Fabrication of Permanent Silver Cement Frit at the Inlet of Micro-Columns: A Significant Progress toward Realization of Disposable Micro-Columns

Hong Kyeong Jung, Miri Mun, Ashraf Ali and Won Jo Cheong*

Department of Chemistry, Inha University, 100 Inharo, Namku, Incheon 22212, South Korea

Received: 14 August 2018; accepted: 17 October 2018

The advent of disposable micro-columns will be a hope of workers of chromatography-related laboratories. A very critical and important requirement is the formation of affordable inlet frits. Welding a metal screen to a column inlet is not recommended because of the risk of damage to stationary phase. In this study, the Tollens probe (silver mirror reaction) was adopted to make affordable frits. Silver is reduced on the particle surface and in an empty space among the particles, forming a solid silver network structure at the column inlet area by injecting the reaction solution into the packed column at a depth of one third (10 cm) of the packed bed (0.5 mm × 300 mm). The silver cement structure was successfully formed, and the silver cement frit endured mobile phase flow well when C18 modified ground silica monolith particles were used to make the packed bed. The formation of the silver cement frit was not successful when the stationary phase based on conventional spherical silica particles was used. Negligible reduction of chromatographic performance by the silver cemented frit was observed. This study serves as the first step toward realization of disposable micro-columns.

Keywords: silver cement frit, silica monolith particles, Tollens probe, disposable micro-column

Introduction

The retaining frit is a part of a column located at the inlet and outlet, and it prevents the loss of stationary phase from the column in the flow of mobile phase. Various shapes and materials have been developed as frits for specific purposes of analysis [1]. Some frits are made of non-metallic materials such as Teflon to minimize the adsorption of biological samples. Porous disc-type frits made of stainless steel are placed in typical chromatography columns. Very thin commercial stainless-steel screen frits are commonly used in micro-columns.

Particularly, monolith columns do not require separate frits since the whole body of monolith with various multi-porous channels functions as a frit itself [2–3]. For more detailed information about monolithic columns, some recent review articles [4–9] may be referred to. Permanent monolith frits for packed columns have recently been employed and have become widespread especially in silica capillary columns [1, 10–12]. Advanced fritting techniques in separation science have been reviewed recently [1].

It has been a kind of stress to workers in the chromatography-related laboratories all over the world to maintain HPLC columns in good shapes. Cheap disposable columns may be very helpful. A disposable column is used only for a day especially for a series of similar samples (routine analysis) and disposed of or used on the purpose of practice in training courses. Disposable columns with moderate separation performance packed with low price stationary phase may be prepared at a much lower price, since most of the rigorous requirements for conventional columns will not be necessary for disposable columns. Disposable columns should be micro-columns of very narrow internal diameter (id) to minimize the required amount of stationary phase since the price of station-

ary phase is quite high. The micro-columns of 0.5-mm id were prepared in this study.

Our lab has been conducting studies on disposable micro-columns [13–17]. Two modes of disposable micro-columns are possible. In the first mode, a separate frit is used. The stationary phase of such a column can be discarded after one day use, and the column can be repacked with a new stationary phase for subsequent use. Disposable frits can be installed whenever the column is repacked. For this purpose, the organic monolith frits encapsulated in polymer tubing had been fabricated and sliced to serve as a cheap frit [16–17].

In the second mode, permanent frits are fabricated at the column inlet and outlet, and the entire column is discarded after being used for about 1 day. We fabricated a column with somewhat improved separation efficiency by welding commercial screen frits to the inlet and outlet of a stainless-steel column [15]. However, such welding technique could be used only for the column packed with bare silica particles but not for the column packed with ligand-modified silica particles due to the fear of damage to the stationary phase. This second mode may be regarded as the real type of disposable micro-columns, but its realization is far from completion yet. In this study, we fabricated a new type of frit by forming a permanent silver network structure in the inlet area of a packed column (0.5 mm × 300 mm) by making use of the Tollens probe reaction.

Experimental

Materials. Silver nitrate, 12 N ammonium hydroxide, acetic acid, phenol, acetophenone, 4-methyl-2-nitroaniline, benzene, toluene, acridine, benzoic acid, and bromobenzene were purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade methanol, acetonitrile, and water were obtained from Avantor (Phillipsburg, NJ, USA). Silica capillary with 50-μm id and 365-μm outer diameter (od) and glass lined

*Author for correspondence: wjcheong@inha.ac.kr.

This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (<https://creativecommons.org/licenses/by-nc/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium for non-commercial purposes, provided the original author and source are credited, a link to the CC License is provided, and changes - if any - are indicated.

stainless-steel tubing (30 cm, 0.5-mm id, and 1.6-mm od) were purchased from Grace (Deerfield, IL, USA). Screen frits (1.6-mm radius and 1- μ m pore size) were purchased from Valco (Houston, TX, USA). A LiChrospher RP-18 (end-capped, 5 μ m, 10 nm) phase was obtained from Merck (Darmstadt Germany). A Luna C18 column (0.5 mm $\times$ 250 mm, 5 μ m, 10 nm) was purchased from Phenomenex (Torrance, CA, USA). The C18 modified silica monolith particles (3.9 μ m) was prepared in our laboratory. We have been studying new stationary phases based on silica monolith particles [18–22], and the stationary phase of this study was prepared according to ref. 22. Briefly, the stationary phase was prepared as follows. A soft silica monolith was prepared by the traditional sol–gel process from a rather dilute reaction mixture, dried, ground smoothly, and calcined. Then, the silica monolith particles are classified into 3 different fractions by vigorous agitation, followed by differential sedimentation. The particles of the lower portion (heavier particles) are collected, dried, and reacted with the C18 derivatizing agent to give the final phase. More detailed procedures may be found in the ref. 22.

Column Packing. In order to pack a column, a commercial screen frit was placed in the outlet of a union (1/16 inch, 0.25 mm bore) installed on a piece of glass-lined stainless-steel tubing (1/16 inch od, 0.5 mm id, 30 cm). Two kinds of stationary phases were used: the C18 modified silica monolith particles (average particle size: 3.9 μ m) and the commercial silica particles (Lichrospher RP-18, 5 μ m). A 95-mg portion of particles was dried at 70 °C in a vacuum oven for 6 h. The slurry was made by suspending the dried particles (95 mg) in 1.35 mL methanol. The slurry was vortexed for 10 min to aid dispersion. The slurry was transferred into the reservoir of the slurry packer with a plastic syringe, and the empty column with a stainless-steel screen frit installed in the outlet union was instantly connected to the reservoir for packing. The initial packing pressure was 15,000 psi for 5 min, and then it was decreased to 12,000 psi and maintained for 10 min with continuous vibration on the column with two Alltech (Deerfield, IL, USA) GC column vibrators operated by two persons. Next, the pressure was further decreased to 10,000 psi and maintained for 30 min without vibration for conditioning of the bed. Finally, the slurry packer was turned off, and the system was left alone until the pressure dropped to 0 psi.

Fabrication of Silver Cement Frit. In order to prepare Tollens' reagent (20 mM), 170 mg of AgNO₃ was dissolved in 9.5 mL water, and then 0.5 mL of 12 N NH₄OH solution was added to form the final complex of [Ag(NH₃)₂]⁺. A 2.0-mL aliquot of this solution was mixed with 8.0 mL acetonitrile (ACN) to make the solution of 20-mM concentration (solution 1). To adjust the pH value of Tollens' reagent, acetic acid was added dropwise through a plastic syringe. The pH value of Tollens' reagent was decreased from 10.25 to 7.0–7.5. The pH meter from Orion (Beverly, MA, USA) was used to measure pH. The second solution of 94 mM D(-)-fructose (180 mg) was prepared in 10 mL of 90 % ACN (solution 2). The two solutions (1 and 2) were mixed in a 1:1 volume ratio to make the final reaction mixture (solution 3).

The reaction mixture was introduced in the packed column to a predetermined depth (10 cm) at the column inlet via a home-made mechanical syringe device for 1 h. The gas tight syringe from SGE (Melbourne, Australia) was used to inject a reaction mixture to the packed column. The column was cultivated at 80 °C for 4 h to carry out silver mirror reaction.

In addition to home-made C18 silica monolith particles, the conventional spherical C18 particles (Lichrosorb RP-18) were also used to pack the columns for comparison purposes. The same procedures of packing and frit formation were applied to both groups.

The chemical equation for the silver mirror reaction is as follows:

Apparatus and Analytical Conditions. A Shimadzu 10-AD pump (Tokyo, Japan) was used to deliver the mobile phase in high performance liquid chromatography. A Valco C14W.06 injector (Texas, USA) with a loop size of 0.06 μ L was used for sample injection. The detector was a UV-2075 UV/Vis detector from Jasco (Tokyo, Japan). A DGU-14A degasser from Shimadzu (Tokyo, Japan) was used to remove the air bubbles in the mobile phase. Chromatograms were collected and processed using Chromate Multicro 2000 version 4.3 (Yullin Technology, Seoul, Korea). An Alltech (Deerfield, IL, USA) slurry packer was used to pack the column. To dry the stationary phase particles, a SH-VDO8 vacuum oven (Saheung, Korea) was used.

The column was connected to a UV detector using a piece of connecting capillary (50 μ m id, 365 μ m od) installed in the 1/16 inch union. The analyte mixture was composed of pure phenol (8.8 μ L), acetophenone (1.4 μ L), 4-methyl-2-nitroaniline (3.2 mg), benzene (29.3 μ L), and toluene (14.6 μ L) and was stored at 4 °C. A 10- μ L aliquot of the mixture was taken and diluted with a 10-mL mobile phase for actual experiments. The mobile phase was 50:50 (v/v, %) acetonitrile–water with 0.1% TFA at a flow rate of 8 μ L/min. Another mixture of components of possible interferences with silver was prepared by dissolving acridine, benzoic acid, and bromobenzene (1 mg each) in 1 mL mobile phase.

Results and Discussion

The Tollens probe reaction was carried out to accumulate reduced silver atoms around the solid phase material. The reaction was activated by mixing a solution of diamine silver (I) complex ([Ag(NH₃)₂]⁺) (Tollens' reagent) with an aldehyde solution under a rather high temperature condition (80 °C). Since the reaction solution was injected into the column at a depth of one third of the packed bed, a solid silver network structure was presumably formed by deposition of reduced silver on the particle surface and in an empty space among the particles from the column end to the one-third depth of the packed bed.

The concentration of Tollens' reagent was adjusted so as to not block the column inlet, and the pH was lowered to 7.0–7.5 so as to not destroy the chemical structure of the stationary phase.

We have found that a unique structure of stationary phase particles is essential to form a silver network structure through a series of tests. High durability of silver network could be achieved by using irregularly shaped particles rather than spherical ones. When a commercial spherical C18 phase (Lichrospher RP-18, 5 μ m) was used, the cross-linked silver cement structure was not firmly formed, and the frit prepared by the silver mirror reaction with the Lichrospher RP-18 packed bed did not withstand the mobile phase flow resulting in an outburst of the stationary phase out of the column. On the other hand, the silver cement structure was successfully formed, and the silver cement frit endured the mobile phase flow well when C18 modified ground silica monolith particles were used to pack the column. It seems that some sufficient inter-particle spaces are essential to fabricate the silver cement network in the packed bed of the particles. Some through-flow channels are formed in the bed packed with the phase based

Figure 1. Formation of silver cement frit

on silica monolith particles, and such through-flow channels seem to make the strong silver cement network. More detailed information on C18 modified silica monolith particles can be found in previous research reports [18–22]. It should be noted that the column separation efficiency (number of theoretical plates, N) of this study is far inferior to that of ref. 22 since micro-columns ($0.5 \text{ mm} \times 300 \text{ mm}$) were packed in this study to minimize the required amount of stationary phase, while narrow bore columns ($1.0 \text{ mm} \times 300 \text{ mm}$) were packed to improve packing quality in ref. 22. However, the column separation efficiency of this study may be good enough for routine analysis.

To check the durability of silver cement frit, the column after frit treatment was connected to the LC pump by re-locating the silver cement frit as the outlet frit. The back pressure on column was monitored, while the flow rate of mobile phase was increased gradually from 0.002 mL/min to 0.025 mL/min . All the columns were tested under the same conditions. The columns packed with C18 modified silica monolith particles maintained the frit structure without any outburst of stationary phase even at a flow rate of 0.025 mL/min . The back pressure was 11 bar at a flow rate of $2 \text{ } \mu\text{L/min}$ and increased up to

92 bar as the flow rate was raised to $25 \text{ } \mu\text{L/min}$. On the other hand, the columns packed with LiChrospher RP-18 couldn't maintain the frit structure even at a low flow rate (0.005 mL/min , column back pressure: 25 bar).

The illustration of formation of silver cement frit on the inlet of packed bed of a micro-column is given in Figure 1. The syringe needle was tightly combined with the column inlet union while injecting the reaction mixture. The SEM images of silver cement frit formed inside the column are shown in Figure 2. The entire cross-section view of the frit is given in Figure 2a. The half cross-section view is given in Figure 2b. The enlarged photographs of silver cement frit are given in Figures 2c and 2d. The aggregated silver atoms are deposited on the surface of monolith particles and in the space among particles to form a solid network structure. The durability seems to arise not from the surface adhesion force but from the 3-dimensional silver cement network since the surface C18 ligand is not well compatible with silver. This silver network acts as a frit by holding stationary particles while letting the mobile phase flow freely.

We determined how this frit affects separation efficiency by examining the performance of the columns with 1 stainless-steel screen frit (outlet) and 1 silver cement frit (inlet) in comparison to the columns with 2 stainless-steel screen frits. The chromatographic data obtained with 5 packed columns are reported. Each column was prepared by packing the particles and installing 2 commercial stainless-steel screen frits. The performance of this column was examined first. Then, the reaction mixture of Tollens probe was applied to the column inlet, and the silver cement frit was formed. The stainless-steel screen frit at the relevant column end was removed, and the chromatographic performance of this column with a silver cement frit was examined. The whole procedure was repeated for 5 columns, and the two sets (denoted as SS and SC) of chromatographic data were compared in Table 1.

According to Table 1, the column separation efficiency obtained with the column of 2 screen frits was comparable to that obtained with the column of 1 screen frit and 1 silver cement frit. Their representative chromatograms are compared

Figure 2. SEM images of silver cement frit: (A) the entire cross-section view, (B) the half cross-section view, and (C and D) the enlarged views. The sizes of scale bar for A, B, C, and D are 500, 100, 10, and $3 \text{ } \mu\text{m}$, respectively

Table 1. Comparison of N values between the case of two screen frits and the case of a screen frit (column outlet) and a silver cement frit (column inlet) for the same column. Five different columns have been examined

Solute		Column #					
		1	2	3	4	5	Average ^b
Phenol	SS ^c	20,300	21,200	21,300	22,300	19,400	20,900 ± 1100
	SC ^d	20,000	21,200	20,300	20,100	21,600	20,600 ± 700
Acetophenone	SS ^c	21,500	22,400	22,500	22,900	22,200	22,300 ± 500
	SC ^d	21,600	23,600	21,100	22,400	22,800	22,300 ± 1000
4-Methyl-2-nitroaniline	SS ^c	21,300	23,100	24,500	23,300	20,800	22,600 ± 1500
	SC ^d	21,600	24,700	21,600	22,900	22,800	22,700 ± 1300
Benzene	SS ^c	20,300	21,200	21,700	22,900	22,800	21,800 ± 1100
	SC ^d	19,400	21,600	20,900	22,700	23,200	21,600 ± 1500
Toluene	SS ^c	20,100	21,300	23,700	23,800	24,300	22,600 ± 1800
	SC ^d	19,900	21,900	21,000	24,400	24,700	22,400 ± 2100
Average ^a	SS ^c	20,700 ± 700	21,800 ± 900	22,700 ± 1400	23,000 ± 600	21,900 ± 1900	22,000 ± 1200
	SC ^d	20,500 ± 1800	22,600 ± 1500	21,000 ± 500	22,500 ± 1500	23,000 ± 1100	21,900 ± 1300

^aAveraged among different analytes.^bAveraged among different columns. The average of the averages was also averaged among different columns.^cThe N data was obtained when the column was installed with 2 stainless-steel screen frits both at the column outlet and inlet.^dThe N data was obtained when the silver cement frit was formed at the column inlet, and the inlet screen frit was removed.

in Figure 3. Negligible reduction of chromatographic performance by the silver cemented frit was observed.

It should be noted that the silver cement frit has been developed as the inlet frit of disposable micro-columns. Such frit may not endure prolonged mobile phase flow. Nevertheless, disposable micro-columns are supposed to be used for a series of routine samples within a day or so, and the silver cement frit is good enough for such purpose.

The micro-column with the silver frit may also be subject to some troubles when it is used in trace analysis of silver ion or fructose (chemicals of silver mirror reaction) or components capable of chemical interactions with them. Disposable columns may have to be used for routine analysis without such

troubles. Silver or silver ions may form complex material with biological polymers (such as proteins, peptides, and saccharides), basic compounds, carboxylic acids, halogen-containing compounds, etc. Leaving out polymeric compounds, three simple analytes were selected to check the possible interferences: acridine, benzoic acid, and bromobenzene. The data of separation efficiency (number of theoretical plates, N), asymmetry factor (As), resolution (Rs), retention time, area, and height count were all obtained.

$$N = 5.54(t_r/W_{1/2})^2$$

$$As = b/a$$

$$Rs = 2(t_{r,b} - t_{r,a})/(W_a + W_b)$$

t_r is the retention time of a single analyte, $W_{1/2}$ is its peak bandwidth at half height, a is the distance from the peak vertical line to the left intersection at 10% height, b is the distance from the peak vertical line to the right intersection at 10% height, $t_{r,a}$ and $t_{r,b}$ are the retention times of a pair of analytes, and W_a and W_b are their base bandwidths.

The data were assembled in Table 2, Table 3, and Figure 4. As shown in Figure 4, the chromatogram obtained with the column of 2 screen frits and the chromatogram obtained with the column of 1 screen frit and 1 silver cement frit look very similar except for the retention times. The retention times obtained with the silver cement frit were systematically less than the counterparts. It is reasonable since the column void volume is reduced by the formation of silver cement frit. According to Table 2, the average N value was 15,600, both for the group of 2 screen frits (group SS) and for the group of 1 screen frit and 1 silver cement frit (group SC). The average asymmetry factors for group SS and group SC were 1.87 and 1.64, respectively, while the average chromatographic resolution

Figure 3. Comparison of chromatograms obtained for common analytes at 214 nm with the column with 2 screen frits (bottom) and the same column with 1 screen frit and 1 silver cement frit (top) in 50:50 (v/v, %) acetonitrile–water with 0.1% TFA at a flow rate of 8 μ L/min. Elution order: phenol, acetophenone, 4-methyl-2-nitroaniline, benzene, toluene**Table 2.** Comparison of the data of column separation efficiency (number of plates, N), asymmetry factor, and resolution (Rs) of troublesome analytes between the group of 5 columns with two stainless-steel screen frits and the group of 5 columns with a stainless-steel screen frit and a silver cement frit

	Column plates number (N)		Asymmetry factor (As)		Resolution (Rs)	
	SS ^a	SC ^b	SS ^a	SC ^b	SS _a	SC ^b
Acridine	9900 ± 1400	11,700 ± 800	2.97 ± 0.26	2.37 ± 0.36		
Benzoic acid	15,800 ± 700	16,000 ± 1400	1.42 ± 0.08	1.31 ± 0.08	2.59 ± 0.11	2.61 ± 0.14
Bromobenzene	21,000 ± 1300	19,200 ± 900	1.23 ± 0.04	1.25 ± 0.07	20.49 ± 0.40	18.40 ± 0.88
Average	15,600	15,600	1.87	1.64	11.5	10.5

^aThe group of columns with 2 stainless-steel screen frits.^bThe group of columns with a stainless-steel screen frit and a silver cement frit.

Table 3. Comparison of the data of retention time, area count, and height count of troublesome analytes between the group of 5 columns with 2 stainless-steel screen frits and the group of 5 columns with a stainless-steel screen frit and a silver cement frit

	Retention time (min)		Area count ($\mu\text{V} \times \text{s}$)		Height count (μV)	
	SS ^a	SC ^b	SS ^a	SC ^b	SS _a	SC ^b
Acridine	8.71 $\pm$ 0.25	8.41 $\pm$ 0.07	199,000 $\pm$ 15,000	183,000 $\pm$ 12,000	13,400 $\pm$ 700	13,600 $\pm$ 800
Benzoic acid	9.66 $\pm$ 0.28	9.24 $\pm$ 0.07	315,000 $\pm$ 13,000	301,000 $\pm$ 13,000	26,700 $\pm$ 700	27,100 $\pm$ 1300
Bromobenzene	18.21 $\pm$ 0.43	16.60 $\pm$ 0.14	202,000 $\pm$ 15,000	197,000 $\pm$ 14,000	10,700 $\pm$ 600	10,900 $\pm$ 700
Average	12.2	11.4	239,000	227,000	16,900	17,200

^aThe group of columns with two stainless-steel screen frits.^bThe group of columns with a stainless-steel screen frit and a silver cement frit.

values for group SS and group SC were 11.5 and 10.5, respectively. The asymmetry factor results imply that the specific adsorption effect of silver cement is less significant than that of bare stationary phase. Thus, it seems that the reduced average resolution of Group SC is mostly owing to the average

reduced retention time (11.4 min vs 12.2 min, Table 3). It is interesting to note that the average area count of group SC was a little smaller (227,000) than that of group SS (239,000), while the average height count of group SC was a little greater (17,200) than that of group SS (16,900). Considering all the observations, it may be concluded that the formation of silver cement frit causes reduced analyte retention but no other significant interference.

For comparison purposes, a commercial column of a similar physical dimension to ours was selected, and its chromatographic performance was examined for our first test mixture. A serious peak overlap (acridine and benzoic acid) was observed for the second test mixture, and thus, only the first test mixture was used for evaluation. The used column was a Phenomenex Luna C18 column (5- μm particle size, 10-nm pore size, 0.5 mm $\times$ 250 mm, carbon load 17.5%). The averages and standard deviations of *N* values based on 3 replicate measurements in 50:50 acetonitrile–water with 0.1% TFA at a flow rate of 8 $\mu\text{L}/\text{min}$ are summarized in Table 4. The relevant chromatogram is shown in Figure 5. The retention

Figure 4. Comparison of chromatograms obtained for possibly troublesome analytes at 214 nm with the column with 2 screen frits (top) and the same column with 1 screen frit and 1 silver cement frit (bottom) in 50:50 (v/v, %) acetonitrile–water with 0.1% TFA at a flow rate of 8 $\mu\text{L}/\text{min}$. Elution order: acridine, benzoic acid, bromobenzene **Figure 5.** The chromatogram obtained with a Luna C18 column (5 μm , 10 nm, 0.5 $\times$ 250 mm) at 214 nm in 50:50 (v/v, %) acetonitrile–water with 0.1% TFA at a flow rate of 8 $\mu\text{L}/\text{min}$. Elution order: phenol, acetophenone, 4-methyl-2-nitroaniline, benzene, toluene**Table 4.** *N* values of the 5 analytes obtained with a commercial column (Luna C18, 5 μm , 10 nm, 0.5 mm $\times$ 250 mm) in 50:50 acetonitrile–water with 0.1% TFA at a flow rate of 8 $\mu\text{L}/\text{min}$

Solute	Run #			
	1	2	3	Average ^b
Phenol	15,100	15,200	14,600	15,300 $\pm$ 300
Acetophenone	16,900	17,800	16,600	17,100 $\pm$ 600
4-Methyl-2-nitroaniline	17,100	18,600	17,100	17,600 $\pm$ 900
Benzene	16,700	17,300	17,000	17,000 $\pm$ 300
Toluene	15,500	15,600	15,500	15,500 $\pm$ 100
Average ^a	16,300 $\pm$ 900	16,900 $\pm$ 1500	16,200 $\pm$ 1100	16,500 $\pm$ 440

^aAveraged among different analytes.^bAveraged among different runs. The average of the averages was averaged among different runs.

time range of Figure 5 is greater than that of Figure 3, since the carbon load of the column of Figure 5 is 17.5%, while that of Figure 3 is only 6.8%. The overall average of N among different runs was found $16,500 \pm 440$ (Table 4). The column length of Figure 5 is 250 mm, while that of Figure 3 is 300 mm. The hypothetical N for a column of 300 mm packed with the Luna C18 phase would be $19,800 \pm 530$.

In order to evaluate whether there is a significant difference in column separation efficiency between our column ($21,900 \pm 1,300$, $n_1 = 5$) and the commercial column ($19,800 \pm 530$, $n_2 = 3$), “ t -test” was carried out.

The t statistic to test whether the means are different can be calculated as follows [23]:

$$t = \frac{\bar{X}_1 - \bar{X}_2}{s_p \cdot \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$$

where

$$s_p = \sqrt{\frac{(n_1 - 1)s_{X_1}^2 + (n_2 - 1)s_{X_2}^2}{n_1 + n_2 - 2}}$$

Here, s_p is an estimator of the pooled standard deviation of the two samples; it is defined in this way so that its square is an unbiased estimator of the common variance whether or not the population means are the same. In these formulae, $n_i - 1$ is the number of degrees of freedom for each group, and the total sample size minus two (that is, $n_1 + n_2 - 2$) is the total number of degrees of freedom, which is used in significance testing. $\bar{X}_1$ and $\bar{X}_2$ are the sample means for the two samples, and s_{X_1} and s_{X_2} are the unbiased estimators of the variances of the two samples. The denominator of t is the standard error of the difference between two means.

The computed t value was 2.62 and the table t values at 90, 95, and 99% confidence levels were 1.90, 2.45, and 3.71, respectively. The separation efficiency of our column was significantly greater than that of the commercial column at 90 and 95% confidence levels. The same conclusion was obtained when the standard deviations among different analytes were used instead.

The “ t -test” was also carried out for the data of Table 1 between the case of two screen frits ($22,000 \pm 1,200$) and the case of a screen frit and a silver cement frit ($21,900 \pm 1,300$). The computed t value was 0.13, and the table t values at 90, 95, and 99% confidence levels were 1.86, 2.30, and 3.36, respectively, proving that there is no significant difference between the two cases. Again, the same conclusion was obtained when the standard deviations among different analytes were used instead.

This study suggests some promising vision in realization of disposable micro-columns in the near future by the incorporation of silver cement frits. Development of a real disposable micro-column with a welded stainless-steel frit at the column outlet and a silver cement frit at the column inlet and its application will be the topic of the next study.

Acknowledgment. This research was financially aided by the Inha University Fund.

References

- Cheong, W. J. *J. Sep. Sci.* **2014**, *37*, 603–617.
- Svec, F.; Frechet, J. M. J. *Anal. Chem.*, **1992**, *64*, 820–822.
- Minakuchi, H.; Nakanishi, N.; Soga, N.; Ishizuka, N.; Tanaka, N. *Anal. Chem.* **1996**, *68*, 3498–3501.
- Ongkudon, C. M.; Kansil, T.; Wong, C. *J. Sep. Sci.* **2014**, *37*, 455–464.
- Liu, K.; Aggarwal, P.; Lawson, J. S.; Tolley, H. D.; Lee, M. L. *J. Sep. Sci.* **2013**, *36*, 2767–2781.
- Rozenbrand, J.; van Bennekom, W. P. *J. Sep. Sci.* **2011**, *34*, 1934–1944.
- Ghanem, A.; Ikegami, T. *J. Sep. Sci.* **2011**, *34*, 1945–1957.
- Hong, T.; Yang, X.; Xu, Y.; Ji, Y. *Anal. Chim. Acta* **2016**, *931*, 1–24.
- Svec, F. *Electrophoresis* **2017**, *38*, 2810–2820.
- Chen, J. R.; Zare, R. N.; Peters, E. C.; Svec, F.; Frechet, J. M. J. *Anal. Chem.* **2001**, *73*, 1987–1992.
- Chen, J. R.; Dulay, M. T.; Zare, R. N.; Svec, F.; Peters, E. *Anal. Chem.* **2000**, *72*, 1224–1227.
- Zeng, S. L.; Chen, C. H.; Santiago, J. G.; Chen, J. R.; Zare, R. N.; Tripp, J. A.; Svec, F.; Frechet, J. M. J. *Sens. Actuator B-Chem.* **2002**, *82*, 209–213.
- Cheong, W. J.; Kang, G. W.; Lee, W. L.; Yoo, J.-S. *J. Liq. Chrom. Rel. Technol.* **2002**, *25*, 1367–1378.
- Cheong, W. J. *J. Chromatogr. A* **2005**, *1066*, 231–237.
- Hong, S. H.; Cheong, W. J. *J. Sep. Sci.* **2016**, *39*, 243–246.
- Park, S. Y.; Cheong, W. J. *J. Sep. Sci.* **2015**, *38*, 2938–2944.
- Park, S. Y.; Cheong, W. J. *J. Sep. Sci.* **2016**, *39*, 1799–1803.
- Ko, J. H.; Baik, Y. S.; Park, S. T.; Cheong, W. J. *J. Chromatogr. A* **2007**, *1144*, 269–274.
- Han, K. M.; Cheong, W. J. *Bull. Korean Chem. Soc.* **2008**, *29*, 2281–2284.
- Faiz, A.; Cheong, W. J. *J. Sep. Sci.* **2014**, *37*, 3426–3434.
- Ali, A.; Cheong, W. J. *Bull. Korean Chem. Soc.* **2017**, *38*, 919–927.
- Ali, A.; Ali, F.; Cheong, W. J. *J. Chromatogr. A* **2017**, *1525*, 79–86.
- Wikipedia article “Student’s t -test”.