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| Complete List of Authors: | Wang, Tingting; Ningbo University of Technology, College of Chemical Engineering<br>Chen, Yihui; Xiangshan Entry-Exit Inspection and Quarantine,<br>Ma, Junfeng; The Johns Hopkins University School of Medicine,<br>Zhang, Xiaodan; Dalian Institute of Chemical Physics, Chinese Academy of Sciences,<br>Zhang, Lihua; Dalian Inst Chem Phys,<br>Zhang, Yukui; Dalian Institute of Chemical Physics, Chinese Academy of Sciences, |
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# Ionic liquid-based zwitterionic organic polymer monolithic column for capillary hydrophilic interaction chromatography

Tingting Wang, *[a] Yihui Chen, [c] Junfeng Ma, [d] Xiaodan Zhang,[b] Lihua Zhang [b] and Yukui Zhang [b]

[a] *College of Chemical Engineering, Ningbo University of Technology, Ningbo 315016, China. E-mail: wangtingting@nbut.edu.cn; Fax: +86 574 65756710; Tel.: +86 574 65756733.*

[b] *Key Laboratory of Separation Science for Analytical Chemistry, National Chromatographic Research and Analysis Center, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, China.*

[c] *Xiangshan Entry-Exit Inspection and Quarantine, Xiangshan 310014, China.*

[d] *Department of Biological Chemistry, The Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA.*

In current study, a novel ionic liquid-based zwitterionic organic polymer monolithic column was developed, by copolymerizing 1-vinyl-3-(butyl-4-sulfonate) imidazolium, acrylamide and *N, N'*-methylenebisacrylamide in a quaternary porogenic solvent consisting of formamide/dimethyl sulphoxide/polyethylene glycol 8,000/polyethylene glycol 10,000, for capillary hydrophilic interaction chromatography. The monolithic stationary phase was optimized by adjusting the amount of monomers in the polymerization solution along with the composition of porogenic solvent. The optimized monolith exhibited excellent selectivity and favorable retention for nucleosides and benzoic acid derivatives. Primary factors affecting the separation efficiency of the monolithic column (including acetonitrile content, pH, and buffer salt concentration in mobile phase) have been thoroughly evaluated. Excellent reproducibility of retention time for five nucleosides was achieved, with relative standard deviations of run-to-run (n=3), column-to-column (n=3) and batch-to-batch (n=3) in the range of 0.18-0.48%, 2.33-4.20% and 3.07-6.50%, respectively.

## 1. Introduction

As a complementary to reversed-phase liquid chromatography (RPLC), hydrophilic interaction chromatography (HILIC) has gained significant popularity for polar compounds separation in recent years. HILIC is characterized as normal-phase chromatography on polar columns in aqueous-organic mobile phases rich in organic solvents. Similar to RPLC, the mobile phase of HILIC dissolves hydrophilic analysts. However, the limited types of sorbents currently available have hampered the separation of polar and hydrophilic compounds with complexity and diversity. To date, several kinds of stationary phases have been developed to serve as the HILIC sorbents [1, 2], among which zwitterionic stationary phase is a very appealing one [3, 4].

Ionic liquid has gained increasing interest in the field of analytical chemistry due to their unique properties such as variable viscosity, negligible vapor pressure, high thermal stability, multiple salvation interaction, and designability of structure and physicochemical properties, etc. Besides being used as mobile-phase additives [5-7], ionic liquids demonstrate almost perfect properties as modifier of silica sorbents in LC separation. A series (~20 types) of surface-confined ionic liquids stationary phase, including the imidazolium zwitterionic stationary phase have been described [8, 9]. In addition, Qiu et al. developed a silica-based 1-alkyl-3-(propyl-3-sulfonate) imidazolium zwitterionic stationary phase, exhibiting multiple retention mechanisms, such as anion-exchange, electrostatic attraction and repulsion interactions, and hydrophobic interaction [10]. However, the applicability of the developed imidazolium zwitterionic stationary phase for HILIC separation was demonstrated only recently. With the IL-modified silica zwitterionic material prepared by the copolymerization of anionic and cationic monomers, Qiu et al. [11] separated polar compounds under the HILIC mode. Shortly afterwards, 1-vinyl-3-(butyl-4-sulfonate) imidazolium was grafted onto the surface of 3-mercaptopropyl modified silica particles by “thiol-ene” click chemistry [12], with the column efficiency measured with cytosine as solute. Of note, silica particles are the main matrix for the preparation of imidazolium zwitterionic stationary phases in these studies, in which excellent

separation efficiency under HILIC mode was observed.

Compared with the traditionally used silica-based packed column, monolithic column is becoming a prevalent approach due to its advantages, such as the easy control of permeability, no need to prepare frits, and higher phase ratios [13]. Several methacrylate-based monolithic columns have been prepared for hydrophilic interaction liquid chromatography separation [14, 15]. Moreover, although several ionic liquid-based monolithic columns have been recently reported, these columns were just used for capillary electrochromatography [16, 17]. The applicability of ionic liquid-based organic polymer monoliths for HILIC separation has been rarely explored [18].

In this study, an ionic liquid-based monolithic stationary phase was synthesized by single-step copolymerization of 1-vinyl-3-(butyl-4-sulfonate) imidazolium (VBSIm), acrylamide (AM) and *N, N'*-methylenebisacrylamide (MBA). The composition of the poly (VBSIm-AM-MBA) monolith was thoroughly optimized. The resulting zwitterionic monolithic column was applied for the separation of nucleosides and benzoic acid derivatives under HILIC mode, with excellent separation efficiency and reproducibility achieved.

## 2. Materials and methods

### 2.1 Reagents and materials

Acrylamide (AM) and *N, N'*-methylenebisacrylamide (MBA) were purchased from J&K Scientific Ltd (Shanghai, China). 1-Vinyl-3-(butyl-4-sulfonate) imidazolium (VBSIm, 99%) was obtained from Shanghai Chengjie Chemical Co., Ltd (Shanghai, China). Azobisisobutyronitrile was purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Acetonitrile (ACN, LC grade) was obtained from Tedia (Fairfield, OH, USA). 3-Methacryloxypropyltrimethoxysiliane was purchased from Sigma (St. Louis, MO, USA). Polyethylene glycol (PEG) with molecular weight 8,000 and 10,000, formamide, dimethyl sulphoxide (DMSO), ammonium formate and formic acid were all purchased from Aladdin (Shanghai, China). Benzoic acid

derivatives including 4-hydroxybenzoic acid (4-HBA), 3-hydroxybenzoic acid (3-HBA), and 3,4- dihydroxybenzoic acid (3, 4-DHBA) were purchased from Aladdin (Shanghai, China). Melamine was obtained from Agro-Environmental Protection Institute, Ministry of Agriculture (Tianjin, China). Nucleosides including adenosine, uridine, cytosine, inosine and cytidine were all purchased from J&K Scientific LtD (Shanghai, China). The water used throughout all experiments was supplied by a Milli-Q system (Millipore, Molsheim, France). The polyimide-coated fused-silica capillaries (150 μm i.d. × 375 μm o.d.) were obtained from Yongnian Optical Fiber Factory (Hebei, China). For optimization of HILIC separation conditions, each benzoic acid derivatives was at concentration of 3.3 $\mu g.mL^{-1}$, and adenosine, uridine, cytosine, inosine and cytidine was at concentration of 5.0, 10.0, 5.0, 10.0 and 10.0 $\mu g.mL^{-1}$, respectively.

**2.2 Instrumentation**

The scanning electron micrographic images of the organic polymer monolithic column were carried out on a Hitachi S-4800 scanning electron microscope (Tokyo, Japan). Evaluation of the monolithic columns were performed on Calmflow-S100 capillary LC system containing UV detector with a 35 nL micro flow cell, one nano valve with two position (Valco Instruments Co., Inc, USA), a vacuum degasser, a binary pump and a data acquisition module (Lumtech, Germany). The permeability of monolithic columns was measured using a high pressure HPLC pump under constant pressure (Dalian Elite Analytical Instruments Co., Ltd, China).

**2.3. Preparation of the monolithic column**

In order to prepare polymer monolith in situ, a fused silica capillary was treated with 3-Methacryloxypropyltrimethoxysiliane according to our previous report [19]. The poly (VBSIm-AM-MBA) monolith was synthesized via one-step polymerization (Fig. 1). To avoid decomposition of VBSIm, 33.4 mg VBSIm was dissolved in 408.0 mg formamide immediately by vortexed. Subsequently, 10.0 mg AM, 20.0 mg MBA, 204.0 mg DMSO, 22.5 mg PEG-8,000 and 40.5 mg PEG-10,000 were added into the

mixture, and then mixed completely homogeneous by ultrasonication. After adding 1.0 mg initiator azobisisobutyronitrile, the solution was vortexed to obtain homogeneous solution, and purged with $N_2$ for 2 min. The pretreated capillary was filled with the mixture, and then plugged at the both ends with silicon rubber for polymerization at 75 °C for 20 h. Finally, the prepared monolithic column was rinsed with methanol to flush out unreacted reagents.

### 2.4. Chromatographic conditions

The HILIC method for benzoic acid derivatives was isocratic with a mobile phase of (A) 6% 20 mmol/L ammonium formate (pH 3.0) and (B) 94% acetonitrile. The HILIC method for nucleosides was isocratic with a mobile phase of (A) 8% 20 mmol/L ammonium formate (pH 4.0) and (B) 92% acetonitrile. The chromatograms of all analytes were set at 214 nm, and the flow rate was 1.2 μL $min^{-1}$. The injection volume was 0.2 μL. The capillary monolithic column was 30 cm total length.

## 3. Results and discussion

### 3.1 Preparation and characterization of the monolithic column

In our previous work, 1-aminopropyl-3-methylimidazolium chloride was successfully grafted onto a poly (glycidyl methacrylate-AM-MBA) monolithic column, which showed a mixed mode of hydrophobicity and anion-exchange capability [20]. In this study, a zwitterionic compound, namely VBSIm, was used to increase the hydrophilicity of the monolithic column. Interestingly, a colorless liquid was obtained when VBSIm was dissolved in formamide immediately. However, with the increase of time (e.g., >3 min), the solution color turned to primrose yellow (the color of 1-vinylimidazole and 1,4-butane). Thus, VBSIm immediately dissolved in the formamide was used to avoid decomposition. Because of the relatively insolubility of MBA in formamide, DMSO was introduced as porogen to enhance the solubility of MBA. To investigate the effect of the composition of the DMSO on the preparation of poly (VBSIm-AM-MBA) monolith, the ratio of formamide/DMSO (w/w) was varied

from 1 (column 1) to 5 (column 3), with the other composition kept constant. As shown in Table 1, only column 2 (with a ratio of formamide/DMSO of 2) yielded a uniform monolithic structure with reasonable permeability, compared to the other two columns whose permeability could not be determined due to the high backpressure of column 1 and a slack continuous bed of column 3.

Since monomers and cross-linkers can often be dissolved well in ternary porogenic solvents, quaternary porogenic solvent systems have rarely been adopted for the preparation of the polymer monoliths [13, 21]. Herein, the effect of the ratio of PEG-8,000/PEG-10,000 (w/w) was investigated (column 2, 4-7). The permeability was increased with the decrease of the ratio of PEG-8,000/PEG-10,000, which is in agreement with a previous report [22], with the maximum column efficiency obtained with a ratio of 25/45. As a result, a ternary porogenic system including PEG-8,000 and PEG-10,000 (with a ratio of 25/45) was used to achieve the best column efficiency.

The ratio of monomers, AM and VBSIm, also influences the permeability and column efficiency. As shown in Table 1, the permeability of column was decreased with the increase of VBSIm composition (column 6, 8-11). When the composition (column 11) of VBSIm was 100%, the monolithic column became almost impermeable that could withstand a pressure high up to 30 MPa. According to its formula, VBSIm contains a butanesulfonic acid group and an imidazole group, which could be assumed as a long carbon chain modified AM. Since chemical modification affects monolith porosity without changing the monolith skeleton integrity [23], an increased amount of VBSIm might lead to an enhanced chemical modification effect and thus high backpressure of the monolithic column. As a result, a 10/33.4 (w/w) of AM/VBSIm was selected in the following studies in order to achieve the best permeability and column efficiency.

In order to further improve column efficiency, we tried to increase the ratio of monomers to porogens. However, an increased ratio also resulted in decreased permeability of certain columns (column 6, 12 and 13; Table 1). Since the polymerization solution with a monomer/porogenic solvent ratio of 63.4/675 (w/w) yielded a monolithic column (column 12) with the highest column efficiency and

excellent permeability. Thus, the composition of polymerization reagents of column 12 was adopted in further experiments. To further evaluate the effect of VBSIm on the HILIC separation efficiency, we prepared a poly (AM-MBA) monolithic column (column 14; Table 1), which showed much lower column efficiency than that of column 12. Apparently, VBSIm plays an important role in the HILIC separation of polar compounds.

The morphology of the optimized poly (VBSIm-AM-MBA) monolithic column was examined by scanning electron microscopy (Fig. 2). The monolithic material was well attached to the inner wall of capillary (Fig. 2A). And the through-pore size was ranged from 0.5 to 2 μm, which could provide high permeability and low backpressure (Fig. 2C). Moreover, the monolithic material contained homogeneous micro-globules and uniform nanopores (Fig. 2B), which could render high separation efficiency.

### 3.2 Chromatographic evaluation of poly (VBSIm-AM-MBA) monolithic column for HILIC

Nucleosides and benzoic acid derivatives, which are commonly used to evaluate the selectivity of HILIC stationary phases [24, 25], were chosen as model compounds to investigate the chromatographic property of the poly (VBSIm-AM-MBA) monolithic column. Log $p$ and p$K_a$ of elected nucleosides and benzoic acid derivatives are shown in Table 2. Since the separation efficiency of HILIC was affected by parameters including organic modifier content, buffer pH and buffer salt concentration in the mobile phase, we optimized these parameters thoroughly.

ACN/water mobile phase is most commonly used for HILIC separation. With a constant salt concentration of 20 mM ammonium formate (pH 3.0), the effect of ACN content on retention factor ($k'$) was investigated. As shown in Fig. 3, retention factor of all nucleosides and benzoic acid derivatives was increased with the increase of the ACN content, indicating a typical HILIC retention mechanism. Nucleosides and benzoic acid derivatives were almost un-retained when the ACN content was less than 75% and 90%, respectively. However, all analytes emerged with broad peaks or were

not eluted when ACN content was higher than 92% for nucleosides and 95% for benzoic acid derivatives. The interesting phenomenon may be explained by the solubility limitations of these analytes [26]. With a mobile phase composed of 92% ACN/20 mM ammonium formate (pH 3.0), the retention factor of nucleosides was higher than that of benzoic acid derivatives, due to the less hydrophilicity/polarity of benzoic acid derivatives. Although 3-HBA has a higher log *P* than 4-HBA (Table 2), the retention factor of 3-HBA was higher than 4-HBA (Fig. 3B), as opposed to hydrophilic mechanism. However, the retention factor was decreased with the increase of p$K_{a2}$ for the benzoic acid derivatives, with log *k'* values plotted against the p$K_{a2}$ values for the benzoic acid derivatives (Supplementary Material, Fig. S1). The results indicate a role of the hydrogen bond of analytes in the retention mechanism, as suggested in a previous report [27]. Since VBSIm also contains a butyl group, a hydrophobic interaction mechanism might be exhibited with lower ACN concentrations (e.g. 60%, 30% and 10%). Nucleosides and benzoic acid derivatives were eluted in the dead time with the mobile phase containing either 60% or 30% ACN. However, benzoic acid derivatives were not eluted in a 50 min run with 10% ACN, although nucleosides were also eluted in the dead time. This phenomenon could be simply explained by hydrophobic interaction mechanism of benzoic acid derivatives. However, since the aim of this work was focused on HILIC separation of polar compounds, we did not intend to investigate the potential hydrophobic interaction mechanism of the poly (VBSIm-AM-MBA) monolith in detail. As a result, to obtain better separation efficiency and peak shape, the ACN content of 92% and 94% was adopted towards nucleosides and benzoic acid derivatives, respectively.

The buffer pH in the mobile phase plays an important role in the separation of nucleosides and benzoic acid derivatives, since it not only determines the state of the analytes in solution as ionic or neutral molecules but also affects the surface charge of the poly (VBSIm-AM-MBA) monolithic column. As shown in Fig. 1, there are several functional groups on the surface of poly (VBSIm-AM-MBA) monolithic column: imidazole group (p$K_a$ 6.7) [28], amine group (p$K_a$ 10.6) [29], and sulfonic group (p$K_a$ 1.2) [21]. With a pH less than 8.6 (p$K_a$-2, amine groups), amine groups

were positively charged, and imidazole groups were partially positively charged. The sulfonic groups were negatively charged with a pH higher than 3.2 (p$K_a$ 1.2 for sulfonic groups). Since the AM and VBSIm were of equal molar ratio in the poly (VBSIm-AM-MBA) monolithic column, the positive charges on the surface of the monolithic column were decreased with the increase of pH value from 3.0 to 6.5.

The effect of the mobile phase pH on the retention factor of nucleosides was investigated in a pH range from 3.0 to 6.5 at 20 mM ammonium formate and ACN/water of 92/8 (v/v). As shown in the Fig. 4A, the retention factor of nucleosides was decreased substantially with the increase of mobile phase pH. Given that the p$K_{a2}$ of adenosine, cytosine and cytidine was 3.82, 4.18 and 4.26, respectively (Table 2), the positive charges were decreased with the increase of the buffer pH. The analytes became neutral when the buffer pH was higher than p$K_{a2}$+2. The charged molecules were more hydrophilic than neutral molecules [27]. With the increase of the buffer pH, the hydrophilicity of adenosine, cytosine, cytidine and monolithic column was decreased, resulting in a decreased retention factor. In addition, the positive charge of analytes could interact with the net positive charge of the monolithic column via repulsive electrostatic interaction. With the increase of pH value, analytes and monolithic column were less positively charged, weakening the repulsive electrostatic interaction. However, the hydrophilicity of analytes and monolithic column might be more remarkable than repulsive electrostatic interaction, contributing to weaker retention of the analytes. On the other hand, since the p$K_{a1}$ of uridine and inosine was 9.39 and 8.8, respectively, uridine and inosine were neutral with a pH ranged from 3.0 to 6.5 (pH<p$K_{a1}$-2). But due to the decreased hydrophilicity of monolithic column with the increase of pH, the retention factors of uridine and inosine were decreased as well.

Fig. 4B shows the retention factor changes of benzoic acid derivatives with the mobile phase pH. The retention factors were increased obviously with the increase of pH. Given that the p$K_{a1}$ value for 4-HBA, 3-HBA and 3, 4-HBA was 4.58, 4.08 and 4.49, respectively, benzoic acid derivatives could be partially deprotonated in the pH range applied. But with a higher pH, they became more negatively charged, leading to

increased electrostatic interaction between some negative analytes and the net positive charges of monolithic column. As a result, increased retention factors were observed with the increased pH from 3.0 to 5.0.

The effect of salt concentration on the retention of nucleosides and benzoic acid derivatives was also investigated, since it not only controls the symmetry of peaks but also affects separation efficiency in HILIC. For the nucleosides analysis, the concentration of ammonium formate (pH 4.0) was varied from 10 to 50 mM, with a constant 92% ACN. For the benzoic acid derivatives analysis, the concentration of ammonium formate (pH 3.0) was varied from 5 to 30 mM, with a constant 94% ACN. As shown in Fig. 5A, with an increase of salt concentration, the retention factors of nucleosides were increased, which might be resulted from the hydrophilic partitioning mechanism [30]. On the other hand, the retention factors of benzoic acid derivatives were increased as the increase of salt concentration from 5 to 20 mM, with the highest retention factors achieved at 20 mM ammonium formate (Fig. 5B), which could also be ascribed to the hydrophilic partitioning mechanism. While the decreasing trend of retention factors can be characteristic of an ion-exchange retention mechanism [31].

### 3.3 Reproducibility

Fig. 6 shows the HILIC chromatograms of nucleosides and benzoic acid derivatives under the optimized separation conditions. Baseline separation for five nucleosides and three benzoic acid derivatives was achieved. Since excellent column stability is a prerequisite for its routine application, the run-to-run reproducibility was evaluated on one poly (VBSIm-AM-MBA) monolithic column for 3 runs, with the RSDs for nucleosides ranged from 0.18% to 0.46% (Table 3). Three poly (VBSIm-AM-MBA) monolithic columns prepared in a signal batch were used to evaluate the column-to-column reproducibility, and the RSDs of retention time for the nucleosides were ranged from 2.33% to 4.20% (Table 3). Batch-to batch reproducibility was also evaluated by columns from three different batches, and the RSDs of retention time for the nucleosides were <6.50%.

## 4. Conclusions

A novel zwitterionic poly (VBSIm-AM-MBA) monolithic column, developed by a one-pot copolymerization approach, was used as a stationary phase for capillary HILIC separation of nucleosides and benzoic acid derivatives. The resulting monolithic column exhibited excellent selectivity, efficient retention, and distinguished quantitative analysis for nucleosides and benzoic acid derivatives under the HILIC mode. Nucleosides were retained on the zwitterionic poly (VBSIm-AM-MBA) monolithic column based on the hydrophilic interaction and repulsive electrostatic interaction mechanisms, while benzoic acid derivatives were well separated based on hydrophilic interaction, electronic interaction and hydrogen bonding mechanisms.

## Abbreviation

| | |
|---|---|
| HILIC | Hydrophilic interaction chromatography |
| RPLC | Reverse phase liquid chromatography |
| AM | Acrylamide |
| MBA | *N, N'*-methylenebisacrylamide |
| VBSIm | 1-Vinyl-3-(butyl-4-sulfonate) imidazolium |
| ACN | Acetonitrile |
| PEG | Polyethylene glycol |
| DMSO | Dimethyl sulphoxide |
| 4-HBA | 4-Hydroxybenzoic acid |
| 3-HBA | 3-Hydroxybenzoic acid |
| 3, 4-DHBA | 3,4- Dihydroxybenzoic acid |

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## References

1 B. Buszewski and S. Noga, *Anal. Bioanal. Chem.*, 2012, 402, 231-247.

2 P. Jandera, *Anal. Chim. Acta*, 2011, 692, 1-25.

3 A. Shen, Z. Guo, L. Yu, L. Cao and X. Liang, *Chem. Commun.*, 2011, 47, 4550-4552.

4 Y. Li, Y. Feng, T. Chen and H. Zhang, *J. Chromatogr. A*, 2011, 1218, 5987-5994.

5 X. H. Xiao, L. Zhao, X. Liu and S. X. Jiang, *Anal. Chim. Acta*, 2004, 519, 207-211.

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

7 M. T. Ubeda-Torres, C. Ortiz-Bolsico, M. C. García-Alvarez-Coque and M. J. Ruiz-Angel, *J. Chromatogr. A*, 2015, 380, 96-103.

8 V. Pino and A. M. Afonso, *Anal. Chim. Acta*, 2012, 714, 20-37.

9 D. S. Van Meter, N. J. Oliver, A. B. Carle, S. Dehm, T. H. Ridgway and A. M. Stalcup, *Anal. Bioanal. Chem.*, 2009, 393, 283-294.

10 H. Qiu, Q. Jiang, Z. Wei, X. Wang, X. Liu and S. Jiang, *J. Chromatogr. A*, 2007, 1163, 63-69.

11 H. Qiu, A. K. Mallik, T. Sawada, M. Takafuji and H. Ihara, *Chem. Commun.*, 2012, 48, 1299-1301.

12 L. Qiao, A. Dou, X. Shi, H. Li, Y. Shan, X. Lu and G. Xu, *J. Chromatogr. A*, 2013, 1286, 137-145.

13 J. Lin, J. Lin, X. Lin and Z. Xie, *J. Chromatogr. A*, 2009, 1216, 801-806.

14 Z. Jiang, N. W. Smith, P. D. Ferguson and M. R. Taylor, *Anal. Chem.*, 2007, 79, 1243-1250.

15 V. Škeříková and P. Jandera, *J. Chromatogr. A*, 2010, 1217, 7981-7989.

16 J. Wang, W. Jia, X. Lin, X. Wu and Z. Xie, *Electrophoresis*, 2013, 34, 3293-3299.

17 Y. Wang, Q. Deng, G. Fang, M. Pan, Y. Yu and S. Wang, *Anal. Chim. Acta*, 2012,

712, 1- 8.

18 Z. Jiang, N. W. Smith, P. D. Ferguson and M. R. Taylor, *J. Sep. Sci.*, 2009, 32, 2544 -2555.

19 T. T. Wang, J. F. Ma, G. J. Zhu, Y. C. Shan, Z. Liang, L. H. Zhang and Y. K. Zhang, *J. Sep. Sci.*, 2010, 33, 3194-3200.

20 T. T. Wang, Y. H. Chen, J. F. Ma, M. J. Hu, Y. Li, J. H. Fang and H. Q. Gao, *Anal. Bioanal. Chem.*, 2014, 406, 4955-4963.

21 B. Gu, Z. Chen, C. D. Thulin and M. L. Lee, *Anal. Chem.*, 2006, 78, 3509-3518.

22 M. Chen, L. Li, B. Yuan, Q. Ma and Y. Feng, *J. Chromatogr. A*, 2012, 1230, 54-60.

23 A. Podgornik, A. Savnik, J. Jančar and N. L. Krajnc, *J. Chromatogr. A*, 2014, 1333, 9-17.

24 D. Moravcová, M. Haapala, J. Planeta, T. Hyötyläinenc, R. Kostiainen and S. K. Wiedmer, *J. Chromatogr. A*, 2014, 1373, 90-96.

25 L. Qiao, S. Wang, H. Li, Y. Shan, A. Dou, X. Shi and G. Xu, *J. Chromatogr. A.*, 2014, 1360, 240-247.

26 H. Qiu, L. Loukotková, P. Sun, E. Tesařová, Z. Bosáková and D. W. Armstrong, *J. Chromatogr. A*, 2011, 1218, 270-279.

27 G. Greco, S. Grosse and T. Letzel, *J. Chromatogr. A*, 2012, 1235, 60-67.

28 J. C. Vander Woude, I. Y. Christlieb, G. A. Sicard and R. E. Clark, *J. Thorac. Cardiovasc. Surg.*, 1985, 90, 225-234.

29 P. Das, A. R. Silva, A. P. Carvalho, J. Pires and C. Freire, *Catal. Lett.*, 2009, 129, 367-375.

30 A. J. Alpert, *J. Chromatogr.*, 1990, 499, 177-196.

31 M. Talebi, R. D. Arrua, A. Gaspar, N. A. Lacher, Q. Wang, P. R. Haddad and E. F. Hilder, *Anal. Bioanal. Chem.*, 2013, 405, 2233-2244.

32 G. P. Cunnngham, G. A. Vidulich and R. L. Kay, *J. Chem. Eng. Data*, 1967, 12, 336-337.

33 H. Vlčková, K. Ježková, K. Stětková, H. Tomšíková, P. Solich and L.Nováková, *J. Sep. Sci.*, 2014, 37, 1297-1307.

34 J. Saurina, S. Hernández-Cassou, R. Tauler and A. Izquierdo-Ridorsa, *Anal. Chim.*

*Acta*, 2000, 408, 135-143.

Fig. 1. Preparatory procedure for the zwitterionic poly (VBSIm-AM-MBA) monolithic column.

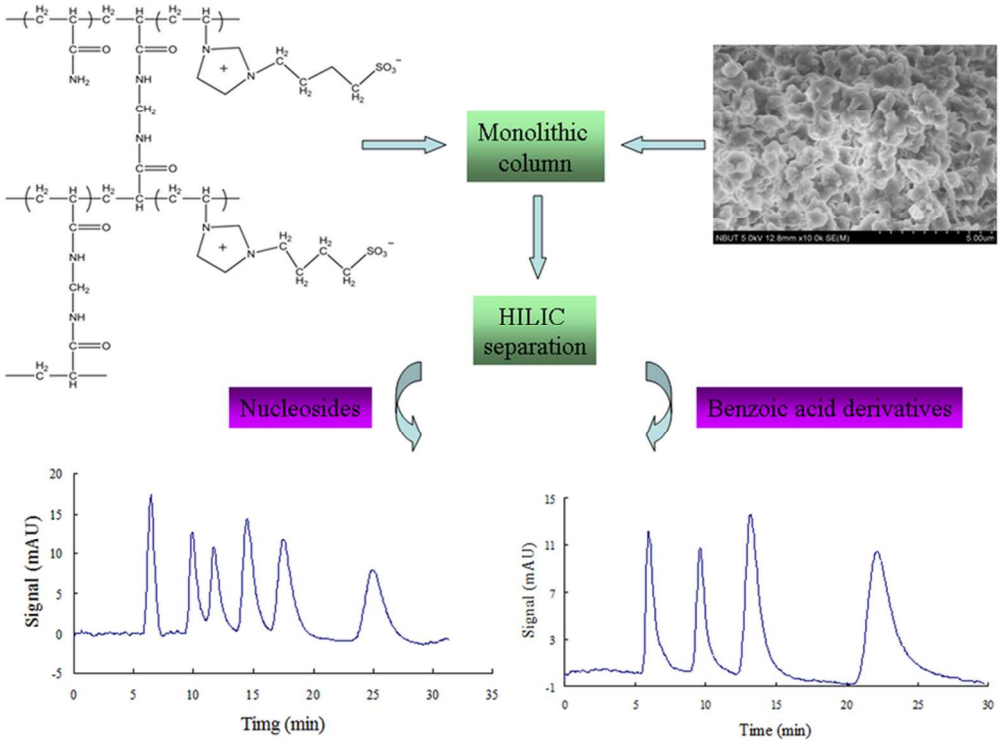
Fig. 2. Scanning electron micrographs of the zwitterionic poly (VBSIm-AM-MBA) monolithic column magnified by 500 (a), 10,000 (b) and 20,000 (c) times.

Fig. 3. Effect of ACN content on retention factors for nucleosides (a) and benzoic acid derivatives (b).

Fig. 4. Effect of buffer pH on retention factors for nucleosides (a) and benzoic acid derivatives (b).

Fig. 5. Effect of buffer salt concentration on retention factors for nucleosides (a) and benzoic acid derivatives (b).

Fig.6. HPLC chromatograms of nucleosides (a) and benzoic acid derivatives (b). Order of peaks for (a): (0) Toluene; (1) adenosine; (2) uridine; (3) cytosine; (4) inosine; (5) cytidine. Order of peaks for (b): (0) Toluene; (1) 4-HBA; (2) 3-HBA; (3) 3,4-DHBA.




Colour graphic
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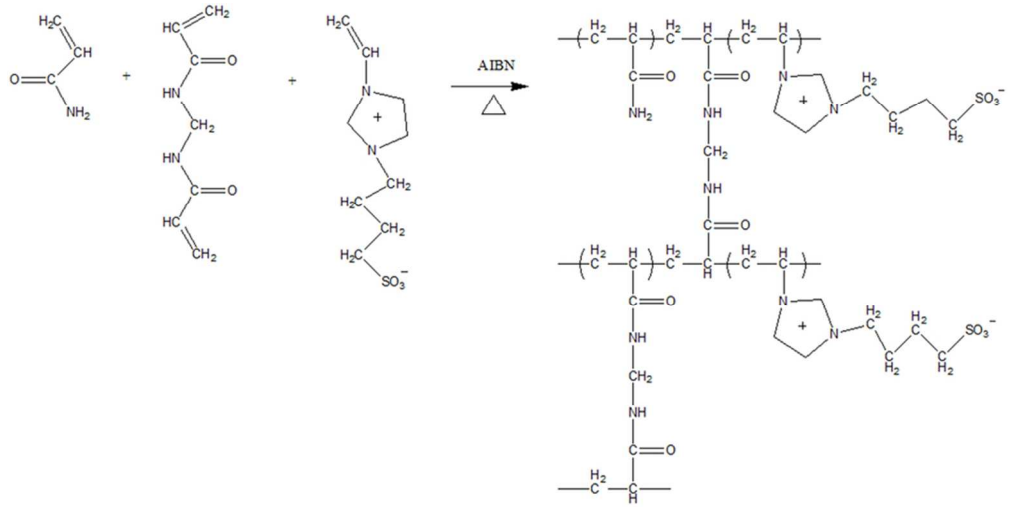


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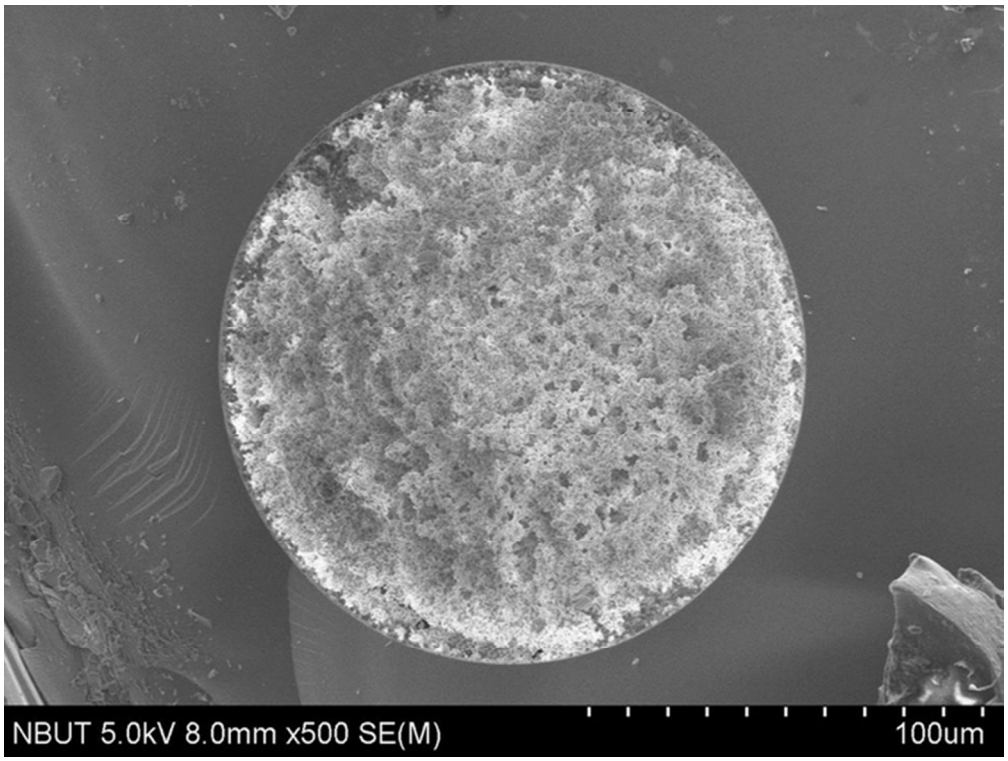


Fig.2a
29x22mm (600 x 600 DPI)

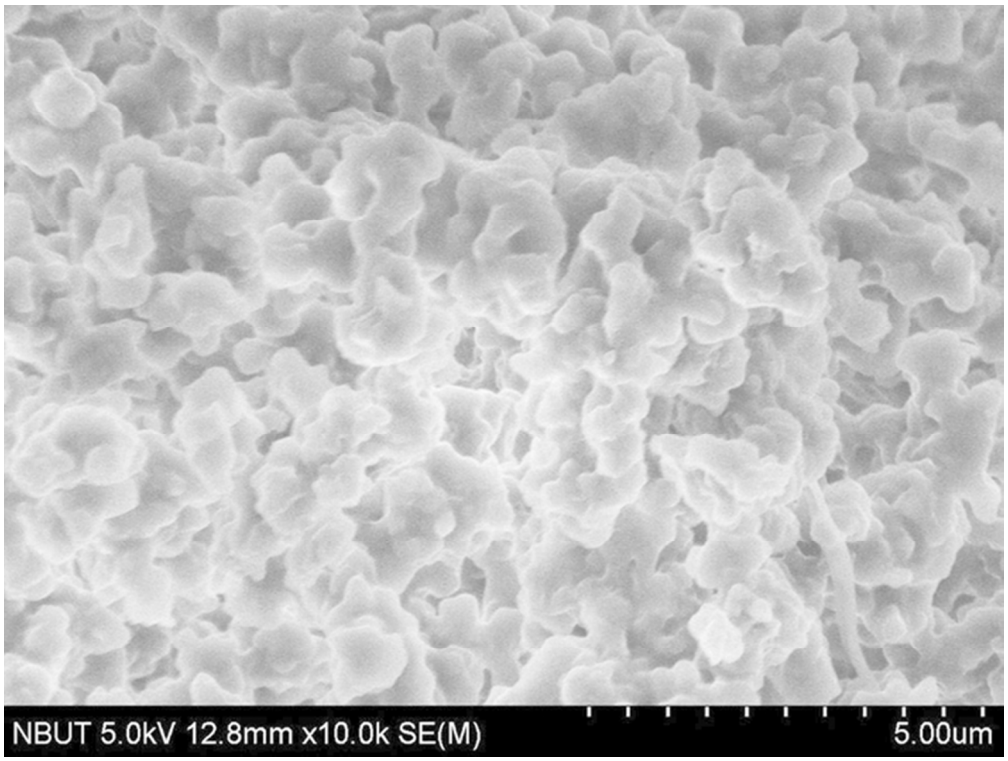


Fig.2b
29x22mm (600 x 600 DPI)

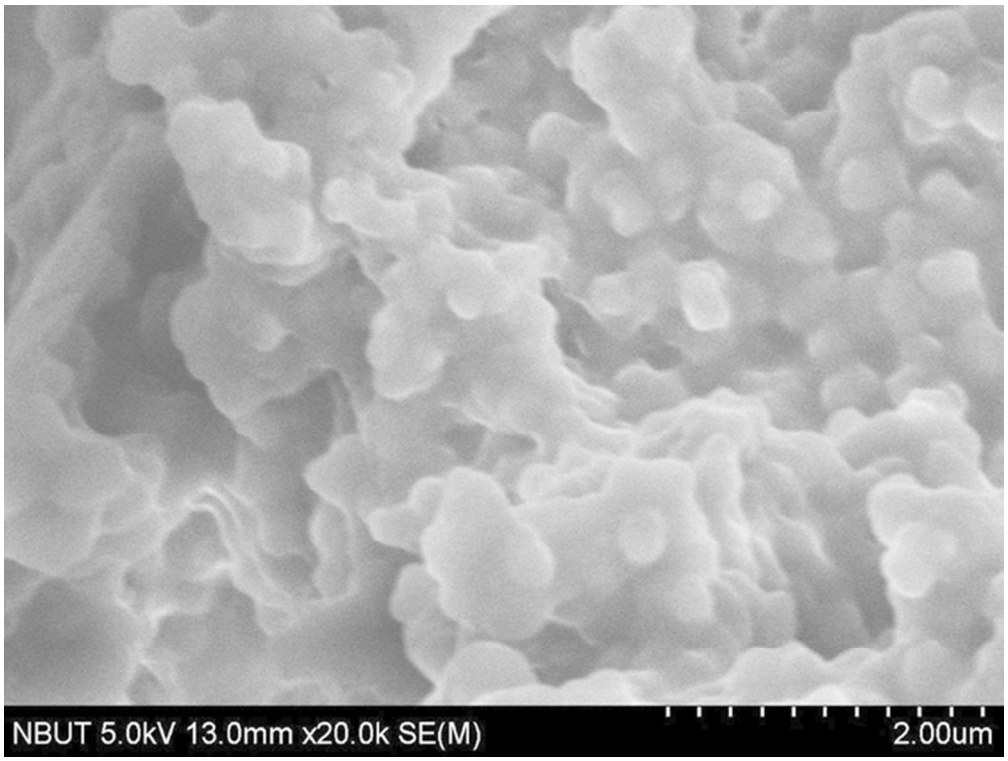


Fig.2c
29x22mm (600 x 600 DPI)

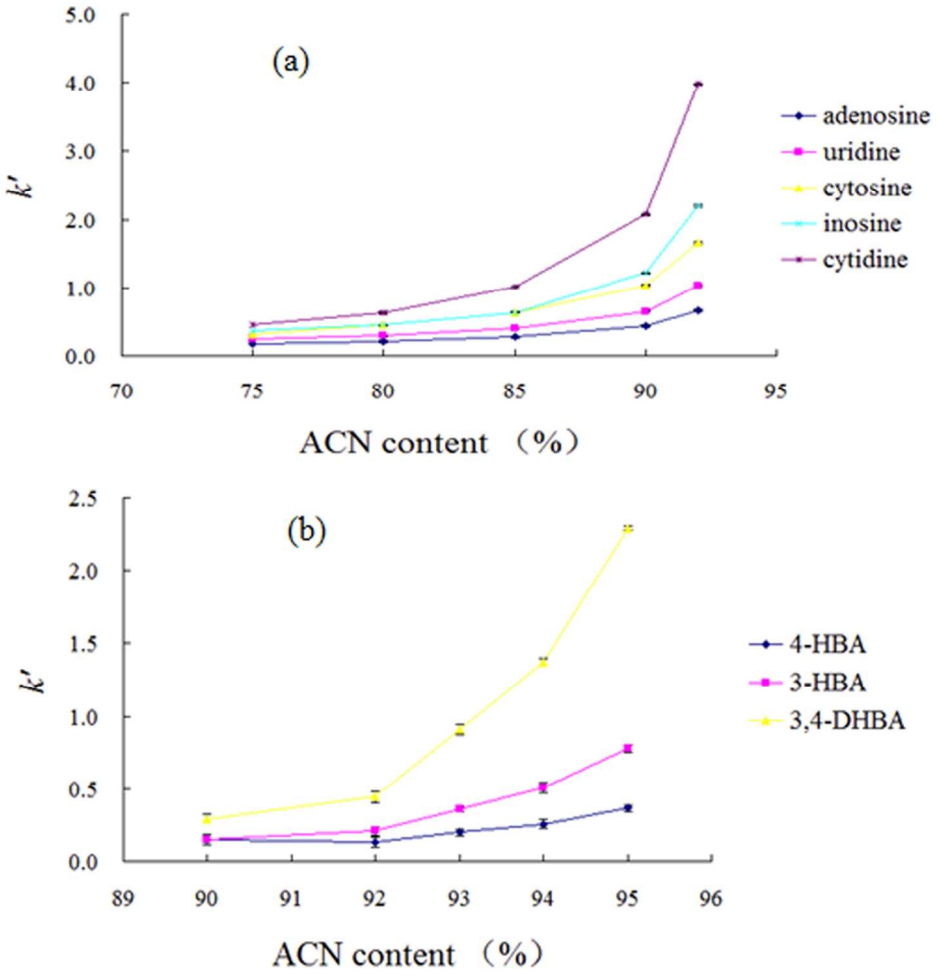


Fig.3
49x48mm (600 x 600 DPI)

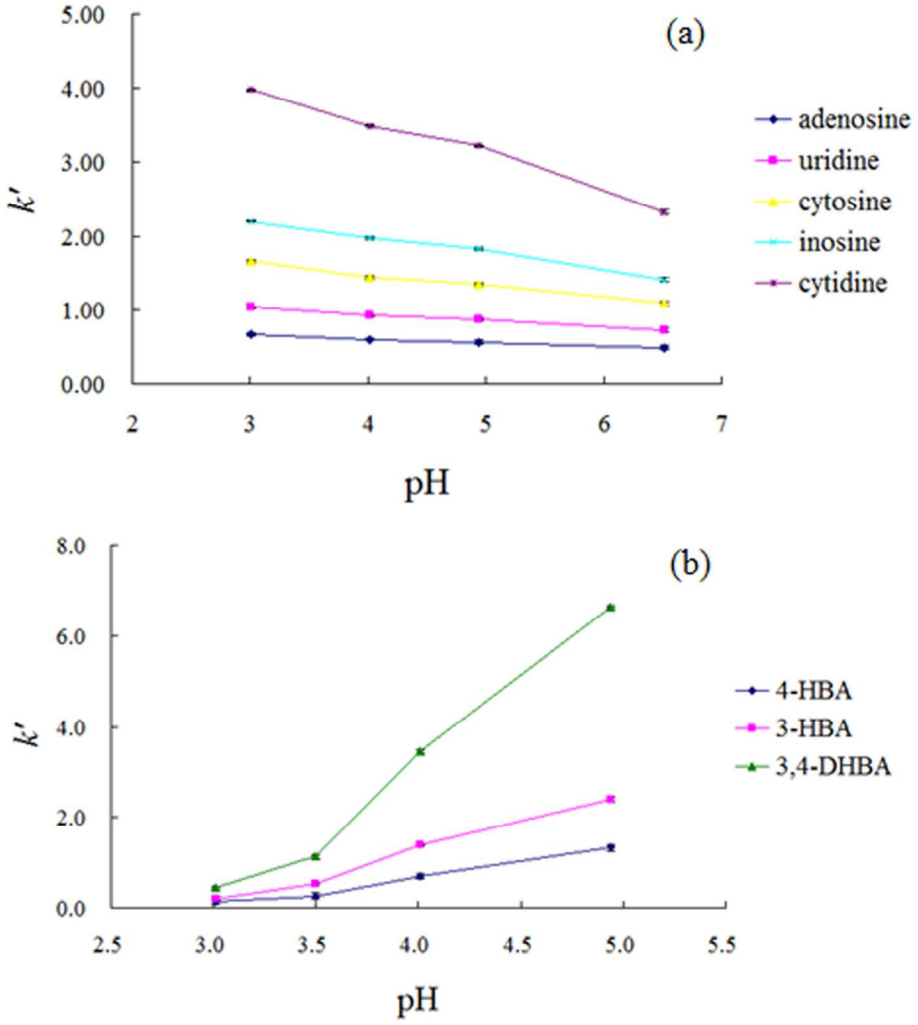


Fig.4
51x53mm (600 x 600 DPI)

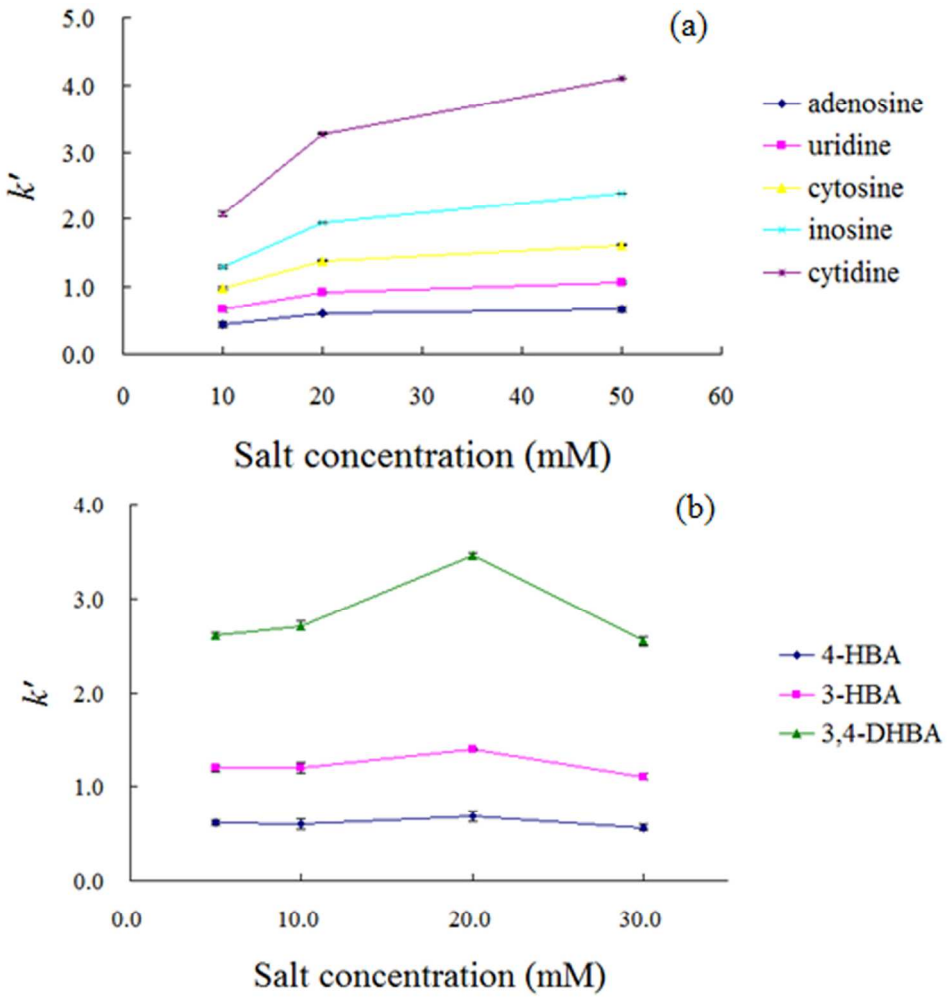


Fig.5
60x60mm (600 x 600 DPI)

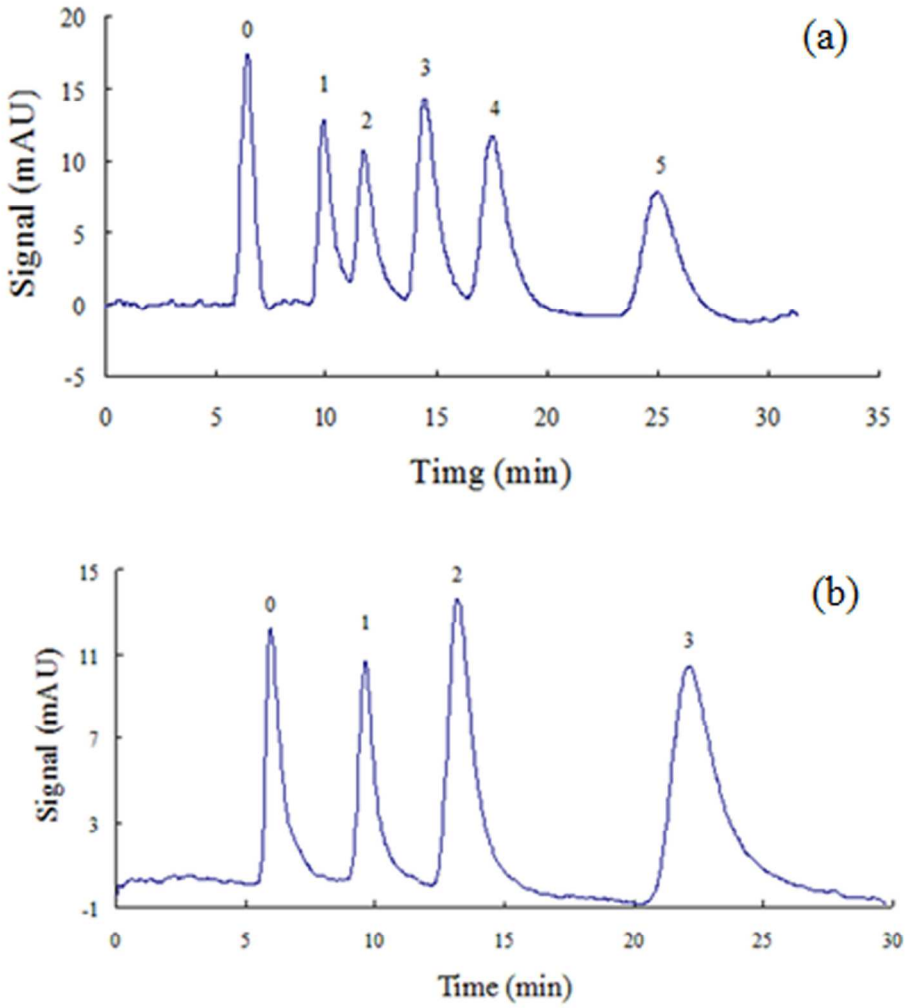


Fig.6
51x54mm (600 x 600 DPI)

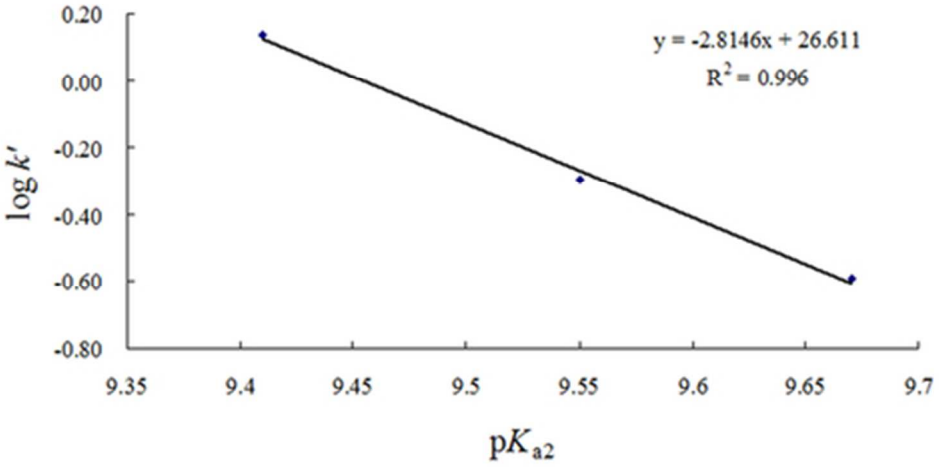


Fig.S1
20x11mm (600 x 600 DPI)

Table 1 Permeability ($K$) and column efficiency ($N$) of the monolithic columns with different composition of the polymerization solution

| Column | Monomer | | | Porogenic solvent | | | | $K$ ($\times 10^{-14}$ $m^2$) | $N_{Melamine}$ (plates/m) | $k'_{Melamine}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| | AM | MBA | VBSIm | Formamide | DMSO | PEG 8,000 | PEG 10,000 | | | |
| 1 | 10 | 20 | 33.4 | 340 | 340 | 45 | 25 | n/a[a] | n/a | n/a |
| 2 | 10 | 20 | 33.4 | 453.3 | 226.7 | 45 | 25 | 33.3 | 2310 | 0.93 |
| 3 | 10 | 20 | 33.4 | 566.7 | 113.3 | 45 | 25 | n/a[b] | n/a | n/a |
| 4 | 10 | 20 | 33.4 | 453.3 | 226.7 | 70 | 0 | 12.0 | 2947 | 0.97 |
| 5 | 10 | 20 | 33.4 | 453.3 | 226.7 | 55 | 15 | 33.6 | 5659 | 0.87 |
| 6 | 10 | 20 | 33.4 | 453.3 | 226.7 | 25 | 45 | 34.0 | 24390 | 0.87 |
| 7 | 10 | 20 | 33.4 | 453.3 | 226.7 | 0 | 70 | 55.1 | 19573 | 0.94 |
| 8 | 14.5 | 20 | 28.9 | 453.3 | 226.7 | 25 | 45 | 113 | 17526 | 0.72 |
| 9 | 8.7 | 20 | 34.7 | 453.3 | 226.7 | 25 | 45 | 9.78 | 2434 | 1.11 |
| 10 | 7.2 | 20 | 36.2 | 453.3 | 226.7 | 25 | 45 | 2.28 | 6331 | 0.85 |
| 11 | 0 | 20 | 43.4 | 453.3 | 226.7 | 25 | 45 | n/a[a] | n/a | n/a |
| 12 | 10 | 20 | 33.4 | 408 | 204 | 22.5 | 40.5 | 29.9 | 33480 | 1.08 |
| 13 | 10 | 20 | 33.4 | 386.8 | 193.4 | 21.3 | 38.4 | 22.3 | 22218 | 1.09 |
| 14 | 43.4 | 20 | 0 | 408 | 204 | 22.5 | 40.5 | 17.2 | 11869 | 0.60 |

Capillary column, 30 cm total length × 150 μm i.d.; Experimental conditions mobile phase: 8% 10 mM ammonium formate (pH 9.0) - 92% ACN (v/v); Flow rate: 1.2 μL/min; Sample for the column efficiency ($N$) determination: 2.5 μg $mL^{-1}$ malamine; The injection volume: 0.2 μL; The permeability ($K$) was measured by using 74/26 (v/v) ACN/water. The viscosity of ACN/water (74/26, v/v) was obtained from Ref. [32]. n/a: the measurements could not be made because the columns were not applicable.

[a] backpressure too high.

[b] Slack, slight detached.

Table 2. Chemical physical data of elected nucleosides and benzoic acid derivatives.

| Name | $pK_{a1}$(acid) | $pK_{a2}$(basic) | Log $P$ [c] |
|---|---|---|---|
| Adenosine | 13.11 [a] | 3.82 [a] | -1.02 |
| Uridine | 9.39 [a] | - | -1.61 |
| Cytosine | 9.00 [a] | 4.18 [a] | -1.71 |
| Inosine | 8.8 [b] | - | -1.91 |
| Cytidine | 13.48 [a] | 4.26 [a] | -1.94 |
| 4-HBA | 4.58 [d] | 9.67 [d] | 1.42 |
| 3-HBA | 4.08 [d] | 9.55 [d] | 1.50 |
| 3,4-HBA | 4.49 [d] | 9.41 [d] | 1.16 |

[a] From Ref. [33].

[b] From Ref. [34].

[c] Calculated using Advanced Chemistry Development (ACD/Labs) Software V6.0.

[d] From Ref. [27].

Table 3 Run-to-run, column-to-column and batch to batch reproducibility expressed as relative standard deviation (RSD) of retention time (n=3).

| Analytes | Run-to-run (%) | Column-to-column (%) | Batch to batch (%) |
|---|---|---|---|
| Adenosine | 0.18 | 2.33 | 3.07 |
| Uridine | 0.20 | 2.83 | 3.28 |
| Cytosine | 0.21 | 3.34 | 3.51 |
| Inosine | 0.29 | 3.39 | 6.50 |
| Cytidine | 0.48 | 4.20 | 5.94 |