Electrochromatographic Evaluation of Diol-Bonded Silica Monolith Capillary Column for Separation of Basic Compounds

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Abstract

A diol-bonded silica monolith capillary column as polar stationary phase was successfully prepared for capillary electrochromatography. The preparation of monolithic stationary phase was based on the individual silica matrix forming and subsequent chemical bonding by (3-glycidoxypropyl) trimethoxysilane to produce the desired function. The diol-bonded silica monolith has been successfully employed in the electrochromatographic separation of alkaloids. The effects of experimental parameters, such as the volume fraction of the organic modifier, pH value, and ionic strength of the buffer on the retention behavior of the solutes were investigated. Column efficiencies greater than 110,000 plates/m for separation of basic compounds were obtained. It was observed that retention of alkaloids on the diol-bonded silica monolith was mainly contributed to a reversed-phase and cation-exchange mechanism, and electrophoresis of basic compounds also played a role in separation.

Introduction

Capillary electrochromatography (CEC) is a powerful separation technique which combines the best features of high-performance liquid chromatography (HPLC) and capillary electrophoresis. The stationary phase has always been the "heart" of CEC; therefore, the preparation of stationary phase in CEC is a very active research area (1–3). As a novel separation media, the monolithic columns have shown great potential because of unique pore structure with high permeability and favorable mass transfer characteristics for the stationary phase (4–6). The main advantage of the monolithic columns is the elimination of the need of retaining frits, which are responsible for drawbacks such as difficult and irreproducible preparation, bubble formation, and unpredictable influence on the electroosmotic flow (EOF) encountered with packed capillaries.

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To date, most of the reported CEC works have been concentrated on the separation of neutral compounds using hydrophobic stationary phase (e.g., C_{18}) in reversed-phase (RP) mode (7–9), which provides relatively satisfactory results for separation of nopolar and neutral analytes. However, analysis of strongly basic analytes has been challenging to method development in RP-CEC for many years because the analysis of basic compounds on traditional silica-based stationary phase often suffers from broadening peaks and serious tailing, which is caused by the secondary interaction between basic solutes and residual silanol groups (10). While end-capping of residual silanol groups helps to alleviate this problem in HPLC, this approach is less suitable for CEC because the silanol groups of the stationary phase are required to generate the EOF.

As complementary to RP stationary phases, polar stationary phases have proved to be very useful in some applications (2). These stationary phases with novel properties provide an alternative approach for highly efficient separation of polar analytes and basic compounds. However, only a few attempts have been made using polar stationary phases for successful applications in the analysis of basic compounds in CEC (2), including bare silica (11–17), fluorinated silica (18), phenylalanine silica (19), poly(2-sulfoethyl aspartamide) silica (20,21), amino and fluorine-silica (22,23), cyano-silica (24) , diethylenetriaminopropyl silica (25), and silica monolithic stationary phases functionalized with 3-(2-aminoethylamino)propyl ligands (26)

(3-Glycidoxypropyl) trimethoxysilane has been usually used as a modifier for capillary walls in CEC (27) and for stationary phases in LC (28). In this work, a novel monolithic silica column containing diol polar groups functionalized with (3-glycidoxypropyl) trimethoxysilane was introduced. The characterization of monolithic stationary phases was performed on pressurized capillary electrochromatography (pCEC) system. pCEC is a powerful separation system in which a mobile phase is driven by both a pressurized flow and an EOF (29,30). The main goal in this study is focused on the initial characterization of the new functionalized diol-bonded silica as polar monolithic stationary phase for CEC, and an electrochromatographic evaluation, through separation of the standard

basic test solutes, which has not been reported so far in our literature search.

Experimental

Reagents and materials

Tetramethoxysilane (TMOS), methyltrimethoxysilane (MTMS), (3-glycidoxypropyl) trimethoxysilane (GPTMS), and poly (ethylene glycol) (PEG, *Mw* 10000) were purchased from Acros Organics (Geel, Belgium). The basic compounds of sinomenine, papaverine, codeine, jatrorrhizine, palmatine, berberine, tetrandrine, and fangchinoline were reference compounds identified by thin-layer chromatography, obtained from the Chinese National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The structures of the model compounds are shown in Figure 1. Buffer solutions were prepared using trishydroxymethylaminomethane (Tris) adjusted with hydrochloric acid (HCl) to different pH values. HPLC-grade acetonitrile (ACN) was supplied by Luzhong Chemical Reagent Corporation (Shanghai, China). 2,6 dimethyl pyridine, sodium hydroxide, HCl, urea, and acetone were obtained from Shanghai Reagent Factory (Shanghai, China). Water used in all of the experiments was doubly distilled and purified by a Milli-Q system (Millipore, Milford, MA). A capillary with 75 µm i.d. × 375 µm o.d. was purchased from Yongnian Optic Fiber Plant (Hebei, China).

Instrumentation

All CEC experiments were performed on a commercially available TriSep-2100 pCEC system (Unimicro Technologies, Pleasanton, CA), which comprised a solvent gradient delivery module, a high-voltage power supply (+30 kV and –30 kV), a variable wavelength UV-Vis detector, a micro-fluid manipulation module with a six-port injector, and a data acquisition module, as described in literature (31). A high-pressure syringe pump was used to provide supplementary flow to the CEC column. The mobile phase is driven by EOF, as well as pressurized flow, and enters into the six-port injection valve. Samples injected are delivered to the injection valve and introduced into the internal 2-µL sample loop, and then carried to the four-port split valve by the mobile phase flow. After splitting in a four-port valve, the flow enters a capillary column under constant pressure stabilized by a backpressure regulator. A negative voltage was applied to the outlet of the column, and the inlet of column was connected to the split valve and grounded. Scanning electron microscopy (SEM) of the silica monolith was carried out on a XL30 scanning electron microscope (Philips, Netherlands)

Column preparation

The procedures of pretreatment of fused-silica capillary and preparation of a hybrid monolith were based on the method described in our previous work (25). The optimal preparation conditions were as follows: 3.6 mL TMOS, 0.4 mL MTMS, 0.88 g PEG, 0.90 g urea, and 10 mL of acetic acid (0.01 M) were mixed together and stirred for 45 min in an ice bath. Then the pretreated capillaries were filled to a certain length with resultant transparent sol by a syringe and sealed with a silicon rubber. Capillaries were aged at 40ºC for 24 h. The mesopores were tailored through the hydrolysis of urea into ammonia by heating at 120ºC for 3 h (32). The capillaries were washed with water and ethanol in order and then dried at 60ºC in the drying oven. The final heat treatment, which was done in a gas chromatography oven at 330ºC for 24 h, allowed organic moieties elimination or withdrawing from the monolith. Before use, an HPLC pump was used to purge dehydrated toluene through silica monolith. A solution of GPTMS (5.0% v/v) and 2,6-dimethyl pyridine (0.25% v/v) as catalyst in dehydrated toluene was pumped through the monolithic silica column and reacted at 110ºC for 1 h; this step was repeated four times. The column was washed with toluene, methanol, and acetone and then purged with helium for 2 h at 50ºC. HCl solution (pH 2.0) was introduced through the column with a syringe pump and reacted at 60ºC for 2 h; finally, water, ACN, and the running buffer flushed the column, sequentially. A detection window was created behind the continuous bed by removing the polyimide coating of fused-silica capillary using a thermal wire stripper. Finally, the column was cut to a total length of 55 cm with an effective length of 30 cm.

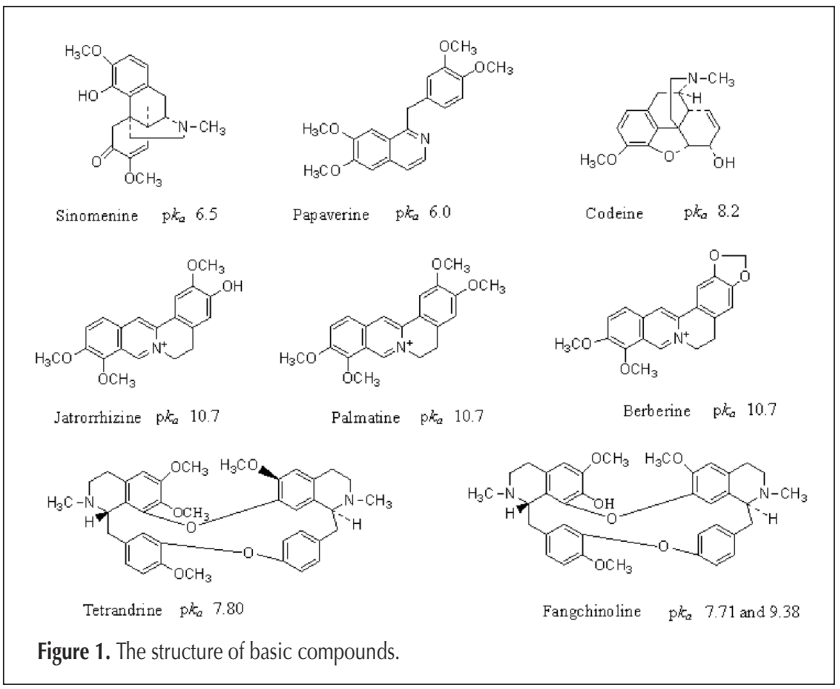

Figure 1. The structure of basic compounds.

Electrochromatography

Tris-HCl buffer had the lowest conductivity and was therefore selected for subsequent experiments (33). All the electrolyte solutions were filtered through a 0.22-µm membrane. In pCEC, the mobile phase was driven by EOF as well as pressurized flow. The major advantage

of the pCEC over pure CEC is that it can increase the speed of the separation and avoid bubble formation with the application of pressure (34). In this work, supplementary pressure (40 psi) was applied to the column inlet during separation of the basic compounds.

To describe the elution of charged solutes in pCEC, the apparent retention factor (k^*) was defined as the following equation (30,35):

$$k^* = \frac{t_r - t_0}{t_0}$$

where t_r and t_0 are the retention time for the retained charged solute and unretained neutral solute, respectively. The acetone was used as the t_0 marker in this work.

Results and Discussion

Column preparation

The SEM photographs of the cross section of the diol-bonded silica monolith prepared in the fused silica capillaries are shown in Figure 2. It can be seen that the monolithic column has the morphology of a continuous silica skeleton and interconnecting macropores. The medium size for macropores characterized by SEM is approximately 2.0 µm, and the skeleton size is approximately 1.0 µm. In addition, the silanol groups at the inner surface of the capillary wall took part in the sol-gel process so that the monolithic sorbent was chemically attached to the capillary wall. The permeability of monolithic silica columns with large domain sizes was much higher than that of a particle-packed column, because the high porosity and the large macropores resulted in high permeability.

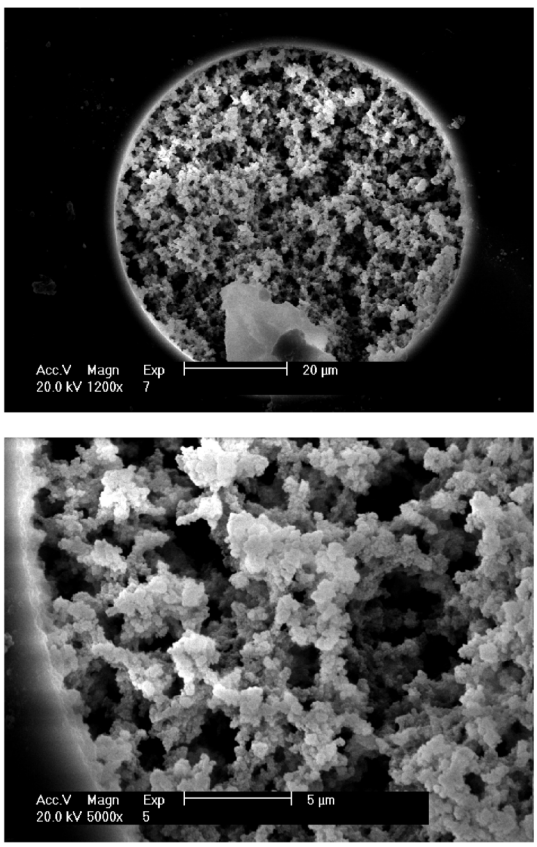

Figure 2. SEM photographs of diol-bonded silica monoliths inside a capillary at magnifications of 1200× (A) and 5000× (B).

The diol-bonded phase consists of a hydrophilic ethanediol structure and a hydrophobic methoxypropyl structure at the upper part and the lower part, respectively; therefore, it appeared that an internal hydrophobic layer hidden by an external hydrophilic layer existed on the support surface of the diol-bonded phase. Such a formation of the diol-bonded phase is usually used in normal-phase mode, but it can also be used in RP mode for separation of polar and ionizable solutes using water–organic mobile phases (36). In our research, GPTMS was used as the chemical modification reagent to prepare the diol-bonded silica monolithic stationary phase. The silanol groups on the skeleton surface of the monolithic silica matrix prepared by the sol-gel process were used for chemical modifications, including two steps. In the first step, the bare silica monoliths were grafted with GPTMS; in a further step, hydrolyzing under acid conditions formed a diol- bonded phase.

Separation of basic compounds

It is well known that separation of basic compounds by RP-CEC is a challenging task, usually with limited success (37–39). Basic mobile phases are not recommended because they are detrimental to most silica-based stationary phases. Analysis of bases in their protonated form is also troublesome, as the peak shape can be very poor. An alternative to RP sorbents is the use of polar stationary phases, which exhibit a different retention mechanism. A polar stationary phase in CEC was successfully used to separate basic compounds (2). Here, we used the diol-bonded silica monolithic stationary phase for separation of basic compounds in the RP-CEC mode. The surface of the diol-bonded silica monolithic column is negatively charged, which results in the direction of EOF from anode to cathode. Therefore, basic compounds will migrate with the direction of EOF, and fast separation of basic solutes can be achieved. The separation mechanism of charged compounds on the monolithic column is the combination of electrophoretic mobility and chromatographic interactions. Figure 3 shows a typical pCEC separation of the basic compounds. As can be seen in the electrochromatogram, all eight alkaloids were baseline separated in 15 min without obvious tailing. High column efficiencies over 110,000 plates/m were obtained for these alkaloids. This result is rather satisfactory, considering that all these alkaloids are bases. The intra-day relative standard deviations (RSDs) (n = 5) and inter-day RSDs (n = 5) for the retention time of eight alkaloids were less than 1.2% and 2.1%, respectively, which indicates the excellent stability of the prepared monolithic column. The reproducibility of column production with the same protocol was assessed by the RSDs for the retention time of these analytes and was found to be less than 7.3% (n = 3).

The content of organic modifier in the mobile phase has a great influence on the resolution and selectivity of polar

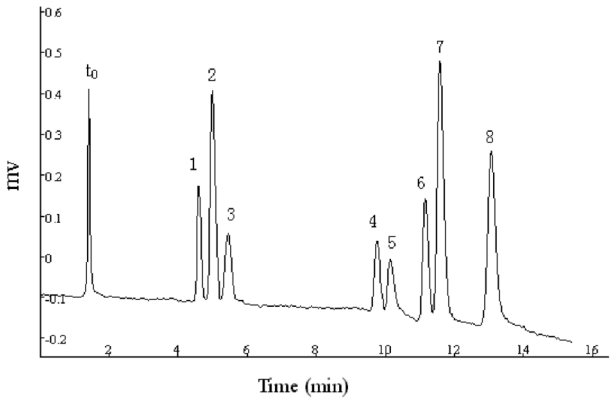

Figure 3. Electrochromatogram of alkaloids on the diol-bonded silica monolithic column. Experiment conditions: capillaries, 55 cm × 75 µm i.d. (30 cm effective length); mobile phase, 10 mM Tris buffer (pH 8.0) containing 70% ACN; voltage, –20 kV; supplement, 40 psi; wavelength, 254 nm; solutes: 1, berberine (1 × 10^{-6} g/mL); 2, jatrorrhizine (3 × 10^{-7} g/mL); 3, palmatine (1.5 × 10^{-6} g/mL); 4, fangchinoline (5 × 10^{-7} g/mL); 5, tetrandrine (5 × 10^{-7} g/mL); 6, sinomenine (1 × 10^{-6} g/mL); 7, codeine (1 × 10^{-6} g/mL); 8, papaverine (5 × 10^{-7} g/mL).

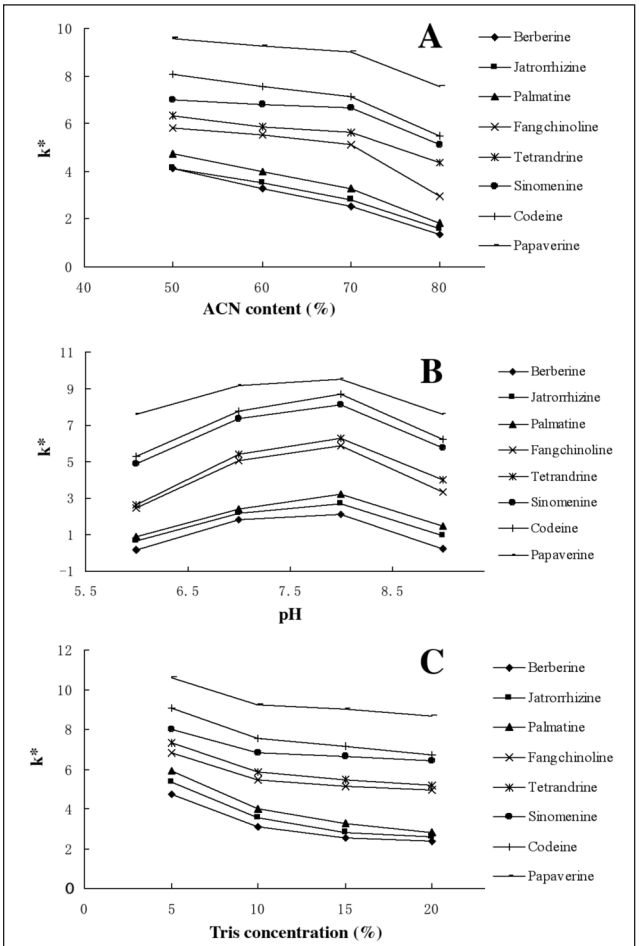

Figure 4. Effect of ACN content (A), pH of buffer (B), and Tris buffer concentration (C) on the retentions of alkaloids. Experiment conditions: mobile phase, 10 M Tris buffer (pH 8.0) containing various contents of ACN (A), 10 mM Tris buffer containing 70% ACN (B), and Tris buffer (pH 8.0) containing 70% ACN with buffer concentrations ranging from 5 to 20 Mm (C); other conditions are the same as in Figure 3.

alkaloids. In this study, the effect of ACN content in the range from 50% to 80% (v/v) on retention was investigated by varying the percentage of ACN in the mobile phase while keeping Tris buffer concentration constant at 10 mM. It can be seen from Figure 4A that the apparent retention factor (k^*) decreased with increasing ACN content in the mobile phase. This suggested that retention was governed primarily by RP mechanism for these solutes.

The mobile phase pH is one of the primary factors to adjust the retention of basic substances on a diol-bonded phase because it not only influences the ionization of the solutes but also the ionization of the surface silanols. Figure 4B shows the retention behavior of eight basic compounds in buffers of different pH in the mobile phase. It was found that the retention increased with increasing pH from 6.0 to 8.0, which may be due to the cation-exchange interaction between positively charged alkaloids and negatively charged residual silanol. The larger the charge density of the residual silanols on the silica surface, the larger the exchange capacity. Thus a relatively strong retention was observed. A further increase of the pH in the mobile phase from 8.0 to 9.0 resulted in a decrease of the retention. The analytes gradually deprotonated and the influence of cation-exchange mechanism lost importance on their retention, and therefore, the k^* values decreased in this scale. Apart from the interaction of these charged alkaloids with surface silanols, the increased contribution of electrophoretic migration also played an important role in the separation. The electrophoretic mobility of basic compounds increased with increasing pH in this scale, also resulting in a decrease of k^* values.

In order to examine the effect of ionic strength, the experiments were performed at various Tris buffer concentrations from 5.0 to 20 mM in the mobile phase containing 70% ACN while keeping pH at 8.0, and the obtained result is shown in Figure 4C. It was observed that the retention of all solutes decreased with increasing Tris buffer concentrations; a trend is obvious when the Tris buffer concentration is reduced to 5.0 mM. This indicated that the same retention mechanism as the cation-exchange mechanism under the experimental conditions due to the ionization of both silanol groups on silica and basic alkaloids.

The effect of the applied voltage on the separation of the eight alkaloids was determined in a mobile phase containing 70% (v/v) ACN, 10 mmol/L Tris buffer (pH 8) at constant pressure. The applied voltage was varied from –10 to –25 kV. As expected, with increasing voltage, the EOF increased and the elution time of the alkaloids increased linearly with no changes in selectivity. Maximum efficiency values and resolution were achieved at an applied voltage of –20 kV.

Conclusions

A new polar stationary phase diol-bonded silica monolithic column for CEC was prepared in fused-silica capillary via sol-gel process. The feasibility of CEC was demonstrated on a diol-bonded silica monolithic stationary phase. The prepared

monolithic column was demonstrated its suitability for separation of basic compounds. The retention mechanisms of basic compounds such as cation-exchange and RP chromatography, on this stationary phase have been observed. Eight alkaloids were baseline-separated within 15 min in CEC with column efficiencies more than 110,000 plates/m, which indicated that diol-bonded silica monolithic columns are effective alternatives for the analysis of basic compounds in CEC.

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