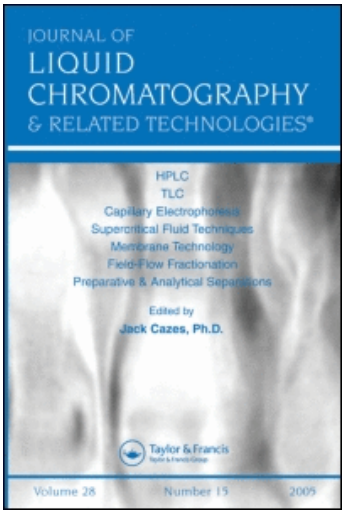

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SHORT, HIGHLY CROSS-LINKED, POLYMER BASED, MONOLITHIC COLUMN FOR CAPILLARY ELECTROCHROMATOGRAPHY

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ABSTRACT

Capillary electrochromatography with a short and highly cross-linked butyl methacrylate based negative charged monolith as stationary phase was developed. The reproducibility of the column in different kinds of mobile phase was investigated. Moreover, the effects of the operational parameters such as, applied voltage, salt concentration, pH, and organic modifier content in the mobile phase on EOF, as well as the retention mechanism of small neutral compounds on such a column were studied systematically.

It was found that the reproducibility and stability of the column is satisfactory in the buffer with very extreme pH values. The retention mechanism of neutral solutes on such

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a column proved to be similar to that of reversed-phase high performance liquid chromatography.

INTRODUCTION

Capillary electrochromatography (CEC) is a powerful technique that combines the strength of capillary electrophoresis and liquid chromatography. Traditionally, CEC has been performed using packed columns. There are a number of drawbacks associated with packed capillaries, such as the time and effort required for packing, the necessary for frits, bubble formation, and fragility of the column. It has been suggested that monoliths, such as porous polymer based monoliths, are more suited to the needs of CEC than packed beds. They do not require frits to hold them in place, they can be made from a variety of monomers (tunable surface chemistry), they readily fill virtually any length of columns.

Studies on polymer based monolithic columns have led to novel ion-exchange, chiral, reversed-phase, etc., type CEC separation systems. Horváth and coworkers had prepared acrylic[1] and styrenic[2,3] monoliths to separate proteins and peptides. Fréchet demonstrated CEC in anion-exchange and normal-mode using monoliths by direct copolymerization of 2-dimethylaminoethyl methacrylate with 2-hydroxyethyl methacrylate and ethylene dimethacrylate.[4] Chiral monolithic columns for CEC were prepared by copolymerization with chiral monomers such as, 2-hydroethyl methacrylate (*N*-*L*-valine-3,5-dimethylanilide)carbamate,[5] quinidine,[6,7] β-cyclodextrin,[8,9] or crown ether.[10] Schweitz et al. had tried molecular imprinted polymer base monolithic column for enantiomer separation.[11,12] Polyacryamide[13,14] and polystyrene[15,16] and polymethacrylate[15,17–22] based monolithic column were prepared by introducing hydrophobic ligands, such as C_4, C_6, C_{12}, C_{18} either as groups pro-existing in the monomers or by derivatization of the polymer. Negatively charged monomers such as, 2-acrylamido-2-methyl-1-propanesulfonic acid, vinylsulfonic acid, and methacrylic acid were introduced to generate cathodal electroosmotic flow (EOF) or positively charged monomers such as, dimethyldiallylammonium chloride and [2-(acryloyloxy)-ethyl]trimethylammonium methyl sulfate to generate anodic EOF. The columns were developed for separations of proteins, peptides, amino acids, alkylbenzenes, poly acromatic hydrocarbons (PAHs), phenols, anilines, phenylenediamines. Thus, the possibility of creating such stationary phases can be considered as established. However, commercial monolith columns for CEC remain scarce.[23,24]

There is still much work to be done to realize the commercialization of the monolith column. In this context, we would like to report on a short and highly cross-linked butyl methacrylate based negative charged monolithic column. The reproducibility and stability of the column in different kinds of mobile phase were investigated. Moreover, the effects of the operational

parameters on EOF, as well as the retention mechanism of small neutral compounds on such a column, were studied systematically. All these are expected to impel the classical use of monolithic columns and better understanding of the mechanism of the CEC separation of neutral compounds on methacrylate based negative charged monolithic columns.

EXPERIMENTAL

Materials

3-(trimethoxysilyl)propyl methacrylate and 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS) were from Acros. Butyl methacrylate (BMA) was from Beijing Donghuan Chemical Reagent Factory. Ethylene glycol dimethacrylate (EGDMA) was from Suzhou Anli Chemical & Engineering Co. Lt. 2,2′-Azobis (2-isobutyronitrile) (AIBN) was supplied by Special Chemical Reagent Factory of Nankai University. Other analytical reagents were from Tianjin Chemical Reagent Co. Lt. Acetonitrile is of HPLC grade. Fused-silica capillary of 100 μm I.D. and 375 μm O.D. with a polyimide outer coating was purchased from Hebei Yongnian Optical Fiber Factory.

Column Preparation

Fused-silica capillary was derivatized with 3-(trimethoxysilyl)propyl methacrylate, according to the basic procedure developed by Hjérten.[25] A mixture of BMA (1 mmol), EGDMA (5 mmol), AMPS (0.6%, wt%) and initiator AIBN (10 mg) were dissolved in porogenic solvent consisting of acetonitrile (90%) and water (10%). The mixture was degassed for 10 min by ultrasonication. The solution was injected into the silanized capillary using a syringe. A 40 cm length capillary was attached to the syringe and filled with the polymerization mixture to a total length of 13 cm. After both ends were plugged with a piece of rubber, the capillary was submerged in a 60°C bath for 48 h. Subsequently, the column was moved out of the water bath and immediately washed with acetonitrile, water, respectively. A detection window was created at the end of the continuous polymer bed by burning out a 2–3 mm segment of the polyimide outer coating.

Capillary Electrochromatography

Capillary electrochromatography separation was performed on a P/ACE system MDQ capillary electrophoresis apparatus (Beckman–Coulter, Fullerton, CA)

equipped with a P/ACE system MDQ UV detector. An IBM personal computer with Beckman P/ACE system MDQ capillary electrophoresis software was used.

The total length of the capillary was 31.2 cm total, effective length (MIP-based stationary phase) 10 cm. The remainders of two ends were cut before the column was installed in a Beckman P/ACE system MDQ capillary cartrige. First, the column was rinsed with mobile phase with 20 p.s.i., then, equilibrated with mobile phase at a voltage of 10 kV with 20 p.s.i. in both vials, until a constant baseline was obtained. The column temperature was 25°C. The samples were injected by 0.5 p.s.i., 3 sec. 20 p.s.i. was applied in both vials in the separation. The electrolyte was composed of acetonitrile and different ratios of buffer with different pH values. The samples were prepared from 10 mM acetonitrile solution diluted with buffer to the desired concentration. All the buffer and sample solutions were made using double distilled water and filtered before use through a 0.2 μm porosity membrane.

RESULTS AND DISCUSSION

Physical Characteristics

The polymerization takes place over a period of 48 hr at 60°C to assure the completeness of reaction, resulting in a highly cross-linked porous polymer (Fig. 1). The most extensively studied materials in the literatures were polymerized with a content of cross-linker <40%. The content of cross-linker in this material is higher than 80%. The high content of cross-linker resisted swelling when the polymer was exposed to different solvents. The flow characteristics were stable over a large range of pH and solvent composition.

To date, the effective length of the monolithic column is no less than 20 cm. The effect length of this material is only 10 cm, utilizing the short end injection method.[26] The column can be rinsed with 20 p.s.i. Electrolyte exchange could be easily carried out by hydrodynamic pumping. It also realized rapid analysis.

Reproducibility and Stability

Reproducibility is a concern in any analytical technique. The reproducibility of this monolithic column was studied. In order to investigate the stability of column in extreme basic and acidic mobile phases, the run-to-run reproducibility of migration time and retention factor of quinoline and anthracene was examined with mobile phase at pH 8.5. The reproducibility of migration time and retention factor of *p*-methoxybenzoic acid with a buffer at pH 2.5 was also

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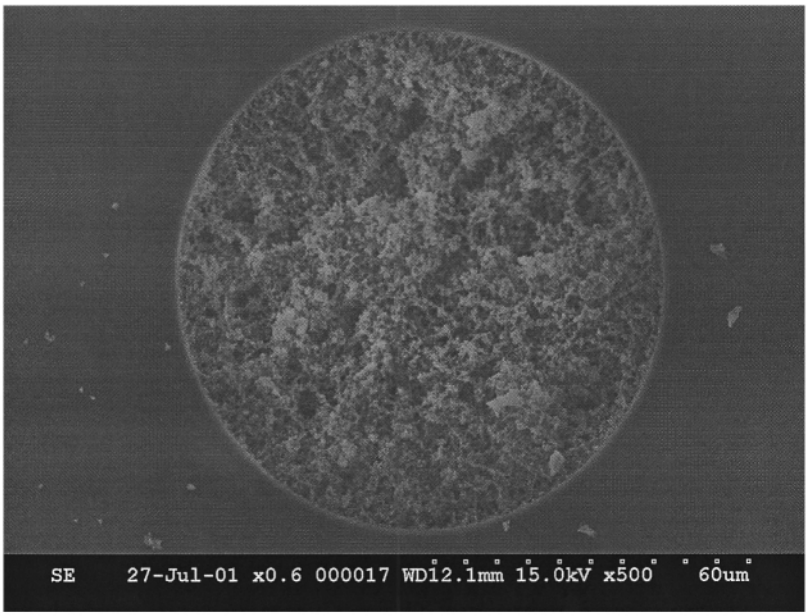

Figure 1. Scanning electron micrograph of polymer filled capillary.

examined. The relative standard deviations (RSDs) of the retention time and retention factor were less than 3% and 4%, respectively. The results obtained are summarized in Table 1. Generally speaking, columns can withstand prolonged exposure to large ranges of pH that the silica-based columns can't tolerate. This reproducibility and stability under a variety of chemical conditions, augurs well for a systematic investigation of the performance of such columns.

Electroosmotic Flow

In our experiments, sulfonic acids of the polymer were introduced to generate EOF. Neutral compound thiourea was used as an EOF marker. The velocity of EOF can be calculated by the quotient of the effective length of the column from the injection end to the detector and the elution time of thiourea.

A study of the effect of applied voltage on the EOF was carried out with voltages ranging from 3 to 10 kV. In our set of experiments, the mobile phase was acetate (0.01 mol/L, pH 7.0)–acetonitrile (60 : 70, v/v). The EOF increases approximately linearly with increasing electric field strength from 100 to 333 V/cm. The regression equation is $v_{eof} = 0.0539v - 0.0568$ with $R^2 = 0.9933$.

Table 1. Reproducibility of Migration Time and Retention Factor of Solutes

Compounds	t_0[a]	RSD, %	t_r[b]	RSD, %	Retention Factor (k')	RSD, %
Quinoline ($n=10$)[c]	3.710	1.4	4.733	1.26	0.277	1.83
Anthracene ($n=9$)[d]	1.681	2.32	3.864	2.04	1.298	1.35
p-Methoxybenzoic acid ($n=8$)[e]	4.646	0.928	5.996	1.91	0.2986	3.32

[a]t_0 is the migration time of thiourea.
[b]$t_r = t - t_0$, t is the migration time of the solute.
[c]Experimental conditions: 5 kV, mobile phase: acetonitrile–0.005 mol/L, pH 8.5 phosphate buffer(30 : 70, v/v).
[d]Experimental conditions: 10 kV, mobile phase: acetonitrile–0.005 mol/L, pH 8.5 phosphate buffer(40 : 60, v/v).
[e]Experimental conditions: 5 kV, mobile phase: acetonitrile–0.005 mol/L, pH 2.5 phosphate buffer(30 : 70, v/v).

This phenomenon indicates that the Joule heat caused by high voltage is not very obviously in this system. The limited Joule heat was removed by the coolant system sufficiently.

The effect of salt concentration on EOF was studied using different ionic strength of electrolyte, 0.001, 0.005, 0.01, 0.04, 0.08, mol/L acetate (pH 7.0)–acetonitrile (30 : 70, v/v). The EOF velocity decreases with increasing the salt concentration. This trend may be caused by the relative thinness of double layer thickness in high ionic strength. We plot the EOF velocity vs. the reciprocal of the square root of salt concentration. The EOF velocity increases almost linearly with the reciprocal of the square root of the salt concentration in 0.001–0.08 mol/L range ($v_{eof} = 0.0123/I^{1/2} + 0.3456$, $R^2 = 0.9846$). This confirmed the results reported in the literature.[27–29] The EOF velocity is directly proportional to the double layer thickness, the double layer thickness is proportional to the reciprocal of the square root of the salt concentration. Thus, the EOF velocity is inversely proportional to the square root of the salt concentration.

The EOF velocity was influenced by the percentage of organic solvent. Figure 2 shows the variation of EOF velocity vs. acetonitrile content. In this set of experiments, ion strength of aqueous buffer acetate (pH 7.0) was kept constant (0.01 M), while the acetonitrile content (v/v) was varied from 20% to 90%. The dependence of EOF velocity on the acetonitrile content is similar to that obtained with a poly(stryrene-co-divinylbenzene-co-methacrylic acid) based monolithic column prepared by Zou et al.[16] and butyl methacrylate based monolithic column prepared by Fréchet.[19] The curve was interpreted as an effect of permitivity and viscosity of the mobile phase.

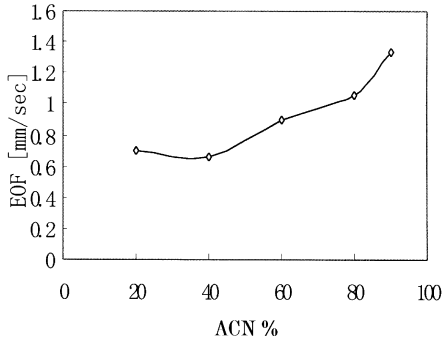

Figure 2. Influence of acetonitrile content on EOF velocity. 10 kV, acetonitrile–0.01 mol/L pH 7.0 acetate buffer.

The effect of pH on the EOF was also investigated and the results are shown in Fig. 3. In this set of experiments, acetonitrile content was kept constant (50%, v/v), 0.1 mol/L acetate buffer in the pH ranged from 3 to 7.0. There is no remarkable change in EOF in the range of tests. This may be due to the shield of the silanol group on the capillary wall by the polymer. In addition, the sulfonic acid in the polymer matrix can deprotonate even in lower pH. Thus, the surface of the polymer exhibits a net negative charge that causes steady EOF in a very wide pH range.

Retention Mechanism of Neutral Solutes

Separation of PAHs and alkyl benzenes were carried out. Baseline separations of the compounds, as shown in Fig. 4, were achieved. The column efficiencies of

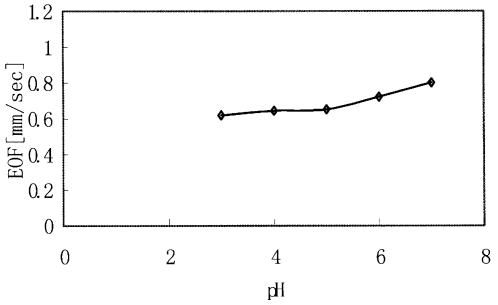

Figure 3. Influence of pH of the buffer on EOF velocity. 10 kV, acetonitrile–0.01 mol/L acetate (50 : 50,v/v).

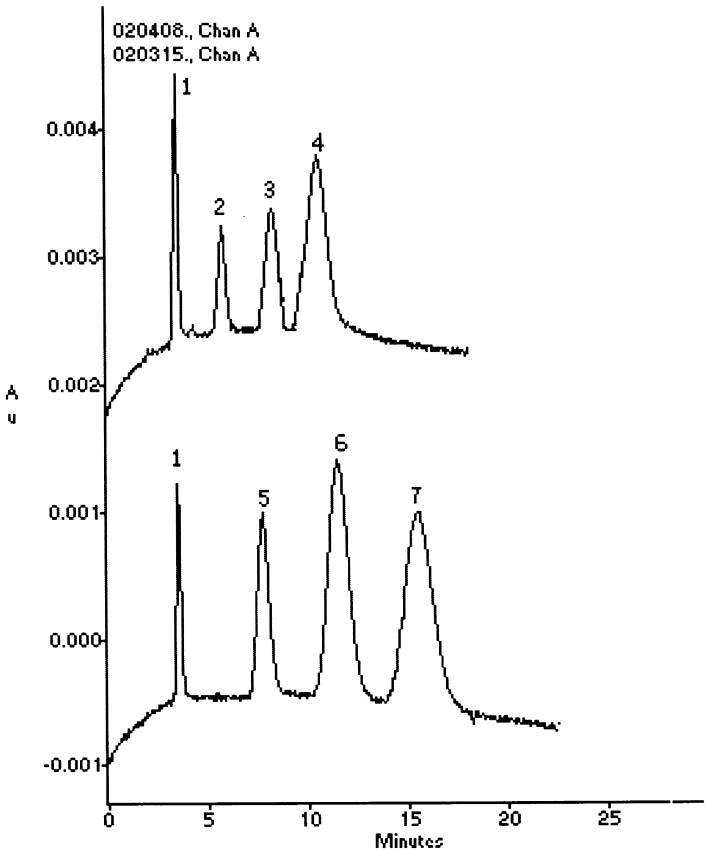

Figure 4. The separation of neutral solutes. 10 kV, mobile phase: acetonitrile–0.01 mol/L, pH 7.0 acetate buffer (46 : 54, v/v). 1, thiourea; 2, toluene; 3, propyl benzene; 4, butyl benzene; 5, naphthalene; 6, fluorene; 7, anthracene.

50,000 plates/m were obtained for unretained thiourea. It seems a little lower than that reported in other similar columns.[16,19] This may be due to the fact that there was not much work on optimizing column preparation procedures.

In CEC, the separation of solutes is based on their partitioning between phases and their differences in electrophoretic mobility. For neutral solutes, the electrophoretic velocity is equal to zero and not involved in the separation process, and chromatographic retention is the dominating factor for the separation process. We examined the effect of varying the acetonitrile content on the separation of PAHs and alkylbenzenes on the column. As can be seen from Fig. 5,

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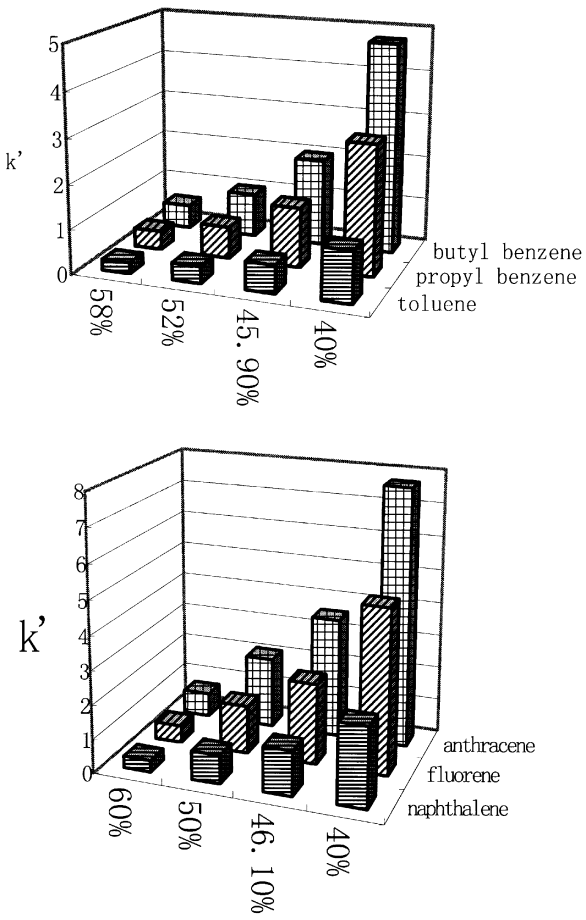

Figure 5. Effect of acetonitrile content on retention factor (k') of neutral solutes. 10 kv, mobile phase: acetonitrile–0.01 mol/L, pH 7.0 acetate buffer.

the capacity factor (k') of each solute decreases with an increase in acetonitrile content. The solute eluted in order of increasing hydrophobicity in the same mobile phase. The displacement of retention with increasing acetonitrile content, as well as the order of retention, provides evidence that the hydrophobic effect is the dominating retention mechanism of the neutral compounds on the column. The relationship between the logarithmic value of the capacity factors of neutral solutes and the volume fraction of organic modifier in the mobile phase (φ) was studied in the range of φ from 0.4 to 0.6, as shown in Table 2. All linear

Table 2. Linear Regression Results Between $\log k'$ and φ of the Solutes

Compounds	Regression Equation	Regression Coefficient
Propyl benzene	$\log k' = 5.2914 - 0.107\varphi$	0.9921
Butyl benzene	$\log k' = 6.2691 - 0.1197\varphi$	0.9917
Naphthalene	$\log k' = 4.7236 - 0.0971\varphi$	0.9981
Fluorene	$\log k' = 6.0496 - 0.1125\varphi$	0.9973
Anthracene	$\log k' = 6.7704 - 0.1191\varphi$	0.9975

Experimental conditions: 5 kV, 0.005 mol/L, pH 7.0 acetate buffer with different ratio of acetonitrile in the mobile phase.

regression coefficient are above 0.99. Typically, it means that the retention mechanism of neutral solutes on CEC columns with highly cross-linked butyl methacrylate polymers as stationary phase is similar to that on RP-HPLC columns.[30]

CONCLUSION

Short highly cross-linked polymer based monolith columns were prepared by in situ copolymerization of butyl metharylate and ethylene glycol dimethacrylate and 2-acrylamido-2-methyl-1-propanesulfonic acid over a period of 48 h at 60°C within a silanized fused-silica capillary. The reproducibility and stability of the column is satisfactory in the buffer with very extreme pH value. Parameters that affected EOF were studied. Separation process of neutral solutes on such monolithic columns is proven to be reversed-phase retention mechanisms. All these imply the potential of the monolithic column to be developed for CEC and allow the development of separation methods for CEC.

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