

# Application of a high-pressure electro-osmotic pump using nanometer silica in capillary liquid chromatography

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## Abstract

A novel designed electro-osmotic pump (EOP) with simple structure was assembled using three 20 cm × 530 μm i.d. fused-silica capillaries packed with 20 ± 5 nm silica grains for capillary liquid chromatography. It was found that the pump could generate pressures over 20 MPa and several μL/min flow rate for most of the liquids being delivered with the applied voltage less than 10 kV. By increasing the pressure, decreasing the applied voltage and the electrical current, the thermodynamic efficiency was about 1–4%. A practical application of the EOP in a 20 cm × 150 μm i.d. 3 μm C<sub>18</sub> fused-silica analytical capillary column demonstrated the applicability of the pump.

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**Keywords:** Electro-osmotic pump; Pump; Nanometer silica; Nanochannel; Capillary liquid chromatography

## 1. Introduction

In recent years, the electro-osmotic pump (EOP) based on the electrically induced osmosis principle is becoming a hot research topic [1–10]. The important feature of the EOP is it could generate pressures from tens to nearly a hundred megapascal with microflow rate at μL/min and sub-μL/min scale pulseless flow [2,3,6,11], which shows a potential application in microcolumn and capillary liquid chromatography. Although features and advantages of downsizing of separation columns have been investigated and demonstrated for a long time [12–16], microcolumn liquid chromatography using separation columns with inside diameters (i.d.) lower than 0.5 mm has not become popular so far. The reasons for slow popularization may lie in the fact that ancillary techniques such as pulseless micropump, gradient elution, sample projector techniques have not been matured in microcolumn liquid chromatography comparing to conventional column.

At the present time and state of the technique among commercially available types of fluid delivery systems, reciprocating and syringe pumps are favored solvent delivery systems for conventional and for microcolumn liquid chromatography. For flows in the order of 1–150 μL/min, reciprocating and syringe pump systems are commercially available, including in most cases devices for binary, ternary, or quaternary gradient formation. These delivery and gradient systems have proven to be reliable down to column i.d.s of approximately 1.0 mm. While for columns with i.d.s less than 500 μm till 50 μm, where the flow rate is in the range of 40 nL–4 μL at pressure of 6–8 MPa, the direct liquid delivery with required accuracy and reproducibility is almost impossible so far. For such flows, at the present time the use of split-flow techniques can be an attractive alternative. These systems are based on the application of packed restrictor columns [17] or flow splitting devices based on a microflow processor concept [18]. It is obvious that the capability of the EOP could compensate the shortage of the mechanical pump. In fact, the mechanic pump such as reciprocating pumps, syringe pumps at μL/min and sub-μL/min domain and pressures above 3 MPa will simply not operate reliably in this scale because of the

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uncontrollable leakage from check valves and dynamic sealing of pistons. Pumps that could deliver high pressure and sub- $\mu\text{L}/\text{min}$  pulseless flow are urgently demanding for microcolumn liquid chromatography, especially for capillary column and nanocolumn liquid chromatography.

In this paper, a novel design EOP with simple structure was designed using three fused-silica capillaries packed with nanometer silica grains. The EOP could generate pressures over 20 MPa and a few  $\mu\text{L}/\text{min}$  flow rate for most of the liquids being delivered with the applied voltage less than 10 kV. By increasing the pressure, decreasing the applied voltage and the electrical current, the thermodynamic efficiency of the pump was near the highest reported. Moreover, the improved EOP has a gas-releasing device being integrated into the pump, which could remove the detrimental bubbles from the mobile phase automatically due to the gravitational action ensuring the mobile phase run continuously and steadily without time limit. A practical application of the EOP in capillary of  $20\text{ cm} \times 150\ \mu\text{m}$  i.d.,  $3\ \mu\text{m}$   $\text{C}_{18}$  fused-silica analytical column has demonstrated that the EOP would find its applications capillary column liquid chromatography.

## 2. Experimental

### 2.1. Design of the electro-osmotic pump

The EOP is schematically shown in Fig. 1. The structure of the EOP was designed as a three-channel pump, which used three  $20 \pm 5\ \text{nm}$  silica (Zhoushan Mingri Nanometre Material Co., Zhejiang, China) packed columns with 20 cm length and  $530\ \mu\text{m}$  i.d. connected in parallel. The average particle diameter was about 20–30 nm by transmission electron microscopy (TEM) analysis (TEM H800: Hitachi, Japan). The prepara-

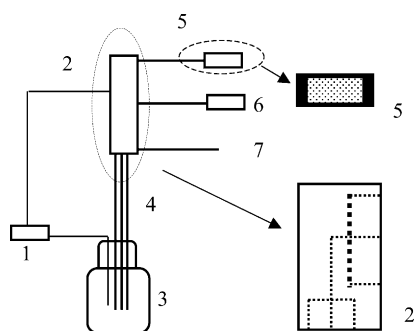


Fig. 1. Schematic diagram of the electro-osmotic pump. (1) A high voltage power supply; (2) a hollow electrode, which was made of stainless steel and connected to the negative pole of the power supply and at the same time used as the grounded electrode; (3) a solvent reservoir, which was connected to the positive pole of the power supply using a Pt wire (0.2 mm diameter) and covered with insulate sheath to prevent from the probably electric shock; (4) packed columns, three electro-osmotic columns connected in parallel; (5) a gas releasing channel, which was made by packing the same nanosilica particles in a stainless steel column; (6) a liquid pressure sensor; and (7) output end of the pumping liquid, to which an analytical system could be connected.

tive steps and the basic structure of the column were just the same as the column used in capillary electrochromatography [5–10]. There are three main methods to pack the electro-osmotic column, the slurry packing method [19], the drying packing method [20], and electrokinetic packing method [21]. In this paper, the slurry packing method was used [19]. The general packing procedures for fabricating packed capillary column involved three steps. First, the frit at one end of fused-silica capillary column was fabricated using a sodium silicate paste mixed with  $2\ \mu\text{m}$  porous silica particles (Dalian Institute of Chemical Physics, Chinese Academy of Sciences, China) and heated in an automatic time-programmed oven (the oven was time-controllable ranging from a few seconds to several minutes, laboratory-made) about  $300\ ^\circ\text{C}$  for about 10 s, then a particle slurry was pumped into the capillary and the filter-like “frit” retained the nanometer silica particles in place and then the second frit was formed by heating the other end of the packed-column with the sodium silicate paste. Column voids often happen in the second frit fabrication process, but should be avoided because they can cause a low fluidic resistance path for backflow. So, the second frit was often formed several times by heating the oven from lower temperature to higher temperature using a sodium silicate paste. The packed columns were dipped into the solvent reservoir directly. The design idea of the EOP was based on the capillary siphonal phenomenon to draw the liquids to the packed columns without additional wetting. When the high voltage power supply was applied to the system the liquid would move to form the electro-osmotic flow (EOF) continuously, and the direction of the EOF was from positive to negative.

### 2.2. Capillary liquid chromatographic separation system

The capillary liquid chromatography system consisted of the electro-osmotic pump, a UV CE-975 (Jasco, Tokyo, Japan) on-column detector, a four-port microvalve injector with an injection volume of 200 nL (VICI, Switzerland), a  $20\text{ cm} \times 150\ \mu\text{m}$  i.d.,  $3\ \mu\text{m}$   $\text{C}_{18}$  fused-silica analytical column (Unimicr Technologies, USA). Data were evaluated with KF-98 Chromatographic Station (Ver. 1.10, Dalian Sci-Tech Instruments, Dalian, China).

### 2.3. Other instruments and reagents

A Jasco PU-1580 intelligent HPLC pump; thiourea, benzene, toluene, naphthalene, biphenyl, anthracene, phenanthrene, and sodium dihydrogenphosphate were purchased from Shenyang Chemical Reagents Factory (Shenyang, China). Methanol and distilled water were of HPLC grade and obtained from Dalian Institute of Chemical Physics, Chinese Academy of Sciences (Dalian, China). Samples containing thiourea (0.1 mM), benzene (0.1 mM), toluene (0.1 mM), naphthalene (0.1 mM), biphenyl (0.2 mM), phenanthrene (0.2 mM), and anthracene (0.2 mM) (Shenyang Chemical Reagents Factory, analytical grade) were prepared in mobile

phase. The reagents were of analytical grade with filtration over a 0.5  $\mu\text{m}$  filter.

#### 2.4. Pressure measurement and flow rate measurement

In order to precisely determine the pressure  $P$  of the EOP, the gas releasing channel 5 and liquid output channel 7 were closed (ref. Fig. 1). Then, the power supply was turned on to make the liquid move from the solvent reservoir to the output end of the pump. The liquid would accumulate at the output of the pump and the hydraulic pressure would be generated with more and more liquid accumulation because of the incompressibility of the liquid. The pressure could be read conveniently from the display board of the liquid pressure sensor 6. In this way, the pressure  $P$  measured was a maximum static hydraulic pressure  $\Delta P_m$  (no net flow). In evaluation of the volume flow rate we have presented an easy method, a set of volume-micrometer was attached to the output of the pump with the liquid output channel 7 opened to the air. Before each measurement, the volume-micrometer was primed to vacuum to collect the liquid output from the pump, and at the same time the stopwatch was used to record the time. Volume flow rate was determined by measuring the volume of the fluid per unit time collecting at the outlet side of the pump. The flow rate  $Q$  measured here was a maximum flow rate  $Q_m$  because the liquid output channel 7 was opened to the air (no back pressure). When the output channel 7 of the EOP was connected to an adjustable resistor (porous channel), the flow rate measured was the flow  $Q$  through adjustable resistor with the head pressure  $\Delta P$ , just the same as the state of the pump connected to a load. All experiments were performed at room temperature. For each experiment, the flow rate and pressure were measured four times under the same conditions.

### 3. Results and discussion

#### 3.1. Characteristic of the EOP

##### 3.1.1. Performance of the EOP

If a potential is applied to the two ends of the packed channel in which the solid phase (small silica particles) is held stationary the liquid moves relative to the surface of the solid phase, this phenomenon is known as electro-osmosis. For a liquid containing ions, the ions within the diffuse layer move in response to the electric field and drag surrounding liquid with them forming an EOF and at the same time a counter flow will be generated. In the packed channel, the interstitial spaces between the particles act like multiple flow passages in parallel. The total behavior of EOF in the packed channel can be estimated by using the behavior of EOF in a single capillary [22,23]. The total flow rate of the packed channel is:

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

where the minus sign means that when the zeta potential  $\zeta$  is negative, the direction of EOF is the same as that of electric field  $E$ ;  $p$  and  $\tau$  are the porosity and tortuosity of the packed channel, respectively;  $I_0$  and  $I_1$  are the zero-order and first-order modified Bessel function of the first kind, respectively;  $A$  is the cross-sectional area of the packed channel,  $\varepsilon$  the dielectric constant,  $\eta$  the viscosity of the liquid,  $a$  the average pore radius of particles in the packed channel,  $L$  the length of the packed channel,  $\Delta P$  the pressure difference along the length of the channel and  $\kappa$  is the reciprocal of the double-layer thickness.

When the counter flow rate inside the channel eventually counter balances the EOF, i.e., the net flow rate is zero, the maximum pressure  $\Delta P_m$  will generate across the porous structure that is obtained from Eq. (1).

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

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

$$Q_m = -\frac{pA\varepsilon\zeta E}{\tau^2\eta} \left(1 - \frac{2I_1(\kappa a)}{\kappa a I_0(\kappa a)}\right) \quad (3)$$

Substituting Eqs. (2) and (3) into (1), a relationship between the flow rate and the pressure of an EOP is obtained:

$$\Delta P = -\frac{\Delta P_m}{Q_m} Q + \Delta P_m \quad (4)$$

For a given length of a packed channel, a given particle material and working fluid, where  $\varepsilon$ ,  $\zeta$ ,  $L$ ,  $a^2$ ,  $\tau^2$ ,  $p$ ,  $A$ , and  $\eta$  are all fixed values, Eqs. (2) and (3) 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 packed channel length under ideal condition. Eq. (2) shows that the pressure is inversely proportional to the square of the particle diameter in the packed channel. Eq. (3) shows that the flow rate is proportional to the cross-sectional area of the packed channel. Moreover, when pure polar organic solvent is used, in which the ion content is negligible, the polarization effects between the negatively charged silica surface and the polar organic molecules under electric field make them behave like ions.

The composition of the pumping fluid has a direct influence on EOF characteristics, different fluids generated different pressures and flow rates when other conditions were kept the same. When the driving voltage of the pump was at 10 kV, for  $3.0 \times 10^{-3} \text{ mol L}^{-1}$  sodium dihydrogenphosphate buffer (pH 7.5), pure methanol and pure methanol–phosphate buffer mixture, the maximum static pressures  $\Delta P_m$  and flow rates  $Q_m$  were about 38 MPa, 6.4  $\mu\text{L}/\text{min}$ ; 28 MPa, 4.5  $\mu\text{L}/\text{min}$ ; 33 MPa, 5.7  $\mu\text{L}/\text{min}$ , respectively.

When the pumping fluid was a pure methanol–phosphate buffer mixture with different proportion, the pressure and flow rate were also shown a good linearity increase as a function of applied voltage. When the driving voltage of the pump

was at 10 kV, for pure methanol–phosphate buffer mixtures (80:20, v/v) and (50:50, v/v), the maximum static pressures  $\Delta P_m$  and flow rates  $Q_m$  were about 36 MPa, 6.1  $\mu\text{L}/\text{min}$  and 33 MPa, 5.7  $\mu\text{L}/\text{min}$ , respectively. It was important because the mobile phase was a mixture in most chromatographic separations.

The EOPs are typically reported to have efficiencies of only a few percent or less. In the recent years, there have been some efforts to improve the performances of the EOP [24–26]. Chen et al. [24] have designed a multi-stage pump to decrease the driving voltage and improve the pressure of the pump. Reichmuth et al. [25] have used a zwitterionic additive, trimethylammonio propane sulfonate (TMAPS), to improve the performance of electrokinetic micropumps around 5.6%. Min et al. [26] have presented an analytical and numerical investigation of the thermodynamic efficiency of electrokinetic pumping. The numerical results have shown that efficiency as high as 15% might be attainable, when using uniform sub- $\mu\text{m}$ -depth microchannels in substrates with moderately high zeta potentials, as well as using electrolytes with low specific conductivity. Actually, it has predicted theoretically the high efficiencies of electrokinetic pumps employing nanochannels. Compared with the electrokinetic or electro-osmotic pump reported [5–9], the thermodynamic efficiency of the nanosilica packed pump was much higher. The use of nanosilica with nanochannels could improve the pressure about 10-fold and flow rates about one-tenth-fold, but the applied voltage as well as the electrical current was much lower. The thermodynamic efficiency was about 1–4%.

### 3.2. Relationship between $\Delta P$ and flow rate $Q$

When no resistance was attached at the output channel 7 of the EOP (no back pressure), we could get the maximum flow rate  $Q_m$ . When the output channel was connected to an adjustable-resistor (porous channel), the flow rate measured was the flow  $Q$  through adjustable-resistor with the head pressure  $\Delta P$ , which would be less than the maximum flow rate  $Q_m$ . In this way, we could obtain a relationship between the flow rate  $Q$  and the pressure  $\Delta P$  of the EOP. Fig. 2 was the relationship between  $\Delta P$  and  $Q$  at the applied voltage of 8 kV and the fluids being pumped were the mixture of  $3 \times 10^{-3} \text{ mol L}^{-1}$  sodium dihydrogenphosphate buffer–methanol (50:50, v/v), the maximum equilibrium static pressure  $\Delta P_m$  was about 26 MPa at  $Q=0$ . The equation was shown in the figure (Eq. (4)).

### 3.3. Application of EOP in capillary liquid chromatography

#### 3.3.1. Gas-releasing device

In the experiment, we found that the linear dependence of pressure and applied electric potential begins to depart from the expected linear behavior at high electric voltage because

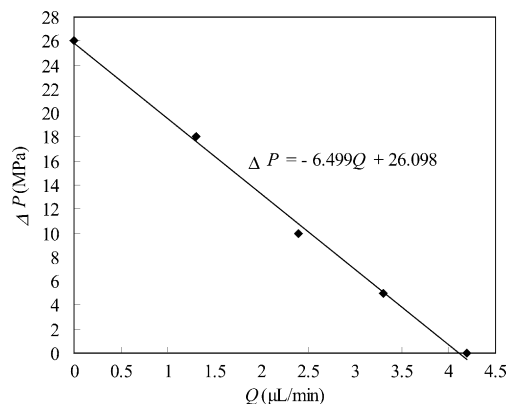


Fig. 2. The relationship between  $\Delta P$  and  $Q$ . Applied voltage: 8 kV. The fluid being pumped was the mixture of  $3 \times 10^{-3} \text{ mol L}^{-1}$  sodium dihydrogenphosphate buffer with pH 7.5 and methanol (50:50, v/v).

of the Joule heating, 25 kV for this pump, for example. In fact, Joule heating and the bubbles in EOP are unavoidable. Gas removal was essential to guarantee the EOP run continuously and steadily. It was very important for the electric driving pump used in capillary liquid chromatography. We have presented a gas-releasing device to remove the bubbles produced from electric reaction at the surface of electrode or gases dissolved in fluid (Fig. 1). The principle of the gas-releasing device was split flow the fluid that contains high content of gases. The inner surface of the chamber was hydrophobic in order to induce the dissolved gas to move and accumulate at upper part of the chamber under pressurized condition. The split flow from the top of the chamber, in which the content of the gases was higher, flows out through a resistor in about 5–10% of the input flow rate. The fluid from the bottom of the chamber contained no bubbles and was used as the output.

#### 3.3.2. Stability of baseline

Fig. 3 demonstrated drifts and noise of the baseline observed for applications of the EOP and a Jasco PU-1580 intelligent HPLC pump on an analytical column of 20 cm  $\times$  150  $\mu\text{m}$  i.d. packed with 3  $\mu\text{m}$  C<sub>18</sub>. Methanol–sodium dihydrogenphosphate buffer of  $3.0 \times 10^{-3} \text{ mol L}^{-1}$  and pH 7.5 (75:25, v/v) solution were supplied from the EOP with the applied voltage 8 kV (flow rate: 0.7  $\mu\text{L}/\text{min}$ ), and the flow rate of the Jasco pump was controlled as 1  $\mu\text{L}/\text{min}$ , followed by monitoring with the UV detector at 254 nm. It was apparent that the noise and drift of the baseline were quite severe when Jasco pump were used, as demonstrated in Fig. 3. Such a severe baseline noise might be caused by the fact that piston moving of the Jasco pump accompanying by pulse flow led to severe effect on a very narrow column. On the contrary, the drift and noise of the baseline were substantially reduced in case the solution was supplied from the EOP, which could output constant fluid velocity. So, the eluent delivery system shown in Fig. 3 (curve 1) was utilized in the following experiments.

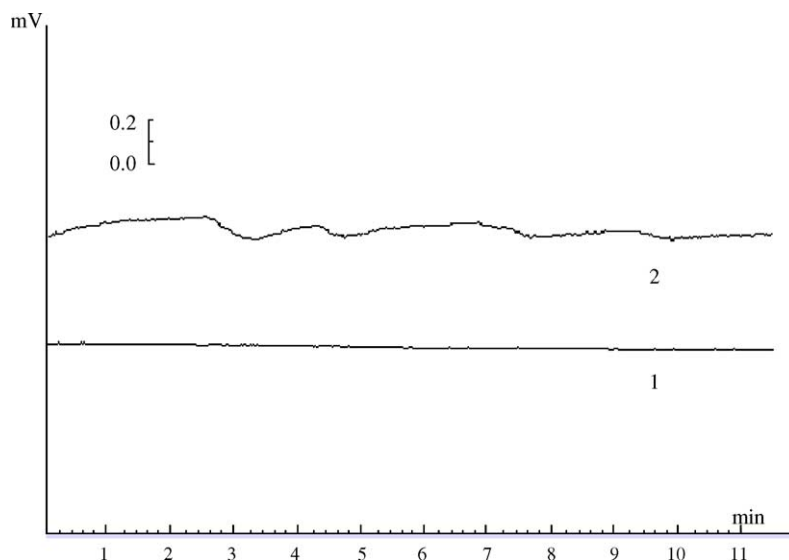


Fig. 3. Drifts and noise of baseline for the EOP as in Fig. 3 (curve 1) and a Jasco PU-1580 intelligent HPLC pump as in Fig. 3 (curve 2). Eluents: methanol–sodium dihydrogenphosphate buffer (75:25, v/v) solution. Applied voltage: 8 kV (flow rate: 0.7  $\mu\text{L}/\text{min}$ ). The flow rate of the Jasco pump: 1  $\mu\text{L}/\text{min}$ . Wavelength of UV detection: 254 nm.

### 3.3.3. Practice application of EOP in capillary liquid chromatography

The sample containing thiourea, benzene, toluene, naphthalene, biphenyl, anthracene, and phenanthrene was separated on a column of 20 cm  $\times$  150  $\mu\text{m}$  i.d. packed with 3  $\mu\text{m}$  C<sub>18</sub>. The theoretical plate number of the column was about 8–10  $\times 10^4/\text{m}$  with EOP driven. The theoretical plate number of the column using the HPLC mode was little lower than that of the same column using the capillary electrochromatography (CEC) mode, which was about 10–12  $\times 10^4/\text{m}$ . The CEC conditions were mobile phase 70% acetonitrile—1 mM 2-(*N*-morpholino)ethanesulfonic acid (MES), applied voltage 10 kV (0.56  $\mu\text{A}$ ), injection 5 kV/1 s, UV detection at 254 nm and the sample contain thiourea, benzyl alcohol, benzaldehyde, and naphthalene, with analysis time of about 15 min.

### 3.3.4. Repeatability of liquid delivery

The repeatability of the peaks retention times in the chromatograms could reflect the performance of the EOP such as the flow rate stability and the flow rate precision of the pump. When chromatographic conditions were methanol–sodium dihydrogenphosphate buffer (75:25, v/v), applied voltage 8 kV, the head pressure about 14.5 MPa and detector UV at 254 nm with analysis time about 12 min, the R.S.D. of the retention times was less than 1% for all the samples separated (0.08–0.85%). When chromatographic conditions were methanol–water (65:35, v/v), applied voltage of EOP 6 kV, the head pressure about 14–15 MPa and detector UV at 254 nm with analysis time about 41 min. The R.S.D. of the retention times was less than 1% for all the samples separated (0.16–0.82%).

## 4. Conclusion

The simple electro-osmotic pump using the nanoparticle packed column has been investigated. By increasing the pressure, decreasing the applied voltage and the electrical current (conductivity decrease), the thermodynamic efficiency was much higher. These improvements would lead to a reduction in voltage and power requirements and would facilitate miniaturization of micro-total-analysis systems ( $\mu\text{TAS}$ ), microfluidically driven actuators and microcolumn liquid chromatography. The EOP was capable of generating high pressures and manipulating minute liquids, exhibiting only a very small dead volume, and can be used for precise handling of fluids for capillary liquid chromatography. The successful application of EOP in 20 cm  $\times$  150  $\mu\text{m}$  i.d., 3  $\mu\text{m}$  C<sub>18</sub> fused-silica analytical column demonstrated the applicability of the pumping system.

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## References

- [1] Y. He, W. Gan, *Chin. J. Anal. Chem.* 26 (1998) 125.
- [2] P.H. Paul, D.J. Rakestraw, U.S. Patent 6019882 (2000).

- [3] P.H. Paul, et al., *Micro Total Analysis Systems*, Kluwer, Dordrecht, 1998, pp. 49–52.
- [4] P.H. Paul, et al., *Micro Total Analysis Systems*, Kluwer, Dordrecht, 2000, pp. 583–590.
- [5] S.L. Zeng, C.H. Chen, J.C. Mikerlsen Jr., J.G. Santiago, *Sens. Actuators B* 79 (2001) 107.
- [6] L.X. Chen, Y.F. Guan, *Chin. J. Chromatogr.* 20 (2002) 115.
- [7] L.X. Chen, J.P. Ma, F. Tan, Y.F. Guan, *Sens. Actuators B* 88 (2003) 260.
- [8] L.X. Chen, J.P. Ma, Y.F. Guan, *Microchem. J.* 75 (2003) 15.
- [9] L.X. Chen, J.P. Ma, Y.F. Guan, *J. Chromatogr. A* 1028 (2004) 219.
- [10] L.X. Chen, Y.F. Guan, J.P. Ma, X. Shu, F. Tan, *Chin. Sci. Bull.* 48 (2003) 2572.
- [11] Y.F. Guan, L.X. Chen, *Chin. Pat.* CN01110506.2 (2001).
- [12] R.P.W. Scott, P. Kucera, *J. Chromatogr.* 125 (1976) 251.
- [13] T. Tsuda, M.V. Novotny, *Anal. Chem.* 50 (1978) 632.
- [14] J.W. Jorgenson, E.J. Guthrie, *J. Chromatogr.* 255 (1983) 335.
- [15] J.P.C. Vissers, A.C. Henk, C.A. Cramers, *J. Chromatogr. A* 779 (1997) 1.
- [16] J.P.C. Vissers, *J. Chromatogr. A* 856 (1999) 117.
- [17] N. Nagae, H. Itoh, N. Numura, T. Takeushita, *J. Microcolumn Sep.* 3 (1991) 5.
- [18] J.P. Chervet, C.J. Mejjivogel, M. Urcem, J.P. Salsmann, *LC-GC* 10 (1992) 140.
- [19] M. Novotny, K.E. Karlsson, *Anal. Chem.* 60 (1988) 1662.
- [20] Y.F. Guan, L.M. Zhou, Z.H. Shang, *J. High Resolut. Commun.* 15 (1992) 434.
- [21] C. Yan, *U.S. Patent* 5453163 (1995).
- [22] C.L. Rice, R. Whitehead, *J. Phys. Chem.* 69 (1965) 4017.
- [23] R.J. Hunter, *Zeta Potential in Colloid Science, Principles and Applications*, Academic Press, New York, 1981, pp. 1–31.
- [24] L.X. Chen, H.L. Wang, J.P. Ma, C.X. Wang, Y.F. Guan, *Sens. Actuators B* 104 (2005) 117.
- [25] D.S. Reichmuth, G.S. Chirica, B.J. Kirby, *Sens. Actuators B* 92 (2003) 37.
- [26] J.Y. Min, E.F. Hasselbrink, S.J. Kim, *Sens. Actuators B* 98 (2004) 368.