

Short communication

Metal tubing/frit with a sintered frit of silica particles and a chromatography column with such tubing/frits

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Received 12 August 2004; received in revised form 22 December 2004; accepted 5 January 2005

Available online 21 January 2005

Abstract

This study is about the metal tubing/frit prepared by treating a powdered silica with a strong base, drying it, scrubbing it into powders, putting it in the tip of metal tubing at a proper depth, and sintering the powders to form a durable sintered frit. This study is also about a liquid chromatographic column prepared by installing the metal tubing/frit at the column inlet and outlet unions and by placing the column main body tubing packed with a stationary phase between the two unions. The metal tubing/frit made according to the description is easily replaced and fixed without disassembling the column when it is necessary. The column of this design could be a cheap substitute for the conventional microcolumn.

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Keywords: Tubing/frit; Sintered frit; Chemical and thermal sintering; Microcolumn

1. Introduction

The liquid chromatography column is composed of three major parts, that is, a column body tubing, end-fitting blocks at both ends of the column body, and a filler as a stationary phase packed in the column. The column body tubing is made of various materials, such as stainless steel, glass-lined stainless steel, polymer, and polymer-coated silica, etc. Porous powders are usually used as stationary phases. Porous silica, alumina, zirconia, and their ligand-attached or polymer-coated products are used, and various kinds of porous polymer powders are also used. The most typical stationary phase at present is octadecyl ligand-attached silica, C18.

Porous disc-type frits are placed in both ends of a column to keep the stationary phase in and to let the mobile phase penetrate in common columns. Such frits are treated very carefully, since frits directly influence chromatographic resolution, and various researches for frits have been being carried out. Frits are installed inside the column main body,

and the frits cannot be removed without disassembling the column. A very special column in view of frit technique is the monolith column. The whole stationary phase of the monolith column is one body with numerous multiple porous channels, thus it does not need any frit since the whole stationary phase functions as a frit [1,2].

Various designs of frits and end fittings have been proposed. For example, polymer encased stainless steel disc frits [3] and trapezoidal disc-type frits [4] were used. Such frits were used to minimize anomalous perturbation of mobile phase flow and consequent column efficiency degradation owing to differences among the inside diameter of column, the diameter of porous frit disc, and the inside diameter of connecting tubing. Another special design is given in a patent [5] where a tough and flexible polymer insert with a central flow channel was proposed to be installed in front of the column inlet frit in order to remove any void volume owing to prolonged use of the column and consequent partial collapse of the stationary phase by just tightening the insert. Cortes et al. [6] proposed to prepare a fixed porous ceramic plug frit at the column end by putting a potassium silicate solution into the end of the column main body and by causing sintering

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on vapor heating. This method was also used in production of other microcolumns [7]. The technique of making fixed frits in the column main body tubing is generally used in production of microcolumns, especially microcolumns made of silica capillary. The initial microcolumn frit designs were, however, non-porous and imperfect. For example, a metal wire whose diameter was slightly smaller than the inside diameter of the silica capillary, or another silica capillary with very narrow I.D. whose O.D. was slightly smaller than the I.D. of column capillary, was used [8]. In addition, a silica capillary column encased in soft polymer tubing with the conventional replaceable frits was proposed [9].

The recent trend of silica capillary microcolumns is shifting swiftly toward monolithic columns where the whole column functions as a large frit. Details of monolith columns have been introduced in some review articles [10–12], and patents of various monolith columns have appeared. A method of preparing fixed frits at both ends of the column by partial heat treatment of packed silica capillary was introduced in the literature [13,14], and a research group made use of such concept, and packed a silica capillary with porous stationary phase powders, and sintered the whole stationary phase by heating with a ring-type electric heating wire, leaving the whole stationary phase combined but the porous spherical structures of the powders unchanged [15]. A monolith column was prepared by putting the dispersed solution of stationary phase powders, water, a solvent, such as alcohols, and a metal alkoxide by a syringe in silica capillary, and by applying heat and vacuum [16]. Piraino and Dorsey [17] compared the performance of several types of frits in capillary electrochromatography.

The conventional arts of preparing frits of liquid chromatography columns described above can be categorized into two techniques. The first one is to place separate frits. In this case, the frit is exclusively installed inside the column end-fitting, thus, if the frit is clogged and needs to be replaced, the column end-fitting should be disassembled. Besides, perturbation of mobile phase flow and consequent reduction of column efficiency can occur if the frit diameter is very larger than the column I.D. or the I.D. of connection tubing. Adopting complicated designs for the frit structure to solve such problems yields cost increase and inconvenience of use. Furthermore, it is very difficult to make miniaturized separate frits for microcolumns with I.D. of 0.5 mm or less whose relative importance has been rapidly increasing.

The other frit technique is to make permanent frits at the column ends or for the whole column without using separate frits. In this case, column repacking is impossible, and the column lifetime ends when the frit is clogged or a crack or void volume develops in the column. First, in the case of permanent end frits only at the column inlet and outlet, cracks in the packing structure are sometimes observed after formation of end frits, and the column efficiency is degraded. Furthermore, if frit clogging happens in the course of forming the inlet frit after packing the column, then the whole column finishes its lifetime without being used at all.

The monolith column in which the whole stationary phase is one porous phase and a huge frit as well, has raised a lot of interest, and has its own advantages and disadvantages. First of all, it is difficult to make a monolith without a void volume in a rigid conventional stainless steel column with I.D. of 1–5 mm, thus formation of monolith is confined in heat-shrinkable polymer tubing [18,19]. Such manufacturing technique seems to suffer from low reproducibility of production. It is relatively easy to make silica capillary monolith column with I.D. of 0.5 mm or less, but lots of labor and time are required for preparation of monolith reactants, formation of monolith, and cleaning the monolith. Furthermore, production reproducibility of silica capillary monoliths is not well known.

There has been no frit technique so far to make permanent frits not in the column main body but in the connection tubing between the column and other devices, such as an injector or detector. If such a frit technique is available, the structure of column end fitting becomes simple, and it is possible to make useful columns at lower prices by using commercial unions. The problem of column efficiency reduction owing to a larger frit diameter compared to the column I.D. is eliminated for this tubing/frit design, and the tubing/frit can be easily replaced without disassembling the main column body when the frit is clogged.

We reported a disposable microcolumn that enabled skipping of sample pretreatment in a previous study [20]. We disposed of the stationary phase after using the column for a day or so and repacked the column with fresh stationary phase. We were unable to suggest a feasible frit technique but a rather tedious one (silica capillary with sintered silica frit) at that time. Thus, this study was motivated.

We have developed in this study the method of producing a durable frit/tubing by sintering porous silica particles chemically and thermally at the tip of metal tubing, and the method of producing a column of a new simple design by installing the frit/tubing at the column inlet and outlet unions.

2. Experimental

2.1. Production of metal tubing/frits with chemically and thermally sintered silica frits

One gram spherical porous 5–10 μ , silica particles with a 6–15 nm pore size are dispersed in a 10 mL 10 wt.% NaOH or KOH solution, and the dispersion is spread over a hard and flat polymer surface as a thin layer with addition of some ethanol, and dried at room temperature under a glass board to protect the dispersion layer from falling dirt. The dried layer is scraped with a polymer scoop and rubbed gently with a round pestle into fine powders. The above steps are necessary to make permeable uniform frits. The powders are then uniformly spread on a flat polymer surface with a depth of 2 mm or so. Metal tubings of 5–10 cm length and 1.6 mm O.D. (0.1 mm I.D.) are located vertically over the fine pow-

ders, and are allowed to hit the surface gently at various spots 50–200 times to get the particles in with a depth of 2–5 mm. The outer wall of the tubing is cleaned with a soft tissue. The tubing with the silica powders filled at the tip is placed in an electric furnace and heated at 500–600 °C for 5 h under nitrogen atmosphere to yield a tubing/frit with a sintered silica frit.

2.2. Silylation of the tubing/frit

In order to increase chemical stability of the frit/tubing and to deactivate the surface silanol groups, the tubing/frit is treated as follows. Fifty tubing/frits are placed in a 250 mL flat bottom flask together with 100 mL hexane and 2 mL trimethylchlorosilane, and the mixture was reacted at the reflux temperature for 24 h. The silylated tubing/frits are taken out and washed with acetone, water, and methanol in series. The pyridine adsorption–retention properties in the deactivated sintered silica tubing/frit prepared by the above process, the untreated sintered silica tubing/frit, and the untreated stainless steel tubing (all with 0.1 mm I.D. and 5 cm length) are measured and compared. Each tubing is connected between a 0.5 μ L Valco injector and an Isco micro UV detector, and a 0.001 M pyridine solution (solvent/methanol) is injected with 100% methanol as the eluent at a flow rate of 0.005 mL/min, and the chromatograms, retention times, peak heights and bandwidths are measured and compared.

2.3. Production of liquid chromatography microcolumn installed with stainless steel tubing/frits

The stainless steel tubing/frit (101b, 5 cm length, 0.1 mm I.D., 1.6 mm O.D.) with the sintered powder frit (10b) is connected to the column outlet union with a nut (103b') and a ferrule (104b) as shown in Fig. 1. The main body column

tubing (100, glass-lined, 30 cm length, 0.5 mm I.D., 1.6 mm O.D.) to be packed with stationary phase powders is connected to the other side of the union, and the open end of the main body column tubing is connected to the column packing apparatus. The C18 stationary phase (5 μ , Alltima C18 and Lichrosorb RP18) of 200 mg is dispersed in 6 mL methanol, placed in an ultrasonic bath for 30 min, and poured into a 4 mL slurry reservoir for immediate column packing. Packing is executed under a high pressure of 14,000 psi for 2 min, followed by conditioning under 8000 psi for 30 min. After completion of packing, the main body column tubing (100) is detached and connected to the column inlet union with a stainless steel tubing/frit to complete the column. In order to save the cost when replacing the tubing/frit, a one-body polymer nut/ferrule can be used instead of a separate stainless steel nut and a ferrule when the tubing/frit is installed as in Fig. 1, thus only the tubing/frit is replaced and the polymer nut/ferrule is reused. The Alltima C18 phase was obtained from Alltech (Deerfield, IL, USA), and the Lichrosorb RP18 phase, from Merck (Darmstadt, FRG). A commercial microcolumn, Luna C18 (5 μ , 0.5 mm I.D., 25 cm length) was purchased from Phenomenex (Torrance, CA, USA) to compare its separation efficiency with those of the above-mentioned home-made microcolumns.

3. Results and discussion

3.1. The microstructure of the silica powders after treating with a strong base followed by drying

In the microstructure of the above powders observed by a scanning electron microscope (Fig. 2), most of the powders maintain their spherical shapes, and they are partially fused and mixed with minute needle crystals of NaOH. The average size of the silica particles is 5 μ .

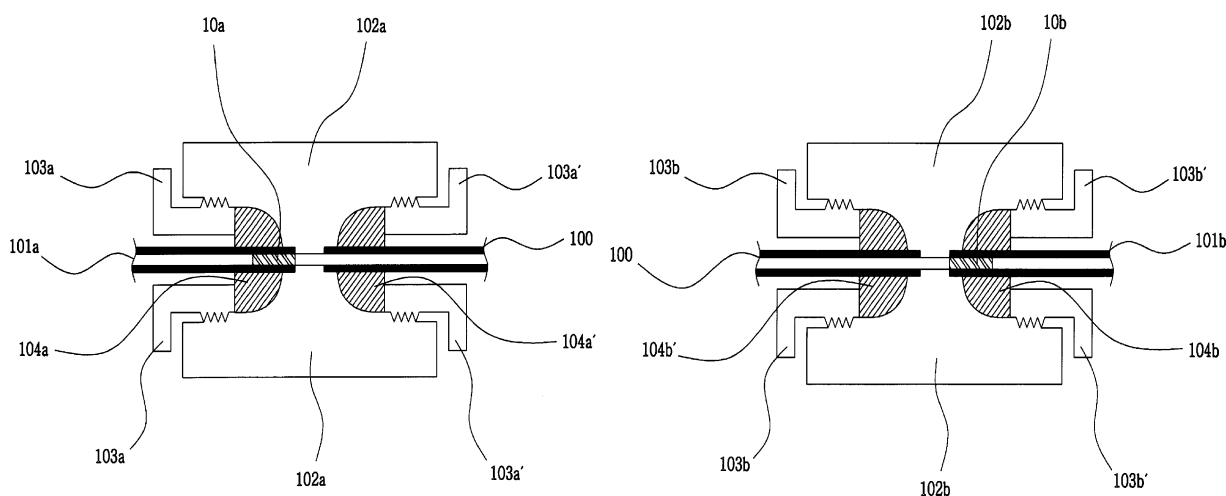


Fig. 1. The drawing of the column structure. 10a, 10b: sintered frit, 100: column main body tubing, 101a: metal tubing of the column inlet, 101b: metal tubing of the column outlet, 102a: column inlet union, 102b: column outlet union, 103a, 103a', 103b, 103b': nuts, 104a, 104a', 104b, 104b': ferrules.

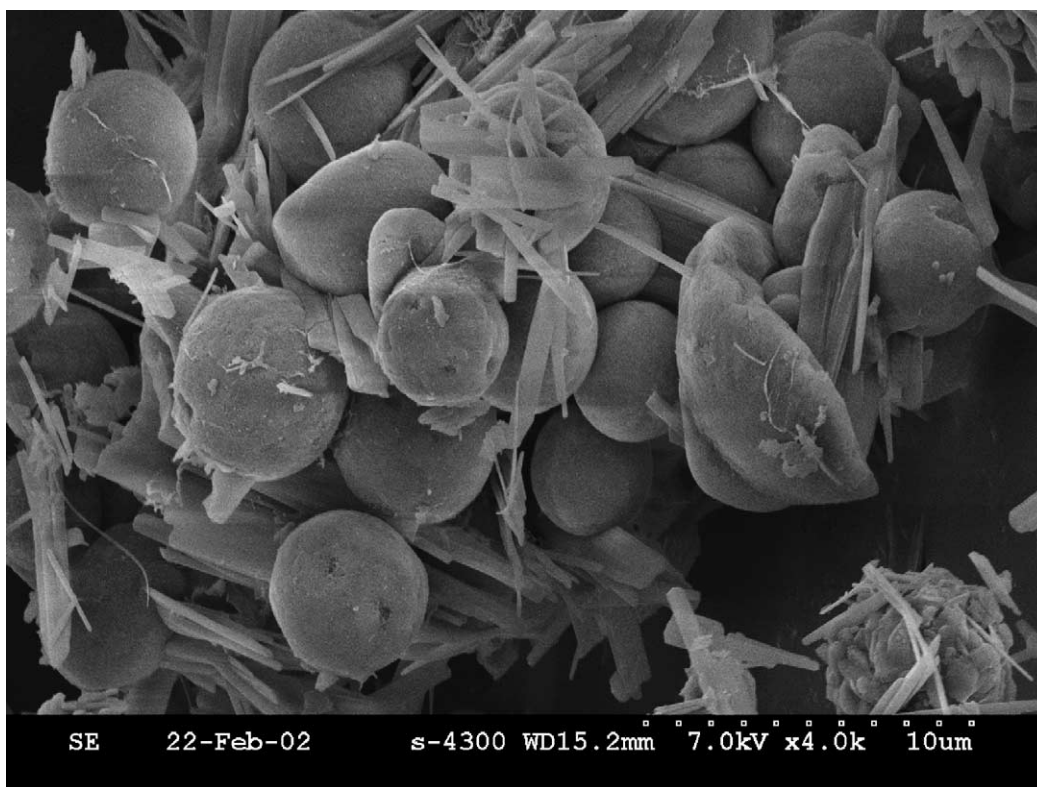


Fig. 2. The SEM photograph of 5 μ silica powders treated by a NaOH solution and dried.

3.2. The structure of the frit/tubing and its endurance

According to Fig. 3 of the cross-section of the tubing/frit, the silica particles are fused one another, and attached to the inner wall of the stainless steel tubing to give a durable sintered silica frit. The frit is strong enough to sustain when a high pressure of 8000 psi is rapidly applied to the tubing/frit. More than 95% of the frits produced on different days survived the 8000 psi pressure for 10 min. The frits also survived the packing process at 14,000 psi since the stationary phase particles were packed instantly over the frit and the actual time under such pressure for the frit was very short. The actual pressure the frits feel in a real system is low since the pressure is applied over the whole length of column, and most of the frits were used successfully for more than 6 months. It has such an excellent permeability that a pressure of only 30–50 psi is developed when the tubing/frit is connected to a LC pump and a flow of 2 mL/min is applied.

3.3. The effect of silylation of the tubing/frit

According to Table 1, silylated tubing/frits show narrower bandwidths, higher peak heights, shorter retention times, and lower asymmetry factors when compared to different type tubings. We can note that not only the silanol groups of sintered silica but also the hydroxyl groups of stainless steel surface are deactivated by silylation based on the results of Table 1. The stainless steel tubing had been treated with 1 M NaOH, 1 M HCl, water, and methanol in series.

3.4. Chromatographic performance of the columns of the new design with the tubing/frits

Separation performance of the microcolumn (packed with 5 μ Alltima C18 or Lichrosorb RP18) was examined. A commercial microcolumn (Luna C18, 5 μ) was also examined for comparison. Two test mixtures were used. One is the

Table 1

Comparison of pyridine retention characteristics in untreated tubing, tubing/frit, and silylated tubing/frit^{a,b}

Tubing	Retention time (min)	Peak height (mV)	Peak bandwidth at half height (cm) ^c	Asymmetry factor
Untreated	0.514 \pm 0.011	6.788 \pm 0.227	1.001 \pm 0.031	1.67 \pm 0.05
Tubing/frit	0.495 \pm 0.022	6.386 \pm 0.207	1.182 \pm 0.092	1.76 \pm 0.15
Silylated tubing/frit	0.448 \pm 0.037	7.771 \pm 0.175	0.956 \pm 0.051	1.22 \pm 0.06

^a The length of each tubing was 5 cm and the eluent was methanol.

^b Averages and standard deviations of 5 different pieces were measured.

^c Measured from the chromatograms in the same scale.

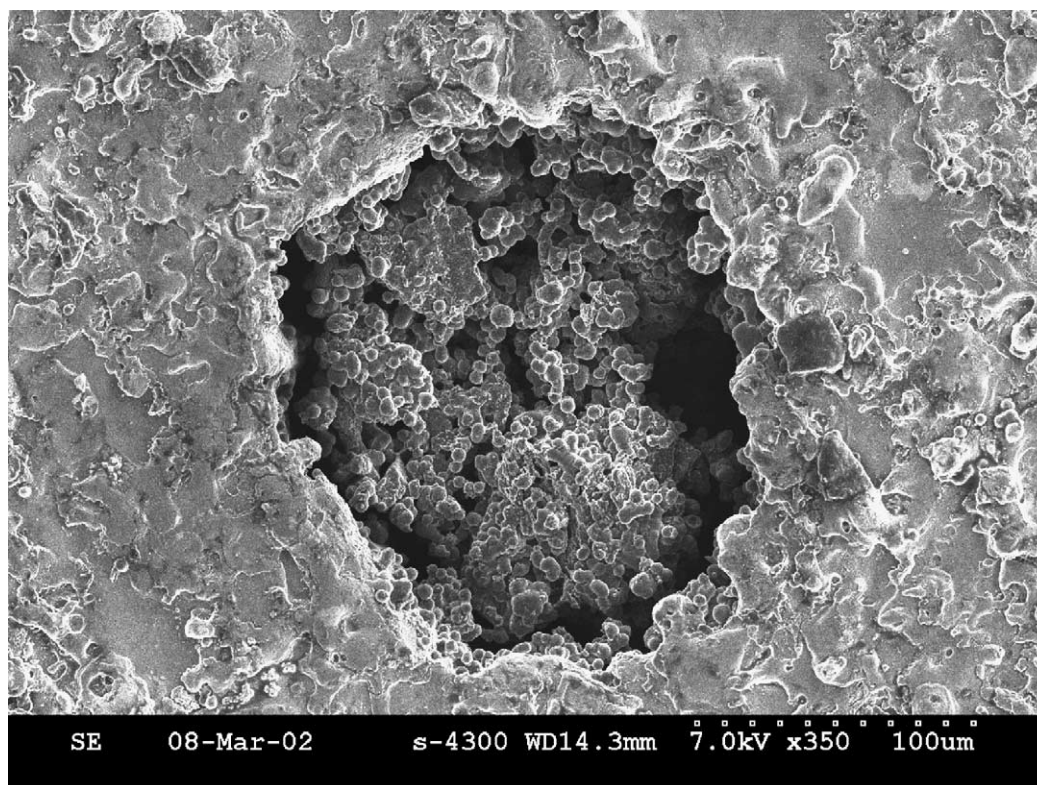


Fig. 3. The enlarged cross-section of the sintered silica frit formed in a stainless steel tubing of 0.1 mm I.D. and 1.6 mm O.D.

mixture of *o*-, *m*-, *p*-nitroanilines (test mix 1). The other (test mix 2) is composed of 4-methoxyphenol, acetophenone, ethylbenzoate, ethylbenzene, acenaphthylene, acenaphthene, phenanthrene, and anthracene. 85/15 (vol%) methanol/water

was used as the eluent for test mix 1, and 90/10 (vol%) methanol/water, for test mix 2. Fifteen measurements were made over 6 months. In order to check reproducibility of the tubing/frit, the inlet and outlet tubing/frits were replaced

Table 2

Comparison of plate heights (μm) of *o*-, *m*-, *p*-nitroanilines eluted in 85/15 (vol%) methanol/water at a flow rate of 10 $\mu\text{L}/\text{min}$ among the home-made microcolumns with tubing/frits and the commercial microcolumn with conventional frits^{a,b}

	Alltima C18 (home-made)	Lichrosorb RP18 (home-made)	Luna C18 (commercial)
<i>p</i> -Nitroaniline	45 \pm 2.5	40 \pm 2.9	68 \pm 7.4
<i>m</i> -Nitroaniline	40 \pm 0.6	41 \pm 3.0	58 \pm 2.3
<i>o</i> -Nitroaniline	30 \pm 0.9	34 \pm 0.8	43 \pm 1.7

^a The relevant chromatograms are shown in Fig. 4.

^b Based on 15 measurements over 6 months.

Table 3

Comparison of plate heights (μm) of the components of test mix 2 eluted in 90/10 (vol%) methanol/water at a flow rate of 10 $\mu\text{L}/\text{min}$ among the home-made microcolumns with tubing/frits and the commercial column with conventional frits^{a,b}

	Alltima C18 (tubing/frit)	Alltima C18 (silica capillary frit) ^c	Lichrosorb RP18	Luna C18	Luna C18 ^d
4-Methoxyphenol	38 \pm 2.9	41 \pm 3.2	36 \pm 1.8	69 \pm 3.9	58 \pm 4.0
Acetophenone	26 \pm 1.6	25 \pm 1.5	33 \pm 1.8	47 \pm 2.7	37 \pm 2.7
Ethylbenzoate	20 \pm 1.0	21 \pm 0.9	28 \pm 1.8	30 \pm 1.1	23 \pm 1.2
Ethylbenzene	15 \pm 0.6	15 \pm 0.7	21 \pm 1.0	24 \pm 0.9	17 \pm 0.9
Acenaphthylene	15 \pm 0.6	14 \pm 0.8	27 \pm 1.4	23 \pm 0.8	15 \pm 0.8
Acenaphthene	14 \pm 0.5	15 \pm 0.7	22 \pm 1.2	19 \pm 0.7	18 \pm 0.9
Phenanthrene	13 \pm 0.5	13 \pm 0.7	23 \pm 1.2	19 \pm 0.7	16 \pm 0.9
Anthracene	15 \pm 0.7	14 \pm 0.8	21 \pm 1.1	19 \pm 0.8	14 \pm 0.9

^a The relevant chromatograms are shown in Fig. 5.

^b Based on 15 measurements over 6 months.

^c The outlet tubing/frit of the Alltima C18 column was replaced by the silica capillary with a sintered silica frit.

^d Eluted in 85/15 (vol%) methanol/water.

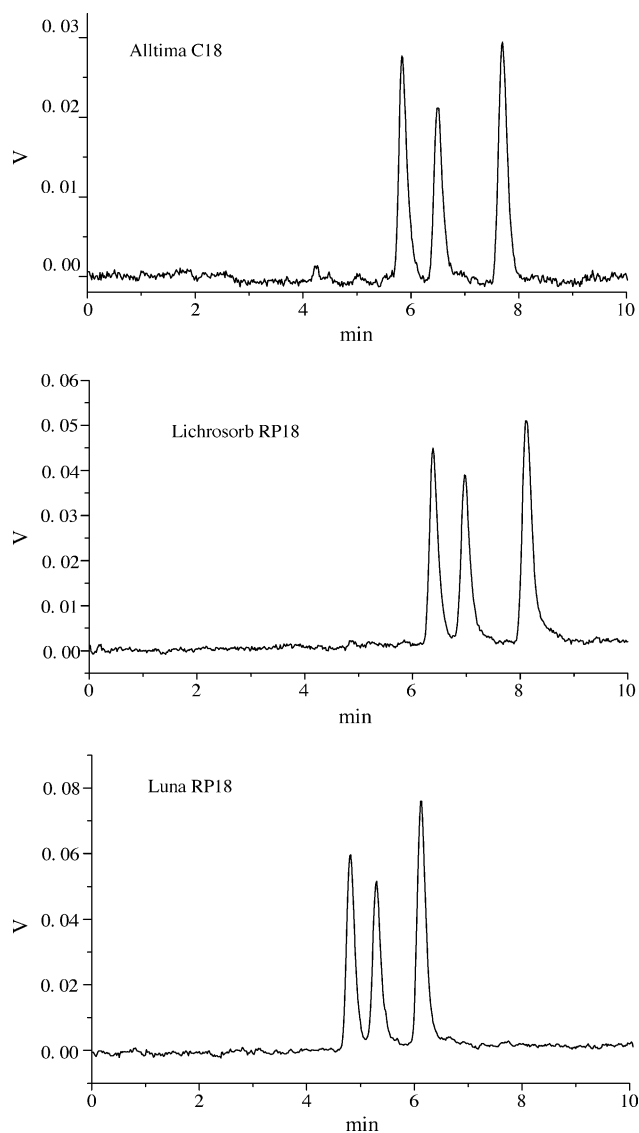


Fig. 4. The chromatograms of test mix 1 obtained by the home-made Alltima C18 phase ($0.5\ \mu$, $300\ \text{mm} \times 0.5\ \text{mm}$), Lichrosorb RP18 ($0.5\ \mu$, $300\ \text{mm} \times 0.5\ \text{mm}$), and the commercial Luna C18 ($0.5\ \mu$, $250\ \text{mm} \times 0.5\ \text{mm}$), in 85/15 (vol%) methanol/water at a flow rate of $10\ \mu\text{L}/\text{min}$. The peaks are eluted in the following order: *p*-nitroaniline, *m*-nitroaniline, *o*-nitroaniline.

with new ones a few times. The retention time reproducibility within a day was found generally better than 1.5% for test mix 2, and better than 3% for test mix 1. The long-term retention time reproducibility over 6 months was found generally better than 4% for test mix 2, and better than 6% for test mix 1.

The measured plate heights of the components of test mix 1 are comparatively assembled in Table 2, and those of the components of test mix 2, in Table 3. The typical chromatograms of *o*-, *m*-, *p*-nitroanilines obtained by home-made and commercial microcolumns are comparatively shown in Fig. 4. The typical chromatograms of test mix 2 are also compared in Fig. 5. Comparing separation efficiency of *o*-, *m*-, *p*-nitroanilines (Table 2 and Fig. 4) and test mix 2 (Table 3 and

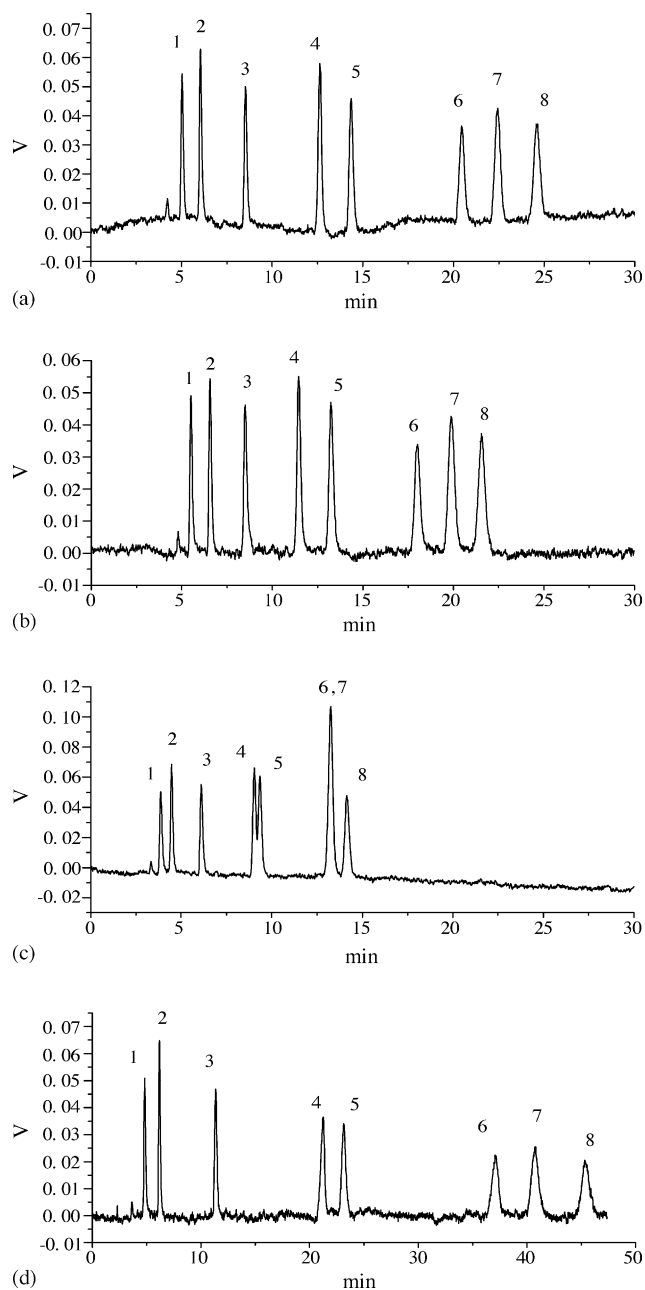


Fig. 5. The chromatograms of test mix 2 obtained by the home-made (a) Alltima C18 phase ($0.5\ \mu$, $300\ \text{mm} \times 0.5\ \text{mm}$), (b) Lichrosorb RP18 ($0.5\ \mu$, $300\ \text{mm} \times 0.5\ \text{mm}$), and (c) the commercial Luna C18 ($0.5\ \mu$, $250\ \text{mm} \times 0.5\ \text{mm}$), in 90/10 (vol%) methanol/water at a flow rate of $10\ \mu\text{L}/\text{min}$. Peaks (1) 4-methoxyphenol, (2) acetophenone, (3) ethylbenzoate, (4) ethylbenzene, (5) acenaphthylene, (6) acenaphthene, (7) phenanthrene, (8) anthracene.

Fig. 5) among home-made and commercial microcolumns, we may conclude that the home-made columns with the tubing/frits show as good separation efficiency as the commercial column. The column characteristics of the commercial column was such that retention in that column was much shorter than retention in other columns and some peaks were overlapped in the eluent of 90/10 vol% methanol/water (Fig. 5).

Its chromatographic efficiency and resolution was improved when 85/15 vol% methanol/water was used instead (Fig. 5d). The above evidences are, however, only necessary, not sufficient to prove the performance of tubing/frit.

In order to make sure that the tubing/frit does not degrade the column efficiency, the outlet tubing/frit of the Alltima C18 column was replaced by the silica capillary with a sintered silica frit [20], and the column separation efficiency was comparatively examined. The sintered silica frit in silica capillary is really tiny and is known to impose no bad effect on separation efficiency. No distinguishable difference was found in column efficiency between the tubing/frit and the silica capillary frit as shown in Table 3.

4. Conclusion

The column of the design of this study could be a cheap substitute for the conventional microcolumn. The tubing/frit of this study may serve a proper frit technique for a disposable microcolumn.

Acknowledgment

Support from InkTec Co. is appreciated.

References

- [1] F. Svec, J.M.J. Frechet, *Anal. Chem.* 64 (1992) 820.
- [2] H. Minakuchi, K. Nakanishi, N. Soga, N. Ishizuka, N. Tanaka, *Anal. Chem.* 68 (1996) 3498.
- [3] L.H. Mott, U.S. Patent 4,399,032 (1983).
- [4] R.W. Allington, A.Y. Tehrani, J.N. Jones, U.S. Patent 4,966,696 (1990).
- [5] P. Shepherd, U.S. Patent 5,227,059 (1993).
- [6] H. Cortes, C.D. Pfeiffer, B.E. Richter, T.S. Stevens, U.S. Patent 4,793,920 (1988).
- [7] H.J. Cortes, C.D. Pfeiffer, U.S. patent 5,679,255 (1997).
- [8] F.J. Yang, U.S. Patent 4,483,773 (1984).
- [9] B.M. Najafabadi, U.S. Patent 5,938,919 (1999).
- [10] J.P.C. Vissers, *J. Chromatogr. A* 856 (1999) 117.
- [11] K. Jinno, H. Sawada, *Trends Anal. Chem.* 19 (2000) 664.
- [12] K.D. Bartle, R.A. Carney, A. Cavazza, M.G. Cikalo, P. Myers, M.M. Robson, S.C.P. Roulin, K. Sealey, *J. Chromatogr. A* 892 (2000) 279.
- [13] R.J. Boughtflower, T. Underwood, C.J. Paterson, *Chromatographia* 40 (1995) 329.
- [14] N.W. Smith, M.B. Evans, *Chromatographia* 38 (1994) 649.
- [15] M. Dittmann, G. Rozing, K.K. Unger, T. Adam, U.S. Patent 5,858,241 (1999).
- [16] R.N. Zare, M.T. Dulay, R.P. Kulkarni, U.S. Patent 6,136,187 (2000).
- [17] S.M. Piraino, J.G. Dorsey, *Anal. Chem.* 75 (2003) 4292.
- [18] H. Minakuchi, K. Nakanishi, N. Soga, N. Ishizuka, N. Tanaka, *Anal. Chem.* 68 (1996) 3498.
- [19] K. Cabrera, *J. Sep. Sci.* 27 (2004) 843.
- [20] W.J. Cheong, G.W. Kang, W.L. Lee, J-S. Yoo, *J. Liq. Chromatogr. Relat. Technol.* 25 (2002) 1367.