Chinese Chemical Letters 31 (2020) 746–750

Contents lists available at ScienceDirect

Chinese Chemical Letters

journal homepage: www.elsevier.com/locate/cclet

Communication

A novel double polymer modified hydrophobic/hydrophilic stationary phase for liquid chromatography

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ARTICLE INFO

Article history:
Received 12 July 2019
Received in revised form 15 August 2019
Accepted 20 August 2019
Available online 22 August 2019

Keywords:
Norbornene imidazolium hexafluorophosphate (NM-MIm-PF_6)
Oligo(ethylene glycol)methacrylate (OEGMA)
Hydrophobic
Hydrophilic
Liquid chromatography (LC)

ABSTRACT

In this paper, norbornene imidazolium hexafluorophosphate (NM-MIm-PF_6) was modified on the surface of aminopropyl silica by ring-opening metathesis polymerization (ROMP), and then oligo(ethylene glycol) methacrylate (OEGMA) were grafted on the surface by atom transfer radical polymerization (ATRP). Some characterizations in this article confirmed that the synthesis of P(NM-MIm-PF_6)-Si-POEGMA (P1-Si-P2) is successful. The P1-Si-P2 can separate sugars, amino acids, sulfonamides in a hydrophilic interaction mode and alkyl benzene, polycyclic aromatic hydrocarbon in a reverse phase mode. The experiment also found that the column has typical characteristics of hydrophobic/hydrophilic separation mechanism. Compared to single hydrophobic C18 column and single hydrophilic Si-NH_2 column, this P1-Si-P2 shows certain advantages.

In LC separation, the column packing is the core [1]. People usually improve the chromatographic performance by modifying the surface of the silica spheres [2]. A material that was modified by two different polymers usually had the dual function of two polymers [3]. Researchers have devised a porous silica sphere introduced by two different properties of polymers, and it was expected to possess different separation properties in LC. A pair of polymer of polyacrylamide and poly(methacrylic acid) potentially responding to stimulation ware introduced onto monosized porous polymer particles to be evaluated as a packing material for LC in Ken Hosoya's work. In this work, the stimulus responses were evaluated by changing temperature or pH to check change of the slope of a Van't Hoff plot [4]. In the work of Cao *et al.*, the separation of peptides proved that the application of double polymer modified poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) capillary monolithic column has good performance [5]. Yuki *et al.* [6] synthesized three novel temperature-responsive polymeric P(NIPAAm-*co*-Phe-OMe5), P(NIPAAm-*co*-Phe-OMe10), and P(NIPAAm-*co*-Trp-OMe5) stationary phase. They studied their chromatographic separation performance and the results indicated that hydrophobic, π-π, and hydrogen bonding interactions all affected the separation mode of the three columns, both temperature-response and molecular recognition characteristics are present in the proposed separation system.

In the choice of polymeric monomer that can be used to modify the surface of silica particles, ionic liquid (IL) has been widely used for its merit. In solid phase extraction, IL can effectively improve the enrichment rate of nucleosides [7], and as a stationary phase for separation, IL can also exhibit good separation performance [8–11]. The application of IL modified silica as a stationary phase is mainly prepared by bonding IL monomer or polymeric IL to the silica surface through a silane coupling agent [12–15]. The ROMP method also has some irreplaceable features [16,17]. Recently, Ye Qian *et al.* [18,19] first synthesized polymeric IL NM-MIm-PF_6 on the surface of the aminopropyl silicon by surface-induced ROMP, which was used as polymer brush for bio-resistance. The polymeric method ROMP is characterized by Grubbs catalyst catalyzed cyclic olefins (such as norbornene and its derivatives) for high activity of ROMP, short polymerization time and high degree of polymerization [20,21]. After the ROMP, the original unsaturation is retained in the chain structure of the polymer, and subsequent modifications can be made [22].

Recently, research on hydrophilic chromatography has attracted more attention. Among them, OEGMA is the best

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https://doi.org/10.1016/j.cclet.2019.08.030
1001-8417/

hydrophilic polymer monomer that can be modified on the surface of silicon spheres [23]. The hydrophilic responsive monomer OEGMA is often used as a synthetic copolymer on the surface of the substrate [24,25]. Hydrophilic oligo(ethylene glycol) chains on the copolymer form H-bonds with solvent molecules at room temperature, whereas the backbones usually lead to a competitive hydrophobic effect and the balance between hydrophilic and hydrophobic moieties in the molecular structure of the copolymers [26]. ATRP is a commonly used method for surface initiated polymerization, which controls the structure of the polymer well by surface-fixed ATRP initiators [27–29]. This technique is characterized by a linear relationship between the thickness of the polymer film and the molecular weight of the free polymer chain [30–33]. More advantages and applications of ATRP have also been reported [34,35].

However, there is no research on the modification of the above two polymers on the surface of silicon spheres in LC. In this article, we first prepared and characterized a dual polymer modified P1-Si-P2 stationary phase and the synthetic process of which was shown in Fig. 1. The stationary phase prepared by grafting the silica surface by ROMP technology using NM-MIm-PF_6 and ATRP technology using OEGMA can exhibit a hydrophilic/hydrophobic dual interaction mode in liquid chromatography separation. By changing the ratio of mobile phase acetonitrile (ACN), the analytical process shows a different mechanism of action. It is a typical hydrophilic mechanism at high acetonitrile ratio, but it exhibits a hydrophobic mechanism at low ACN ratio. The two interaction mechanisms undergo a transition at a certain ratio between the higher ACN ratio and the lower ACN ratio.

In reversed phase liquid chromatography (RPLC), the retention and selectivity of hydrophobic analyte depend on the type of stationary phase and the eluent conditions. Choosing the optimum chromatographic conditions is not only important for chromatographic separations, but also of great importance to the retention mechanism of reversed phase chromatography. So the influences of the retention of polycyclic aromatic hydrocarbons on P1-Si-P2 were systematically investigated by changing different chromatographic conditions, including organic solvent content and column temperature, as illustrated in Fig. 2. First, the effect of organic solvent content on retention was studied by changing the volume fraction of methanol (MeOH) in mobile phase. As shown in Fig. 2a, when the percentage of MeOH was between 40% and 90%, the retention time of the test analytes was decreased by increasing MeOH concentration, indicating that P1-Si-P2 possessed the typical reversed phase chromatography retention characteristics [36].

Column temperature is another reason that can affect retention of hydrophobic analytes in P1-Si-P2 and its impact is related mainly to the enthalpy of transfer of the analytes between stationary and mobile phases. The Van't Hoff equation (Eq. (1)) is often utilized to explore the relationship between column temperature and retention factor:

$$\ln k = -\frac{\Delta H^{\theta}}{RT} + \frac{\Delta S^{\theta}}{R} + \ln\phi \quad (1)$$

where ΔH^{θ} and ΔS^{θ} are standard molar enthalpy and standard entropy change between stationary/mobile phase and analytes, respectively. R is the universal gas constant, k is the retention factor, ϕ is the phase ratio and T is column temperature in Kelvin [37]. The retention on P1-Si-P2 was investigated from 5 °C to 45 °C with an interval of 10 °C. As illustrated in Fig. 2b, as the temperature rises, the viscosity of the mobile phase decreases, which is beneficial to mass transfer and reduces the retention time of all analytes. In addition, the weaker the hydrophobicity, the shorter the retention time, and the stronger the hydrophobicity, the longer the retention time. Both of these result in differences in the slope of analytes that differ in hydrophobicity.

Commercial C18 columns are considered to be the most hydrophobic columns found today. Therefore, the contrast between P1-Si-P2 and commercial C18 columns is very important.

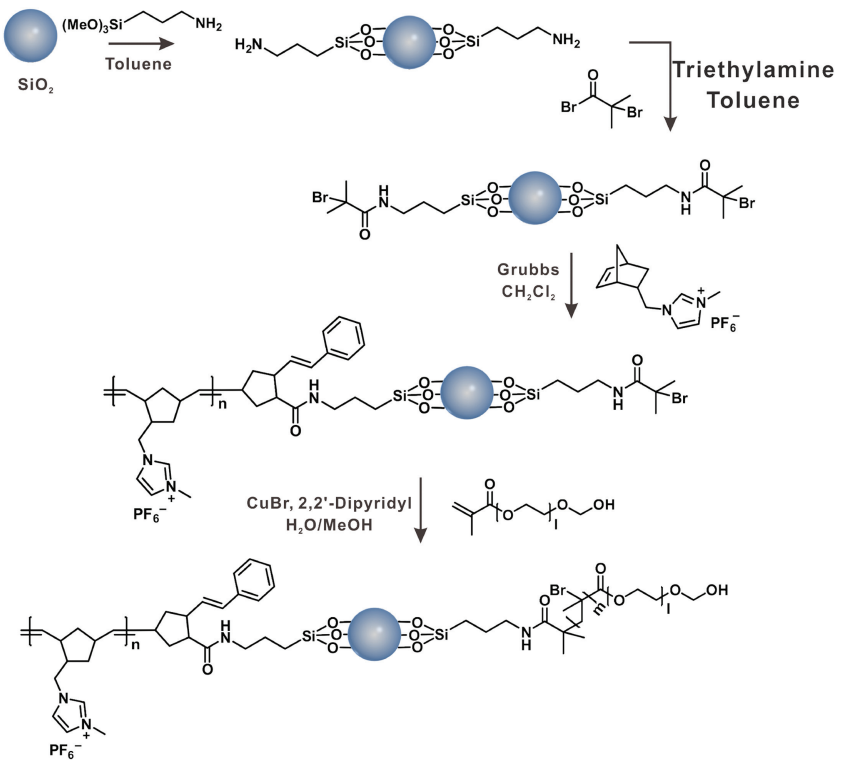

Fig. 1. Synthetic process of P1-Si-P2.

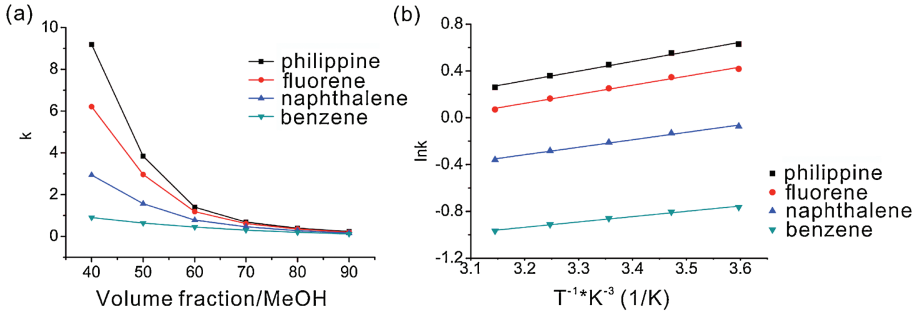

Fig. 2. Effect of volume fraction of MeOH (a) and column temperature (b) on the retention of polycyclic aromatic hydrocarbons in the P1-Si-P2 column. Mobile phase: (a) H_2O with different ratio of MeOH, (b) MeOH/H_2O (60/40, v/v). UV detection at 254 nm. Column temperature was 25 °C; Flow rate: 1.0 mL/min.

The hydrophobic separation performance of this P1-Si-P2 column was evaluated by comparing the separation performance of hydrophobic analytes such as four polycyclic aromatic hydrocarbons and six kinds of alkylbenzenes on two columns, and the results are shown in Fig. 3. First, the polycyclic aromatic hydrocarbons were separated in RPLC mode, and the chromatograms are shown in Figs. 3a and b. The four polycyclic aromatic hydrocarbons can be separated on P1-Si-P2 columns and commercial C18 column with consistent peak order and effective retention and symmetrical peak shape. We obtained different resolutions of polycyclic aromatic hydrocarbons by adjusting the proportion of MeOH in the mobile phase in the commercial C18 column. It can be seen from Fig. 3b that the baseline separation has been reached when the proportion of MeOH in the mobile phase is 70%, and the retention time increases as the proportion of MeOH decreases. We can also infer that when separating polycyclic aromatic hydrocarbons on the C18 column under the mobile phase conditions of Fig. 3a, a longer separation time will be obtained. In summary, within a certain range, the four polycyclic aromatic hydrocarbons have a consistent peak order on the P1-Si-P2 column and the strongly hydrophobic commercial C18 column, indicating that they have the same mechanism with commercial C18 column. The separation mechanism of P1-Si-P2 column is called the hydrophobic interaction mechanism but much weaker than that of the C18 column. By gradient elution, the six alkylbenzenes can be effectively separated on the P1-Si-P2 column and have a peak order consistent with the commercial C18 column. It can be seen that the peak order of alkylbenzene is positively correlated with the electron donating ability of the alkyl side chains. We can see from Fig. 4a that when the proportion of MeOH in the mobile phase is 32%, benzene and toluene can be eluted in 6 min. Compared to commercial C18 column (Fig. 4b), when the MeOH ratio is 55%, the elution time of benzene and toluene is as long as 18 min, and we can also see from the Fig. 4b that as the MeOH ratio decreases, the elution time increases. Therefore, we can infer that P1-Si-P2 column has a weaker hydrophobicity than the commercial C18 column, which can also be verified from the characterization of the contact angle (Figs. S2a in Supporting information).

According to our characterization of the contact angle (Figs. S2c and S2d in Supporting information) of the stationary phase packing, P1-Si-P2 column possessed the performance of hydrophilic interaction liquid chromatography (HILIC) [38]. To investigate the chromatographic performance of this P1-Si-P2 stationary phase, amino acids were chosen to explore the effct of ACN contenton retention. Plot of k *versus* volume fraction of ACN in mobile phase were shown in Fig. 5a. It was found that the retention of amino acids decreased with the ACN content from 10% to 40%, and then increased with the ACN content increasing from 40% to 60%, which meant P1-Si-P2 stationary phase switched from RPLC to HILIC. When ACN content exceeded 40% in mobile phase, the stationary phase was occupied by a water-rich layer thus hydrophilic interaction emerged [39]. This P1-Si-P2 stationary phase had the typical characteristics of hydrophobic/hydrophilic chromatography [40].

For hydrophilic columns, column temperature is another cause of the retention of hydrophilic analytes in P1-Si-P2, the effect of which is primarily related to the transfer enthalpy of the analyte between the stationary phase and the mobile phase. To verify this rule, the retention on P1-Si-P2 was investigated at 10 °C intervals at 10 °C to 50 °C. As shown in Fig. 5b, the increase in column temperature does not result in a significant change in retention time, and it can be seen that under this chromatographic condition, the temperature does not have a significant effect on the interaction between the analyte and the mobile phase. However, we can find that the amino acids containing different R groups have a small response to different temperatures, and the amino acids containing the R group of the benzene ring structure basically have the characteristics that the retention factor increases as the column temperature increases. Furthermore, varying degrees of adsorption interaction across the retention mechanism results in small differences in the slope of different analytes.

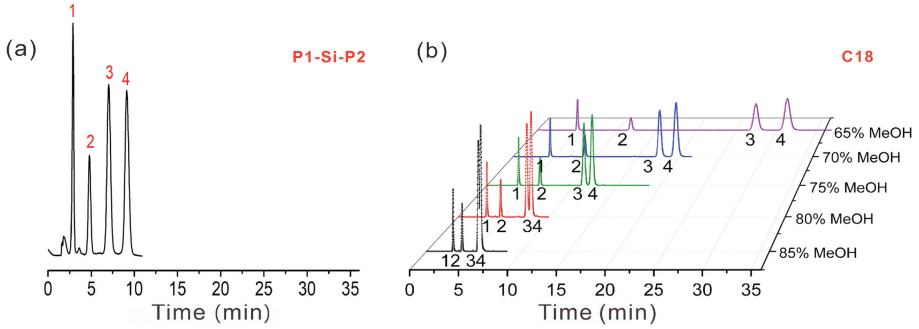

Fig. 3. Separation of four polycyclic aromatic hydrocarbons with P1-Si-P2 (a) and commercial C18 column (b). Mobile phase: (a) MeOH/H_2O (55/45, v/v), (b) MeOH/H_2O (85/15, 80/20, 75/25, 70/30, 65/35, v/v). Analytes: 1. benzene, 2. naphthalene, 3. fluorene, 4. philippine. UV detection at 254 nm. Column temperature: 30 °C. Flow rate: 1.0 mL/min.

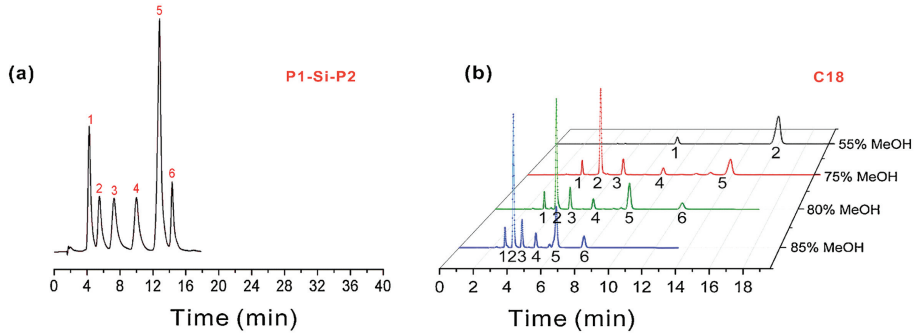

Fig. 4. Separation of six alkylbenzenes with P1-Si-P2 (a) and commercial C18 column (b). Mobile phase: (a) gradient elution: 6 min, MeOH/H_2O (32/68, v/v); 14 min, MeOH/H_2O (63/37, v/v). (b) MeOH/H_2O (85/15, 80/20, 75/25, 55/45, v/v). Analytes: 1. benzene, 2. toluene, 3. ethylbenzene, 4. propylene, 5. butylbenzene, 6. pentylene. UV detection at 254 nm. Column temperature: 30 °C. Flow rate: 1.0 mL/min.

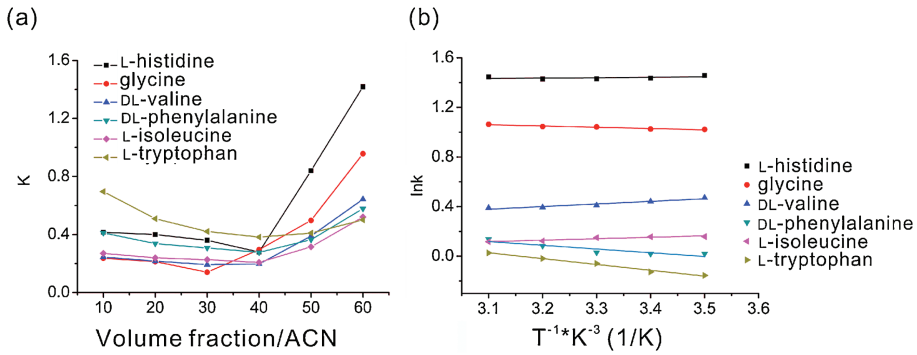

Fig. 5. Effect of volume fraction of ACN (a) and column temperature (b) on the retention of amino acid in the P1-Si-P2 column. Mobile phase: (a) H_2O with different ratio of ANC; (b) ACN/H_2O (60/40, v/v). UV detection at 254 nm. Column temperature was 25 °C; Flow rate: 1.0 mL/min.

The order of peaks of these amino acids in P1-Si-P2 is consistent with the order of peaks on commercial amino silica (Fig. 6a shows the separation of four amino acids on P1-Si-P2). It is well known that the interaction mechanism of commercial amino columns is HILIC, so it can be preliminarily concluded that their separation mechanism on P1-Si-P2 is a hydrophilic interaction mechanism. To discuss the retention behavior of sugars on P1-Si-P2 column, 10 sugars were used as analytes. The separation of the 10 sugars is shown in Fig. 6b. Since the structure of sugars is very similar, it is difficult to achieve complete separation of various sugars in chromatographic separation, so we usually adopt a gradient elution method in the separation process. Finally, we also found that under the same mobile phase conditions, 10 sugars on P1-Si-P2 obtained the same peak order with the commercial amino column, and the interaction force on P1-Si-P2 was obviously weaker than commercial columns. It also shows that P1-Si-P2 column has a weaker hydrophilicity than the aminopropyl column.

The drug is generally hydrophilic and hydrophobic [41], so we also selected several sulfonamides to evaluate the separation performance of the P1-Si-P2 column and compared it with the separation on Si-NH_2. In order to explore the qualitative analysis and quantitative analysis of a component in the drug for the treatment of inflammation (Supporting information) [42], we also studied the separation of sulfadiazine on P1-Si-P2. From the Fig. 7, we can visually see that under the same chromatographic separation conditions, when the ACN content is 40% in the mobile

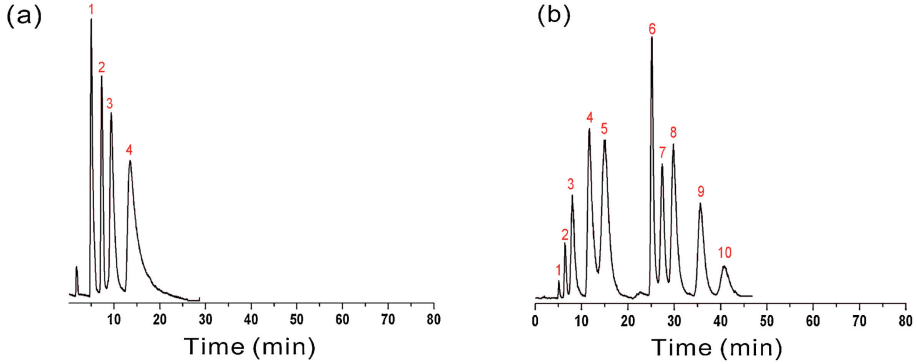

Fig. 6. (a) Separation of four amino acids on P1-Si-P2. Analytes: 1. L-methionine, 2. L-alanine, 3. L-serine, 4. L-histidine. Mobile phase: ACN/ H_2O (80/20, v/v). Evaporative light scattering (ELS) detector: gas flow: 2.0 L/min, tube temperature: 115 °C. Column temperature: 25 °C. Flow rate: 1.0 mL/min. (b) Separation of ten sugars with P1-Si-P2. Analytes: 1. D-ribose, 2. xylose, 3. DL-arabinose, 4. D-fructose, 5. glucose, 6. sucrose, 7. maltose, 8. lactose, 9. D-pinetriose, 10. raffinose. Mobile phase: Gradient elution: 18 min: ACN/H_2O (93/7, v/v), 20 m in. ACN/H_2O (87/13, v/v). ELS detector: gas flow: 2.0 L/min, tube temperature: 115 °C. Column temperature: 30 °C. Flow rate: 1.0 mL/min.

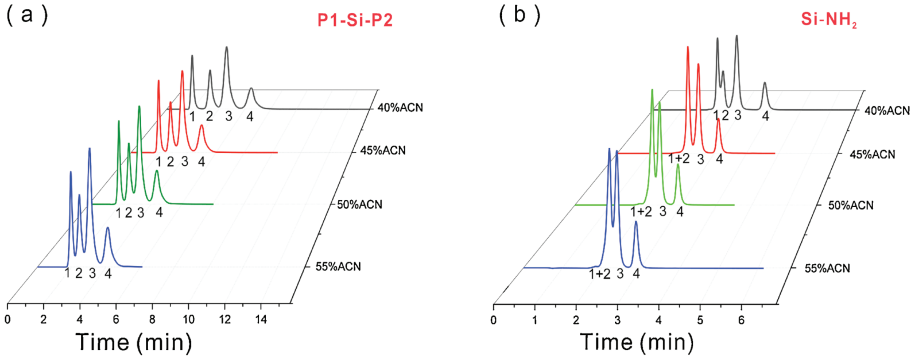

Fig. 7. Separation of four pyrimidines with P1-Si-P2 (a) and commercial Si-NH_2 (b). Mobile phase: ACN/H_2O (40/60, 45/55, 50/50, 55/45, v/v). Analytes: 1. thymine, 2. sulfadimethyl pyrimidine, 3. sulfamethazine, 4. sulfadiazine. UV detection at 254 nm. Column temperature: 30 °C. Flow rate: 1.0 mL/min.

phase, a baseline separation of four pyrimidines can be obtained on P1-Si-P2 (Fig. 7a) but incomplete separation was obtained on Si-NH_2 (Fig. 7b).

In this work, a novel hydrophobic/hydrophilic stationary phase was prepared by modifying the polymerized complex on the surface of the silica. This work demonstrates the feasibility of a chemically modified ROMP and ATRP polymerization process on the surface of silica particles. By exploring the effects of different chromatographic conditions on P1-Si-P2 retention, we can conclude that the retention mechanism of the stationary phase is based on the partitioning mechanism and adsorption interaction. In the separation of the P1-Si-P2 column, it can separate the hydrophobic mixture by the hydrophobic interaction mechanism in the RPLC mode and the hydrophilic mixture by hydrophilic interaction mechanism in the HILIC mode. It is hoped that this new stationary phase will be used to separate and analyze other hydrophobic/hydrophilic compounds to address more practical problems in chromatographic separations.

Acknowledgment

This work was supported by the National Natural Science Foundation of China (Nos. 21575148, 21575149, 21505146).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.cclet.2019.08.030.

References

[1] M. Catani, O.H. Ismail, F. Gasparrini, et al., Analyst 142 (2017) 555–566.
[2] T. Yasmin, K. Mueller, J. Porous. Mat. 23 (2016) 339–348.
[3] D. Aferrari, S. Fermani, P. Galletti, et al., Langmuir 29 (2013) 1938–1947.
[4] F. Kitagawa, M. Kamiya, K. Otsuka, J. Chromatogr. B 875 (2008) 323–328.
[5] Q. Cao, Y. Xu, F. Liu, F. Svec, J.M.J. Frechet, Anal. Chem. 82 (2010) 7416–7421.
[6] Y. Hiruta, R. Kanazashi, E. Ayano, T. Okano, H. Kanazawa, Analyst 141 (2016) 910–917.
[7] H.H. Li, T. Cheng, S.X. Wang, X.Y. Zhu, H.X. Zhang, Talanta 153 (2016) 71–78.
[8] M. Sun, J.J. Feng, S.J. Liu, et al., J. Chromatogr. A 1218 (2011) 3743–3749.
[9] M.J. Rodriguez, H. Nan, M. Varona, et al., Anal. Chem. 91 (2019) 505–531.
[10] Y. Wang, M.L. Tian, W.T. Bi, K.H. Row, Int. J. Mol. Sci. 10 (2009) 2591–2610.
[11] L. He, W. Cui, Y. Wang, et al., J. Chromatogr. A 1522 (2017) 9–15.
[12] K. Hu, W. Zhang, H. Yang, et al., Talanta 152 (2016) 392–400.
[13] H.D. Qiu, L.C. Wang, X. Liu, S.X. Jiang, Analyst 134 (2009) 460–465.
[14] V. Pino, A.M. Afonso, Anal. Chim. Acta 714 (2012) 20–37.
[15] S. Kowalska, B. Buszewski, J. Sep. Sci. 29 (2006) 2625–2634.
[16] Y. He, F.M. Benedetti, S. Lin, et al., Adv. Mater. 31 (2019) 1807871.
[17] J.H. Swisher, J.A. Nowalk, T.Y. Meyer, Poly. Chem. 10 (2019) 244–252.
[18] L. Qiao, W. Lv, M. Chang, X. Shi, G. Xu, J. Chromatogr. A 1559 (2018) 141–148.
[19] Q. Ye, T.T. Gao, F. Wan, et al., J. Mater. Chem. 22 (2012) 13123–13131.
[20] M.J. Allen, K. Wangkanont, R.T. Raines, L.L. Kiessling, Macromolecules 42 (2009) 4023–4027.
[21] B.O. Ozturk, B. Sariaslan, S.K. Sehitoglu, J. Organomet. Chem. 822 (2016) 13–19.
[22] X.L. Wang, Q. Ye, T.T. Gao, J.X. Liu, F. Zhou, Langmuir 28 (2012) 2574–2581.
[23] X. Chen, J. Pang, G. Zhou, Micro. Nano. Lett. 10 (2015) 310–314.
[24] M.K. Khang, J. Zhou, Y. Huang, A. Hakamivala, L. Tang, RSC Adv. 8 (2018) 34625–34633.
[25] B. Mendrek, A. Fus, K. Klarzynska, et al., Polymers 10 (2018) 1255.
[26] Q. Wang, S. Liu, W. Sheng, N. Guang, X. Li, Macromol. Res. 23 (2015) 607–617.
[27] S. Mu, J. Guo, C. Yu, Y. Gong, S. Zhang, Prog. Chem. 27 (2015) 539–549.
[28] J. Chen, M. Liu, Q. Huang, et al., Chem. Eng. J 337 (2018) 82–90.
[29] Q. Huang, M. Liu, J. Chen, Q. Wan, J. Tian, et al., Appl. Surf. Sci. 419 (2017) 35–44.
[30] Q. Huang, J. Zhao, M. Liu, et al., J. Taiwan Inst. Chem. E 86 (2018) 174–184.
[31] G. Zeng, T. Chen, L. Huang, et al., J. Taiwan. Inst. Chem. E 82 (2018) 205–213.
[32] L. Huang, S. Yang, J. Chen, et al., Mat. Sci. Eng. C-Mater. 94 (2019) 270–278.
[33] L. Huang, M. Liu, L. Mao, et al., Appl. Surf. Sci. 434 (2018) 204–210.
[34] M. Liu, G. Zeng, K. Wang, et al., Nanoscale 8 (2016) 16819–16840.
[35] X. Zhang, Q. Huang, F. Deng, et al., Appl. Mater. Today 7 (2017) 222–238.
[36] G.E. Berendsen, L. Degalan, J. Chromatogr. A 196 (1980) 21–37.
[37] Z.G. Hao, B.M. Xiao, N.D. Weng, J. Sep. Sci. 31 (2008) 1449–1464.
[38] S. Foorginezhad, M.M. Zerafat, Appl. Surf. Sci. 464 (2019) 458–471.
[39] P. Jandera, Anal. Chim. Acta 692 (2011) 1–25.
[40] Q. Wu, L.X. Chen, J. Gao, et al., Talanta 194 (2019) 105–113.
[41] S.I. Kang, Y.H. Bae, J. Control. Release 80 (2002) 145–155.
[42] L. Song, H. Zhang, T. Cai, et al., Chin. Chem. Lett. 30 (2019) 863–866.