rate

Factors affecting the pressure ΔP and flow rate Q of the EOP are all shown in Eqs. (5)–(8). For a given packed column and working fluid, the equations indicate that the pressure and flow rate are proportional to the strength of the electric field, E (in the pump studied, $E = V/L = V/25$ cm). The easiest way to change the pressure or flow rate of a given pump is to control the applied voltage. As shown in Figs. 2 and 3, the pressures and flow rates were proportional to the applied voltage. For different working fluids, the ΔP–V or Q–V relations were varied depending on the properties of the fluid (see Figs. 2 and 3). The relationships were linear when heat dispatch of the EOP columns was sufficient and no over-heating of the columns was happened. As the applied voltage of the EOP went even higher, for example, from 25 kV (1 kV/cm) to 30 kV (1.2 kV/cm), so as to yield higher pressure and flow rate, we found that the ΔP–V linear relation began to fall with 2 mM potassium dihydrogenphosphate buffer because of the joule heating (data were not shown in the figures).

The flow rate of the EOP can be also increased by using packed columns of larger cross-section area (see Eq. (5)). The best way to do so is to use several columns connected in

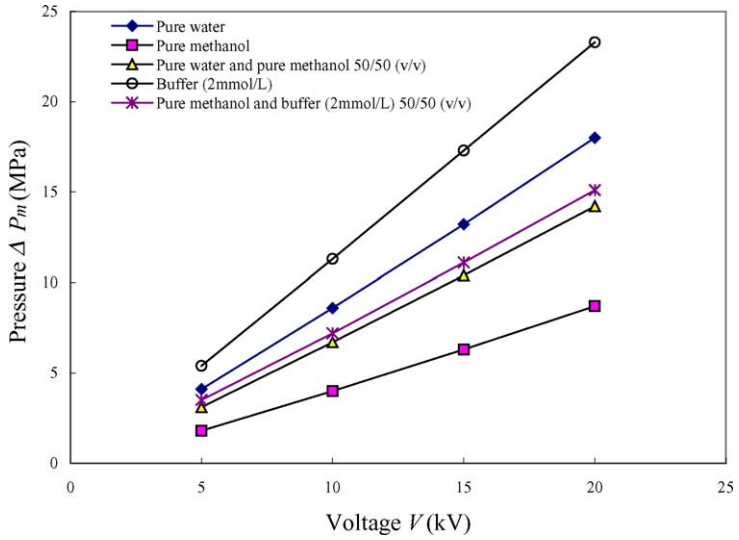

Fig. 2. Relation between the maximum pressure, ΔP_m, and applied voltage V (kV). The solvents are pure water, pure methanol, 2 mM potassium dihydrogenphosphate buffer and their mixtures.

parallel to improve the heat dispatch of Joule heating. That was the reason that we used three columns in parallel for the EOP being studied.

4.2. The relationship between ΔP and Q

Eq. (8) expresses the relationship between the pressure ΔP and the flow rate Q of an EOP. The maximum flow rate, Q_m, was measured at pressure ΔP equal to zero (no load resistance), while the maximum pressure ΔP_m was measured at flow rate equal to zero (load resistance $\rightarrow \infty$). When the resistance of a load is above zero, a flow through the load with a certain flow rate Q will generate a pressure ΔP across the load. Fig. 4 demonstrated the ΔP–Q relationships of pure water and the mixture of water–methanol 50:50, v/v) at the applied voltage of 20 kV. The ΔP–Q relationships were dependent on the properties of the liquid, and were linear within the experimental conditions as shown in the Fig. 4. When the fluids being pumped were the mixture of water–methanol (50:50, v/v), for example, the maximum static pressure ΔP_m was about 14 MPa at $Q = 0$, and 12 MPa at $Q \approx 2.6$ μl/min, and so on. It is the same when a capillary HPLC column was connected to the output end of the pump (see Fig. 1). Different from mechanical pump, the flow rate of mobile phase through the capillary HPLC column was determined by Eq. (8), i.e., the characteristics of the EOP, the load resistance and the properties of mobile phase.

The pressure and flow rate generated by the EOP can meet the requirement of μ-HPLC with column i.d. of less

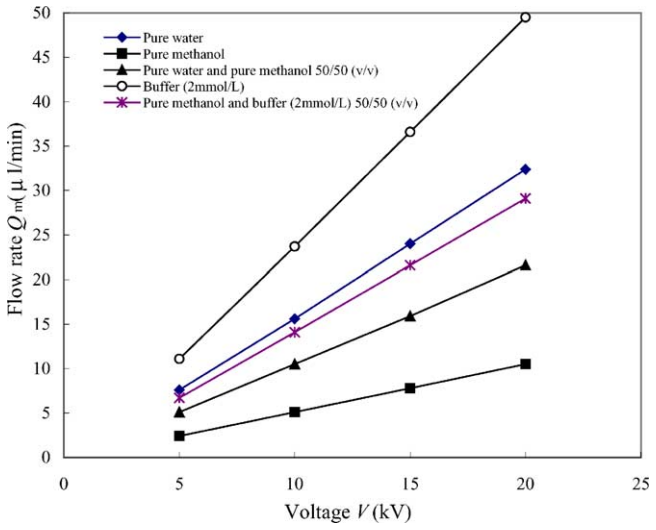

Fig. 3. Relation between the maximum pressure, Q_m, and applied voltage V (kV). The solvents are pure water, pure methanol, 2 mM potassium dihydrogenphosphate buffer and their mixtures.

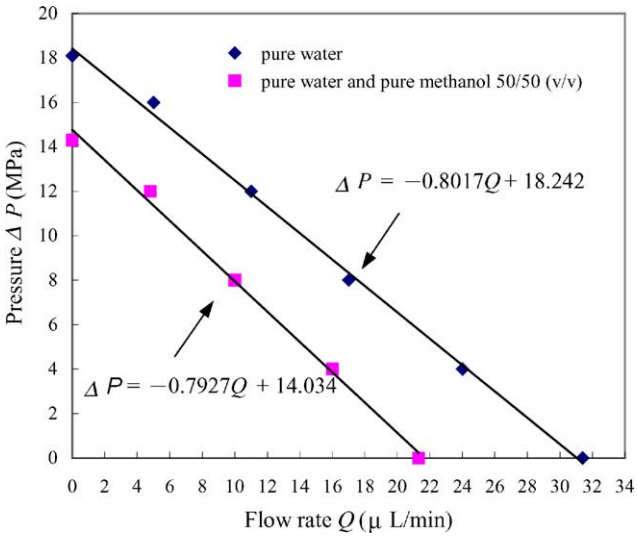

Fig. 4. The relationship between ΔP and Q at $V = 20$ kV.

Table 1
The changes of solvent mixture composition before and after pumping

Mixtures (detector)	Components	A_{peak} (Be) (mean, $n = 4$)	R.S.D. (%)	A_{peak} (Af) (mean, $n = 4$)	R.S.D. (%)	Relative error of A_{peak} (%)
Organic solvent (FID)	Methanol	25.74	2.7	25.28	2.7	−1.79
	Ethanol	32.65	2.7	33.82	2.1	+ 3.58
	Acetonitrile	41.61	0.45	40.90	0.87	−1.71
Methanol water (TCD)	Water	63.44	0.40	63.65	0.60	+ 0.33
	Methanol	36.56	0.74	36.35	1.05	−0.57

A_{peak} is unitary peak area; A_{peak} (Be) the peak area before pumping, and A_{peak} (Af) the peak area after pumping.

than 530 μm for the commonly used mobile phase in reverse phase liquid chromatography.

4.3. The mobile phases before/after pumping

The mobile phase delivered by the EOP was in direct contact with the surface of packing particles and the wall of the channels without any diaphragms as in Refs. [13,21]. When the solution was a mixture of two or more different polar organic solvents, or a mixture of water and polar organic solvent, the EOP could also generate flow and high pressure. The $Q_m V$ relations in Fig. 3 were dependent upon the property of solvent. We were suspicious about the possible molecular discrimination of the EOP when mixed solvents were driven and different molecules passed through the packed electroosmotic column. To verify the suspicion, a mixture of methanol, ethanol and acetonitrile, and a mixture of water and methanol, was measured before and after pumping by the GC instrument, and the results were shown in Table 1. The data in the Table 1 proved that there was no obvious change of composition within our experimental error during the electro-pumping process.

4.4. Application of an EOP in μ-HPLC

In order to evaluate the performance of the EOP for a practical application, we used the EOP as the pump for mobile phase delivery in the μ-HPLC system. A mechanical pump was also used instead of the EOP on the same μ-HPLC system under identical chromatographic conditions except the pumping system. The sample containing thiourea, benzene, toluene, naphthalene, biphenyl, anthracene and phenanthrene was separated on a column of 15 cm × 320 μm i.d. packed with 5 μm C_{18}. The chromatograms obtained by EOP driven and mechanical pump driven were shown in Figs. 5 and 6, respectively. The sequence of the peaks was the same and the retention times of the peaks were about the same in the two chromatograms, which demonstrated that there was no visible property change of the mobile phase composition during the electro-driven process of the EOP. Note that when the EOP was used, all the peaks were baseline separated; while the peaks in Fig. 6 were wider than in Fig. 5 and had some overlap between peaks 6 and 7, although the mobile phase composition and flow rate were the same. The number of

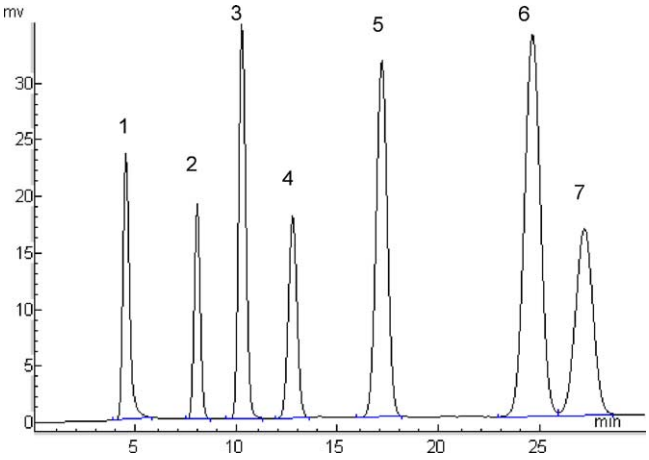

Fig. 5. Chromatogram of a test mixture by using the EOP. Conditions: mobile phase, methanol/water (80:20, v/v); applied voltage of EOP, 20 kV; separation column, 15 cm × 320 μm i.d. (C_{18}, 5 μm); injector, 200 nl; detector, UV at 254 nm. Peaks: 1, thiourea; 2, benzene; 3, toluene; 4, naphthalene; 5, biphenyl; 6, phenanthrene; 7 anthracene.

theoretical plates of the column was about 2.3–3.2·10^4/m with EOP driven, and 1.4–2.3 × 10^4 m^{-1} with mechanical pump delivery. The loss of column efficiency was attributed to the fluctuation of flow rate caused by the mechanical

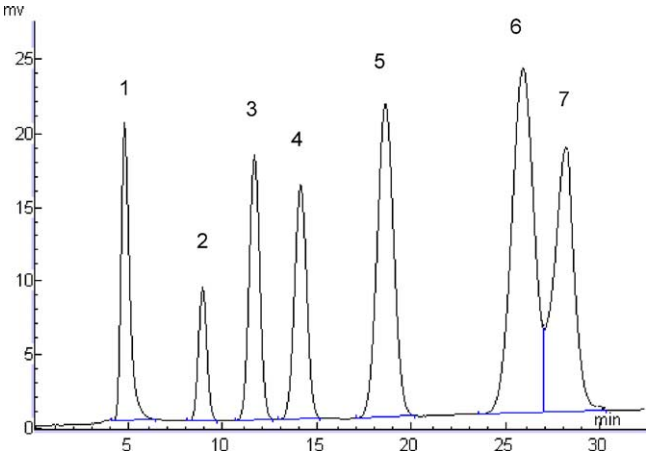

Fig. 6. Chromatogram of a test mixture by using the mechanic pump (Jasco PU-1580 intelligent HPLC pump). Conditions: flow rate at 3 μl/min, using split technique to adjust the head pressure similar to that when using the EOP; other conditions are the same as Fig. 5; the sequence of the peaks is the same as those in Fig. 5.

Table 2
Comparison of repeatability of retention time, t_r, by using the EOP and the mechanical pump

Sample	EOP		Mechanical pump	
	t_r (min) (mean, $n = 4$)	R.S.D. (%)	t_r (min) (mean, $n = 4$)	R.S.D. (%)
Thiourea	4.531	0.77	4.917	3.58
Benzene	8.059	0.66	8.792	1.91
Toluene	10.278	0.66	11.262	2.59
Naphthalene	12.776	0.65	13.678	2.52
Phenanthrene	17.172	0.58	18.312	1.88
Biphenyl	24.568	0.60	25.340	1.90
Anthracene	27.094	0.63	27.826	1.87

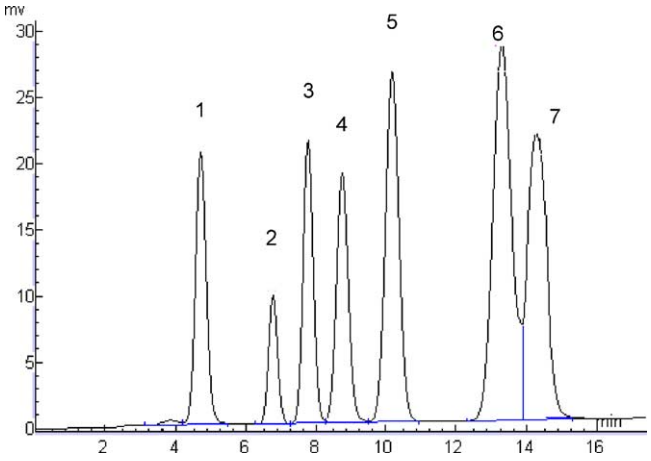

Fig. 7. Chromatogram of a test mixture by using the EOP. Conditions: mobile phase: methanol-water (85:15, v/v); applied voltage of EOP: 20 kV; other conditions as in Fig. 5; peak identities: 1, thiourea; 2, benzene; 3, toluene; 4, naphthalene; 5, biphenyl; 6, phenanthrene; 7, anthracene.

pump at μl/min flow rate range. We adjusted the mobile phase to methanol–water (85:15, v/v), and ran the same sample again. The chromatogram was demonstrated in Fig. 7. The total analysis time was less than 16 min in sacrificing the separation of peaks 6 and 7. This experiment proved that with EOP the composition of the mobile phase could be adjusted according to the requirement of separation.

4.5. The reproducibility of the retention

The retention time t_r of peaks in the chromatograms reflected the flow rate stability of the EOP, since the t_r depended upon the mobile phase flow rate when other conditions were kept constant. The results were shown in Table 2 for both EOP and mechanical pump delivery of mobile phase. The error of t_r expressed in RSD% was within 0.8% with EOP as pump for all compounds tested, while the error of t_r reached 3.6% with the mechanical pump.

5. Conclusion

In this paper, a concise theory on a packed column-based EOP was presented. The ΔP_m is proportional to the length of the packed column (or internal resistance of the column) and the Q_m is proportional to the cross-section area of the column. Both ΔP_m and Q_m are proportional to the applied voltage. The Q and ΔP are related to the ΔP_m and Q_m and the resistance of the load, and are proportional to the applied voltage. We did not observe composition changes of mobile phases before/after pumping within our experimental error. A comparative study of the EOP with a mechanical μ-HPLC pump in capillary chromatography proved that the EOP is advantageous over the piston pumps for capillary liquid chromatography because of pulsation-free liquid delivery property of EOP. High pressure, low volumetric flow, non-stop, pulsation free, friction free, dynamic sealing free and mechanical driven free of fluids delivery are the characteristics of the EOP, which shows great potential in the application of μ-HPLC and other microfluidic systems. The EOP studied was for use in μ-HPLC with separation columns of $\leq$530 μm i.d. When lower flow rate and higher pressure is required, the EOP should be modified as to use longer packed channel or finer packing particles.

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