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PREPARATION OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC COLUMNS FROM GLASS-LINED STAINLESS-STEEL TUBING

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SUMMARY

High-performance liquid chromatographic columns were prepared by packing nominally 10–20- μm silica into glass-lined stainless-steel columns, employing the so-called high-viscosity packing method. Cyclohexanol was used to prepare the silica slurry, containing 11% (w/w) of solid material. The slurry was pumped into the columns at 6000 p.s.i. A similar process was used to prepare columns made of precision-bore stainless-steel tubing. The columns were tested, and those made of glass-lined tubing were found to be about 1.5 times more efficient than those prepared under identical conditions but made of precision-bore stainless-steel tubing. Scanning-electron microscopic photographs revealed that the inner wall of the former columns was smoother than that of the latter and was free of deep axial grooves, and this is believed to be the main factor that gives more efficient columns.

INTRODUCTION

Many papers have been published on the preparation of efficient high-performance liquid chromatographic (HPLC) columns containing particles smaller than 20 μm , employing either the balanced-density slurry packing method¹⁻⁴ or the high-viscosity packing method⁵. The effect of the particle size, column diameter and the method of packing on the efficiency of the columns obtained has been studied by various workers. Asshauer and Halász⁵ found that much better results were obtained if tubes with specially drilled inner walls were used to prepare the columns. Recently, glass-lined stainless-steel tubes of O.D. $\frac{1}{8}$ in. have become available from Scientific Glass Engineering (SGE) (North Melbourne, Australia). A study was therefore initiated to establish if the glass lining had any effect on the efficiency of the columns compared with those packed into regular $\frac{1}{8}$ -in. O.D. precision-bore stainless-steel tubes.

EXPERIMENTAL AND RESULTS

A nominal 10–20- μm silica fraction, Kieselgel HR (Merck, Darmstadt, G.F.R.),

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classified on an Alpine Air Classifier (Alpine MZR, Augsburg, G.F.R.) and sedimented several times in $10^{-3} N$ aqueous ammonia solutions to free it from fines, was used. A Classimat Particle Analyser (Leitz, Wetzlar, G.F.R.) was used to determine the particle size distribution of the fraction, shown in Fig. 1. Freshly distilled cyclohexanol was used to prepare slurries containing 11% (w/w) of the above silica, dried at 120° for 6 h. The slurry was homogenized in an ultrasonic bath for 10 min while applying a low-speed mechanical agitator as well. The packing was carried out according to the procedure of Asshauer and Halász⁵, the slurry being pressurized to 6000 p.s.i. with the aid of a Waters Model 6000 pump (Waters Assoc., Millford, Mass., U.S.A.). The columns were made of $1/8$ -in. O.D., 2.0-mm I.D. glass-lined stainless-steel tubing (SGE) and $1/8$ -in. O.D., 2.0-mm I.D. precision-bore stainless-steel tubing (Cat. No. 96-000078-02; Varian Aerograph, Walnut Creek, Calif., U.S.A.), cut to lengths of 25 cm. After preliminary experiments, the original $1/4$ -in. Swagelok column terminator and ferrule system, delivered with the SGE glass-lined tube columns, was modified and a new PTFE ring sealing system, applied a modified $1/8$ -in. Swagelok terminator, was developed that gave a considerable reduction in the dead volume.

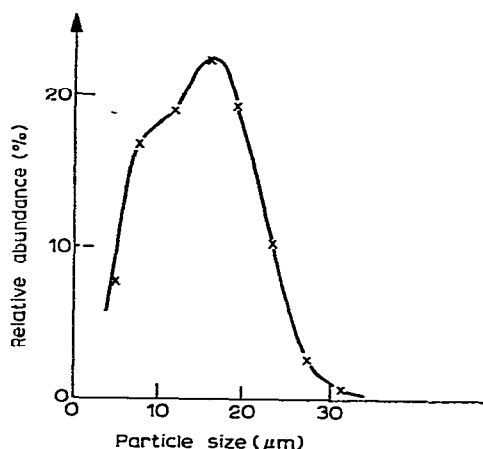


Fig. 1. Relative particle size abundance of the silica fraction.

The columns were thoroughly cleaned before packing according to the procedure suggested by Karger *et al.*⁶. Under the conditions described, the preparation of each column took *ca.* 1.5 h. Once the packing procedure was completed, a small glass-fibre filter disk (Type GF/F, Whatman, Maidstone, Great Britain) was placed on top of the columns. The columns were conditioned by pumping through them about 250 ml of methanol, 150 ml of acetone, 150 ml of acetone-cyclohexane (1:10) and finally 500 ml of 50% water-saturated *n*-hexane.

A three-component sample containing chloroform ($k'_{av} = 0.8$), phenanthrene ($k'_{av} = 0.8$) and nitrobenzene ($k'_{av} = 3.5$) was used to evaluate the columns. A 5- μ l volume of the test sample was injected with a Waters Model U6K injector. Extra-column contributions to the peak broadening were found to be negligible. Plate counts were determined at flow-rates in the range 0.5–5.0 ml/min. The linear equation sug-

gested by Halász and Naefe⁷ for this range was used to correlate the plate height values and the linear velocity values:

$$H = A + Cu \quad (1)$$

where H is the plate height (μm), u the linear velocity (mm/sec) and A and C are constants.

TABLE I

COMPARISON OF THE COEFFICIENTS IN EQNS. 1 AND 2 AND THE H VALUES OBTAINED AT 10 mm/sec WITH IDENTICALLY PREPARED GLASS-LINED TUBES AND PRECISION-BORE STAINLESS-STEEL COLUMNS, TOGETHER WITH VALUES OBTAINED BY ASSHAUER AND HALÁSZ⁵.

Type of column	A_{av} (μm)	C_{av} (sec)	H^* (μm)	$A_{S_{av}}^2$
Precision-bore stainless-steel	126	$6.8 \cdot 10^{-3}$	208	1.98
Glass-lined tube	75	$9.5 \cdot 10^{-3}$	154	2.16
Asshauer and Halász ⁵	121	$1.0 \cdot 10^{-3}$	139**	1.4

* At 10 mm/sec.

** Calculated.

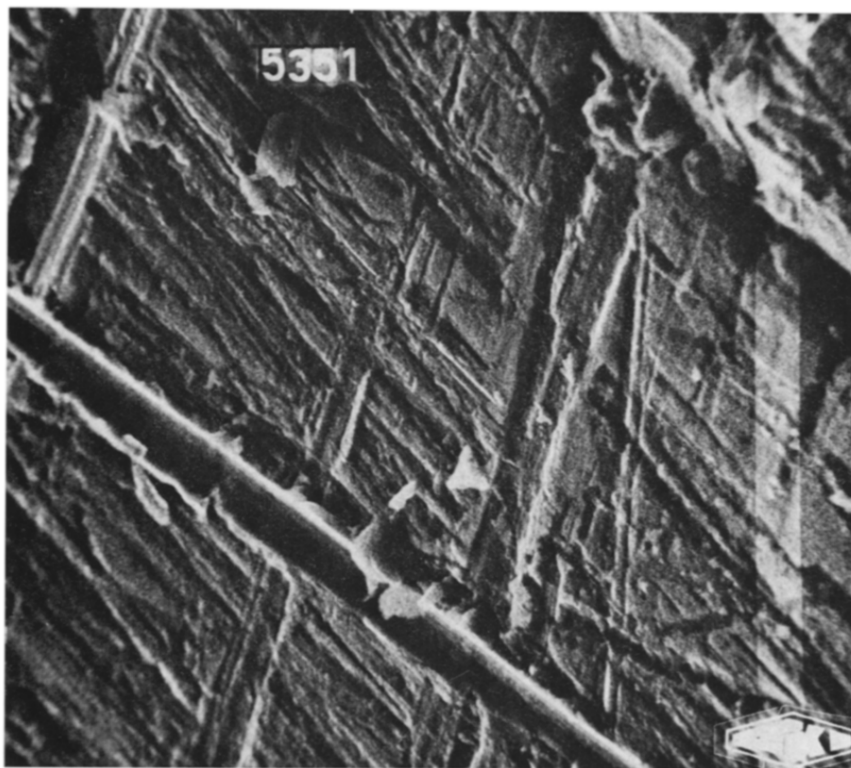


Fig. 2. Scanning-electron microscopic photograph of the precision-bore stainless-steel tubing (magnification $\times 1000$).

Also, the peak asymmetry coefficient, A_s , of Asshauer and Halász⁵:

$$A_s^2 = \left(\frac{b}{a}\right)^2 \quad (2)$$

where b is the distance from the position of the peak maximum to the peak tail and a is the distance from the position of the peak maximum to the front line of the peak, measured at 10% of the peak height, was determined. Table I shows the A , C , A_s^2 and H values obtained at $u = 10$ mm/sec (average for five columns) for each type of column. For comparison, values obtained by Asshauer and Halász are also shown.

DISCUSSION

It can be seen that the columns made from glass-lined tubing and packed under conditions identical with those for columns made from precision-bore stainless steel tubing, are about 1.3–1.5 times more efficient than the latter. Although the silica and the packing method used are far from the optimum and thus several chance side-effects may have a significant influence, the difference in the efficiencies is clear. It was thought that the inner walls of the tubes might provide the explanation, so several columns were washed, short sections were cut off and scanning-electron microscopic photographs were taken. Fig. 2 shows that there are deep axial grooves in the preci-



Fig. 3. Scanning-electron microscopic photograph of the glass-lined stainless-steel tubing (magnification $\times 1000$).

sion-bore stainless-steel tubing and the surface gives an impression of general roughness, while in Fig. 3, showing the glass-lined tubing, an absolutely smooth surface with no micron-sized discontinuities can be seen.

CONCLUSION

It has been shown that under the conditions described, more efficient HPLC columns could be made from glass-lined tubing than from precision-bore stainless-steel tubing; the difference is believed to be due to the smooth, flat surface of the former. Having modified the commercially available terminator system of the glass-lined tubing, no further practical problems or disadvantages were encountered while working with the columns packed into glass-lined SGE tubing.

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NOTE ADDED IN PROOF

Recently $\frac{1}{4}$ -in. O.D. (4 mm I.D.) glass-lined tubing has become available from SGE. Columns were packed using this material and $\frac{1}{4}$ -in. O.D. (4.5 mm I.D.) stainless-steel tubing (Varian, Cat. No. 96-000079-02). Columns packed into the glass-lined tubing were again much better. Scanning-electron microscopic photographs were very similar to Figs. 2 and 3.

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