Surface treatment effects in microcolumn liquid chromatography

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ABSTRACT

Packed fused-silica columns with several surface pretreatments are prepared for micro liquid chromatography. Untreated fused silica is compared to polymethylhydrosiloxane-deactivated tubing as well as a polymer-coated commercially available capillary GC column. Columns are packed under identical conditions with 5-μm reversed-phase material. The chromatographic and physical properties of these columns are examined. Surface pretreatments have minimal effects on the chromatographic characteristics of packed fused-silica columns but substantial effects on the physical capabilities.

INTRODUCTION

Microcolumn liquid chromatography is a rapidly growing analytical technique. From its beginning in the mid 1970s [1], miniaturization of high-performance liquid chromatography (HPLC) has held great promise. One of the most important developments for micro chromatography came about with the invention of the fused-silica column [2]. This led to the landmark work of Takeuchi and Ishii [3] and Yang [4]. In these papers, high efficiency (100 000 theoretical plates) columns are prepared with reasonable operating pressures. Since that time, fused silica has been the most widely used column material, mainly due to its smooth inner wall and flexibility.

In transferring steel column packing technology to fused-silica columns, a major problem is encountered. The most popular steel column packing technique [5] employs a large flow-rate, constant pressure pump. Steel columns are typically packed at 8000 to 12 000 p.s.i. This packing pressure is applied instantaneously and held for a designated period of time (30–60 min). The high pressure is required to create a stable, highly efficient column bed. Fused silica, however, fails at much lower packing pressures.

The purpose of this work is to examine the chromatographic and physical properties of packed fused-silica columns with different wall pretreatments. Column efficiency, permeability and activity illustrate chromatographic characteristics while pressure and packing tests demonstrate physical parameters.

EXPERIMENTAL

Apparatus

The liquid chromatograph consisted of an EM Science MACS 500 microbore pump. Injections were made with a Rheodyne 7520 0.2-μl fixed loop valve and an on-column injection volume of 0.08 μl was achieved with a timed split. Detection was performed by a Hewlett-Packard 1050 multiple wavelength detector fitted with a micro flow cell. Column connection were made with PEEK fittings from Upchurch Scientific (Oak Harbor, WA, U.S.A.). Column packing and pressure testin employed a Chemco Econopacker CPP-085 high-pressure pneumatic pump. Gas chromatography (GC) was performed on a Hewlett-Packard 5890A system.

Materials

All solvents were Burdick and Jackson (Muskegon, MI, U.S.A.) HPLC grade and used as received. The packing material was 5-μm spherical ODS Hypersil from Shandon (Cheshire, U.K.). Untreated fused-silica tubing (0.30 mm I.D. $\times$ 0.450 mm O.D.) and the coated column (Ultra 2) were acquired from Hewlett-Packard.

Column preparation and evaluation

The untreated fused silica and the Ultra 2 column (0.52 μm film thickness) were used as received. The polymethylhydrosiloxane (PMHS) column blanks were prepared as described by Kong *et al*. [6] and gave a nominal film thickness of 0.03 μm. In order to examine the activity of the column blank material, a gas chromatographic analysis was performed. A 1 m length of each type of column blank was installed in a HP5890A GC apparatus and 1-octanol was used as the test probe. Peak asymmetry was measured according to Lee *et al*. [7]. For the chromatographic evaluation, 50 mg of packing material was slurried in 200 μl of 2-propanol. The slurry was then suspended and placed into a steel reservoir. Packing was performed at 3000 p.s.i. liquid pressure with acetonitrile as the displacement fluid in a downwards technique [5]. For the pressure test the column blank was connected to the packing pump and an old packed HPLC column for a source of backpressure. No packing material was used and the pressure ramped from 3000 to 9000 p.s.i. at a rate of 1500 p.s.i./min. The packing test used the same slurry as mentioned previously but 6000 p.s.i. of displacement fluid pressure was used.

RESULTS AND DISCUSSION

Chromatographic testing

Fig. 1 shows the typical surface composition of the three column materials. Untreated fused silica is comprised of acidic surface silanols (both geminal and vicinal), inert siloxane bridges, and highly acidic hydrogen bonding sites. The PMHS surface consists of a thin film of methyl–hydrogen silicone polymer that can be chemically bound to the surface or crosslinked via reactive Si–H bonds. Because of the relatively thin film and the possibility of unreacted surface silanols, the PMHS material may still be capable of polar interactions with the solutes. The polymer coated material consists of the untreated fused silica covered with an adhered film of 5% phenylmethylpolysiloxane (SE-54).
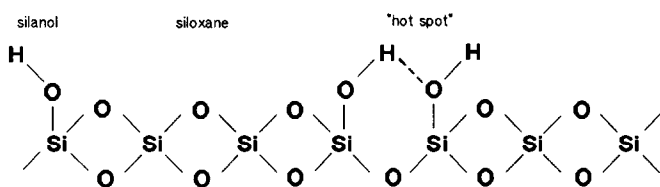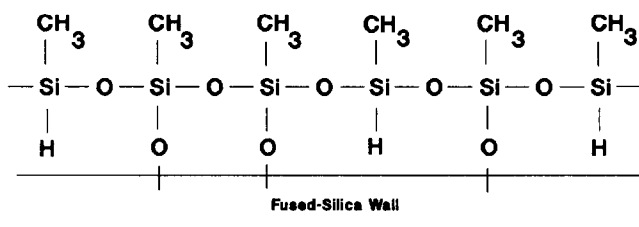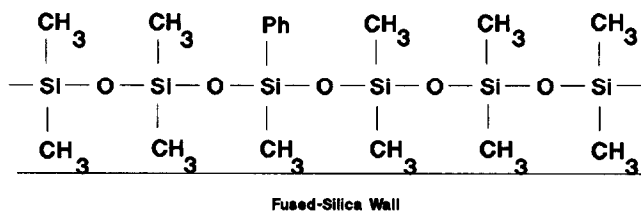
Fig. 1. Surface composition of column blanks.

The results of the gas chromatographic analysis are given in Table I. A statistically significant difference is obtained for each column type. However, the difference is minimal and in the transition from GC to HPLC, several factors decrease the column wall activity. First, the decrease in solute diffusion coefficient by three to four orders of magnitude minimize the chance for solutes to interact with the wall. In addition, the presence of acetonitrile and water as the mobile phase can decrease wall activity. These polar mobile phases can adsorb to the active sites on the surface and essentially eliminate them. Perhaps the greatest factor occurs when the column is packed with stationary phase. The number of silanols remaining on the packing material is at least 1000 times greater than the amount of silanols on the column wall.

TABLE I

COLUMN BLANK ACTIVITY BY GC

Sample: 1-octanol; column temperature: 90°C; injection temperature: 175°C; detector temperature: 250°C; split: 190 to 1.

Column material	Peak asymmetry $\pm$ S.D. ($n = 3$)
Untreated	1.22 ± 0.03
PMHS	1.16 ± 0.04
Ultra 2	1.09 ± 0.03

The column resistance factor, Φ, was also determined. Column resistance is defined by Snyder and Kirkland [5] as

$$\Phi = \frac{0.1 \, \Delta P \, t_0 \, d_p^2}{n \, L^2 \, 10^9}$$

where ΔP = pressure drop (bar); t_0 = dead time (s); d_p = particle diameter (μm); n = viscosity (cP) and L = column length (m). To make a qualitative comparison, the values of Φ for each column type are calculated and normalized to the value obtained for the untreated fused silica. The results are given in Table II. The untreated and PMHS columns are extremely similar in term sof column resistance. Since the film thickness is so small in the PMHS column, it is anticipated that its contribution to backpressure will be negligible. In the polymer-coated column, however, a much thicker film is present. Most siloxane polymers used as GC phases are very viscous and present a considerable effect on column backpressure. The crosslinked polymeric layer creates drag and thus, retards the passage of mobile phase. This is verified by the experimental results as the column resistance for the polymer-coated column increases by 30% over the untreated column. An additional effect that could decrease column permeability is the elasticity of the polymeric film. As suggested by Verzele *et al.* [8], the packing particles may be able to press into the column coating and obtain a slightly greater particle density at the walls. Normally, the wall imposed packing structure is less dense than the particle imposed packing structure [9]; therefore, flow velocities near the wall are greater than in the center of the column. In any case, the use of a coated, crosslinked GC column for the blank material will increase the column resistance. For short columns, this poses no significant problem. Packed capillary

TABLE II

MICROCOLUMN RESISTANCE (Φ)

Column material	Relative resistance
Untreated	1.00
PMHS	1.01
Ultra 2	1.34

TABLE III

WALL COATING EFFECTS ON CHROMATOGRAPHY

Adapted from ref. 8. k' = Capacity factor; A_s = peak asymmetry; H = plate height.

Compound	Superox-20M			RSL-300			RSL-150		
	k'	A_s	H	k'	A_s	H	k'	A_s	H
Phenol	0.47	1.65	0.044	0.47	1.80	0.037	0.48	1.74	0.037
Benzaldehyde	0.84	1.49	0.041	0.84	1.32	0.037	0.86	1.64	0.033
NNdpT[a]	1.11	1.46	0.031	1.10	1.56	0.026	1.13	1.51	0.027
Toluene	2.64	1.21	0.014	2.62	1.26	0.012	2.71	1.28	0.011
Ethylbenzene	3.99	1.18	0.012	3.87	1.25	0.011	4.03	1.30	0.010

[a] N,N-diethyl-p-toluamide.

columns, though, are typically used in long lengths for greater efficiency. In this instance, an increase in column backpressure may not be so tolerable.

The effects of the polymer layer on retention, asymmetry and plate height are illustrated by the research of Verzele and co-workers [8]. The results of their study are given in Table III.

The coated polymers are of different polarities, ranging from a non-polar polymethylsiloxane to a polar polyethylene glycol. Note that the capacity factor for each solute does not change with the different polymers. In addition, the asymmetry factors for each probe are quite similar on the various columns. The largest relative standard deviation (R.S.D.) for peak asymmetry is only 10.8% for benzaldehyde. All other R.S.D. values are below 5%. While the wall coating is different for each column, the type and strength of interaction between solute and micropacked column (both wall and stationary phase) are relatively constant. Finally, the plate height (H) is given for each case. A comparison of H for ethylbenzene on each column shows that, theoretically, optimum efficiency is attainable. For 5-μm particles, the reduced plate height, h, should give a value of 2. The average value for these columns is 2.25. Consequently, the use of an inner wall coating is not significantly detrimental to the chromatographic performance of the microcolumn.

Physical testing

The second aspect of this study involves physical testing of the column types to elucidate the effect of surface pretreatment. Two specific tests are performed, a blank column pressure test and a column packing test. After each test is performed an empirical assessment of flexibility is made.

The pressure test results are given in Table IV. The untreated fused silica repeatedly failed at 8250 p.s.i. Since stainless-steel HPLC fittings are standardized to withstand up to 6000 p.s.i., the absolute strength of the fused silica appears to be sufficient. In terms of flexibility, the fragments break upon bending but the polyimide remains intact. The PMHS material also failed at this pressure. When the pieces were flexed, they break crisply. Not only the fused silica weakened but the polyimide exterior coating is also brittle. Because the film is too thin to provide additional mechanical stability, the column blank fails at the same pressure as untreated fused silica. Perhaps the temperature curing of the PMHS column causes the polyimide to

TABLE IV

FUSED-SILICA BLANK TEST

Conditions: acetonitrile as pumping fluid; ramp from 3000 to 9000 p.s.i. at 1500 p.s.i./min and hold at 9000 p.s.i.

Column material	Highest attainable pressure (p.s.i.)
Untreated	8250
PMHS	8250
Ultra 2	>9000

weaken. The polymer coated column blanks, however, demonstrate far greater tensile strength under high pressure. At 9000 p.s.i. none of the test columns failed. In terms of flexibility, the Ultra 2 column is as resilient as an untested piece of the same material.

The packing test results are given in Table V. Ten columns of each type of material are made by typical packing conditions and the percentage of failures are listed. The untreated fused silica has a very high failure rate. The reason behind this is partially explained by Han and Armstrong [10]. Electron microscopy shows that cracks are formed by the packing process. Han and Armstrong suggest that the cracking could be caused by particle-induced scoring of the inner walls, the high pressure of packing, or localized high pressure points. If the cracks are formed by the high pressure of packing, then the pressure test should give 6000 p.s.i. as the highest attainable pressure. Since this is not the case, this explanation can be rejected. If the cracks are caused by localized high pressure points, then the various column materials should have similar failure rates. While 90% of the untreated columns failed, only 30% of the PMHS columns failed. These column materials have the same highest attainable pressure but not the same column success rate. The thin deactivation layer adds mechanical strength to the column; however, the walls are not completely protected from the particle-induced scoring. This scoring phenomenon appears to be the main culprit of column failure. Essentially, the packing particle scratches the inner wall and the hydrated fissure then propagates radially. The relatively thick film of the Ultra 2 column almosts eliminates this problem as evidenced by its high success rate.

TABLE V

PACKING TEST

Conditions: constant pressure packing at 6000 p.s.i.; slurry ratio: 1 g/4 ml; slurry solvent: isopropanol; displacement solvent: acetonitrile.

Column material	Failures (%)
Untreated	>90
PMHS	30
Ultra 2	10

CONCLUSIONS

The first aspect of this study demonstrated that the wall coating of the column blank before packing does not significantly diminish the chromatographic performance of the microcolumn. However, the wall coating does substantially improve column flexibility and durability.

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