Analyst

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/analyst

Facile preparation of polyvinyl alcoholcoated SiO_2stationary phases for high performance liquid chromatography

ShunliJi,[a]Feifang Zhang,[*a]Shengjie Wu, [a]Bingcheng Yang,[*a]Xinmiao Liang[a,b]

A facile way to prepare polar stationary phase for hydrophilic interaction chromatography (HILIC) was proposed by coating polyvinyl alcohol onto silica particle (PVA-Sil). A water or organic solvent-insoluble permanent PVA coating on the silica particle surface can be formed simply by dipping silica particles into hot PVA solution and then settled from solution, leaving a thin layer of PVA coating and frozen in freezer. The PVA-Sil shields silica core from solution erosion to some degree and the pH tolerance range was extended to 9.5 from 8.0 for bare silica. PVA-Sil demonstrated good hydrophilic property for several kinds of polar compounds and ~57000/m of plate count was achieved. This way can also be extended as a universal way to prepare various stationary phases with exchangeable functionalities by doping desired ingredient in PVA solution.

*Corresponding Author: 130 Meilong RD, Pharmacy School, East-China Univ. Sci. Tech., Shanghai 200237, China. Tel/fax, +86-21-64250622; Email, zhangff@ecust.edu.cn (F. Zhang); bcyang@ecust.edu.cn (B. Yang); [b] Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, 116023, China

1. Introduction

Since Alpert coined the term hydrophilic interaction chromatography (HILIC) in 1990, it has been widely recognized as a distinct chromatographic mode for separation of highly polar compounds.[1] There compounds are poorly retained on common reverse phase liquid chromatography (RPLC) column or may not be compatible with the mobile phase used in normal phase liquid chromatography (NPLC). HILIC can be regarded as a hybrid of RPLC and NPLC to some degree, which uses similar polar stationary phases to NPLC and similar mobile phase to RPLC. The use of organic-rich mobile phase in HILIC makes it well suited with mass spectrometry (MS), which would be greatly helpful for sensitive analysis of biological compounds.

Along with increasing popularity of HILIC, the development of novel HILIC stationary phases has received much attention, and some of them have been commercially available in the past decade.[2-4] Most HILIC stationary phases consist of silica gels chemically bonded with polar functional groups, which can be generally divided into three categories according to the charge characteristics of functional groups, namely, neutral (i.e. diol-, amide-),charged (i.e. amine- and triazole) and Zwitterionic.[3] Recently we have developed a serial of novel silica gel-based HILIC stationary phases, such amide-,[5]amine-,[6]triazole[7] and Zwitterionic.[8-10] In addition,

other types of non-silica gels with high chemical stability were also investigated, e.g. TiO_2,[11] ZrO_2,[12-14] MgO[15] and graphitic carbon.[16]

Among HILIC stationary phases, bare silica gel itself can be used as HILIC stationary phase and in fact remains popular for the analysis of biological samples.[3, 17] The major drawbacks of bare silica gel lie in its poor stability at high pH value and unwanted interaction from residual silanol groups, which is especially obvious for separation of some basic compounds, in which irreversible adsorption always occurs, then leading to serious tailing or poor reproducibility. In addition, obvious retention difference of bare silica from different sources was also observed, probably due to their different purity or different preparation procedures.[4, 18] Obviously this will lead to difficulty for the development of analytical methods. An approach to address these problems is to generate a hydrophilic polymer coating onto silica gel.[3, 19] The coating can effectively protect bare silica gel against erosion of aggressive solvents. A diol coating has been prepared by cross-linking of one of diol groups through an ether linkage to produce a polymer layer on silica gel surface.[20] A polyvinyl alcohol (PVA) coating-based stationary phase has also been commercially available.[4] It demonstrated highly hydrophilic property and has found some applications in super fluid chromatography,[21] NPLC,[22] and HILIC.[23] It should be noted that the preparation of

commercial PVA stationary phases were based on chemical bonding [21, 22, 24] and an obvious disadvantage of such way is complicate procedures and lengthy preparation process.

PVA coating was also extended for capillary electrophoresis (CE),[25]which was formed by physically thermal polymerization inside the inner wall of fused silica capillary. The coating was observed to greatly eliminate unwanted electrostatic adsorption of basic protein onto the silica wall. Hydrophilic PVA coating prepared for CE separation was also reported via chemical bonding.[26-28] Hassan et al. has reported a simple method to prepare insoluble PVA gel formed predominantly via crystallites by freezing and thawing of aqueous PVA solutions.[29] In the present study, such method has been extended to prepare PVA-coated silica stationary phase (PVA-Sil) simply by dipping silica particles into hot PVA solution and then settled from solution, leaving a thin layer of PVA coating and finally frozen in freezer. The performance of PVA-Sil has been in detail evaluated and the data indicated that it behaved well under HILIC mode. Another desirable feature of this way is that it can be extended as a universal way to prepare varying stationary phases with exchangeable functionalities by doping desired ingredient in PVA solution.

2. Experimental

2.1 Reagents and materials

Spherical silica was purchased from Fuji (Fuji Silysia Chemical Ltd., Japan,5 μm particle size; 10 nm pore size; 300 m^2 /g surface area). PVA (MW 1750±50) was from Ling Feng(Shanghai, China). Formic acid (≥98%) and ammonium formate (≥99%) were obtained from ACROS (USA). Ethanol and acetonitrile were from J.T Baker (USA). Acetonitrile, formic acid, ammonium formate, ethanol were of chromatographic grade. The other chemical reagents were of analytical grade. The chemical structure of the analytes used for evaluating stationary phase was provided in the Scheme 1 in the supplementary information (SI). Ultrapure water was provided from a Milli-R04 purification system (Millipore, Germany).

2.2 Instrumentation

The KH5200B ultrasonic bath was obtained from Hechuang ultrasonic instrument Co., Ltd. (Jiangsu, China. 200W, 50 Hz). The chromatographic experiments were performed on a Waters Alliance HPLC consisting of a 2695 separation unit, automatic injector and a 2998-UV absorbance detector. Unless otherwise stated, 1 mL/min of the flow rate and 254 nm of absorbance wavelength were used.

The pore structure and surface area were performed on a TriStar II 3020^{TM} Surface Area and Pore Analyzer (Micromeritic, USA). The FT-IR spectrum was

recorded on a spectrum 100 (PerkinElmer, USA). The morphology of PVA-Sil was observed by a scanning electron microscope (SEM) (FESEM NOVA NanoSEM 450, FEI, USA). The solid phase nuclear magnetic resonance (NMR) was characterized on an AVANCE DRX-500 (Bruker, Germany).

2.3 Preparation and packing of PVA-Sil

The general preparation route of PVA-Sil was shown in Fig. 1.0.08g/mL PVA solution was prepared by dissolving 4g PVA in 50 mL water, then stirred by a magnetic stir bar and heated in water bath with temperature of 90 °C until PVA was completely dissolved and a homogeneous solution was obtained. Then 3 g silica gel was mixed with PVA solution above and then homogenized in an ultrasonic bath for 5 min at room temperature to allow PVA to be coated onto silica gel. The solution driven by vacuum pump was filteredvia4$^{\#}$sand core funnel with pore size of 3-4 μm, and the obtained solids were frozen at -20 °C for 1 h, followed by vacuum dried at 60 °C for 30 min. Finally the white powder PVA-Sil was obtained.

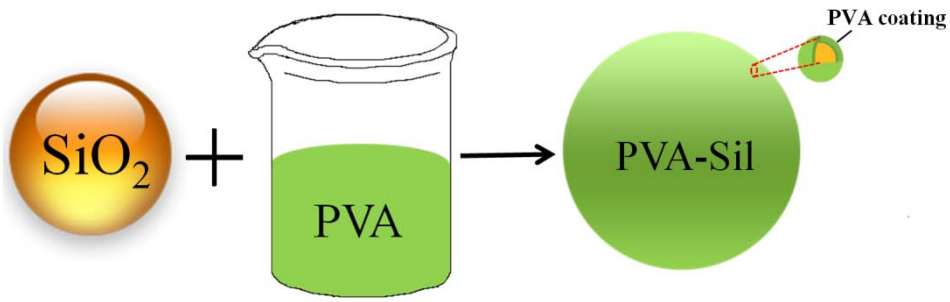

Fig. 1. Schematic diagram of preparation route of PVA-Sil.

Detail preparation procedures see the text.

PVA-Sil stationary phase was slurry-packed into a stainless-steel column (4.6 mm i.d. ×150 mm length) using methanol as slurry and propulsion solvent. In addition, a homemade bare silica gel column (4.6 mm i.d. ×150 mm length) using the same silica gel with PVA-Sil was also prepared for comparison.

The column evaluation parameters such as plate number and the tailing factor were directly provided by the software of Waters Alliance HPLC system.

3. Results and discussion

3.1 General optimization of preparation conditions

The main operation parameter affecting the performance of PVA-Sil was the concentration of PVA solution. Dilute PVA solution may lead to thin layer of formed PVA coating, and vice versa. The effect of PVA solution concentration was explored as provided in the SI-Fig. 1. The optimal PVA concentration was found to be 0.08 g/mL in terms of plate number and peak shape. Higher concentration of PVA solution (0.1 g/mL in this case) did not produce better results as expected, which probably resulted from its high viscosity, then leading to uneven coating. In fact, much higher concentration could not be explored since it was difficult to perform filtration and other operation step due to high viscosity of PVA solution. Thus 0.08 g/mL was chosen as optimal concentration of PVA solution in the further experiment.

3.2 Characterization of PVA-Sil

The morphology of PVA-Sil was characterized by SEM as shown in SI-Fig. 2. The monodispersity and spherical shape of PVA-Sil particles were kept well. PVA-Sil and bare silica were firstly characterized by FTIR, as shown in SI-Fig. 3. The IR spectra of bare and PVA-Sil are very similar, but the peaks at 2926.1 cm^{-1} and 2854.7 cm^{-1} corresponding to –C-H are characteristic peaks of PVA-Sil, indicating the introduction PVA groups after coating. The elemental analysis data (shown in SI-Table 1) also indicated the existence of PVA coating onto silica surface due to obvious increase of carbon content of PVA-Sil relative to bare silica. The solid phase ^{13}C-CP/MAS NMR spectrum (see SI-Fig. 4) provided more direct evidence for characterizing the introduced PVA coating. The peaks at 66.01 ppm and 39.94 ppm were attributed to–C-OH and $–CH_2$, respectively. In addition, the difference between PVA-Sil and bare silica was also reflected by their titration curves (provided in SI-Fig.5). It can be seen that the pH values of PVA-Sil solution during titration was generally higher than those of bare silica.

From SI-Table 1, it can be seen that slight decrease of surface area of PVA-Sil relative to bare silica particles were found. It probably resulted from that some micropores of bare silica particles were occupied by PVA coating while it would not

influence the separation performance, as proved below. The mechanical test of the column by measuring the backpressure against the applied flow rate was shown in SI-Fig. 6.The backpressure of PVA-Sil column was ~20% lower than that of bare silica column. It means that the permeability of PVA column was ~1.24-fold higher than that of bare silica column, which would be helpful for fast analysis. In addition, near-ideal linear relationship between the flow rate and the backpressure indicated that PVA-Sil had good mechanical stability in the tested range of flow rate.

Typically the pH value of bare silica column can tolerate is8.0, while PVA-Sil was proved to bear at least pH 9.5, as illustrated in Fig. 2. When the eluent volume for the mobile phase with pH 9.5 was over than 2000-fold column volume, ~33% decrease of the plate count for bare silica was observed and ~19% increase of retention time, indicating bare column was basically broken down. By contrast, no observable change was found for PVA-Sil column when the elution volume was >3500-fold column volume, indicating its high stability. The stability of PVA-Sil at extreme pH value (pH 11.0 in this case) was also explored, as provided in SI-Fig. 7. The column behaved well when the elution volume was ~2000-fold column volume. When further increasing elution volume, the change of both retention time and plate count was observed. This indicated PVA-Sil still had limited lifetime in the solution

with extremely high pH value. This was probably caused by the existence of tiny flaw of PVA coating, which could not completely prevent the erosion of hydroxide to silica gel.

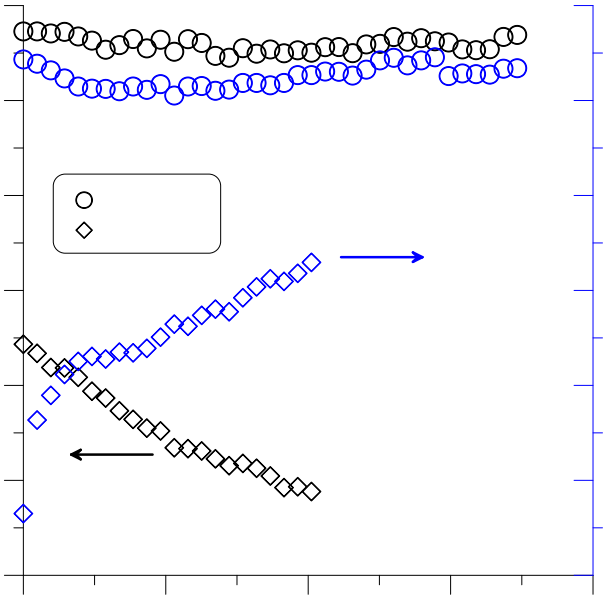

Fig. 2 Chemicalstability of PVA-Sil and bare silica in the solution at pH 9.5.

Conditions: column, 4.6 mm i.d. ×150 mm length; mobile phase: ACN/triethylamine solution(v/v=90/10); model analyte, uridine; detection wavelength, 254 nm; flow rate, 1.0 mL/min; injection volume, 10μL; column temperature, 30°C.

3.3Chromatographic evaluation of PVA-Sil for HILIC

Since PVA is highly hydrophilic, PVA-Sil would be much suitable for HILIC stationary phase. Here several types of highly polar analytes were chosen to evaluate its performance. A typical chromatogram was shown in Fig. 3 for separation of five nucleosides. Baseline separation and good peak shape for tested analytes was

observed under HILIC mode. By contrast, bare silica demonstrated less retained for five nucleosides and poor resolution was observed as well. In addition, indirect comparison was also made with commercial PVA column was used for separation of nucleosides (see ref. 3). It can be seen that stronger retention and much similar (or even better) performance of PVA-Sil relative to commercial one was observed.

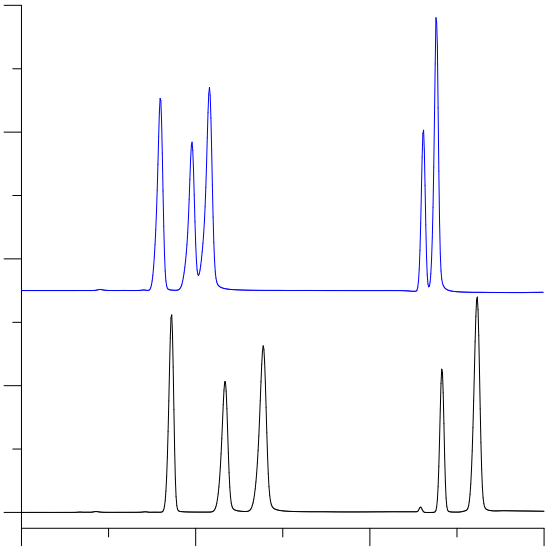

Fig. 3 Typical chromatogram of nucleosides separated by bare silicaand PVA-Sil. Conditions: mobile phase: A: ACN; B: H_2O; C: 250 mM NH_4FA (pH 3.1). 0-5 min, 90% A/4% B/6% C; 5-5.5 min, 75% A/19% B/6% C; 5.5-15 min, 75% A/19% B/6% C;Peak identification (from left to right), A: uracil, B: 5-methyl uridine, C: uridine, D: cytosine, E: cytidine.

The advantage of PVA-Sil over bare silica has also been highlighted for separation of small base compounds. It is known that the surface of silica gel bears negative charge in the typical pH range of HILIC due to the dissociation of silanol

groups, while the base compounds bear positive charge due to its protonization. Thus strong electrostatic interaction between silica gel and base compounds always leads to serious tailing or irreversible adsorption when bare silica is used for separation of base compounds. Such unwanted electrostatic interaction can be eliminated for PVA-Sil since PVA coating shields silanol groups from the basic organic compounds. It has been proved by the separation of four basic organic compounds, as provided in Fig.4. Excellent peak shape and resolution for four model basic organic compounds was observed by PVA-Sil column and high separation efficiency (e.g. ~57000/m of plate count for diprophylline) was achieved as well. In contrast, only three peaks were observed in less than 10 min and peak distort of the third peak occurred for bare silica column. Similarly, significant difference was also observed for separation of several acidic organic compounds, as shown in SI-Fig. 8. The utility of PVA-Sil for simultaneous separation of 8 cephalosporins was demonstrated in Fig. 5. PVA-Sil column demonstrated good separation for these analytes.

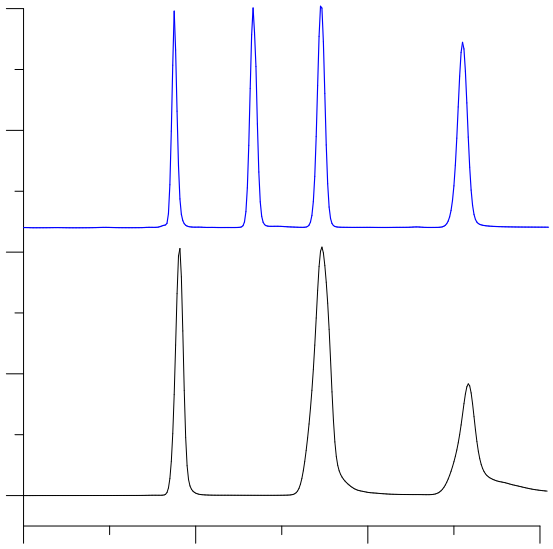

Fig. 4 Separation of small molecular bases on bare silica and PVA-Sil.

Conditions: mobile phase: ACN/ 250 mM NH_4FA (pH, 6.0) =94/6; other conditions same to Fig.3.

Peak identification: A, diphenylamine, B, caffeine, C, theophylline, D, diprophylline.

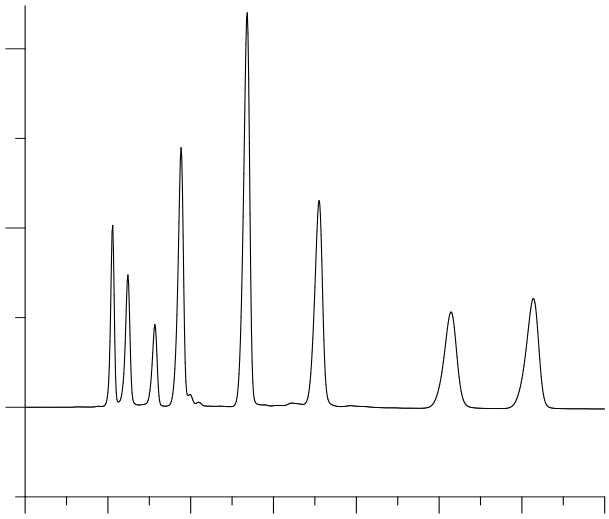

Fig. 5 Separation of eight cephalosporins on PVA-Sil column.

Conditions: mobile phase, A: ACN; B: H_2O; C: 250 mM NH_4FA (pH 5.7). 0 min, 80% A/14% B/6% C, 4 min, 72% A/22% B/6% C; 15 min,65% A/29% B/6% C;other conditions same to Fig. 3.

Peak identification: 1: cefamandolenafate; 2, cefuroxime sodium; 3, cefazolin sodium; 4, ceftizoxime sodium; 5, cefixime, 6, cefotiam hydrochloride; 7, cefepimedihydrochloride; 8,ceftazidime.

Relative standard deviations (RSD) of retention time for intra-day and inter-day assay on PVA-Sil column were less than 0.4% and 0.7%, respectively. Its long-term stability was also explored by continuously flushing the column for 100 days and the RSD of plate count and retention time for three model analytes were less than 1.9% and 1.0%, respectively, as provided in Fig. 6. This indicated good long-term stability of PVA-Sil column. In addition, 3 batch columns were prepared under the same conditions and RSD of retention time and plate number were 3.6% and 3.9%, respectively, which indicated good preparation reproducibility of such way.

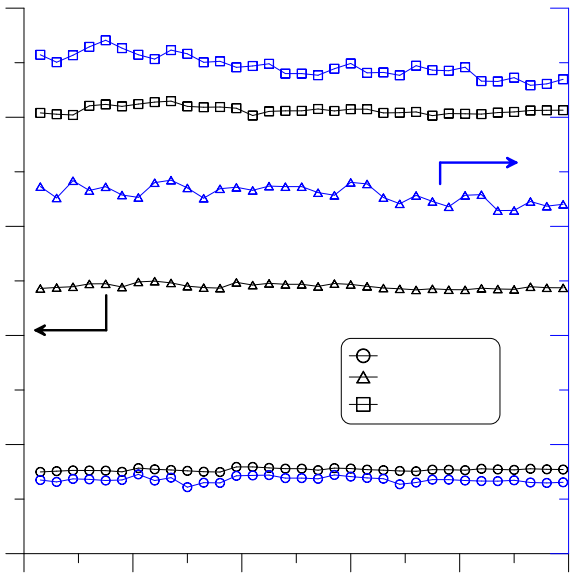

Fig. 6 Long-term stability of PVA-Sil for separation of model organic bases.

Conditions same to Fig. 4.

3.4Mechanism of PVA-Sil for separation of polar compounds

The possible separation mechanism of PVA-Sil for polar compounds was explored by selecting five nucleosides as model compounds. Firstly, the effect of water content in mobile phase on retention behavior of nucleosides was investigated by varying the water content in the mobile phase. The capacity factors of model compounds (lnk) were plotted against the water content ($ln\varphi_{H2O}$) in the mobile phase in SI-Fig.9.Clearly, the retention factors of all analytes decreased with the increase of water content. Such behavior was in accordance with typical HILIC mechanism suggested by Alpert, in which the retention was based on hydrophilic partitioning of analytes between bulk eluent and a water-rich layer immobilized on the surface of stationary phase.[1] In addition, it can be seen from SI-Fig.9that perfectly linear relationship between lnk and $ln\varphi_{H2O}$ was observed for all compounds and the linear correlation coefficients were over than 0.9997 (except uracil, its coefficient was 0.9852). This was consistent with the retention behavior of water-soluble vitamin on Diol column in previous report.[30]

The addition of buffer salt in mobile phase is often used to improve the retention properties of compounds in HILIC. Herein, the effect of concentration of buffer

solution was also investigated by varying the percentage of ammonium formate in the mobile phase, as provided in SI-Fig.10. The retention of several model nucleosides slightly increased with the increase of salt concentration probably due to enhanced polarity of the rich water layer, which matched well with some previous reports.[6, 9] In addition, the effect of the pH value of mobile phase was also studied, as shown in SI-Fig.11. Generally, the pH value had less impact on the retention in the tested pH range. This was different from the behavior of bare silica and further highlighted the role of PVA coating.

3.5Extension for preparation other modes of stationary phases

The way described here is based on physical polymerization manner and it can be extended to be a universal way to prepare varying stationary phases with exchangeable functionalities by doping desired ingredient in PVA solution. Such idea has been demonstrated by preparation of two stationary phases applied for RPLC and ion chromatography (IC) by doping little amount of sodium dodecyl sulfate (SDS) and polyethylenimine (PEI) in PVA solution, respectively. SDS, a surfactant containing an alkyl straight chain, functions to be the sites of reverse phase interaction, which is similar to ODS always used in RPLC. And PEI molecule contains lots of primary, secondary and tertiary amino groups, which can function as ion exchange

sites. Both stationary phases were abbreviated here as PVA-SDS and PVA-PEI, respectively. Their preparation routes were much similar to that of PVA-Sil except that PVA solution with addition of little amount of SDS or PEI was used other than pure PVA solution used for PVA-Sil. In the process of PVA polymerization, SDS or PEI was integrated into the network of PVA polymer. Thus PVA-SDS and PVA-PEI could be used for RPLC and IC stationary phase, respectively. The separation of six polycyclic aromatic hydrocarbons by PVA-SDS was shown in SI-Fig. 12. Their elution order followed the mechanism of reverse phase interaction. In addition, due to the existence of PVA coating, PVA-SDS demonstrated mixed-mode mechanism of HILIC and RPLC, which would be useful for both polar and hydrophobic analytes. From SI-Fig.13, four inorganic anions with ultraviolet absorption property could be well separated and the separation mechanism was proved to be ion exchange by the phenomenon of the decrease of retention factor with the increase of eluent concentration.

4. Conclusions

A facile way to prepare PVA coating-based stationary phase for HILIC was proposed. The preparation procedure is simple and readily to be performed. PVA-Sil has wider pH tolerance range and eliminates unwanted interaction from silanol groups of bare

silica gel. By replacing silica gel into other kind of gels with high chemical stability such as TiO_2 or ZrO_2 or Carbon, it can also be used to prepare novel stationary phase with much wider pH tolerance. Such work will be presented in the near future. In addition, the way described here can be extended to prepare varying stationary phases with exchangeable functionalities by doping desired ingredient in the PVA solution. Webelieve it will find more applications either for the analysis of polar compounds or for fast preparation of novel stationary phases.

Acknowledgements

The research was sponsored by National Natural Science Foundation of China (No. 21177040, 21322502) and the Ministry of Science and Technology of China (2012YQ090229).

AppendixList of the explanation of the abbreviations in the text

Abbreviation	Full name
PVA	Polyvinyl alcohol
HILIC	Hydrophilic interaction chromatography
RPLC	Reverse phase liquid chromatography
NPLC	Normal phase liquid chromatography
MS	Mass spectrometry
CE	Capillary electrophoresis
SEM	Scanning electron microscope
NMR	Nuclear magnetic resonance
PVA-Sil	PVA-coated silica stationary phase
IC	Ion chromatography

SDS	Sodium dodecyl sulfate
PEI	Polyethylenimine
ODS	Octadecylsiyl

References

1. A. J. Alpert, *J. Chromatogr. A*, 1990, 499, 177-196.
2. P. Hemström and K. Irgum, *J. Sep. Sci.*, 2006, 29, 1784-1821.
3. Y. Guo and S. Gaiki, *J. Chromatogr. A*, 2011, 1218, 5920-5938.
4. P. Jandera, *Anal. Chim. Acta*, 2011, 692, 1-25.
5. G. B. Shen, F. F. Zhang, B. C. Yang, C. H. Chu and X. M. Liang, *Talanta*, 2013, 115, 129-132.
6. Y. J. Liu, Q. Du, B. C. Yang, F. F. Zhang, C. H. Chu and X. M. Liang, *Analyst*, 2012, 137, 1624-1628.
7. F. F. Zhang, Y. J. Liu, J. Wei, Z. M. Guo, B. C. Yang and X. M. Liang, *J. Sep. Sci.*, 2011, 34, 796-799.
8. H. Guo, R. Liu, J. Yang, B. Yang, X. Liang and C. Chu, *J. Chromatogr. A*, 2012, 1223, 47-52.
9. A. J. Shen, Z. M. Guo, X. M. Cai, X. Y. Xue and X. M. Liang, *J. Chromatogr. A*, 2012, 1228, 175-182.
10. A. J. Shen, Z. M. Guo, L. Yu, L. W. Cao and X. M. Liang, *Chem. Commun.*, 2011, 47, 4550-4552.
11. T. Zhou and C. A. Lucy, *J. Chromatogr. A*, 2010, 1217, 82-88.
12. B. Yan, C. V. McNeff, F. Chen, P. W. Carr and A. V. McCormick, *J. Am. Ceram. Soc.*, 2001, 84, 1721-1727.
13. Y.-Q. Feng, Q.-H. Zhang, S.-L. Da and Y. Zhang, *Anal. Sci.*, 2000, 16, 579-583.
14. R. Kucera, P. Kovarikova, M. Klivicky and J. Klimes, *J. Chromatogr. A*, 2011, 1218,

6981-6986.

15. Z. Zhang, Y. Zheng, J. Chen, Q. Zhang, Y. Ni and X. Liang, *Adv. Funct. Mater.*, 2007, 17, 2447-2454.

16. M. F. Wahab, M. E. A. Ibrahim and C. A. Lucy, *Anal. Chem.*, 2013, 85, 5684-5691.

17. B. Dejaegher, D. Mangelings and Y. V. Heyden, *J. Sep. Sci.*, 2008, 31, 1438-1448.

18. B. A. Olsen, *J. Chromatogr. A*, 2001, 913, 113-122.

19. X. T. Peng, B. F. Yuan and Y. Q. Feng, *J. Sep. Sci.*, 2011, 34, 3123-3130.

20. P. B. Explore Luna HILIC: Discover HPLC Polar Retention and http://www.phenomenex.com.

21. C. West and E. Lesellie, *J. Chromatogr. A*, 2006, 1110, 200-213.

22. J. Nordbäck, E. Lundberg and W. W. Christie, *Marine Chem.*, 1998, 60, 165-175.

23. N. S. Quiming, N. L. Denola, S. R. B. Samsuri, Y. Saito and K. Jinno, *J. Sep. Sci.*, 2008, 31, 1537-1549.

24. L. Quinton, K. Quinton, A. Baillet and P. Chaminad, *J. Sep. Sci.*, 2006, 29, 390-398.

25. M. Gllges, M. H. Kleemiss and G. Schomburg, *Anal. Chem.*, 1994, 66, 2038-2046.

26. D. Belder, A. Deege, H. Husmann, F. Kohler and M. Ludwig, *Electrophoresis* 2001, 22, 3813-3818.

27. L. Xu, X. Y. Dong and Y. Sun, *Biochem Eng J.*, 2010, 53, 137-142.

28. M. Mori, T. Yamamoto, M. Kaseda, S. Yamada and H. Itabashi, *J. Chromatogr. B*, 2012, 887-888, 1-7.

29. C. M. Hassan, P. Trakampan and N. A. Peppas, in *Water Soluble Polymers Solutions Properties and Applications*, ed. Z. Amjad, Plenum Press, New York, 1998, pp. 31-40.

30. A. E. Karatapanis, Y. C. Fiamegos and C. D. Stalikas, *J Sep Sci.*, 2009, 32, 909-917.

Graphic abstract

A facile physical way to prepare polyvinyl alcohol coating-based silica stationary phase for HPLC was proposed.