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Covalently bonded polysaccharide-modified stationary phase for *per* aqueous liquid chromatography and hydrophilic interaction chromatography

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ABSTRACT

The mixed sulfated/methacryloyl polysaccharide derivative was prepared and successfully immobilized onto the surface of porous silica particles by polymerization. Polysaccharide derivative was calculated as 10.33% in the stationary phase prepared. The new stationary phase (PMSP) showed both hydrophilic interaction (HILIC) and *per* aqueous liquid chromatography (PALC) characteristics. The effects of column temperature, the water content, pH and ion strength of mobile phase on the retention time of test compounds in highly aqueous eluents were investigated to evaluate the PALC features of PMSP. The column efficiency is about 31,000 plates/m for benzoic acid in water/ACN (97/3, v/v) mobile phase at a flow rate of 1.0 mL/min. Compared with C18 column, the PMSP had shorter retention time for weak polar and non-polar compounds, but also showed stronger retention for strong polar compounds. It indicated that PALC was a suitable mode of chromatography as replacement of HILIC and complementarity of reversed-phase liquid chromatography (RPLC).

1. Introduction

Hydrophilic interaction liquid chromatography (HILIC) becomes an important and popular analytical technique for the separation of polar compounds and highly hydrophilic compounds [1–6]. Similar to normal phase liquid chromatography (NPLC), polar compounds are more strongly retained on HILIC column, but the non-aqueous mobile phase in NPLC is replaced by an aqueous–organic mixture with water as the strongly eluting solvent [2–4,7–9]. There is still a debate on the retention mechanism of HILIC [10,11]. Thus it might be a complex retention process composed of partitioning of solutes between a surface water layer and the bulk mobile phase, surface adsorption and electrostatic interactions [12–14]. In HILIC, a high percentage of acetonitrile (ACN) (70–95%) is often used. ACN is ranked as hazardous solvent and has negative influence on the environment, partly responsible for acid rain [15,16]. Chromatographers need pay special attention to the use of bulk hazardous solvents in LC analysis. It has been concerned to replace ACN by other benign solvents [17–19].

Green analytical chemistry has recognized momentum not only in the academic world but also in industrial and pharmaceutical laboratories, e.g. in recent years [15,16]. *Per* aqueous liquid chromatography (PALC) which was also called as reversed HILIC was named by Sandra et al. [15]. In PALC, mobile phases contain a high percentage of water and stationary phase is reversed to non-polar to separate polar compounds [20]. Gritti et al. [16] assessed the possible alternative PALC as a replacement of HILIC processes for the analysis and separation of polar compounds. Pereira et al. [15] illustrated the features of PALC with the analysis of catecholamines, nucleobases, acids, and amino acids. Gritti et al. [21] investigated the transition from the HILIC to the PALC adsorption mechanism for pyridine on a column packed with shell particles of neat porous silica.

In this work, we prepared a new bonded polysaccharide-modified stationary phase. *Gynostemma pentaphyllum* Makino is a perennial liana and grows widely in Southern China. It was reported that the GPP was composed of rhamnose and xylose with molar ratio as 1:12.25 [22]. The mixed sulfated/methacryloyl polysaccharide derivative was firstly prepared and immobilized onto the surface of porous silica particles by radical co-polymerization reaction in ionic liquid. Due to the existence of hydroxyl and sulfated groups in sulfated/methacryloyl GPP molecules, the obtained material (PMSP) was rendered both PALC and HILIC characteristics and was stable enough while contacting with water-rich mobile phase for a long time. In PALC mode, highly aqueous (90–100%) eluents were mainly studied. This not only helps to eliminate or reduce the problem associated with hazardous solvents, but also makes green LC possibilities. In addition, the separation of phenolic compounds, peptides and nitrogen-containing polar compounds on the PMSP and C18 column was demonstrated in the PALC mode.

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2. Experimental

2.1. Reagents and materials

The crude GPP was from Shanxi Lixin Biotechnology Co. (Shanxi, China). Chlorosulfonic acid (CSA), pyridine and N,N-dimethylformamide (DMF) were purchased from Gansu Yinguang Chemical Industry Co. (Gansu, China). Spherical silica (5 μm particle size; 10 nm pore size; 320 $m^2 g^{-1}$ surface area) was purchased from Fuji Silysia Chemical (Aichi, Japan). 3-(Methacryloyloxy)-propyltrimethoxysilane (MPTMS) was obtained from Alfa Aesar (Karlsruhe, Germany). Phosphorous trichloride (PCl_3) was purchased from Shanghai Chemical Reagents (Shanghai, China). Methacrylic acid (MAA) and azobis (isobutyronitrile) (AIBN) were from the Tianjin Chemicals Corporation (Tianjin, China). 1-Butyl-3-methylimidazolium chloride (ionic liquid) was from Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences (Lanzhou, China).

Vitamin B_2 (VB_2), vitamin B_5 (VB_5), vitamin B_6 (VB_6), and caffeine were purchased from Sigma (St. Louis, MO, USA). Melamine was obtained from J&K Chemical Ltd. (Beijing, China). Benzoic acid, phenol, catechol, hydroquinone, resorcinol and p-aminophenol were purchased from Tianjin Guangfu Chemical Reagent Co. (Tianjin, China). Five peptides including Val-D-Pro-Gly-Leu, tert-Leu-D-Pro-Gly-Leu, phenylglycine-D-Pro-Gly-Leu, cyclohexylglycine-D-Pro-Gly-Phe and Phe-D-Pro-Gly-Phe were kindly provided by School of Life Sciences at Lanzhou University. ACN of HPLC grade was from Dima Technology (Richmond Hill, VA, USA). All other reagents were of analytical-reagent grade (Tianjin Chemicals, China) and purified water from a Milli-Q system was used throughout the experiments.

2.2. Preparation of mixed sulfated/methacryloyl GPP derivative

The crude GPP was purified according to Refs. [23,24]. Its purity was up to 94%. Molecular weight of GPP was 9.3 kDa. It was characterized by IR and ^{13}C NMR.

IR (KBr, cm^{-1}): 3500 (OH), 2950 (CH), 1300 (C–O–C), 1610 and 750 (C_6H_6).
^{13}C NMR (D_2O, 600 M, 40 °C) δ (ppm): 62.4 (C^6), 72.1 (C^4), 74.2 (C^2), 75.4 (C^5), 78.4 (C^3), 102.1 (C^1).

Sulfated modification of GPP GPP (0.5 g) was suspended in 35 mL anhydrous DMF at room temperature and kept stirring for 30 min. Sulfation reagent (7 mL CSA in 25 mL anhydrous pyridine) was added dropwise and the mixture was then allowed to react at 40 °C for 2 h. After that, the mixture was cooled to room temperature and neutralized by NaOH solution. Then the reaction mixture was dialyzed against distilled water for 48 h to remove pyridine, salt and potential degradation products. Later, the sulfated GPP was isolated as the insoluble fraction with ethanol. The product (yield ~24.1%) was characterized by IR and ^{13}C NMR.

IR (KBr, cm^{-1}): 3500 (OH), 2980 (CH), 1610 and 750 (C_6H_6), 1300 (C–O–C), 1250 (S=O), 818 (C–O–S).
^{13}C NMR (D_2O, 600 M, 40 °C) δ (ppm): 62.5 (C^6), 68.9 (C^6), 72.1 (C^4), 74.2 (C^2), 75.3 (C^2), 75.9 (C^5), 78.3 (C^3), 94.7 (C^1), 98.4 (C^1).

Preparation of mixed sulfated/methacryloyl GPP derivative Sulfated GPP (0.5 g) was firstly suspended in 35 mL anhydrous DMF and stirred until it dissolved (about 3 h). Then 0.5 mL methacryloyl chloride (MC) prepared with MAA and PCl_3 was added to the solution. The reaction mixture was stirred under a N_2 atmosphere for 10 h at 60 °C. Later the mixture was cooled to room temperature and the obtained GPP derivative was isolated as the insoluble fraction with ethanol. The product (yield ~40.7%) was characterized by IR and elemental analysis (Table 1).

Table 1
Elemental analysis characterization of polysaccharide-modified stationary phase.

Elemental content (%)	C	H	S
Sulfated/methacryloyl GPP	17.87	3.23	6.10
MPTMS-silica	9.01	1.55	0
Polysaccharide stationary phase	10.82	1.54	0.63

IR (KBr, cm^{-1}): 3500 (OH), 3000 (CH), 1735 (C=O), 1610 and 750 (C_6H_6), 1300 (C–O–C), 1250 (S=O), 815 (C–O–S).

2.3. Immobilization of MPTMS on silica surfaces

The silica (1.5 g) was suspended in 30 mL anhydrous toluene, and 1.5 mL MPTMS was added with a stirring. The reaction mixture was heated under reflux with a N_2 atmosphere at −100 °C for 12 h. Then the obtained MPTMS-bonded silica was filtered and intensively washed with dichloromethane, acetone and methanol respectively, and then dried under vacuum at 60 °C overnight. The IR spectrum displayed 1730 (C=O) and 1640 (C=C) cm^{-1} peaks. Elemental analysis was shown in Table 1.

2.4. Polymerization [25–27]

Sulfated/methacryloyl GPP derivative (0.5 g) was firstly dissolved in 25 mL 1-butyl-3-methylimidazolium chloride with stirring for 5 h at 50 °C. Then the MPTMS-bonded silica (1.5 g) was added to the solution. The polymerization was performed with the initiator AIBN (1.0 wt.% of monomer) under N_2 atmosphere at 65 °C for 24 h. The final product was filtered, intensively washed with methanol and water respectively, and then dried under vacuum at 40 °C overnight. The obtained material was characterized by elemental analysis (Table 1).

Mass of polysaccharide derivative/100 g phase calculated from the results of elemental analyses (mass %) in Table 1 was 10.33.

The routes for synthesis of the new separation material were shown in Fig. 1.

2.5. Instruments and Chromatographic evaluation

The chromatographic system consisted of a Varian 210 high-performance liquid chromatographic pump (Palo Alto, CA, USA), a Varian 325 UV–vis detector, and a Varian Star chromatographic workstation. ^{13}C NMR spectrum in D_2O was recorded with a Bruker AVANCE 600 MHz spectrometer (Rheinstetten, Germany). IR spectra were obtained on a Nicolet 20 NEXUS 670 FT-IR (Ramsey, MA, USA) using KBr pellets. Elemental analysis was measured on a Vario EL elemental analysis system (Hanau, Germany). Molecular weight of sulfated derivatives was determined by high-performance gel-filtration chromatography (HPGFC) on a Waters 2695 instrument equipped with three Ultrahydrogel columns 500, 250, 120 (300 mm × 7.8 mm) and a Waters Refractive Index Detector (RID).

The new separation material was slurry-packed into a 150 mm × 4.6 mm I.D. stainless steel column with methanol as slurry medium and tetrachloromethane–methanol mixture was used as eluent at 41.4 MPa pressure. C18 column (150 mm × 4.6 mm, 5 μm particle diameter, 10 nm pore size) was from Lanzhou Zhongkeantai Corporation (Lanzhou, China). The chromatographic evaluations were carried out at room temperature (25 ± 2 °C). The flow rate was 1.0 mL/min. A set of test probes with a concentration of 20 μg/mL was prepared in

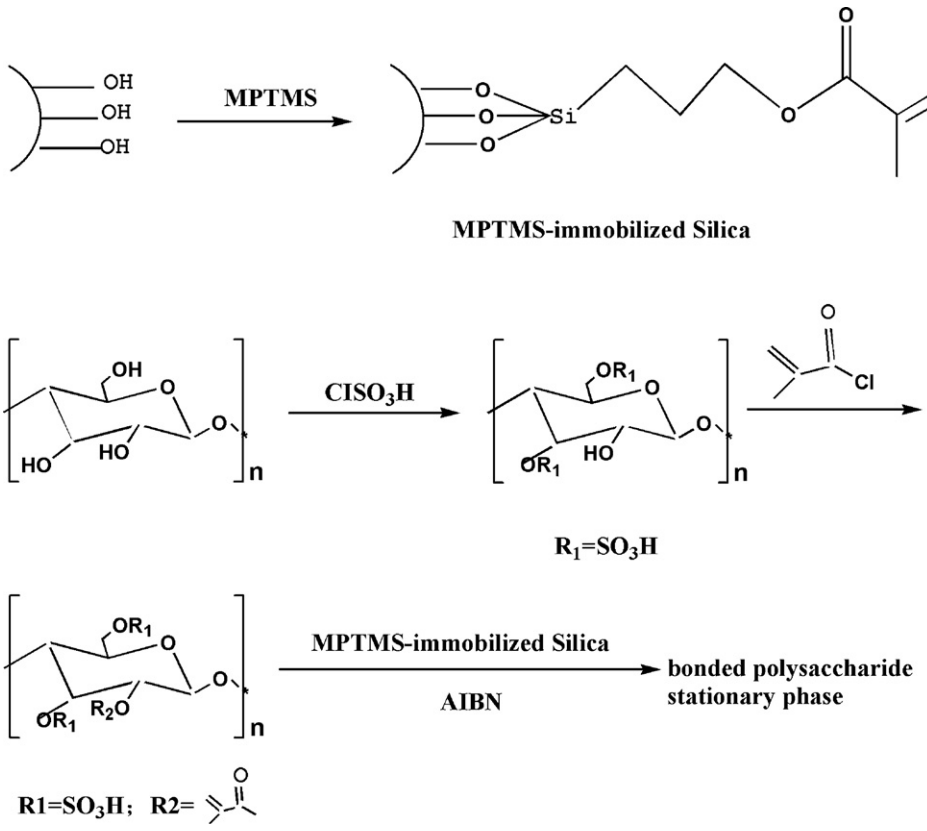

Fig. 1. Synthesis routes for the new separation material.

water/acetonitrile (1/1, v/v). The dead time was 1.5 min, which was determined by injecting 5 μL methanol with water/ACN (1/1, v/v) as mobile phase [28]. Each measurement was replicated three times.

3. Results and discussion

3.1. Retention properties in the HILIC mode

Solvent strength in the eluent is probably the most significant factor on retention of compounds, because hydrophilic interaction is enhanced by decreasing the polarity of the eluent. Benzoic acid and VB_6 were chosen as the test compounds. When ACN content was 90, 97 and 98% in the mobile phase, the retention times were 1.85, 2.60 and 3.26 min for benzoic acid and were 2.13, 3.10 and 3.58 min for VB_6 respectively. The retention change of benzoic acid and VB_6 clearly demonstrated the HILIC property of the PMSP due to hydroxyl and sulfated groups on the silica surfaces.

3.2. Retention properties in the PALC mode

For practical, economical and ecological reasons, we were more interested in PALC. We compared the peak shape in the PALC and HILIC conditions at a flow rate of 1.0 mL/min. The similar retention times were obtained in the PALC mode with 75% water in mobile phase and HILIC mode with 3% water for VB_6 (k' = 1). The peak shape of VB_6 in PALC had no significant difference compared with in HILIC (the peak asymmetry factors were 1.13 and 1.18 in the HILIC and PALC, respectively). So the present study showed that PALC was possible as a retention mode alternative to HILIC for the separation of polar compounds.

3.2.1. Effect of water content in mobile phase on retention

Six strong polar and weak polar compounds including melamine, VB_2, VB_6, caffeine, benzoic acid and hydroquinone were selected as test probes to investigate the PALC properties of the PMSP. Structures and physical properties of six test compounds could be seen in Fig. 2. The volume fractions of water in mobile phases (60, 75, 90, 95, 96, 97, 99 and 100%) were studied. The retention factors (k') of six test probes were plotted against the volume fraction of water in the eluent as shown in Fig. 3.

The compounds exhibited typical PALC behaviors of increasing retention with increasing water content in the mobile phase on the PMSP. The retention time of all six compounds leveled off initially as the water content increased from 60 to 90%, and then increased dramatically when the water content further increased to 100%. It showed that the retention of the test probes was extremely sensitive to any small variation of ACN content in water-rich eluents. The possible reason might be that the surfaces of the PMSP were significantly saturated with water and ACN, resulting in slight decrease of the retention factors of compounds with the water content of 90–60% in mobile phase. But within the ACN concentration ranges of 0–10%, small variation of ACN content could lead to the drastic composition change of the adsorbed eluent multilayer onto PMSP. So the retention factors were strongly sensitive to little change of mobile phase with the bulk water concentration [16,20,29].

The column efficiency were about 24,000, 10,000, 18,000, 25,000, 31,000 and 11,000 plates/m for melamine, VB_2, VB_6, caffeine, benzoic acid and hydroquinone in water/ACN (97/3, v/v) mobile phase, respectively.

3.2.2. Effect of column temperature on retention

Column temperature is also known to be an important influential factor for retention and selectivity on the retention of compounds in LC [30]. The relationship between retention factor

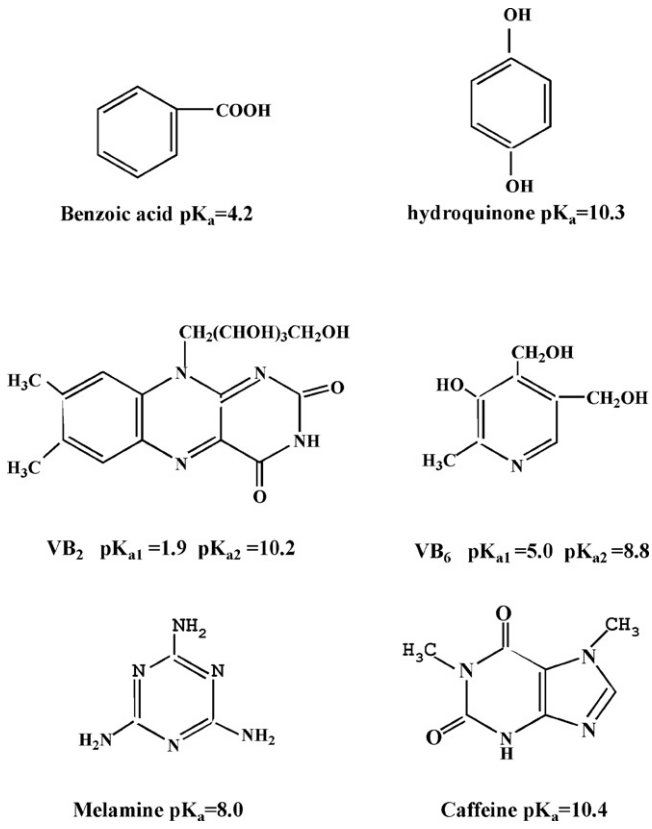

Fig. 2. Structures and physical properties of six test compounds.

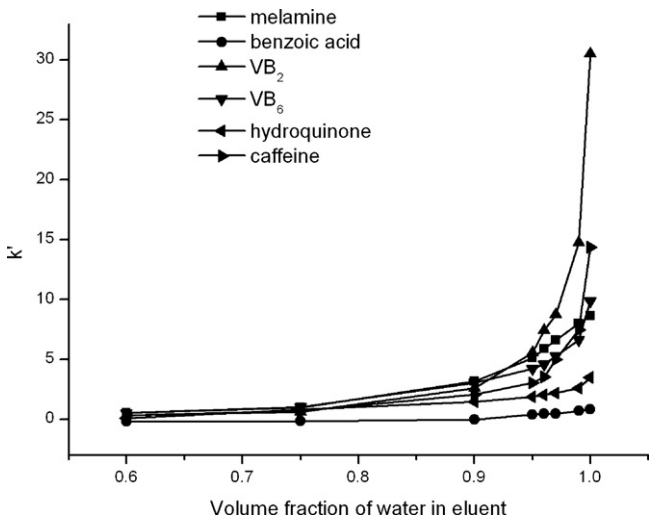

Fig. 3. Effect of the water volume fraction on retention in the eluent on the PMSP. Mobile phase: water/ACN; UV detection: 260 nm.

(k') and column temperature (T) in RPLC is often described by van't Hoff equation [31]:

$$\ln k' = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} + \ln \phi$$

where k', ΔH°, ΔS°, R, T and ϕ are the retention factor for the solute, the standard partial molar enthalpy of transfer, the standard partial molar entropy of transfer, the gas constant, the absolute temperature and the phase ratio, respectively. In this study, the retention data for the model compounds were used to construct van't Hoff plots with column temperature varying (20, 30, 40, 50 and 60 °C) in water/ACN (98/2, v/v) mobile phase. These non-linear plots were often assumed that the enthalpy and entropy of transfer changed with temperature according to literature [3,12]. It could be found the test probes showed a loss of retention upon increasing the column temperature. Due to the k' value is strongly dependent on the column temperature, increasing column temperature can lead to decrease of k' because of the higher solubility of the analytes in the mobile phase.

3.2.3. Effect of mobile phase pH on retention

Mobile phase pH plays an important role in retention and selectivity in LC by influencing solute ionization in the eluents. Fig. 4 shows that the effect of the mobile phase pH on the PALC retention by changing the pH of ammonium formate aqueous solutions at 6.3, 5.0, 4.3, 3.5 and 2.8 before mixing with acetonitrile while keeping the concentration of ammonium formate constant at 10 mM. Benzoic acid showed the most dramatic decrease in retention from pH 2.8 to 6.3, because benzoic acid was dissociated gradually with the increase of pH. For VB_2, caffeine, and hydroquinone with pK_{a1} ~1.9, ~10.4 and ~10.3, there were no significant changes in ionization in the pH range studied. So the retention time remained essentially unchanged. The retention times of melamine and VB_6 with pK_a ~8.0 and ~5.0 remained almost unchanged in the pH range from 6.3 to 5.0, but from 5.0 to 2.8 retention time slightly decreased. The reason might contribute to be more protonated in low pH and less hydrophobicity.

3.2.4. Effect of ionic strength of mobile phase on retention

The effect of salt concentration on the retention of six compounds in the PALC retention process was also investigated by varying the concentration of ammonium formate at 10 mM, 25 mM and 50 mM, respectively (Table 2). For VB_2, VB_6, melamine and caffeine, the retention decreased on the PMSP with the increase of salt

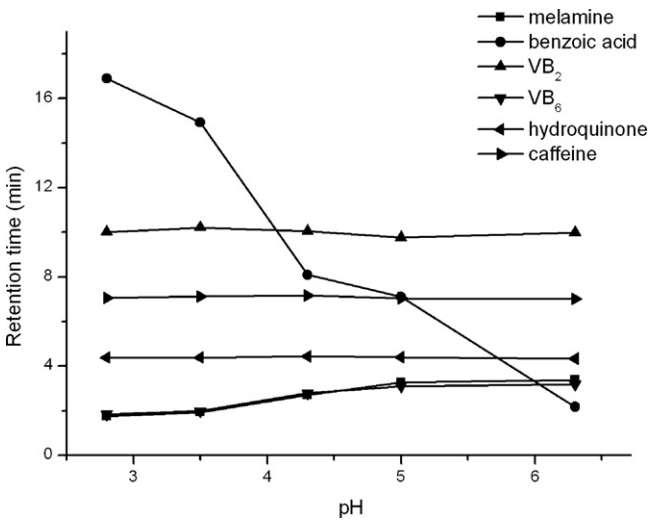

Fig. 4. Effect of pH on retention on the PMSP. Mobile phase: water/ACN (95/5, v/v) containing 10 mM ammonium formate; UV detection: 260 nm.

Table 2
Retention time of the model compounds at different ammonium formate concentrations in the mobile phase[a].

Concentration (mM)	Hydroquinone	Benzoic acid	VB_2	VB_6	Melamine	Caffeine
10	4.33	2.17	9.98	3.19	3.37	6.95
20	4.37	2.20	9.71	2.84	3.10	6.93
50	4.45	2.25	9.70	2.77	2.83	6.89

[a] Mobile phase: ACN/ammonium formate solution (5/95, v/v); pH = 6.3; UV detection: 260 nm.

concentration. This result was consistent with the involvement of ion exchange interaction between the basic compounds and sulfated groups on the PMSP. Higher salt concentrations increased the eluting strength of the mobile phase and weakened ion-exchange interaction, thus leading to decreasing retention. The retention time of the acids (benzoic acid and hydroquinone) increased slightly along with the increasing concentrations of salt. It could be due to the increase in hydrophobic interactions.

3.3. Application

We investigated the separation ability of the PMSP in PALC mode. Mixtures of ACN and water (water % >90%) were used as the mobile phases and neither supporting salts nor buffers were added to the eluent. In order to assess PALC process on the PMSP, C18 column was also used in this study.

3.3.1. The separation of phenolic compounds

A mixture of phenolic compounds including phenol, catechol, hydroquinone, resorcinol and p-aminophenol was selected as model compounds. As could be seen in Fig. 5, good separation was obtained with pure water as the mobile phase within 25 min. The results showed that PALC process depended on the position and the number of the hydroxyl groups. Compared with C18 column, overall analysis times reduced by half and the order of catechol and resorcinol was reversed. The result showed the interaction of phenolic compounds with the PMSP was weaker than that with C18 column. The unexpected wider peak for p-aminophenol may attribute to ion-exchange capacity of PMSP.

3.3.2. The separation of peptides

We also investigated the separation of five peptides on the new stationary phase and C18 column under the same chromatographic conditions. No peak appeared within 40 min on C18 column. Five peptides were retained and separated in water-rich eluents on the PMSP (Fig. 6).

3.3.3. The separation of nitrogen-containing compounds

To demonstrate the special selectivity of the PMSP, a mixture of five polar nitrogen-containing compounds was used for further evaluation. The compounds were difficult to retain and separate on C18 column. However, good separation was obtained with 96% aqueous eluent on the PMSP (Fig. 7).

The PMSP has been used for about 3 months (about running over 600 h) in our laboratory and had no obvious change of column efficiency. So, the PMSP had high column stability in the PALC mode.

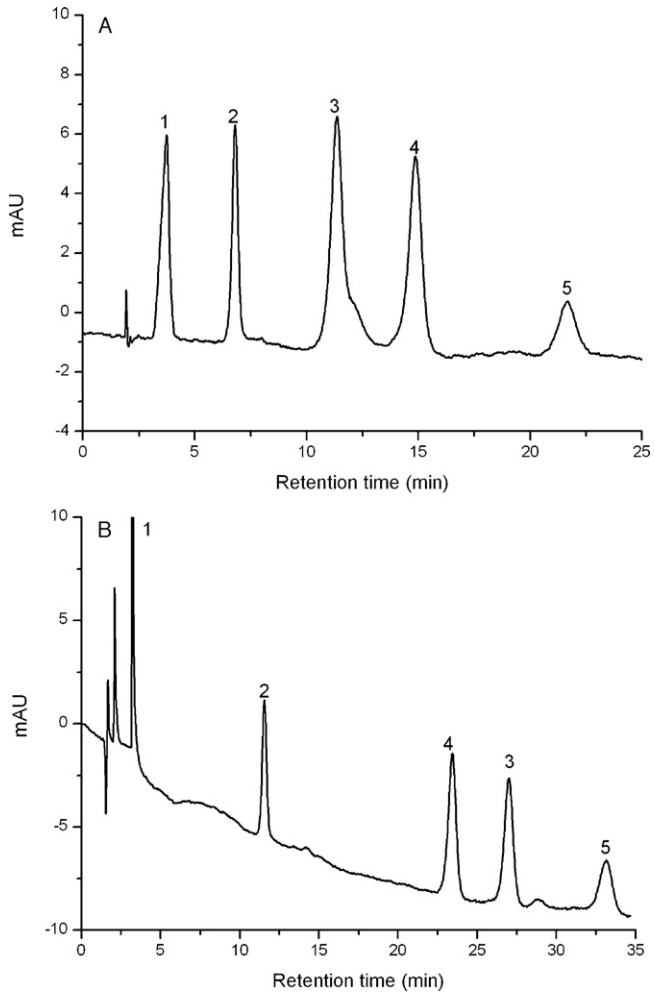

Fig. 5. Separation of phenolic compounds. (A) On the PMSP and (B) on C18 column. Mobile phase: 100% water; UV detection: 260 nm. Solutes: (1) p-aminophenol, (2) hydroquinone, (3) catechol, (4) resorcinol, and (5) phenol.

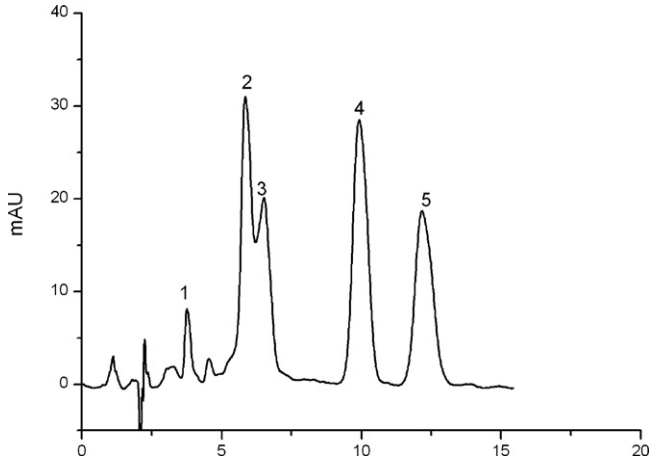

Fig. 6. Separation of five peptides on the PMSP. Mobile phase: water/ACN (92/8, v/v); UV detection: 220 nm. Solutes: (1) Val-D-Pro-Gly-Leu, (2) tert-Leu-D-Pro-Gly-Leu, (3) phenylglycine-D-Pro -Gly-Leu, (4) cyclohexylglycine-D-Pro-Gly-Phe, and (5) Phe-D-Pro-Gly-Phe.

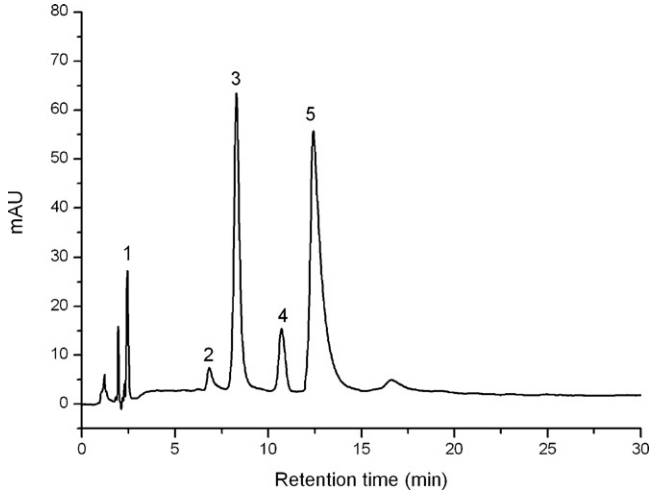

Fig. 7. Separation of nitrogen-containing compounds on the PMSP. Mobile phase: water/ACN (96/4, v/v); UV detection: 260 nm. Solutes: (1) VB_5, (2) caffeine, (3) VB_6, (4) melamine, and (5) VB_2.

4. Conclusion

In this paper, a new polysaccharide-modified stationary phase was prepared, characterized and evaluated. The PMSP exhibited HILIC and PALC properties, and PALC could provide similar retention as HILIC for polar compounds. The features of PALC on the PMSP were illustrated through investigating the effects of column temperature, the water content, pH and ion strength of mobile phase on the retention time of test compounds. And phenolic compounds, peptides and nitrogen-containing polar compounds were respectively separated on the new material in the PALC mode. Compared with C18 column, the PMSP had shorter retention time for weak and non-polar compounds and showed stronger retention for strong polar compounds. In the framework of green chromatography and the current shortage of ACN, PALC could be a suitable mode of chromatography as replacement of HILIC and complementarity of RPLC.

Acknowledgements

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References

[1] J. Randon, S. Huguet, C. Demesmay, A. Berthod, J. Chromatogr. 217 (2010) 1496.
[2] T. Ikegami, K. Tomomatsu, H. Takubo, K. Horie, N. Tanaka, J. Chromatogr. A 1184 (2008) 474.
[3] Y. Guo, S. Gaiki, J. Chromatogr. A 1074 (2005) 71.
[4] M. Liu, E.X. Chen, R. Ji, D. Semin, J. Chromatogr. A 1188 (2008) 255.
[5] B. Bard, P.A. Carrupt, S. Martel, J. Med. Chem. 52 (2009) 3416.
[6] D.V. McCalley, J. Chromatogr. A 1217 (2010) 858.
[7] S.C. Churms, J. Chromatogr. A 720 (1996) 75.
[8] H. Tanaka, X. Zhou, O. Masayoshi, J. Chromatogr. A 987 (2003) 119.
[9] D.V. McCalley, J. Chromatogr. A 1217 (2010) 3408.
[10] A.J. Alpert, J. Chromatogr. 499 (1990) 177.
[11] T. Yoshida, J. Chromatogr. A 811 (1998) 61.
[12] P. Hemstrom, K. Irgum, J. Sep. Sci. 29 (2006) 1784.
[13] T.A. Blake, T.L. Williams, J.L. Pirkle, J.R. Barr, Anal. Chem. 81 (2009) 3109.
[14] D.V. McCalley, U.D. Neue, J. Chromatogr. A 1192 (2008) 225.
[15] A.S. Pereira, F. David, G. Vanhoenacker, P. Sandra, J. Sep. Sci. 32 (2009) 2001.
[16] F. Gritti, A.S. Pereira, P. Sandra, G. Guiochon, J. Chromatogr. A 1217 (2010) 683.
[17] W. Bicker, J. Wu, M.L. Riekkola, W. Lindner, J. Sep. Sci. 31 (2008) 2971.
[18] K. Hartonen, M.J. Riekkola, Trends Anal. Chem. 27 (2008) 1.
[19] R. Smith, Anal. Bioanal. Chem. 385 (2006) 419.
[20] B.A. Bidlingmeyer, J.K. Del Rios, J. Korpi, Anal. Chem. 54 (1982) 442.
[21] F. Gritti, A.S. Pereira, P. Sandra, G. Guiochon, J. Chromatogr. A 1216 (2009) 8496.
[22] S.L. Song, L.G. Ji, H. Liang, W.L. Wang, J. Chinese Med. Mater. 31 (2008) 454.
[23] T. Chen, J. Wang, Y. Li, J. Shen, T. Zhao, H. Zhang, Glycoconjugate J. 27 (2010) 635.
[24] T. Chen, B. Li, Y. Li, C. Zhao, J. Shen, H. Zhang, Carbohydr. Polym. 83 (2011) 554.
[25] X. Chen, F. Qin, Y. Liu, X. Huang, H. Zou, J. Chromatogr. A 1034 (2004) 109.
[26] P. Franco, A. Senso, C. Minguillon, L. Oliveros, J. Chromatogr. A 796 (1998) 265.
[27] C. Minguillón, P. Franco, L. Oliveros, P. Lopez, J. Chromatogr. A 728 (1996) 407.
[28] Z. Guo, Y. Jin, T. Liang, Y. Liu, Q. Xua, X. Liang, A. Lei, J. Chromatogr. A 1216 (2009) 257.
[29] T. Zhou, A.L. Charles, J. Chromatogr. A 1187 (2008) 87.
[30] Z. Hao, B. Xiao, N. Weng, J. Sep. Sci. 31 (2008) 1449.
[31] T.L. Chester, J.W. Coym, J. Chromatogr. A 1003 (2003) 101.