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Note

Packing of 3-μm particle ODS silicas using hexanol-1–methylene chloride (1:1) as a slurry medium in high-performance liquid chromatography

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In high-performance liquid chromatography (HPLC) microparticulate silicas with particle sizes of 5 and 10 μm have been packed widely and effectively by the balanced density technique[1,2], the viscosity method[3] and the low viscosity method with a single-component solvent *e.g.*, hexane[4], chloroform[4], methanol[5] or carbon tetrachloride[6,7].

Recently silicas with particle sizes $\leqslant$ 3 μm have become more attractive as microparticulate packings than the 5- or 10-μm silicas[8,9,10] because they give better resolution in HPLC. However, some difficulties have been recognized in the slurry packing procedures.

Previously, we reported that hexanol-1 can be used as a high potential slurry medium for packing 5-μm octadecylsilane (ODS) silicas or 5-μm silver-coated silicas[11,12]. The use of hexanol-1 is extended to 3-μm ODS silicas. Though 3-μm ODS silicas are well-dispersed in hexanol-1, the slurry made therefrom did not achieve high efficiencies, presumably due to the insufficiently controlled viscosity of the slurry. However, we found that the use of hexanol-1–methylene chloride (1:1) as a slurry medium easily enabled to make highly resolvable columns packed with 3-μm ODS silicas with a constant-flow pump conventionally used in HPLC systems.

This note describes a high-efficiency slurry packing procedure for 3-μm ODS silicas, which provides a powerful technique in HPLC.

EXPERIMENTAL

Adsorbents

Develosil ODS-3 (3 μm, 20% loading; Nomura Chemical, Seto-City, Japan) and Unisil QC-18 (5 μm, 20% loading; Gasukuro Kogyo, Tokyo, Japan) were used without further modifications.

Solvents

Commercially available chromatographic and reagent grade solvents were used.

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HPLC systems

The chromatographic system consisted of a plunger pump (Type SF 0396-57; Milton Roy, Philadelphia, PA, U.S.A.), a 350 kg/cm^2 pressure gauge (Umetani Seiki, Osaka, Japan), a bellows-type damper (Type DAM, Umetani Seiki) and a 20-μl syringe-loading sample injector (Rheodyne Model 7125). The effluent was monitored at 254 nm with a UV detector (UVLOG; Oyobunko, Tokyo, Japan).

Column packing

A mixture of 2.0 g (8.0 g)* of the 3-μm ODS silicas and 15 ml (30 ml) of hexanol-1–methylene chloride (1:1) was sonicated for 2 min. The slurry obtained was placed in a 15-ml (30-ml) reservoir connected to a 15 cm × 4.6 mm I.D. (25 cm × 8.0 mm I.D.) analytical column. Methylene chloride was pumped through the column at a flow-rate of 3.0 ml/min (4.5 ml/min) using a 800 kg/cm^2 high-pressure pump (NS-800-15DX; Nihon Seimitsu, Tokyo, Japan). It took 5–6 min (8–9 min) to replace the slurry medium with the pressure of the packing system at 400 kg/cm^2. Then 50 ml of methylene chloride were pumped through the column at the same pressure, followed by methanol at a pressure of 450 kg/cm^2 for 60 min.

Similarly the 5-μm ODS silicas were packed into a 15 cm × 4.6 mm I.D. (25 cm × 8.0 mm I.D.) column using *n*-hexanol-1–chloroform (1:1) as the slurry medium. Chloroform was pumped through the column at a flow-rate of 8.0 ml/min (15.0 ml/min), followed by methanol at a pressure of 450 kg/cm^2.

RESULTS AND DISCUSSION

Fig. 1 shows a chromatogram of a mixture of polyaromatic hydrocarbons on the 3-μm silica column using acetonitrile–water (7:3) as eluent. Furthermore, a value

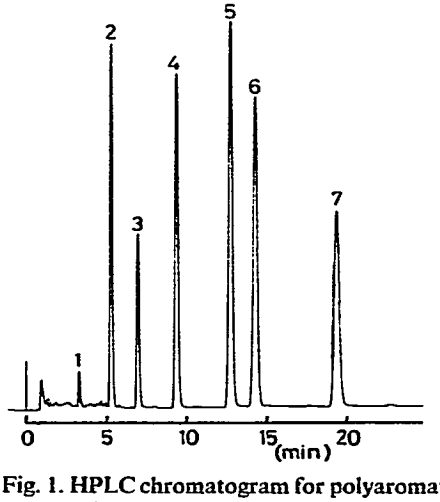

Fig. 1. HPLC chromatogram for polyaromatic hydrocarbons. Column, Develosil ODS-3 (3 μm); flow-rate, 1.5 ml/min; UV detection, 254 nm, 0.16 a.u.f.s. Peaks: 1 = benzene; 2 = naphthalin; 3 = biphenyl; 4 = phenanthrene; 5 = fluoranthene; 6 = pyrene; 7 = chrysene.

* Values in parenthesis in this section correspond to those for a semi-preparative column.

TABLE I

COMPARISON OF HETP FOR CHRYSENE IN HPLC BETWEEN 3-μm AND 5-μm ODS COLUMNS

Column size	*Gel particle size (μm)*	*Slurry medium*		*Column pressure (kg/cm²)***	*Flow-rate of eluent (ml/min)*	*HETP (μm)*
		*Mixture**	*Flow-rate (ml/min)*			
15 cm × 4.6 mm I.D.	3		3.0	400	1.2	8.8, 8.5, 7.9
	3	H–MC (1:1)	3.2	550	1.2	11.1
	3		3.5	650	1.2	15.0
	5	H–CF (1:1)	8.0	400	1.2	15.0
25 cm × 8.0 mm I.D.	3	H–MC (1:1)	4.5	400	2.5	8.3
	5	H–CF (1:1)	15.0	400	2.5	12.5

* H = Hexanol-1; MC = methylene chloride, CF = chloroform.

** Pressure when slurry medium is replaced completely with methylene chloride or chloroform.

for the height equivalent to a theoretical plate (HETP) was measured on the basis of the chrysene peak on the analytical column packed with the 3-μm silicas at varying column pressure (400–650 kg/cm²) or at varying flow-rates of the slurry medium (3.0–3.5 ml/min).

From Table I it can be seen that the lowest HETP values for chrysene, (8.8, 8.5 and 7.9 μm) were obtained with good reproducibility at a slurry medium flow-rate of 3.0 ml/min. The capacity factors were 13.2, 13.5 and 13.4, respectively.

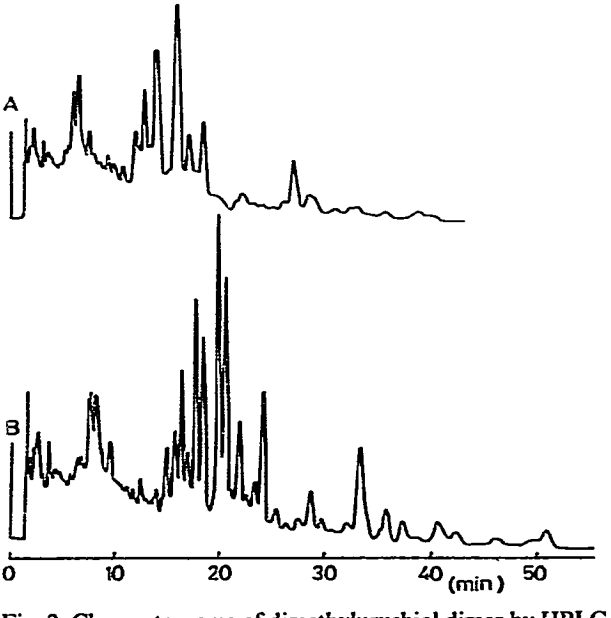

Fig. 2. Chromatograms of dimethylurushiol dimer by HPLC. A, 3-μm ODS silicas; B, 5-μm ODS; eluent, acetonitrile; column, 15 cm × 4.6 mm I.D. Other conditions as in Fig. 1.

Comparable HETP values were obtained for the 3-μm ODS preparative column (see Table I).

The HETP values for chrysene using the 5-μm ODS column, were 15 μm and 12.5 μm for the 15 cm × 4.6 mm I.D. and the 25 cm × 8.0 mm I.D. column, respectively. The 5-μm ODS column is clearly inferior in resolution compared to the 3-μm ODS column.

Fig. 2 illustrates the difference in the resolution of dimethylurushiol dimer[13] (separated from Japanese Lac by gel permeation chromatography) by HPLC using the 3-μm and 5-μm ODS columns packed in this work. It can be seen that higher resolution is attained for the 3-μm ODS column when compared to the 5-μm one.

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