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COMPARING BOROSILICATE AND SODA-LIME GLASS FOR FABRICATING OPEN-TUBULAR LIQUID CHROMATOGRAPHIC COLUMNS

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SUMMARY

A comparison was made between reversed-phase, open-tubular liquid chromatographic columns fabricated from borosilicate glass (modified by an electro-etching technique) and from soda-lime glass (modified with sodium hydroxide). Non-uniform etching was observed throughout the length of borosilicate glass columns. Soda-lime glass columns exhibited slightly higher retention (k') of test solutes, but the column modification procedure was plagued by a high incidence of column clogging. Borosilicate glass will probably be the best material for fabricating efficient open-tubular columns with inner diameters of 10 μm or less, primarily due to the high incidence of column clogging during column modification procedures for soda-lime glass.

INTRODUCTION

Many advances have been made in the field of open-tubular liquid chromatography (OTLC) since it was first reported by Nota *et al.*[1] in 1970 and later developed by Ishii and co-workers[2–5]. Chromatographic theory has been derived that describes LC separations performed in microcapillary open-tubular columns[6,7]. This theory indicates that the dimensions of OTLC columns will have to be reduced to 10 μm or less before the column efficiency surpasses that of packed LC columns of the same length. These theoretical studies also indicate that these columns require new or modified detection methods due to minute column dimensions and low volumetric flow-rates (< 1 μl/min).

Early work in the field of OTLC emphasized the development and evaluation of chemically coated and chemically bonded columns of large dimension ($d_c > 30$ μm, were d_c is the inner diameter of the column). Separations demonstrating several hundred thousand theoretical plates were achieved, but these separations required very long columns and lengthy analysis times, due to the inefficiency of large-bore OTLC columns. Detection was usually achieved by measuring the absorbance of eluted solutes in a capillary quartz tube that was connected to the end of the column.

Once it had been adequately demonstrated that open-tubular columns could be fabricated and used to achieve impressive separations, emphasis in the field shifted from column technology to the development of sensitive, low-volume detectors that would be suitable for use with the smaller, more efficient columns ($d_c < 10\ \mu m$). Sensitive detection with columns of $d_c < 15\ \mu m$ has been demonstrated by electrochemical[8,9] and laser-induced fluorescence[10–14] detection methods. Both detection methods can be performed on-column, thus eliminating any band broadening of chromatographic peaks that may be introduced by using a flow-cell attached to the end of the column.

Now that suitable detection methods are available, more emphasis needs to be placed on column technology. Various glass types and chemical modification procedures have been used to fabricate OTLC columns, but there have not been any detailed comparisons to determine if any of the fabricated columns are superior to the others. Likewise, satisfactory discussion is lacking for problems that are encountered with the chemical modification procedures for different glass types. Comparisons of column performance between columns that have been reported in the literature are usually not possible, since the associated column evaluation data and chromatograms usually involve the separation of different test solutes, using different mobile phases, and with columns of differing inner diameters.

OTLC columns should have a bonded stationary phase, rather than coated, if stable and reproducible performance is to be achieved. In addition, the columns should have the largest concentration of bonded stationary phase on the surface that is possible. The capacity factor (k') is directly related to the concentration of stationary phase. Reversed-phase columns that have a low concentration of stationary phase will require an increase in the water content in organic–water mobile phases to obtain satisfactory solute retention. This can create serious sample solubility problems, because the polarity of the sample solvent must be similar to that of the mobile phase or the sample will travel through the column as a "plug" and not mix appreciably with the surrounding mobile phase. Non-extractable, bonded polymeric stationary phases (such as those used in supercritical-fluid chromatography) would seem to be ideal for use with OTLC columns. Unfortunately, these phases exhibit very poor retention of non-polar solutes [such as polycyclic aromatic hydrocarbons (PAH)] because none of the stationary phases that have been synthesized contain the long hydrocarbon chains that are necessary for high retention. Ishii and co-workers[5,15] observed much higher retention of PAH compounds on an octadecyl-bonded-phase OTLC column than they did with columns that had bonded, cross-linked stationary phases of SE-54, OV-1, OV-17, and OV-25. Columns that have been dynamically modified with long-chain quaternary ammonium bromides also exhibit decreased solute retention, compared to ODS-bonded columns[16]. This decrease may be due to smaller surface coverage by the stationary phase in dynamically modified silica columns, as opposed to columns with bonded stationary phase.

The previous references illustrate the importance of selecting a glass type and chemical modification procedure that will provide maximum surface coverage of bonded stationary phase groups. The glass type and chemical modification procedure that is used should be selected so that a maximum concentration of surface silanol groups are obtained. The optical properties and flexibility of fused-silica tubing would make it appear to be ideal for fabricating OTLC columns; but, unfortunately, this glass has too few silanol groups and the surface composition of the glass is too free from leachable impurities to generate a sufficient number of silanol groups to form a monolayer-bonded-phase column with high solute retention.

Borosilicate and soda-lime glasses are widely used to fabricate OTLC columns. This is because both glass types are commercially available in suitable dimensions for drawing columns and because both glass types contain enough surface impurities (B_2O_3 in borosilicate glass, and Na_2O and CaO in soda-lime glass) that can be chemically leached to form a surface with a large number of silanol groups. Ref. 17 is an excellent source of information concerning the chemical structure of various glass types and the effects that various chemical reagents have on the surface of these glasses. Treatment of the glass surface with dilute sodium hydroxide appears to be the best method for modifying soda-lime glass[2,18], while an electro-etching technique appears to be the best method for chemically modifying borosilicate glass[19].

This report will compare the degree of solute retention that is obtained with ODS-bonded OTLC columns that have been fabricated from borosilicate glass (chemically modified by the electro-etching technique) and soda-lime glass (chemically modified with sodium hydroxide). The advantages and disadvantages of each glass type and its associated chemical modification procedure will be discussed.

EXPERIMENTAL

Borosilicate and soda-lime glass capillary tubing (Vitro Dynamics, Rockaway, NJ, U.S.A.) were drawn to a finished size of *ca.* 600 μm O.D. × 30 μm I.D. with a glass drawing machine (Model GDM-1B, Shimadzu, Kyoto, Japan).

Soda-lime glass columns were chemically etched by filling the column with 0.3 *M* sodium hydroxide, sealing the ends in a flame, and then heating the column at 50°C or 65°C for a specific length of time. Precautions must be taken to minimize the amount of carbonate (formed from dissolved carbon dioxide) in the sodium hydroxide solution. Insoluble calcium carbonate can form when calcium oxide is leached from the glass surface, and it can accumulate to plug the column irreversibly. After the etching treatment was completed, the column was rinsed with water (until the column effluent was neutral), methanol, and 0.1% (v/v) hydrochloric acid in methanol (until the column effluent was $<$pH 3). The column was then dehydrated with purified nitrogen gas at 140°C for several hours.

Borosilicate columns were chemically etched by an electro-etching technique[19]. The borosilicate glass columns were first heated at 600°C for 24 h to enhance the surface concentration of leachable B_2O_3 groups. After heating, the column inlet was attached to a pressurized reservoir, and a phosphate buffer solution (0.2 *M*, pH 7) was passed through the column. The column (excluding inlet and exit ends) was immersed in a dilute hydrochloric acid bath (2 *M*, 85°C) during this procedure. The column exit protruded from the bath and was immersed in a small beaker containing phosphate buffer solution. A 3-kV potential was placed across the glass capillary by placing a carbon electrode in the hydrochloric acid bath and another one in the "exit end" beaker that contained the phosphate solution. With some columns, the column ends and direction of phosphate buffer solution flow were reversed midway through the etching procedure. When the columns were cut into segments and the k' for each elevated, the segment that was originally connected to the pressurized phosphate buffer reservoir was called the "inlet" segment while the remaining segments were identified as "middle" and/or "exit". After the electro-etching procedure was completed, the columns were rinsed and dehydrated by the previously described procedure.

Octadecyl-bonded columns were prepared by allowing the generated surface silanol groups of the glass to react with dimethyloctadecylchlorosilane (Petrarch Systems, Bristol, PA, U.S.A.). The columns were filled with a 5% (w/w) solution of dimethyloctadecylchlorosilane in toluene; the column ends were sealed in a flame, and the column was heated at 140°C for 1 h. The column was then rinsed with toluene, blown dry with purified nitrogen gas, and then rinsed overnight with acetonitrile.

Column connections[10,20,21] and the on-column injection procedure[10,21] that were used have been described. The mobile phase [acetonitrile–water (50:50)] was delivered at constant pressure with a syringe pump (Model 314, ISCO, Lincoln, NE, U.S.A.). A 2-μm pore size filter was placed between the pump and column inlet to filter particulates from the mobile phase.

Laser-induced fluorescence was used for detection. A He/Cd laser (Model 3112-10S, Omnichrome, Chino, CA, U.S.A.) provided approximately 15 mW of continuous wave output power at 325 nm. Detection was achieved either on-column or in a fused-silica flow-cell (50 μm I.D.) attached to the end of the column. UV-grade lenses were used to focus the incident light beam and to collimate the emitted fluorescence. The fluorescence signal was passed through 380-nm and 400-nm cut-off filters before being detected with a photomultiplier tube (PMT) biased at 500 V. The PMT current was displayed on a photometer, and the signal was electronically filtered to reduce high-frequence noise before the output was displayed on a strip-chart recorder. The entire detection and chromatographic system was mounted on a vibration-damping optical table.

RESULTS AND DISCUSSION

Capacity factor (k') correction

The degree of column etching was measured by observing the retention of the test solute, 9-methylanthracene. The value of k' observed by flow-cell detection will differ from the true k' observed by on-column detection. This is due to the additional time that the solute spends traveling in the flow-cell until it is detected. This error can be significant if the flow-cell is long or if the mobile phase linear velocity inside the flow-cell is very slow. An equation was derived that allows the true column k' to be calculated when a flow-cell is used for detection.

$$k' = \frac{t_R^* - t_m^*}{t_m^*} = \frac{t_R - t_m}{t_m^*} \qquad (1)$$

$$t_m^* = t_m \frac{L_c r_c^2}{L_c r_c^2 + L_f r_f^2} \qquad (2)$$

where k' is the true column capacity factor, t_R^* is the true elution time for a retained solute, t_m^* is the true elution time for mentioned solute, t_R is the observed elution time for a retained solute (column + flow-cell), t_m is the observed time for a nonretained solute to be eluted, L_c is the column length, r_c is the column internal radius, L_f is the flow-cell length, and r_f is the internal radius of the flow-cell. Capacity factor values that were measured in a flow-cell were corrected by eqn. 1.

Borosilicate columns

The electro-etching technique for borosilicate glass is not well understood. Columns can be etched without the applied electrical potential, but the results are not as good or consistent. The author had noted in previous work[10] that the degree of etching was not uniform throughout the column, but did not adequately investigate and document this observation. The initial investigators of this technique apparently did not notice this deleterious effect[19]. In this work, an attempt was made to determine the severity and cause of this effect in the hope to correct this problem.

Experimental studies showed that the etch is greatest at the inlet end of the column (the end that is connected to the phosphate buffer reservoir) and rapidly decreases towards the exit end of the column. This was demonstrated by electro-etching the column for a given length of time, bonding the ODS stationary phase to the column, then breaking the column into two or three parts of equal length, and determining the retention (k') of the test solute for each section. The degree of retention is related to the surface concentration of stationary phase groups. These data are presented in Table I (along with additional data generated by various modifications of the procedure). The inlet portion of the column always exhibited greater

TABLE I

EVALUATION OF ELECTRO-ETCHED BOROSILICATE COLUMNS

Original column length (m)	*k′ for 9-methylanthracene*				*Polarity*	*Etching time (h)*
	Inlet	*Middle*	*Exit*	*Overall*		
10.5	0.53	0.18	0.09		–	31
12	0.41	0.15	0.05		–	24
9	2.60	1.58	0.58		–	29
10	1.04	0.33	0.16		–	23
9.9	0.30	0.10	0		–	26
10.1	0.47	0.17	0.06		–	27
9.3	0.55	0.16	0.09		–	26
8.8	0.23	0.07	0.07		None	26
10.5				0.22	–	26
3				0.81	–	26
8	0.67		0.18		–	30
8	0.70		0.16		–	30
9*	0.56		0.17		–	19
10*				0.40	–	17
10*				0.38	–	17
10.5*				0.22	–	17
8	0.26		0.18		+	31
8	0.54		0.13		+	29
8	0.32		0.09		+	30

* Etching direction reversed after time indicated, total etching time is twice the time indicated.

retention of the test solute. The inlet portion averaged a k' value of about 0.50, the middle part about 0.15, and the end about 0.10, when the column was broken into three equal parts. Several columns were evaluated without breaking the column into parts, and an overall k' value of about 0.30 was observed.

The polarity of the applied potential was changed from negative to positive, but this did not have any significant effect on the process. In another modification, the column ends were switched midway through the electro-etching procedure to account for differences in the applied potential throughout the length of the column (the electric charge dissipates as it travels through a capillary). When the column ends were switched, the original inlet was immersed into the phosphate reservoir beaker containing the carbon electrode, the original exit end was attached to the phosphate buffer reservoir, and the buffer flow through the column was then reversed in direction. If the cause of the non-uniform etch is the charge dissipation through the column, then reversing the column orientation should produce an additional etch reversed from the initial one. This was not the case (see data in Table I), since the modification in the procedure did not have any significant effect. This observation leads the author to believe that the problem may be due to the diffusion of the leachable B_2O_3 groups from the glass surface back into the bulk of the glass before the etching procedure is completed. Several attempts were made to verify this by reheating the glass to 600°C after etching, followed by additional etching in the reverse direction. This should bring the B_2O_3 groups back to the surface where they can be leached from the glass by reversing the direction of the etch. Unfortunately, this theory could not be tested, since the glass became too brittle to handle after the second heat treatment.

Soda-lime columns

Soda-lime columns were etched with dilute sodium hydroxide. The capacity factor (k') for 9-methylanthracene is shown for a variety of columns, etched for

TABLE II

EVALUATION OF SODA-LIME COLUMNS ETCHED WITH SODIUM HYDROXIDE

Etching with 0.3 M sodium hydroxide		*k' for 9-methylanthracene*
Temperature (°C)	*Time (days)*	
50	2.8	0
50	3.1	0.04
50	7.8	0.19
50	10.10	0.19
50	10.4	0.56
50	11.0	0.48
65	4.0	0.16
65	4.0	0.19
65	5.0	0.50
65	5.8	0.60
65	5.8	0.98
65	6.0	0.80
65	7.0	0.45

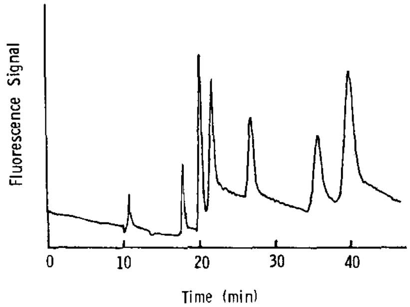

Fig. 1. Separation of a six-component mixture of polycyclic aromatic hydrocarbons. Column: 4.2 m × 30 μm I.D., soda-lime, bonded with ODS. Mobile phase: acetonitrile–water (50:50). Identity of solutes and amount of each (in order of elution): riboflavin (dead-time marker), anthracene (1.3 ng), fluoranthene (0.53 ng), pyrene (1.3 ng), 1,2-benzanthracene (0.78 ng), 2,3-benzofluranthene (0.3 ng), benzo[*a*]pyrene (1.0 ng). Laser-induced fluorescence detection performed on-column.

differing lengths of time and at different temperatures (Table II). The best columns exhibited a retention (k') of the test solute between 0.5 and 1.0. Column clogging was a severe problem with this procedure. A 75% failure was observed when the columns were subjected to the etching times necessary for achieving $k' > 0.4$. Nearly all of the columns that became clogged did so during the final rinse and conditioning with acetonitrile after the silylating procedure had been completed. Conditioning the column with different solvents did not help. Apparently, portions of the column wall were dislodged and formed plugs in the column when highly etched columns were conditioned with polar solvents.

Comparisons

Each glass type and modification procedure has its advantages and disadvantages. Very few borosilicate columns became clogged during modification procedures. Unfortunately, these columns exhibit non-uniform etching, and only the first few meters of the column exhibit significant k'. Soda-lime columns exhibit greater solute retention and can be produced in longer lengths. Column clogging makes the fabrication of soda-lime columns with adequate solute retention very difficult. Column clogging will increase as column dimensions are reduced to $d_c < 10$ μm. It is very doubtful that OTLC columns with adequate retention can be fabricated on a routine basis from soda-lime glass etched with sodium hydroxide. Borosilicate glass has the most promising future for fabricating efficient OTLC columns of $d_c < 10$ μm.

Separation

The separation of a six-component mixture of polycyclic aromatic hydrocarbons is presented in Fig. 1. The mixture was separated with a 4.2 m × 30 μm I.D. ODS-bonded column with on-column, laser-induced fluorescence detection. Although the separation efficiency and analysis time are not impressive, it should be considered that the separation was achieved with a relatively inefficient 30-μm I.D. column. Theoretically, the same separation could be achieved in 4.5 min with a 10-μm I.D. column that has the same k' values for the solutes and is operated at the same mobile-phase linear velocity, or in 1.2 min with a 5-μm I.D. column. The goal of future work will be to produce efficient columns with $d_c < 10$ μm.

Added note

A new chemical etching technique for borosilicate glass was reported by St. Claire and Jorgenson at the *Pittsburgh Conference and Exposition on Analytical Chemistry and Applied Spectroscopy, Atlantic City, NJ, March 10–14, 1986*. This new etching technique reportedly produced uniformly etched columns that had capacity factor values similar to those produced by the electro-etching technique.

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