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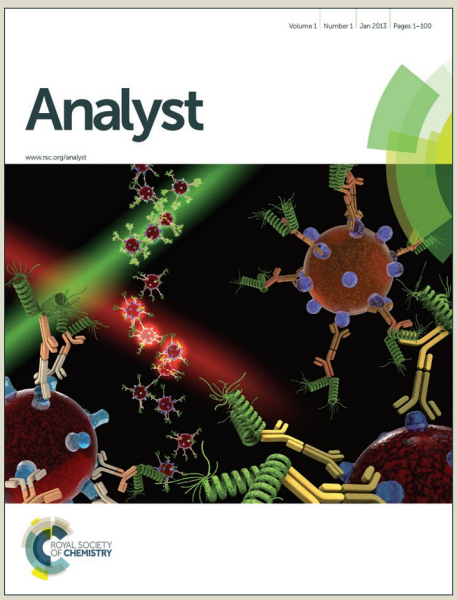





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# A polyvinyl alcohol-coated silica gel stationary phase for hydrophilic interaction chromatography

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

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**Multiple layers of polyvinyl alcohol (PVA) coating are generated onto silica gel by thermal immobilization to form a stationary phase applied for hydrophilic interaction liquid chromatography (HILIC). It offers an easy way to manipulate the thickness of PVA coating and the obtained stationary phase demonstrated high efficiency and high chemical stability.**

Along with the increasing popularity of HILIC for separation of highly polar compounds,[1-3] the development of HILIC stationary phases has received much more attention.[4-9] The majority of HILIC stationary phases are based on silica gel, which is much similar to the situation of RPLC.[10] Silica gel itself can function as HILIC stationary phase while its major drawbacks include limited pH tolerance (typically<8) and some unwanted interaction from residual silanol groups.[11] A solution to address these problems is to generate a hydrophilic polymer coating onto the silica gel.[12, 13] Polyvinyl alcohol (PVA) as a hydrophilic polymer has found applications in many fields.[14, 15] PVA-based stationary phase has been proved to be a good option in HILIC and it has been commercial available. These stationary phases are obtained via chemical bonding and lengthy preparation procedures are always involved.[14, 16] More recently, we proposed a facial physical way to prepare PVA-coated silica gel stationary phase (PVA-Sil), in which a thin layer of PVA coating was formed simply by dipping silica particles into a hot PVA solution, then settled from this solution, and polymerized in a freezer.[17] It demonstrated efficient separation for several polar compounds and overcame inherent drawbacks of bare silica gel to a great degree. While such method could not manipulate the PVA thickness and only one layer can be formed, leading to possibly insufficient coating to silica gel. Here, thermal polymerization approach other than freezing way was explored to prepare PVA-Sil (for convenience, it was termed as PVA-Sil-T@PVA-Sil-F for freezing way) and such idea was inspired by a previous report,[18] in which a layer PVA coating for capillary electrophoresis (CE) is formed inside the fused silica capillary by thermal polymerization, aiming to effectively reduce unwanted electrostatic adsorption of basic proteins onto the silica wall. An obvious advantage of such thermal immobilization approach over previously freezing way is to offer an easy way to manipulate the coating thickness simply by multiple repetitive coating. The thick coating would produce better protection of silica gel inside against the erosion of aggressive mobile phase with high pH value, then leading to improved lifetime of PVA-coated silica stationary phase.

The preparation of PVA-Sil-T was similar to that previously described with slight modification.[17] Briefly, a PVA solution is freshly prepared by dissolving 8% (w/v) PVA in boiling water. Silica gel with given amount was added into PVA solution, followed by ultrasonic treatment for 5 min. The suspended solution was filtered by 4# sand core funnel with a pore size of 3-4 μm to leave a thin layer of PVA coating onto the silica gel. The final immobilization was achieved by heating the obtained solids at 140 °C at $N_2$ atmosphere for 1h. If multiple layers of coating are needed, repeat the procedures above. The preparation route was illustrated in Fig. 1. 2.5 g PVA-Sil-T was in slurry packed into a stainless steel column (150 mm length × 4.6 mm i.d.) with methanol as both slurry and propulsion solvent under pressure of 7000 psi.

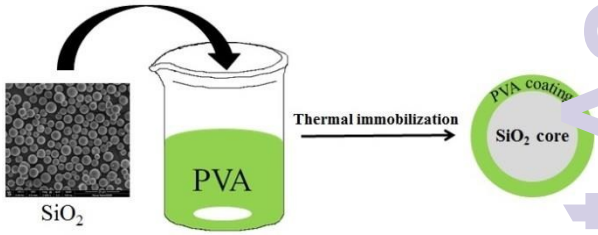


**Fig. 1**. Schematic diagram of preparation route of PVA-Sil-T

The fabrication of multiple layers of PVA coating is an easy way to manipulate its thickness. PVA-Sil-Ts with one and more layers coating were firstly characterized by the elemental analysis, as shown in Support Information, SI-Table 1. The average carbon percentage of PVA-Sil-T increased with the increase of PVA layers, e.g. C% of PVA-Sil-T with three and two PVA coating was ~2.5-fold and ~1.5-fold than that of PVA-Sil-T with one layer of PVA coating, respectively. The correlation can be instructive for manipulating the thickness of PVA coating. This may lead to better protection effect for silica gel inside. To prove this, the silica gels with and without PVA coating were immersed in ACN/triethylamine solution (v/v, =80/10, pH=11) stirred at 300 rpm for 72 h at 25 °C. Under such

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alkaline condition, the silica gel will be eroded, then leading to net decrease of silica gel mass and at the same time the increase of relative carbon percent of PVA-coated silica gel. The data were provided in Table 1. Net decrease of PVA-Sil-T with three, two and one layer of PVA coating is ~13.5-fold, ~3.4-fold and ~1.9-fold lower than that of bare silica gel. Obviously, this will be helpful for extending pH tolerance range of silica gel when increasing PVA thickness. In addition, the monodispersity and spherical shape of PVA-Sil-T were kept well relative to bare silica gel, as indicated by SEM provided in Fig. 2. The characterization of PVA-Sil-T by a $^{13}$C-CP/MAS NMR and IR further indicated the introduction of PVA coating onto silica gel (details see SI-Fig.1 and SI-Fig.2).

**Table 1** Chemical stability test of PVA-Sil-T.

| Stationary phases | *m* (g) | $m_t$(g) | Q（%） | C% |
|---|---|---|---|---|
| Bare silica gel | 0.2402 | 0.1752 | 27.1 | / |
| PVA-Sil-T (1-layer) | 0.2411 | 0.2077 | 13.9 | 4.38 |
| PVA-Sil-T (2-layer) | 0.2430 | 0.2236 | 8.0 | 5.69 |
| PVA-Sil-T (3-layer) | 0.2482 | 0.2432 | 2.0 | 8.02 |

Note：The test was as follows. PVA-Sil-Ts were immersed into ACN-triethylamine solution (pH =11, v/v = 80/10), then stirred at 300 rpm for 72 h at 25 °C. After centrifuging at 6000 rpm for 10 min, then removing the supernatant, the left amount of solid was weighed by analytical balance. The erosion amount of silica gel was calculated as $Q = \frac{m - m_t}{m} \times 100\%$, where *m* (g) and $m_t$(g) is the initial and final mass of silica gel, respectively.

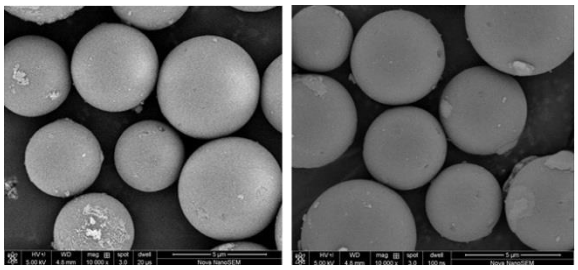

**Fig. 2** Scanning electron micrographs of bare silica gel (left) and PVA-Sil-T (right)

Although more layers of PVA coating has significant effect on the chemical stability of PVA-Sil, it has less impact on the chromatographic separation, as shown in SI-Fig. 3. Slight difference of the retention time of model analytes was observed for PVA-Sil-T with 1-3 layers of PVA coating. This can be speculated that only the outer PVA layer functions to be responsible for separation, the inner layers do not. Small decrease of column efficiency was observed for PVA-Sil-T with more layers. An interesting thing should be noted that lower operation pressure of PVA-Sil-T with more layers of PVA coating was found, e.g. ~35% decrease of backpressure of PVA-Sil-T with three layers relative to that with one layer. This is consistent with what observed previously.[17] For convenience, the following chromatographic evaluation was performed on PVA-Sil-T with one layer. A typical chromatogram were shown in Fig.3. Good separation of nucleosides by PVA-Sil-T was observed with high efficiency (e.g. ~75000/m of plate count for cytosine). In comparison, PVA-Sil-T demonstrated comparable or better performance relative to other two columns. In addition, an indirect comparison with a commercial PVA-based column (YMC Corp., http://www.ymc.co.jp/en/download/pdf/pdf03.pdf) was made, as shown in SI-Fig.4. Stronger retention and comparable resolution was observed for PVA-Sil-T relative to commercial one. The advantage of PVA-Sil-T was also exhibited for separation of organic bases, which always have poor separation on bare silica gel due to their strong electrostatic interaction. Such interaction could be effectively eliminated by introduction of PVA coating, as well proved previously [17]and in SI-Fig. 5. Four bases were well separated with good shape on PVA-Sil-T. Baseline separation of organic acids by PVA-Sil-T was also provided inSI-Fig.5. The retention mechanism of these polar analytes on PVA-Sil-T was found to follow the typical HILIC mode, as illustrated in SI-Fig.6.

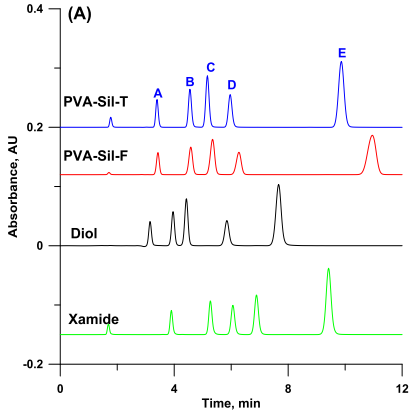


**Fig. 3**. Typical chromatogram of nucleosides by PVA-Sil-T Conditions: mobile phase: A, ACN; B, $H_2O$; C, 250 mM $NH_4FA$ (pH 3.5). 90% A/4% B/6% C; injection volume, 10 μL; column temperature, 30 °C. Peak identification: A, uracil (30 μg/mL), B, 5-methyl uridine (80 μg/mL), C, uridine (30 μg/mL), D, adenine (70 μg/mL), E, cytosine (400 μg/mL). Peak order is same for all tested columns.

The chemical stability of PVA-Sil-T was explored by observation of the shift of retention time of model analytes over a long period of time under relatively harsh mobile phase containing 75% acetonitrile-25% of 20 mM $NH_4OAc$ (pH 7.8) referring to the test method described previously.[19] Three model analytes including adenine (neutral), nicotinic acid (negatively charged) and benzyltrimethylammonium chloride (BTMA) (positively charged) were chosen. Over 35h continuous use for PVA-Sil-T with one layer of PVA coating, the retention times of model analytes were almost constant, the RSDs were in the range of 0.087%-0.96%, as shown in Fig.4A. At the same time, the operation pressure of the column was almost constant. By contrast, the RSDs of retention time of the analytes above on bare silica gel (Fig.4B) ranged from 0.31% to 1.91%, while the operation pressure had a significant increase (~2.2-fold higher). The column was obviously almost broken down, which may result from the column blockage due to the erosion of bare silica gel. These data proved good stability of PVA coating and also the protection effect of the coating for silica gel. Run-to-run reproducibility of PVA-Sil-T was also measured by consecutive injections (provided in SI-Fig.7). Intra-day and inter-day relative standard deviation (RSD) of retention time were less than 0.21% (n=6) and 0.32% (n=3), respectively, indicating good running stability of PVA-Sil-T. Moreover, the long-term stability of PVA-Sil-T column was continuously tested for over 3 months and no obvious deterioration was observed, indicating its high chemical stability. This was also proved by PVA-Sil-F described previously.[17] In addition, the approach to make PVA-coated silica gel described here is simple, leading to good batch-to-batch reproducibility, as indicated by the

RSD values of the retention time and the plate counts of the columns (n=3) were 1.1% and 4.5%, respectively.

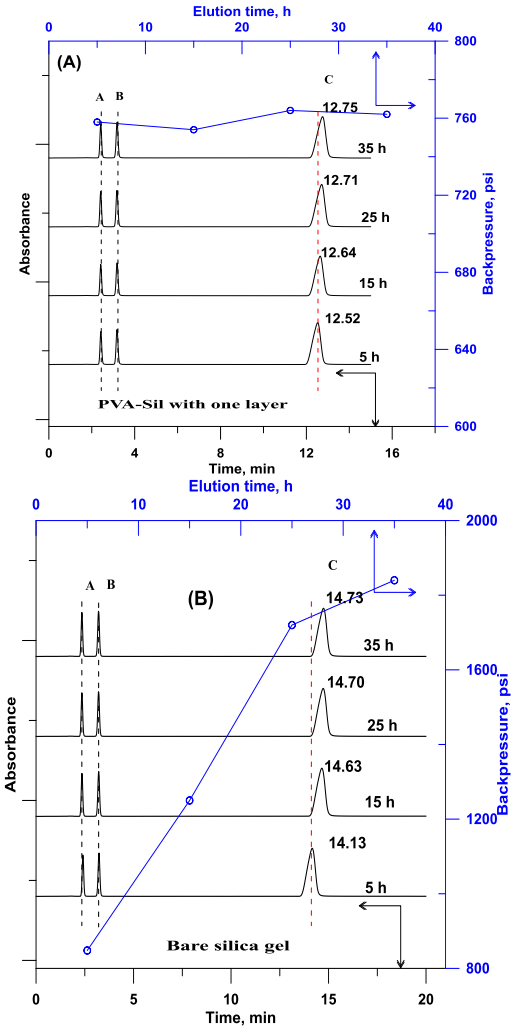


**Fig. 4** Retention drift on PVA-Sil-T and bare silica gel. Conditions: mobile phase: 75% acetonitrile -25% of 20 mM $NH_4OAc$, (pH 7.8, adjusted by ammonia water); model analytes, adenine(A), nicotinic acid (B) and BTMA (C). Other conditions same to Fig. 3.

As an economic and "green" alternative to classical HPLC, subcritical fluid chromatography (SubFC) has aroused much interest in the pharmaceutical industry. PVA-Sil-T operated in the mode of SubFC was also demonstrated by separation of three model compounds (acetophenone, caffeine, and thymine), shown in SI-Fig.8. Three model compounds could be well separated and high separation efficiency was obtained, e.g. the plate count of caffeine was ~48050 plate/m.

In short, a facile and green way to prepare PVA-coated silica gel stationary phase was proposed by thermal immobilization PVA onto the surface of silica gel. It is easy to be implemented and offers an easy way to manipulate the coating thickness. The obtained stationary phase demonstrated good chemical stability and hydrophilicity. It believes it will find useful applications for separation of polar analytes in HILIC or in SFC.

The research was sponsored by National Natural Science Foundation of China (No. 21322502, 21477037). It also thanks for the support from Outstanding young talent cultivation fund of ECUST.

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