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A Novel C18 Reversed Phase Organic-Silica Hybrid Cationic Monolithic Capillary Column with Ionic Liquid as Organic Monomer via "One-Pot" Approach for Capillary Electrochromatography

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Abbreviations: IL, ionic liquid**;** **$VC_{18}HIm^{+}Br^{-}$**, 1–vinyl–3–dodecylimidazolium bromide**;** **VTES**, triethoxyvinylsilane; **TEOS**, tetraethyl orthosilicate; **EGDMA**, ethylene dimethacrylate; **i-PrOH**, isopropanol; **n-BuOH**, n–Butanol; **MeOH**, methanol; **EA**, element analysis; **DDW,** doubly deionized water; **cLC**, capillary liquid chromatography

Keywords: Capillary electrochromatography**,** Hybrid cationic monolithic column**,** Ionic liquid, One-pot, Reversed phase

ABSTRACT

A novel C18 reversed phase (RP) organic-silica hybrid cationic monolithic capillary column with ionic liquid (IL) as organic monomer has been fabricated by "one-pot" approach for capillary electrochromatography (CEC). Through copolymerization, the IL 1-vinyl-3-octadecylimidazolium bromide ($VC_{18}HIm^{+}Br^{-}$) was successfully anchored into the monolithic matrix which was formed through polycondensation of tetraethyl orthosilicate (TEOS) and triethoxyvinylsilane (VTES). Several experimental variables, which were essential to the preparation of the columns, such as TEOS/VTES ratio, content of H_2O and supermolecule template, amount of IL and polycondensation temperature were studied in detail, and three control columns were prepared to compare with this prepared novel hybrid monolithic column. Separation of various neutral, charged and basic analytes as well as protein sample on the $VC_{18}HIm^{+}Br^{-}$ hybrid monolithic column and control columns was achieved by CEC. It was found that the prepared hybrid monolithic column possessed its own superiority in separation. Besides, the retention mechanism of neutral analytes on this column was typical reversed phase chromatographic retention mechanism, and the separation of charged compounds depended on the combination of electrophoretic mobility, ionic exchange interaction and hydrophobic interaction. Moreover, the prepared hybrid monolithic column also settled the problem of peak tailing for separating the basic analytes, and the separation of egg white demonstrated its potential in proteome analysis.

1. Introduction

With the rapid development of analytical chemistry, more complex mixtures are needed to be separated. The requirement of novel separation systems with excellent separation ability is becoming more urgent. Capillary electrochromatography (CEC) demonstrates its distinct merits in satisfying the requirement as it combines various merits of both high performance liquid chromatography (HPLC) and capillary electrophoresis (CE).[1,2] As the "heart" of the CEC system, the capillary columns provide the driving force as well as the separation medium.[3,4] Consequently, the preparation of CEC columns makes phenomenal progress in the past few decades. The monolithic columns, the most promising CEC columns, have been attracting more attention due to theirs unique properties including easy preparation, good permeability and fast mass transfer.[5-7] According to the types of monolithic matrix, the monolithic columns are mainly categorized into three major types: silica-based monolithic columns, organic polymer-based monolithic columns and organic-silica hybrid monolithic columns.[8,9] Considered to be the new burgeoning monolithic columns, the organic-silica hybrid monolithic columns combine the advantages of two former classes columns,[10,11] and have been becoming the primary monolithic columns recently.

Since Malik et al. fabricated a hybrid monolithic column via sol-gel reaction for the first time,[12] many approaches have already been developed in the preparation of organic-silica hybrid monolithic columns. The advanced "one-pot" approach is one of the most widely utilized methods currently which contains two thermal treatments,[13] the sol-gel process occurs at low temperature to achieve uniform porous monolithic matrix, and the subsequence copolymerization occurs at a relatively high temperature

to make the monomer incorporate into the monolithic matrix. This new method overcomes one drawback of traditional sol-gel reaction that the functional monomer must be silane reagent,[14,15] making it possible to use ionic liquids (ILs) as organic monomers.

ILs are a new type molten salts with many unique physical and chemical characteristics,[16,17] including low melting point, non-volatility, high ionic conductivity, available design and high thermal stability, which make them easy to be utilized in chemical analysis field. So far, ILs have been applied as mobile phase additives to improve the shape of chromatographic peak and the separation efficiency.[18,19] In the preparation of monolith, ILs also have been used as stationary phase coatings to reverse the direction of electroosmotic flow (EOF) for better separation of analytes.[20,21] Besides, Li et al.[22] have reported that the ILs can assistant to form simultaneously the through pores and mesopores during the sol–gel reaction. Yan et al.[23] used ILs as pore template and to reduce gel shrinkage in the preparation of molecularly imprinted silica-based hybrid monoliths for chiral separation. Recently, ILs are used as the functional monomers for preparation of the organic polymer-based monolithic columns.[24] Liu et al.[25] prepared a PIL modified (PImC8-silica) hybrid monolithic column with IL as functional monomer which was succeed in separated aromatic hydrocarbons, alkylbenzenes and phenols.

Although the applications of ILs in monolith were reported, ILs were mostly used as assisted component to reverse the EOF or tailor mesopores in order to improve the separation efficiency. Few previous works were reported by using ILs as functional monomer, and the preparation processes were tedious which always contained two steps. Firstly, a silica monolithic column was prepared, and then ILs were pumped into the prepared column for further modification. Here, an organic-silica hybrid

monolithic capillary column with IL as functional monomer was fabricated via "one-pot" approach. This work not only simplified the preparation of ILs monolith, but also used ILs to provide both function groups to enhance the selective of neutral compounds and charged groups to reverse the EOF in CEC mode.

As we know, octadecylsilane (C18) stationary phase is one of the most widely used non-polar phase owe to its great resolving ability for a wide range of analytes.[26] Hence, 1-vinyl-3-octadecylimidazolium bromide ($VC_{18}HIm^{+}Br^{-}$) was chosen in this work and the $VC_{18}HIm^{+}Br^{-}$ hybrid monolithic column was successfully prepared via "one-pot" approach. Three control columns were prepared to investigate the superiority of the prepared $VC_{18}HIm^{+}Br^{-}$ hybrid column. Moreover, a series of characterizations and chromatographic experiments indicated the prepared $VC_{18}Him^{+}Br^{-}$ hybrid monolithic capillary columns possess a promising prospect for broad applications.

2. Experimental

2.1. Chemicals and materials

1-vinyl-3-octadecylimidazolium bromide ($VC_{18}HIm^{+}Br^{-}$) was purchased from Lanzhou Institute of Chemical Physics (Lanzhou, China). Tetraethyl orthosilicate (TEOS), Ethylene dimethacrylate (EGDMA) and Triethoxyvinylsilane (VTES) were obtained from Sigma (St. Louis, MO, USA). Azobisisobutyronitrile (AIBN) was purchased from Tianjin Chemical Reagent Factory (Tianjin, China) and recrystallized in ethanol before utilization. Cetyltrimethylammonium bromide (CTAB) was purchased from Aobox Biotechnology Co., Ltd. (Tianjin, China). Isopropanol (i-PrOH) and n–Butanol (n-BuOH) were obtained from Sinopharm Chemical Reagent

Co., Ltd. (Shanghai, China). Doubly deionized water (DDW, 18 MΩ cm^{-1}) used throughout the experiment was manufactured by a Milli-Q system (Millipore Corporation, USA). HPLC-grade methanol (MeOH) and acetonitrile (ACN) used as mobile phases were purchased Merck KGaA (Darmstadt, Germany). Fused-silica capillary (100 μm i.d., 375 μm o.d.) was purchased from Refine Chromatography Ltd. (Hebei, China).

2.2. Apparatus

The Fourier transform infrared (FT-IR) spectra with KBr pellets (4000–400 cm^{-1}) were achieved by a Vector 22 spectrometer (Bruker, Germany). Scanning electron microscopy (SEM) images of the monoliths were obtained on a SU1510 SEM (Hitachi, Japan). Elementary analysis (EA) was performance on Vario MACRO cube (ELEMENTAR, German). The capillary liquid chromatography (cLC) experiments were performed on an Agilent 1100 LC system (Agilent, USA). CEC experiments were performed on P/ACE MDQ CE system (Beckman-Coulter, USA) equipped with a UV detector which was controlled by Beckman ChemStation software.

2.3. Procedures for cLC and CEC

All data obtained were based on three runs. The mobile phase must be filtered by a 0.22 μm membrane and degassed by ultrasonic before used on cLC.

The $VC_{18}HIm^{+}Br^{-}$ hybrid monolithic capillary columns must be conditioned for at least 30 min on cLC with buffers prior to the CEC experiment. Every time before separation, the columns were equilibrated until an unfluctuating current was achieved. All the solutions loading into the CE system must be filtered with 0.22 μm membrane and degassed by ultrasonic. The total separation was carried out at room temperature.

2.4. Preparation of the $VC_{18}HIm^{+}Br^{-}$ hybrid monolithic capillary column

According to the "one-pot" process reported previously,[13] the fused-silica capillary must be pretreated to make the capillary inner wall expose more Si-OH for the subsequent attachment of monolith. Firstly, the fused-silica capillary was handled with 1.0 M NaOH for 12 h, DDW for 30 min, 1.0 M HCl for 12 h, DDW for another 30 min, MeOH for 30 min, respectively, and then dried in a nitrogen stream at room temperature for further use.

The prepolymerization mixture containing i-PrOH (500 μL), n-BuOH (100 μL), water (110 μL), TEOS (300 μL), VTES (200 μL), CTAB (5 mg), AIBN (5 mg), 1.0 M ammonia water (50 μL) and $VC_{18}HIm^{+}Br^{-}$ (150 mg) was stirred and sonicated for 10 min, respectively, to obtain homogenous solution at room temperature. The solution was artificially poured into 36 cm long pretreated capillary to a suitable length with syringe. After both ends of the capillary were sealed with rubbers, the capillary was incubated at 40 ℃ water for 12 h for polycondensation of TEOS and VTES, and then the capillary was continuously incubated at 60 ℃ water for another 12 h for incorporation of $VC_{18}HIm^{+}Br^{-}$. At last, the prepared monolithic capillary columns were connected to the cLC and rinsed with i-PrOH and n-BuOH to remove CTAB and other residuals, and a detection window was made at the end of capillary columns.

Several control columns were prepared to compare with the prepared $VC_{18}HIm^{+}Br^{-}$ hybrid column, and the preparation of control columns was showed in Supporting Information.

3. Results and discussion

3.1. Optimization of synthetic conditions for the $VC_{18}HIm^{+}Br^{-}$ hybrid monolithic capillary column

As shown in Table 1, several experimental variables such as TEOS/VTES ratio, content of H_2O, amount of supermolecule template (CTAB) and polycondensation temperature were investigated in detail.

In the processes of hydrolysis and polycondensation of alkoxysilanes, the effect of TEOS/VTES ratio on the formation of monolith was investigated by varying its volume ratios from 300/150 to 300/250. As seen from Table 1, the low content of VTES (column A2, TEOS/VTES = 300/150) would decrease the content of C=C double bonds which was essential to the subsequent bond of $VC_{18}HIm^{+}Br^{-}$, while the high content of VTES (column A3, TEOS/VTES = 300/250) would result in layering phenomenon and poor permeability of monolith, and the mobile phase was hard to be pumped through the column A3. Only when the volume ratio was at 300/200 (column A1), can a uniform and non-transparent monolith with excellent permeability be produced.

Because of the vital effect on the hydrolysis of alkoxysilanes, the content of water was also optimized and the results were reflected by column B1 (110 μL), column B2 (50 μL) and column B3 (140 μL). As shown in Table 1, the matrix of column B2 detached seriously due to the incomplete hydrolysis of alkoxysilanes without enough water. However, with content of water increasing, the reaction solution would become more and more incompatible for the high hydrophobicity of IL. So the reactant solution of column B3 seriously stratified which led to the failing formation of monolith. In contrast, the matrix of column B1 was acceptable and the 110 μL water could ensure not only the complete hydrolysis of alkoxysilanes but also the stability of the reactant system.

The CTAB was used as both surfactant and supramolecular template in the process of sol-gel reaction. According to Yan et al's work,[27] it proved that the formation of organic-silica hybrid mesostructure was the result of the delicate balance of two competitive processes-organizations of the template and polymerization. A series of columns C1 (5 mg CTAB), column C2 (3 mg CTAB) and column C3 (9 mg CTAB) in Table 1 were produced to study the effect of CTAB on the formation of monolith. The results showed that the low amount of CTAB resulted in the poor permeability of the column, while the high one substantially deteriorated the efficiency of the column. Correspondingly, the matrix of column C2 was inhomogeneous and that of column C3 was slacked. Only the column C1 exhibited a desirable monolith. Hence, 5 mg CTAB was proved to be the best condition in this reactant system.

As the temperature had significant effect on the formation of monolithic matrix, columns D1, column D2, column D3 were fabricated at 40 ℃, 35 ℃, 45 ℃, respectively, to research the tendency of the temperature effect. It was found the monolithic matrix of column D2 （35 ℃）was nonrigid and seriously detached from the capillary inner wall, which attributed to the incomplete polycondensation of the alkoxysilanes. With the increase of temperature, the structure of monolith gradually became solid. When the temperature was 40 ℃, a uniform monolithic matrix (column D1) tightly bonded onto the capillary inner wall was achieved. Continuously increasing the temperature to 45 ℃, the monolith (column D3) became too solid to allow the mobile phase flow through. Accordingly, the 40 ℃ was employed in this work.

In order to ensure the amount of the $VC_{18}HIm^{+}Br^{-}$, the μ_{EOF} and k' were chosen as evaluating standard. The μ_{EOF} was calculated by the equation $\mu_{EOF} = L_e L_t/(Vt_0)$, where L_t is the total length of column, L_e is the effective length of column, V is the applied

voltage, t_0 is the elution time of unretained compound (thiourea), and the k' was calculated by the equation $k' = (t_r - t_0)/t_0$, where t_r is the retention time of analyte, t_0 is the elution time of unretained compound (thiourea). During the experiment, it was found the maximum dissolution of $VC_{18}HIm^+Br^-$ in the optimal solution was 200 mg. Hence, a number of columns with 100 mg, 125 mg, 150 mg, 200 mg $VC_{18}HIm^+Br^-$ were prepared to confirm the best amount of $VC_{18}HIm^+Br^-$. According to the results, with the amount of $VC_{18}HIm^+Br^-$ increasing, the μ_{EOF} decreased obviously from 2.53 $\times 10^{-4}$ cm^2 v^{-1} s^{-1} to 1.33 $\times 10^{-4}$ cm^2 v^{-1} s^{-1}, and the columns prepared with 200 mg $VC_{18}HIm^+Br^-$ were even blocked, owing to the fact that overfull IL had an adverse effect on the permeability of the column. On the contrary, the k' for benzene increased from 0.671 to 0.989. In consideration of the common effect of $VC_{18}HIm^+Br^-$ on μ_{EOF} and k', 150 mg $VC_{18}HIm^+Br^-$ would be the best choice in this work.

Due to high hydrophobic property of the functional monomer $VC_{18}HIm^+Br^-$, i-PrOH and n-BuOH were chosen as the solvent, and a highly stable transparent reactant system was achieved when the volume ratio of i-PrOH/n-BuOH was 500/100 (v/v). The concentration of ammonia water played an important role in the sol-gel reaction. It was found the matrix could be formed after 12 h reaction when the concentration of ammonia water was 0.5 M, but the prepared matrix was non-rigid. With the concentration of ammonia water increasing, the matrix became more and more rigid, and the rate of condensation increased as well. When the concentration was 2 M, the reaction mixture quickly became solid, and there was no enough time to inject the mixture into the capillary. It was proved that the optimal concentration was 1 M in this work.

3.2. Characterization of the optimized the $VC_{18}HIm^+Br^-$ hybrid monolithic capillary column

The FT-IR was used to prove the anchor of $VC_{18}HIm^{+}Br^{-}$ to the monolithic matrix. As showed in Fig. 1, some characteristic peaks of $VC_{18}HIm^{+}Br^{-}$ were observed clearly at 2927, 2850 cm^{-1} ($-(CH_2)_n-$), 1650 cm^{-1} (C=C) and 1550 cm^{-1} (C=N) in Fig. 1(I). Comparing with the other two FT-IR photographs, the IL characteristic peaks of $-(CH_2)_n-$ at 2929, 2851 $cm^{-1}$and C=N at 1552 cm^{-1} shown in Fig. 1(III) were not appeared in Fig. 1(II) indicating that the $VC_{18}HIm^{+}Br^{-}$ groups were successfully incorporated into the silica-based monolith.

The EA results of the monoliths with different amount of $VC_{18}HIm^{+}Br^{-}$ were showed in Table. 2. According to the results, the monolith prepared without $VC_{18}HIm^{+}Br^{-}$ only contained 0.08% nitrogen which may result from the residuals of CTAB and AIBN. Correspondingly, the nitrogen proportion of $VC_{18}HIm^{+}Br^{-}$ monolith was increased apparently (1.18%), and with the amount of $VC_{18}HIm^{+}Br^{-}$ increasing, the N% also increased from 1.18% to 1.66%. As a consequence, the results proved the successful copolymerization of $VC_{18}HIm^{+}Br^{-}$ and the monolith.

The SEM photographs of $VC_{18}HIm^{+}Br^{-}$ hybrid monolithic capillary column were shown in Fig. 2. It can be seen that a uniform porous monolithic matrix tightly anchored to the inner capillary wall through the 600 times magnification condition. Besides, with 6000 times magnification, the matrix was constructed by plenty of small particles, which could increase the rate of mass transfer due to the increasing of contact area with samples during the separation.

With thiourea as the EOF maker, the relationship of mobile phase pH and the EOF of was investigated. According to the Fig. 3, the prepared hybrid column could maintain strong anodic EOF in a wide range of pH (3.0–11.0), which was due to the existence of strong cationic imidazole groups. The permeability of the $VC_{18}HIm^{+}Br^{-}$ hybrid monolithic capillary columns was investigated by the Darcy's Law[28] B_0 =

$F\eta L/(\pi r^2 \Delta P)$, where F is the flow rate of the mobile phase, η is the viscosity of the mobile phase, L is the effective length of column, r is the inner radius of the column, and ΔP is the pressure drop of the column. Using ACN (η = 0.38 cP) as the mobile phase, the permeability of the hybrid monolithic column was calculated to be 5.28 × 10^{-14} m^2. The mechanical stability of the $VC_{18}HIm^+Br^-$ hybrid monolithic capillary column was examined by connecting columns to cLC using ACN as the mobile phase. As the results showed in Fig. 4A, with the flow rate ranged from 0.5 μL min^{-1} to 10 μL min^{-1}, the backpressure increased linearly from 11 bar to 185 bar with relation factor of 0.9999. The column efficiency of the $VC_{18}HIm^+Br^-$ hybrid monolithic capillary columns was evaluated by Van Deemter curve, which was shown Fig. 4B. Using toluene as test sample, A minimum plate height of 5.58 ± 0.22 μm corresponding to 179211 ± 7060 theoretical plates per meter was obtained.

In order to prove the advantages of this work, a series of control columns (the preparation showed in Supporting Information) were prepared to compare with the $VC_{18}HIm^+Br^-$ hybrid monolithic capillary column. According to the Supplementary Fig. 1 (Supporting Information), the prepared hybrid monolithic capillary column could form a stable EOF, and the direction of EOF in the column was reversed. The control column 2 and 3 also formed EOF under reversed voltage, while there was no EOF in control column 1, which may result from no charged group on the matrix. The results indicated the IL was the essential factor in the formation of EOF. Under the same calculating condition with the $VC_{18}HIm^+Br^-$ hybrid monolithic column, the permeability of control column 1, 2 and 3 were calculated to be 8.06 × 10^{-14} m^2, 4.32 × 10^{-14} m^2 and 7.59 × 10^{-14} m^2, respectively, and the relation factor for backpressure and flow rate of control column 1, 2 and 3 were determined as 0.9996, 0.9986, 0.9992, respectively. Due to absence of EOF, the column efficiency of control column 1

cannot be achieved, while that of control column 2 and 3 were calculated to be 136798 ± 5677 theoretical plates per meter and 110879 ± 4139 theoretical plates per meter. Accordingly, the IL was the essential factor on the formation of EOF and the $VC_{18}HIm^{+}Br^{-}$ hybrid monolithic column prepared by "one-pot" approach possessed its own superiority in the permeability, mechanical stability and column efficiency.

The repeatability and reproducibility of the $VC_{18}HIm^{+}Br^{-}$ hybrid monolithic capillary columns were investigated through the relative standard deviation (RSD) of the retention time for benzene. the RSD of the run-to-run (n = 7) and day-to-day (n = 4) repeatability were 0.48% and 1.16%, respectively, and the RSD of the column-to-column (n = 3) and batch-to-batch (n = 3) were 3.02% and 3.94% respectively indicating that the hybrid monolithic capillary columns via "one-pot" approach owned not only stable separation repeatability but also satisfied reproducibility.

3.3. Chromatographic evaluation of the optimized $VC_{18}HIm^{+}Br^{-}$ hybrid monolithic capillary column

A mixture of alkylbenzenes was used to investigate the separation properties of the $VC_{18}HIm^{+}Br^{-}$ hybrid monolithic capillary columns by CEC. As shown in Fig. 5A, the alkybenzenes were well separated and the analytes were eluted in the order of thiourea < benzene < toluene < ethylbenzene < propylbenzene < butylbenzene according to their polarity from high to low, and with the increase of separation voltage, the separation time reduced rapidly. In addition, the effect of different ACN content on the retention factors of alkylbenzenes was investigated and the results were exhibited in Fig. 5B. It was found that the retention factors of alkylbenzenes decreased with the content of ACN increasing in the buffer. Consequently the

separation of alkylbenzenes on the $VC_{18}HIm^{+}Br^{-}$ hybrid monolithic capillary columns mainly based on typical reversed phase chromatographic retention mechanism

The amino acids mixture (aspartic acid pI = 2.77, glutamic acid pI = 3.22, L-phenylalanine pI = 5.48, glutamine pI = 5.65 and L-proline pI = 6.30) was chosen to investigate the separation of charged analytes on the $VC_{18}HIm^{+}Br^{-}$ hybrid monolithic capillary columns. As shown in Fig. 6A, the amino acids were baseline separated with the elution order aspartic acid < glutamic acid < glutamine < L-proline < L-phenylalanine. In order to acquaint the retention mechanism of the charged mixture, the same amino acids mixture was separated in different pH, salt concentration and content of ACN. In the Supplementary Fig. 2a, due to the only negatively charged amino acids was the aspartic acid at pH 3.0, its electrophoretic migration was identical to EOF resulting in the headmost elution of the aspartic acid. When pH of the mobile phase was 5.0, the glutamic acid also became negatively charged. Thus, it brought the reduction retention time of glutamic acid and the improvement of separation, which was showed in Supplementary Fig. 2b. At last, with the continuous increase of pH to 7.0, all the amino acids became negatively charged. Correspondingly, the retention times of all the amino acids shortened obviously which was consistent with Supplementary Fig. 2c. Through the change of salt concentration, the retention mechanism of ionic exchange was studied. Through the Supplementary Fig. 3a, it can be seen the amino acids were separated, when the salt concentration was 40 mM. As the increase of salt concentration to 50 mM, the retention time of all the amino acids decreased and the peak of aspartic acid overlapped with glutamic acid. When the salt concentration was 60 mM, the retention time of all the amino acids continued to decrease, and the separation also deteriorated obviously. Since the ionic exchange interaction could be suppressed by higher salt

concentration to some extent, the results could demonstrate that ionic exchange existed in the separation process. What' more, the results of different ACN content effect on the separation were showed in Supplementary Fig. 4. The retention time of all the amino acids decreased with the increase of the ACN content, which indicated the hydrophobic interaction was also one of the mechanisms during the separation. In summary, the retention mechanism of the prepared $VC_{18}HIm^{+}Br^{-}$ monolithic capillary columns for charged compounds is the combination of electrophoretic mobility, ionic exchange interaction, and hydrophobic interaction.

Since the separation of basic compounds is always suffered from peak tailing due to the nonspecific absorption between basic analytes and silica monolithic matrix in previous reports.[29] The $VC_{18}HIm^{+}Br^{-}$ monolithic capillary columns were also applied to separate the basic compounds (methimazole, aniline, gramine, 1,2-diphenyl hydrazine). As shown in Fig. 6B, the basic compounds were baseline separated with good peak shape. The conventional peak tailing problem didn't appeare as a result of the repression of positively charged imidazole groups on the nonspecific absorption mentioned above.

The control columns were also applied to separate the same neutral, charged and basic analytes under the same condition with the prepared hybrid monolithic column, and the results and interpretations were showed in Supporting Information (the data of control column 1 cannot be obtained for no EOF). As see from the Supplementary Fig. 5, the separation of alkylbenzenes on the control columns was undesirable. Although the retention of benzene, toluene, ethylbenzene and propylbenzene on control column 2 was acceptable, the peak broadening of butylbenzene was serious, and the column efficiency of control column 3 for the alkylbenzenes was not high. According to Supplementary Fig. 6, the glutamine and L-proline was not separated by

the control column 2, while all the amino acids was separated on the control column 3, but the peak of the aspartic acid and glutamic acid was too close and the shape of all the peaks was asymmetrical. As to the basic compounds, the peak tailing problem was not appear which was showed in Supplementary Fig. 7. However, the retention for the basic compounds was weak on the control columns compared with the hybrid monolithic column prepared with "one-pot" in this work. Hence, it can be achieved that the comprehensive separation ability of $VC_{18}HIm^{+}Br^{-}$ hybrid monolithic capillary column was outstanding.

To evaluate the potential proteome analysis of the prepared column, the $VC_{18}HIm^{+}Br^{-}$ hybrid monolithic capillary columns were further applied to separate egg white. As shown in Fig. 7, it can be found 7 major peaks were detected with no organic solvent and additives in mobile phase, which was friendly for protein, demonstrating potential separation of the prepared columns on the proteins compared with the previous report.[30]

4. Conclusion

The $VC_{18}HIm^{+}Br^{-}$ hybrid monolithic capillary columns with desirable morphology were obtained in this work. Moreover, the prepared columns were applied in the analysis of various neutral, charged and basic analytes as well as protein sample, and further compared with a series of control columns. All these results indicated that the fabricated columns would be of great potential in separation area.

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5. Reference

1. I. Krull, A. Sebag and R. Stevenson, *J. Chromatogr., A,* , 2000, 887, 137-163.

2. J. Knox and I. Grant, *Chromatographia*, 1987, 24, 135-143.

3. M. Pursch and L. C. Sander, *J. Chromatogr., A*, 2000, 887, 313-326.

4. Q. Tang and M. L. Lee, *Trac-trend Anal. Chem.*, 2000, 19, 648-663.

5. F. Svec, E. C. Peters, D. Sýkora and J. M. Fréchet, *J. Chromatogr., A*, 2000, 887, 3-29.

6. H. Zou, X. Huang, M. Ye and Q. Luo, *J. Chromatogr., A*, 2002, 954, 5-32.

7. M. M. Wang, H. F. Wang, D. Q. Jiang, S. W. Wang and X. P. Yan, *Electrophoresis*, 2010, 31, 1666-1673.

8. I. Gusev, X. Huang and C. Horváth, *J. Chromatogr., A*, 1999, 855, 273-290.

9. G. Guiochon, *J. Chromatogr., A*, 2007, 1168, 101-168.

10. M. Wu, R. a. Wu, Z. Zhang and H. Zou, *Electrophoresis*, 2011, 32, 105-115.

11. L. Bai, H. Liu, Y. Liu, X. Zhang, G. Yang and Z. Ma, *J. Chromatogr., A*, 2011, 1218, 100-106.

12. J. D. Hayes and A. Malik, *Anal. Chem.*, 2000, 72, 4090-4099.

13. H. Lin, J. Ou, Z. Zhang, J. Dong, M. Wu and H. Zou, *Anal. Chem.*, 2012, 84, 2721-2728.

14. M. Wu, R. a. Wu, F. Wang, L. Ren, J. Dong, Z. Liu and H. Zou, *Anal. Chem.*, 2009, 81, 3529-3536.

15. Z. Zhang, M. Wu, R. a. Wu, J. Dong, J. Ou and H. Zou, *Anal. Chem.*, 2011, 83, 3616-3622.

16. H. Ohno, M. Yoshizawa and W. Ogihara, *Electrochimi. Acta*, 2004, 50, 255-261.

17. T. Welton, *Chem. Rev.*, 1999, 99, 2071-2084.

18. M. Ruiz-Angel, S. Carda-Broch and A. Berthod, *J. Chromatogr., A*, 2006, 1119, 202-208.

19. W. Zhang, L. He, Y. Gu, X. Liu and S. Jiang, *Anal. Lett.*, 2003, 36, 827-838.

20. W. Qin, H. Wei and S. F. Y. Li, *J. Chromatogr., A*, 2003, 985, 447-454.

21. M. Borissova, M. Vaher, M. Koel and M. Kaljurand, *J. Chromatogr., A*, 2007, 1160, 320-325.

22. J. Chen, P. Zhang and L. Jia, *J. Chromatogr., A*, 2011, 1218, 3699-3703.

23. H. F. Wang, Y. Z. Zhu, X. P. Yan, R. Y. Gao and J. Y. Zheng, *Adv. Mater.*, 2006, 18, 3266-3270.

24. Y. Wang, Q. L. Deng, G. Z. Fang, M. F. Pan, Y. Yu and S. Wang, *Anal. Chim. Acta*, 2012, 712, 1-8.

25. H. Han, Q. Wang, X. Liu and S. Jiang, *J. Chromatogr., A*, 2012, 1246, 9-14.

26. Z. Zhang, H. Lin, J. Ou, H. Qin, R. a. Wu, J. Dong and H. Zou, *J. Chromatogr., A*, 2012, 1228, 263-269.

27. L. Yan, Q. Zhang, J. Zhang, L. Zhang, T. Li, Y. Feng, L. Zhang, W. Zhang and Y. Zhang, *J. Chromatogr., A*, 2004, 1046, 255-261.

28. R. D. Stanelle, L. C. Sander and R. K. Marcus, *J. Chromatogr., A*, 2005, 1100, 68-75.

29. X. Wang, K. Ding, C. Yang, X. Lin, H. Lü, X. Wu and Z. Xie, *Electrophoresis*, 2010, 31, 2997-3005.

30. Q. Qu, C. Gu, Z. Gu, Y. Shen, C. Wang and X. Hu, *J. Chromatogr., A*, 2013.

435 **Fig. 1.** FT-IR spectra of (I) IL ($VC_{18}HIm^{+}Br^{-}$), (II) the silica-based monolith without
436 IL and (III) the $VC_{18}HIm^{+}Br^{-}$ hybrid monolith.

437 **Fig. 2.** SEM images of $VC_{18}HIm^{+}Br^{-}$ hybrid monolithic capillary column with
438 optimal condition. Magnification: (A) ×6000 and (B) ×600.

439 **Fig. 3.** Effect of pH on EOF of the $VC_{18}HIm^{+}Br^{-}$ hybrid monolithic capillary column.
440 Experimental conditions: column dimension, 20 cm × 100 μm i.d.; injection, −0.5 psi
441 for 5 s; mobile phase, 30 mM phosphate buffer (H_3PO_4 – $Na_2HPO_4$for pH 3.0–5.0,
442 Na_2HPO_4–NaH_2PO_4 for pH 5.0−9.0, Na_2HPO_4–NaOH for 9.0−11.0) containing 30%
443 ACN; void time marker of EOF, thiourea; separation voltage, −10 kV; detection
444 wavelength, 214 nm.

445 **Fig. 4.** (A) The mechanical stability of the $VC_{18}HIm^{+}Br^{-}$ hybrid monolithic capillary
446 column and (B) relationship between linear velocity and the plate height of the
447 prepared column. Experimental conditions: (A) column dimension, 20 cm × 100 μm
448 i.d.; flow rate 0.5 μL min^{-1}-10 μL min^{-1} for ACN; (B) column dimension, 20 cm ×
449 100 μm i.d.; injection, −0.5 psi for 5 s; mobile phase, ACN/30 mM acetic acid buffer
450 at pH 3.0 = 30/70 (v/v); separation voltage, from −4 kV to −10 kV; detection
451 wavelength, 214 nm.

452 **Fig. 5.** (A) Separation of alkylbenzenes on the $VC_{18}HIm^{+}Br^{-}$ hybrid monolithic
453 capillary column at different separation voltage by CEC and (B) effect of ACN
454 content in the mobile phase on the retention factors of alkylbenzenes. Solutes: (A), (0)
455 thiourea, (1) benzene, (2) toluene, (3) ethylbenzene, (4) propylbenzene, (5)
456 butylbenzene. Experimental conditions: column dimension, 20 cm × 100 μm i.d.;
457 mobile phase: (A) ACN/30 mM acetic acid buffer at pH 3.0 = 40/60 (v/v), (B)
458 different content ACN in 30 mM acetic acid buffer at pH 3.0; injection, −0.5 psi for 5

459 s; separation voltage: (A) different voltage, (B) −10 kV; detection wavelength, 214
460 nm.

461 **Fig. 6.** (A) Separation of amino acids and (B) basic compounds on the $VC_{18}HIm^{+}Br^{-}$
462 hybrid monolithic capillary column by CEC. Solutes: (A) (1) aspartic acid, (2)
463 glutamic acid, (3) glutamine, (4) L-proline, (5) L-phenylalanine; (B) (1) methimazole,
464 (2) aniline, (3) gramine, (4) 1,2-diphenyl hydrazine. Experimental conditions: column
465 dimension, 20 cm × 100 μm i.d.; injection: (A) −1.0 psi for 15 s, (B) −0.5 psi for 5 s;
466 separation voltage: (A) −5 kV, (B) −10 kV; detection wavelength: (A) 190 nm, (B)
467 214 nm; mobile phase: (A) 40 mM H_3PO_4-Na_2HPO_4 buffer at pH 4.4, (B) ACN/30
468 mM H_3PO_4-Na_2HPO_4 buffer at pH 5.0 = 40/60 (v/v).

469 **Fig. 7.** Separation of egg white on the $VC_{18}HIm^{+}Br^{-}$ hybrid monolithic capillary
470 column by CEC. Experimental conditions: column dimension, 20 cm × 100 μm i.d.;
471 injection, −10.0 psi for 15 s; separation voltage, −3 kV; detection wavelength, 210 nm;
472 mobile phase, 40 mM H_3PO_4-Na_2HPO_4 buffer at pH 4.0.

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Table 1. Effects of Synthesis Parameters on the Formation of the $VC_{18}HIm^{+}Br^{-}$ Monoliths

Column	VTES μL	Water μL	CTAB mg	Temp °C	Surface morphology	Permeability ($\times10^{-14}$ m^2)
A1 (B1, C1, D1)	200	110	5	40	homogeneous non-transparent	5.28
A2	150	110	5	40	homogeneous semi-transparent	3.82
A3	250	110	5	40	Stratified	blocked
B2	200	50	5	40	detached	/*
B3	200	140	5	40	/**	/**
C2	200	110	3	40	inhomogeneous transparent	1.98
C3	200	110	9	40	slacked	15.64
D2	200	110	5	35	nonrigid	/*
D3	200	110	5	45	homogeneous non-transparent	blocked

a) Other components of the prepolymerization mixture: TMOS, 300 μL; i-PrOH, 500 μL; n-BuOH, 100 μL; IL, 150 mg; AIBN, 5 mg; 1 M ammonia water, 50 μL. ACN was used as mobile phase and the flow rate was 0.5 μL/min when calculating the permeability.

b) "/*" represents the monolith was washed out and "/**" represents the failure formation of monolith.

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Table 2. The element analysis of the monolith

Column	Element proportion		
	N [%]	C [%]	H [%]
1 （0 mg $VC_{18}HIm^{+}Br^{-}$）	0.08	17.37	2.84
2 （100 mg $VC_{18}HIm^{+}Br^{-}$）	1.18	21.61	3.38
3 （125 mg $VC_{18}HIm^{+}Br^{-}$）	1.39	25.09	3.97
4 （150 mg $VC_{18}HIm^{+}Br^{-}$）	1.66	27.76	4.37

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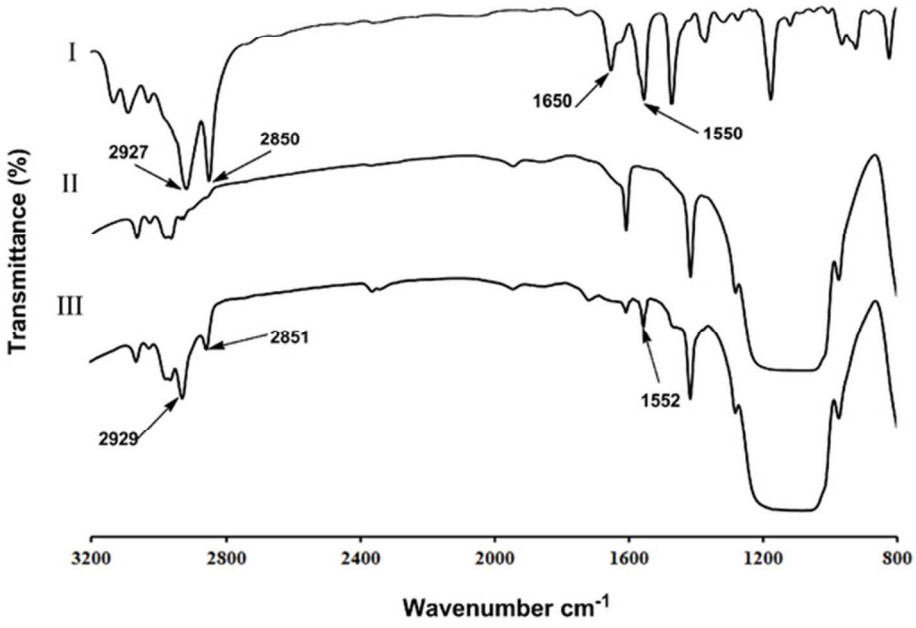

Fig. 1. FT-IR spectra of (I) IL (VC18HIm+Br−), (II) the silica-based monolith without IL and (III) the VC18HIm+Br− hybrid monolith.
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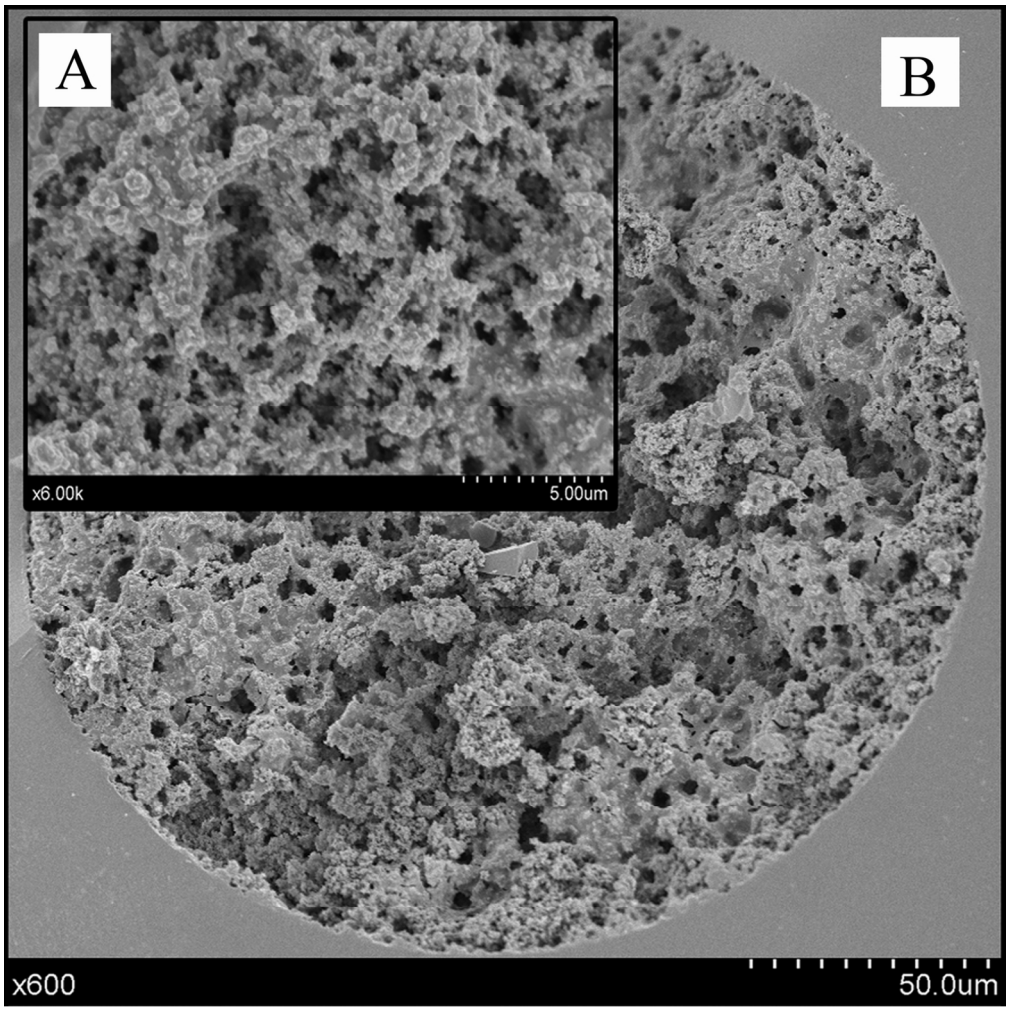
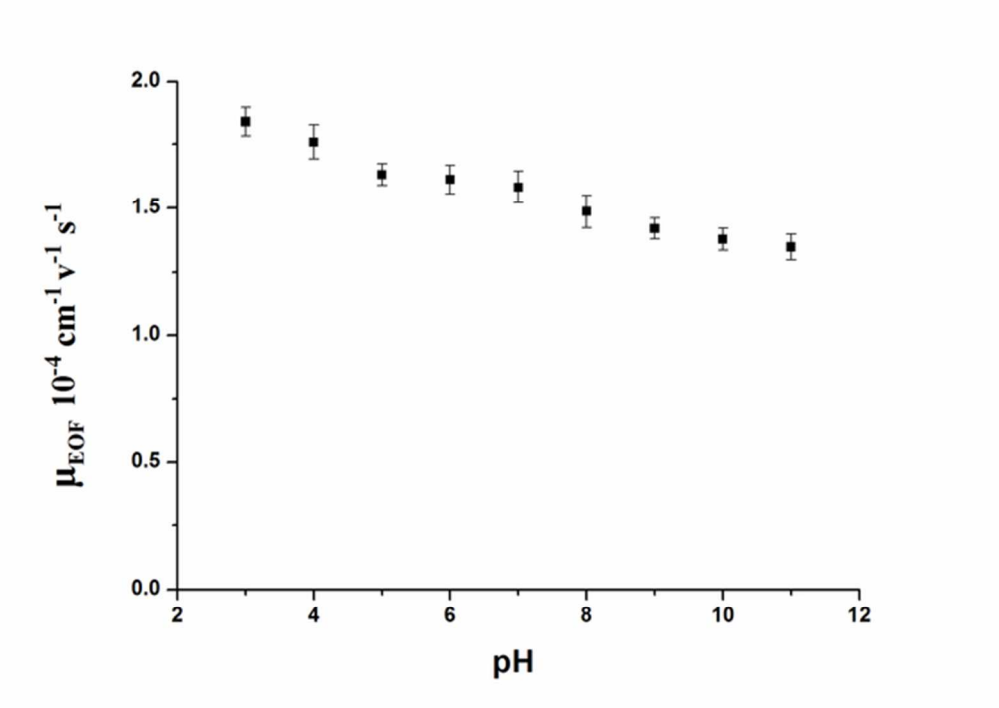


Fig. 2. SEM images of VC18HIm+Br− hybrid monolithic capillary column with optimal condition. Magnification: (A) ×6000 and (B) ×600.
63x63mm (600 x 600 DPI)



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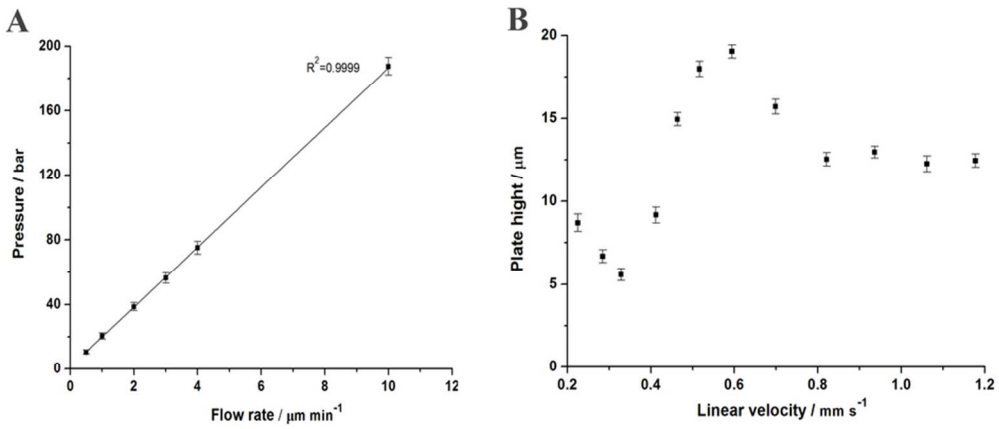

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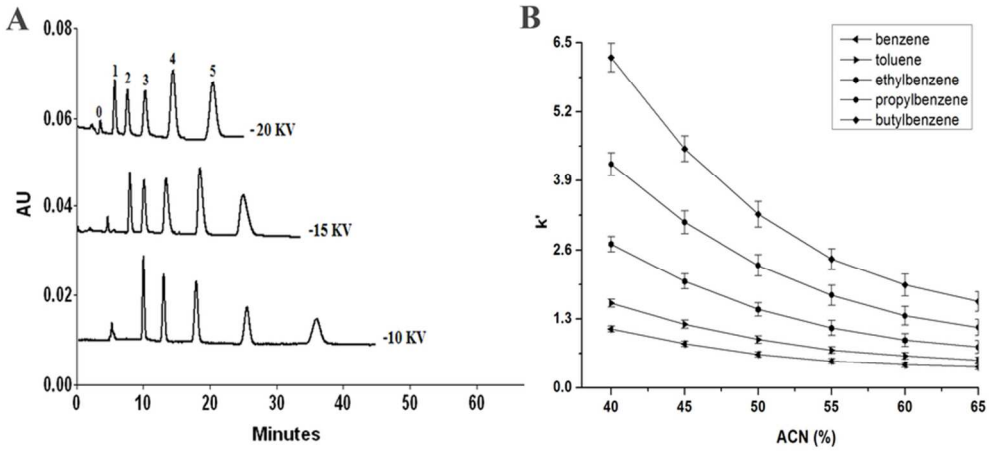

Fig. 5. (A) Separation of alkylbenzenes on the VC18HIm+Br− hybrid monolithic capillary column at different separation voltage by CEC and (B) effect of ACN content in the mobile phase on the retention factors of alkylbenzenes. Solutes: (A), (0) thiourea, (1) benzene, (2) toluene, (3) ethylbenzene, (4) propylbenzene, (5) butylbenzene. Experimental conditions: column dimension, 20 cm × 100 μm i.d.; mobile phase: (A) ACN/30 mM acetic acid buffer at pH 3.0 = 40/60 (v/v), (B) different content ACN in 30 mM acetic acid buffer at pH 3.0; injection, −0.5 psi for 5 s; separation voltage: (A) different voltage, (B) −10 kV; detection wavelength, 214 nm.
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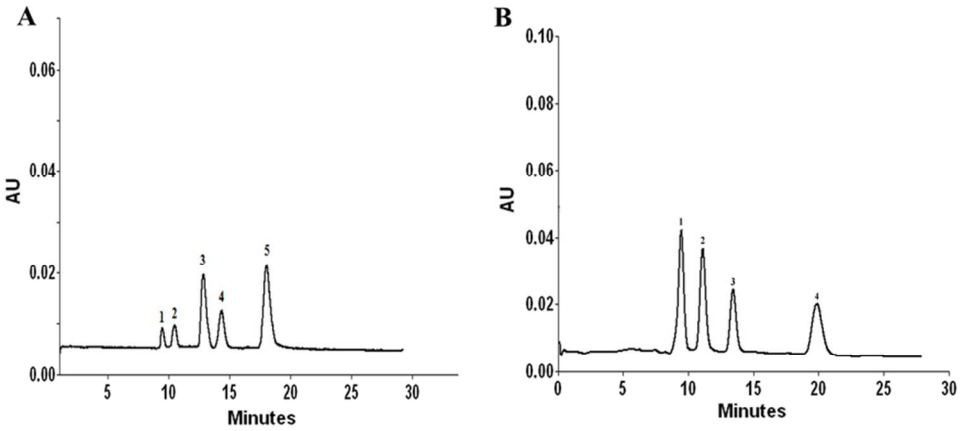

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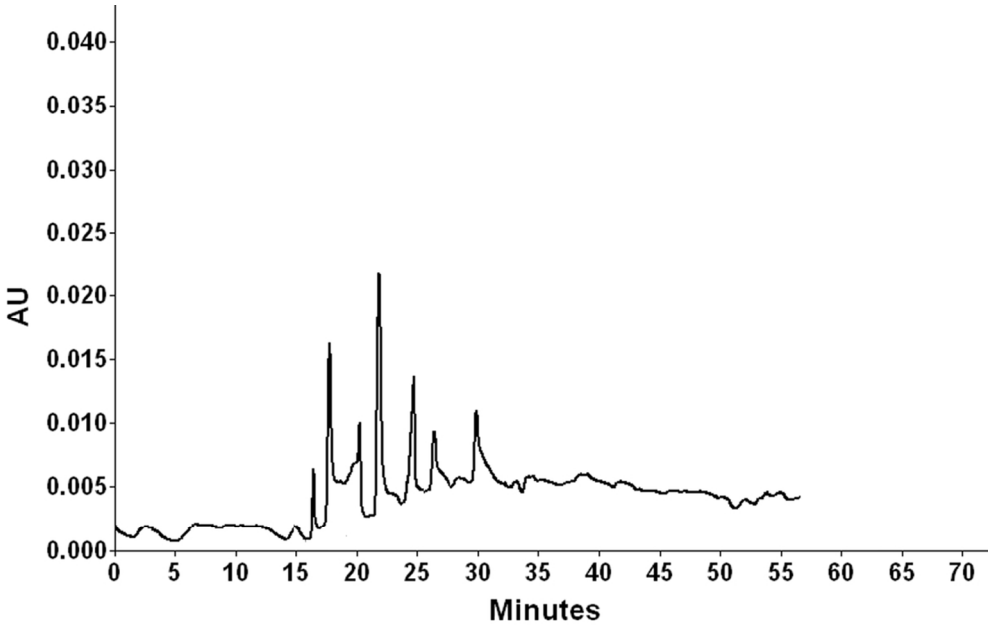

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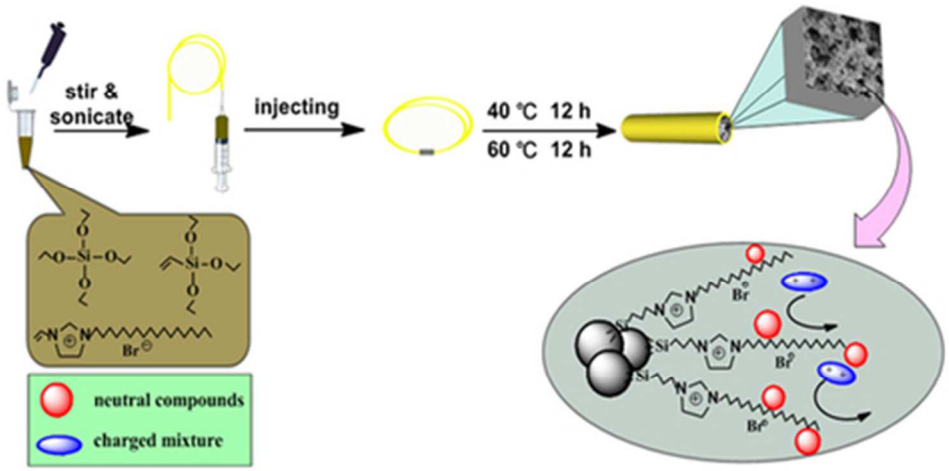

39x19mm (300 x 300 DPI)

Textual Abstract:

A novel IL hybrid monolithic column with great potential in separation has been fabricated via “one-pot” approach for capillary electrochromatography