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Single step on-column frit making for capillary high-performance liquid chromatography using sol–gel technology

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Abstract

One step frit-making in packing fused-silica capillary column of high-performance liquid chromatography was developed using sol–gel technology. Frit fabrication procedure was quite simple without sintering. On-column frit was formed through gelling of sol solution with packing materials, silica gel, and jointing the particles together with capillary wall through bonded and immobilized networks. Solvent types and proportions in sol solution were selected. And the sol solution compositions as well as amount of silica gel particles were also optimized to achieve maximum strength. Such an on-column frit of 250 μm in diameter is capable of resistant packing pressure up to 500 bars in ultra-sonic bath action. Chemical resistance to solvents and extreme pHs were also tested. Scanning electron micrograms of the frit profile showed that the evolving sol–gel network joined particles to each other and onto the column wall. Routine runs in reversed-phase mode, the frits of several columns proved to be effective enough to resist pressures without collapse.

Keywords: Frit making; Sol–gel technology; Capillary columns

1. Introduction

Capillary liquid chromatographic columns are most widely used in micro/nano-HPLC separations. High sample capacity and availability of various packing materials in contrast to open tubular capillary columns, packed capillary columns are mainly employed in high-performance liquid chromatography and capillary electrochromatography(CEC). Various problems for packing capillary columns remain to be solved. The most common problem is frit-making. Although variety of frit-making methods have been developed [1–9], it is difficult to prepare a high mechanically strong and high permeable end-frit. Frits made by polycondensation of potassium silicate and formamide [1–3] may cause some problems for permeability and chemical inertness. Sintering packing material or together with sodium silicate was often used for end-frit making [3,9]. However, a partially intense heating in sintering process can easily destroy the structure of particles and lead to some active sites on surface of the frit and may cause permeability problems. Sometimes further deactivation is necessary [9,10]. This heating process can also result in capillary fragile at the frit location. Also, the sintering process involves an uncontrollable temperature heating, reproducibility of the frits making are not easy to be obtained.

Recently, monolithic column making method

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using sol–gel technology were developed by Dulay et al. [11]. Packing material, octadecylsilica (ODS) was employed to form a porous monolith column. Tang and Lee [12–14] made sol–gel type monolithic columns using CO_2 slurry packing of ODS particles. Columns made in this manner were inert and efficient in CEC separations. A similar work, silicate-entrapped columns, was carried out [15]. Columns packed with reversed-phase material subjected to silicate entrapment could change retention properties and enhance the separation speeds and efficiencies. Some other monolithic columns with continuous porous bed were also widely studied [16–18] in avoid of frit makings. On-column frits making after slurry packing using sol–gel method was also developed to avoid of high packing pressure [19]. Monolithic column makings without frits are indeed quite convenient methods. However, in some cases, packing material covered by a layer of silica-based gel network might change the retention behaviors in various degrees. Some methods of filling up packing materials were not compacted as it is done by traditional high pressure packing process. Therefore, to develop a high mechanic strength and reproducible frit-making method is still necessary to use plenty of available packing materials in micro-column making.

In this work, we present an approach to making on-column frit that is capable of resistance up to 500 bars of packing pressure using capillary diameter as large as 250 μm. The method used silica gel particles, instead of ODS or some other chemical bonded stationary phases, highly mechanical strength, permeability, and reproducibility have been achieved.

In comparison with sintering methods, frit making at lower temperature did not destroy the particle structure of the frit and protect capillary from breakage on heating spot. Since the frit making used silica gel, the sol–gel reactions formed a high intensive network on particle surfaces, and mechanical high strength has been achieved. Also, because frit making involved sol–gel reactions on silica gel surfaces, chemically inertness was suggested to be efficient [12]. The frit making process does not involve sintering at uncontrollable temperature. Reproducible on-column frits are easy to be made. A strong mechanical structure, stability and permeability of the frits was also demonstrated in this paper.

2. Experimental

2.1. Materials and chemicals

Fused silica capillaries (250-μm I.D.; 380-μm O.D.) and (75-μm I.D.; 360-μm O.D.) were purchased from Polymicro Technology (Phoenix, AZ, USA). Packing materials, 5-μm octadecylsilyl (Zorbax bp-ODS) and spherical silica gel (Zorbax bp-Sil), were obtained from Du Pont Co (Wilmington, DE), Zorbax 300SB-C8 5-μm from Hewlett-Packard, and Partisil 5 ODS-3 5 μm particles from Whatman (NJ, USA). Trifluroacetic acid (TFA) was got from Merck (Munich, Germany), methyltriethoxysilane (MTES) and tetramethoxysilane (TMOS) from Institute of Rubber Product (Shanghai, China). Methanol, acetonitrile were HPLC grade from Fisher Scientific (Fairlawn, NJ), methylene chloride (CH_2Cl_2), acetone purchased from Shanghai Chemical factory (Shanghai, China) were all analytical grade regents. The test compounds of aromatic ketone was synthesized products of Organic Laboratory in Fudan University.

2.2. Instrumentation

Shimadzu LC-4A HPLC pump was used for slurry column-packing. Hewlett-Packard model 1100 quaternary pumping system was used for evaluation of column performance. Split injection of sample and delivering appropriate amounts of mobile phases was achieved with a six-port valve via splitter of capillary restrictor. On-column detection was carried out using Waters 484 Tunable Absorbance Detector (Waters, MA, USA) with a modification.

2.3. Frit preparation

Producing a sol–gel-derived frit involved the preparation of the sol solution and the formation of gel by polymerization. The optimized composition of the sol solution was prepared as follows: Add 100 μl of TFA, 75 μl of MTES, 10 μl of water, 200 μl of methylene chloride sequentially to a vessel and mix uniformly, then suspend 0.15 g of silica gel (5 μm Zorbax BP-Sil or equivalent) in the solution by stirring. A several millimeters segment of the resulting sol solution was drawn into the capillary at a desired position. Then, lie the capillary on a plane to avoid the sol-segment moving during dryness. The capillary should be previously rinsed with methylene chloride and dried with nitrogen. The gelling procedure was expected to complete within 30 min for polymerization and crossing-linking reaction in the capillary at temperature 30°C. An additional 6 h drying in oven at 100°C or overnight drying at ca. 50°C under infrared light is necessary to ensure the complete formation of monolith structure of network with enough mechanical strength.

The procedure of slurry packing with reversed-phase particles (ODS) was referred to those reported before [3,20–24]. Maximum 500 bars were applied for packing columns of 18–40 cm long. After packing, columns were kept in ultrasonic bath at this pressure for about 1 h, then, stopped the sonication and decreased pressure slowly until reached atmospheric pressure.

The inlet frit-making is also quite simple. After solvent evaporating by warming up the column end, the inlet end of the capillary were dried and dipped into the fresh sol solution, the solution came into the column packing bed for few millimeters and resulted in the formation of inlet-frit after drying.

An on-column detection window could be made immediate after the outlet-frit by using a electric heating wire to burn off the capillary polyimide coating.

3. Results and discussion

3.1. Mechanism of the sol–gel approach

Sol gel reaction is as follows: (1) Acid catalytic hydrolysis of the precursor; (2) Polycondensation of hydrolyzed product; (3) Condensation of silica molecules to the evolving sol–gel network. The goal of this research was to optimize above reaction process, and to develop a reproducible on-column-frit with good mechanical stability and sufficient permeability. To achieve this goal, the preparation of the sol solution is of key importance. In general, tetramethoxysilane (TMOS) is commonly used as sol–gel precursor due to its 3-dimensional strong structure [12]. However, to our knowledge, frits made by TMOS are easy to shrink and crack of the gel network during drying. This, in turn, may weaken the general strength of the prepared frit. Methyltriethoxysilane has some advantages over TMOS [8]. Its gelling results in a more flexible network and mechanically stronger on-column frit.

In this approach, methylene chloride was used as organic solvent for solvating the precursor. Methanol, acetone, and some other solvents were also usable. Methylene chloride is the best one due to its weak polarity and moderate volatility. Solvent evaporating either fast or slow can affect the formation of gelling network. On the other hand, the relative proportion of methylene chloride to other ingredients is also an important factor that affects strength of the final frit. Too high percentage of methylene chloride would diluted sol solution and lead to forming a rather weak thin layer of sol–gel network. Too low percentage of solvent would result in low permeability and excessive contraction of the gel network.

TFA served as the acid catalyst to accelerate the hydrolysis of the precursor. TFA containing 5–10% water was more effective for acceleration of the gelling process. Excessive TFA was evaporated in drying.

Reaction temperature is another important factor to influence the final strength. Higher than 30°C of the reaction temperature, sol solution is gelling rapidly. So, frit-making has to be completed in few minutes. On the other hand, sol–gel reaction is much slower as temperature is lower than 20°C. Long time delay for gelling reaction may cause solvent loss and lead to forming non-uniform gel network. It also may weaken the frit strength. Therefore, the best operation is filling sol solution at 20–25°C, and gelling at 30°C.

Generally speaking, various ODS packings can also be used in making frits. However, their mechanic strength of the frits is weaker than that of silica gel particles. It is probably due to much less free silanol groups on surface of the ODS particles were remained. Even so, strength of the ODS frits was quite different. For instance, frits made by Zorbax bp-ODS particles can resist much higher pressure than that of ODS-3 particles from Whatman. Anyway, both of them are still strong enough for inlet-frits.

3.2. Characterization of frit by S.E.M.

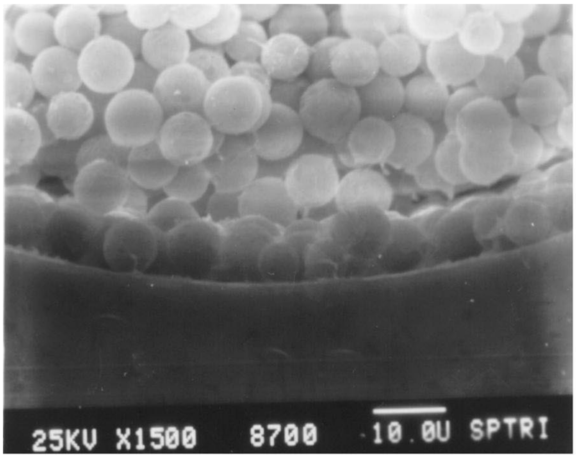
In practice, silica gel particles as well as the column wall, were coated by sol solution. After polymerization of hydrolyzed product of the MTES, 3-dimensional networks were formed and anchored onto both of the packing surfaces and column inner surface through chemical bondings. It provides high mechanic strength for tolerating packing pressure up to 500 bars for 2–3 mm-length frit on a 250 μm I.D. capillary. Strength of a frit increases with decrease of capillary inner diameters. It is believed that such a frit for 75-μm-I.D. capillary can resist higher pressure than 500 bars.

Fig. 1A shows a part of cross-sectional view of 250-μm-i.d capillary on-cloumn frit. Near the capillary wall, packing particles are tightly fixed onto the column to form a high stable and mechanically strong layer. In Fig. 1B, some bridge structures that formed by the sol–gel network could be visible. The joining points between particles were thicker. Such a thin layer 3-D network structure enhances resistance of high pressure. The figure also shows that the frit made using sol–gel technology is quite similar to a normal packing bed. Therefore, these features both for good mechanical stability and sufficient permeability are essential to on-column-frits.

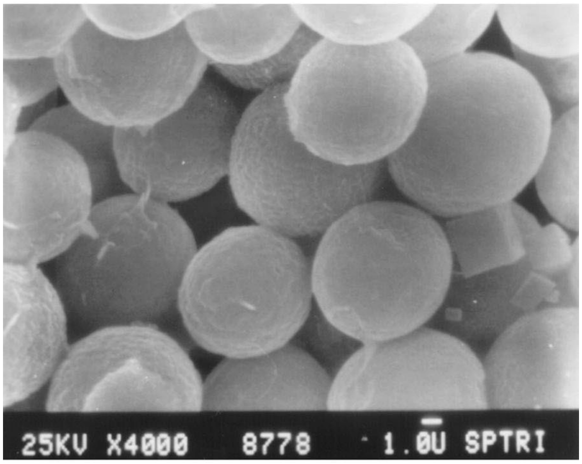
3.3. Frit performance test

3.3.1. Permeability test

It is rather difficult to measure the permeability for 2–3 mm frits. So, frits of 11/13 mm long were made and the permeability was measured. For the 11 mm frit, while flow-rate of pure methanol at 0.1 ml/min, pressure drops were 36 bars. Flow rate increased up to 0.25 ml/min, inlet column pressure approaches 91 bars. So, the permeability of the frit could be estimated to be 12.8 to 13.0 $mm^2\ s^{-1}\ bar^{-1}$ according to [25] (while flow-rate=0.1 ml/min and porosity=0.8 for 5 μm totally porous particles, linear velocity, u, was calculated to be 42.4 mm/s). The average permeability was about 12.9 $mm^2\ s^{-1}\ bar^{-1}$. Meanwhile, the average value for the13 mm frit was 12.8 $mm^2\ s^{-1}\ bar^{-1}$. Additional two columns with 2 mm frits and 10/40 mm packing beds were made using identical packing material, 5-μm Zorbax bp-Sil. Their permeability was also estimated to be 12.2 and 12.4 $mm^2\ s^{-1}\ bar^{-1}$. For instances, the column of 2 mm frit and 40 mm packing bed, while the flow-rate at 0.1 ml/min, pressure drop reached 144 bars. Its permeability was about 12.4 $mm^2\ s^{-1}\ bar^{-1}$. Therefore, relative permeability of the frits to packing bed is about 1.05. This permeability is quite reasonable. Frits with slightly higher permeability are probably less compact than that for packing bed.



(A)



(B)

Fig. 1. Scanning Electron Micrograms of sol–gel-derived and SIL-based frit on 250-μm-I.D. capillary. (A) Characterization of the cross-section view. Packing particles tightly fixed onto the column to form a high stable and mechanically strong layer with a magnification of 1500×. (B) An indication of some bridge structures formed from sol–gel network (magnification: 4000×).

3.3.2. Column efficiency

Five aromatic ketones were used to test separation efficiency of capillary column with sol–gel frit. Fig. 2 shows that the five compounds are nice peak shapes. Theoretical plate heights were 13.8 and 15.2 µm for acetophenone and fluorenone, respectively. Such column efficiency is normal for columns of 5 µm particles. It has been proved that the frits are stable and effective in routine reversed-phase operations. Hundreds of samples, traditional Chinese medicine, proteins, and their digests, were analysed using on-column UV detection and LC–ESI-MS analysis. The separation efficiency is high and eluting peaks are quite normal. Fig. 3 is a separation profile for tryptic digestion of protein TCS (trichosanthin) which was extracted from the leaves of *Trichosanthes kirilowii* Maxim., a traditional Chinese medicine.

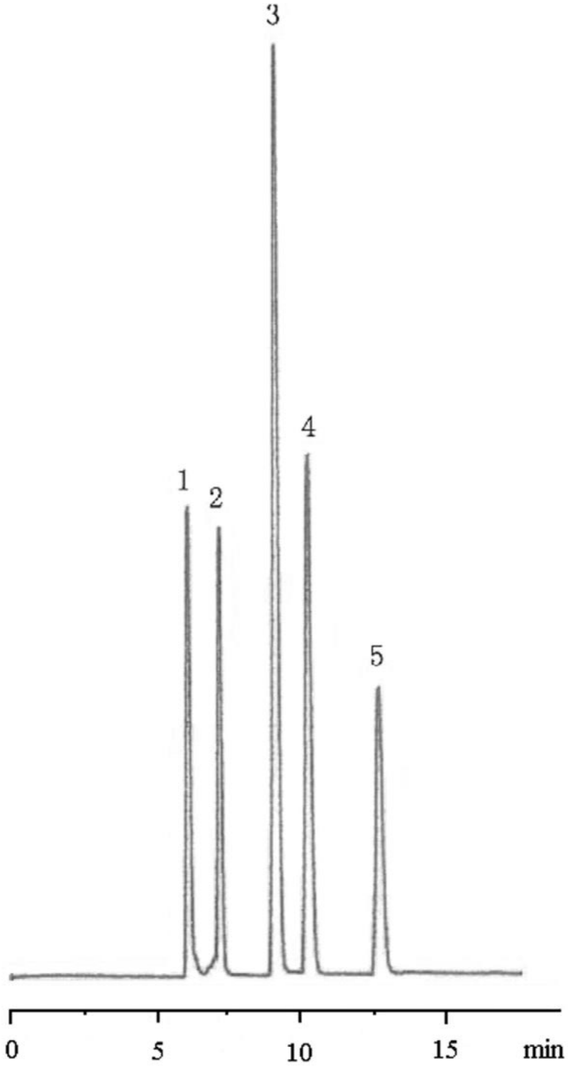


Fig. 2. LC separation of five aromatic ketones on a reversed-phase column with sol–gel frit. Conditions: column: 24 cm×250 µm I.D. packed with Zorbax bp-ODS 5-µm particles. Isocratic elution: methanol/water (85:15); total flow-rate: 0.2 ml/min (column:1.9 µl/min), split ratio 105:1; UV on-column detection at 280 nm. Peak identities: (1) *o*-nitro acetophenone, (2) acetophenone, (3) dibenzyl ketone, (4) diphenyl ketone, (5) fluorenone.

3.3.3. Frit stability

The frits were run at allowed pH range of the ODS packings (pH 2–9). There was no evident effect on the frits' strength. Extremely low and high pHs were also tested. The frits were tolerable for flushing 1 ml 0.1 *M* HCl (pH 1.3) and 1 ml 0.05 *M* NaOH (pH 12.4), respectively, in 10 min. On the other hand, some typical solvents were tested. In most cases, they were proved to be stable for mechanical strength after hours rinses. Methylene chloride may slightly weaken the frit strength during flushing, because two out of ten testing frits were destroyed during the flushing.

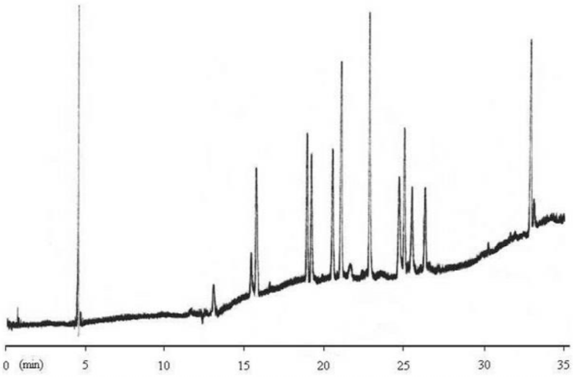


Fig. 3. Peptides profile of the trichosanthin (TCS) tryptic digestion. Trypsin and TCS (1:50) in 0.1 *M* NH_4HCO_3 solution reacted at 37°C overnight. Chromatographic gradient elution was carried out. Solvent A: water contained 0.1% TFA, solvent B: acetonitrile with 0.1% TFA. Elution condition was keeping 100% A for 2 min, then increased B linearly up to 45% in 25 min; further increased B to 80% in 5 min and kept for 5 min. Column: 30 cm×250 µm I.D. packed with Zorbax 300SB-C8 5 µm particles. Flow rate: 0.2 ml/min (column: 2.35 µl/min), split ratio: 80:1. On-column UV detection at 210 nm.

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References

[1] H.J. Cortes, C.D. Pfeiffer, B.E. Richter, T.S. Stevens, J. High. Resolut. Chromatogr. Commun. 10 (1987) 446.
[2] J.H. Knox, I.H. Grant, Chromatographia 32 (1991) 317.
[3] B. Behnke, E. Grom, E. Bayer, J. Chromatogr. A 716 (1995) 207.
[4] C. Yan, D. Schanfelberger, F. Erni, J. Chromatogr. A 670 (1994) 15.
[5] C. Yan, R. Dadoo, H. Zhao, R.N. Zare, D.J. Rakestraw, Anal. Chem. 67 (1995) 2026.
[6] C. Yan, R. Dadoo, R.N. Zare, D.J. Rakestraw, D.S. Anex, Anal. Chem. 68 (1996) 2726.
[7] C. Fujinoto, Y. Fujise, Anal. Chem 68 (1996) 2753.
[8] L.A. Colon, Y. Guo, A. Fermier, Anal. Chem 69 (1997) 461A.
[9] Y. Chen, G. Gerhardt, R. Cassidy, Anal. Chem. 72 (2000) 610.
[10] B. Behnke, J. Johansson, S. Zhang, E. Bayer, S. Nilsson, J. Chromatogr. A 818 (1998) 257.
[11] M.T. Dulay, R.P. Kulkarni, R.N. Zare, Anal. Chem. 70 (1998) 5103.
[12] Q. Tang, B. Xin, M.L. Lee, J. Chromatogr. A 837 (1999) 35.
[13] Q. Tang, M.L. Lee, J. High. Resolut. Chromatogr. Commun. 23 (2000) 73.
[14] Q. Tang, N. Wu, M.L. Lee, J. Microcol. Sep. 12 (2000) 6.
[15] G. Chirica, V.T. Remcho, Electrophoresis 20 (1999) 50.
[16] C. Fujimoto, J. High. Resolut. Chromatogr. Commun. 23 (2000) 89.
[17] C.K. Ratnayake, C.S. Oh, M.P. Henry, J. High. Resolut. Chromatogr. Commun. 23 (2000) 81.
[18] K. Cabrera, D. Lubda, H.-M. Minakuchi, K. Nakanishi, J. High. Resolut. Chromatogr. Commun. 23 (2000) 93.
[19] M. Schmid, F. Bauml, A.P. Kohne, T. Welsch, J. High Resol. Chromatogr. 837 (1999) 35.
[20] C. Borra, S.M. Han, M. Novotny, J. Chromatogr. 355 (1987) 75.
[21] W. Shi, H. Zou, J. Zhang, L. Dong, Y. Zhang, Chin. J. Chromatogr. 14 (1996) 351.
[22] N.W. Smith, M.B. Evans, Chromatographia 38 (1994) 649.
[23] H. Rebscher, U. Pyell, Chromatographia 42 (1996) 171.
[24] R.J. Boughtflower, T. Underwood, C. Paterson, Chromatographia 40 (1995) 329.
[25] V.R. Meyer, Practical High-performance Liquid Chromatography, John Wiley & Sons Ltd, New York, 1988.