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Note

Simple method for improving the efficiency of liquid chromatographic columns filled with soft gels

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Unexpected changes in separation efficiency are sometimes observed in liquid chromatography with columns filled with soft or semi-rigid organic gels. A detailed study of this phenomenon showed that it is connected with changes in size of the swollen gel particles of the column packing. These changes are caused by the injection of solutes that reduce the swelling of the gel considerably or by accidental penetration of air into the gel bed. The practical aspects of these effects are discussed here.

EXPERIMENTAL

The experiments were performed on a simple home-made low-pressure apparatus[1]. Glass columns (15–28 mm I.D.) were filled by the classical low-pressure slurry technique[2]. The volumes of the gel beds (V_t) were 200–400 cm^3. Air and liquid samples were injected into the columns from a 2-cm^3 loop of the injector. The columns were washed with a large volume (*ca.* V_t) of the de-gassed eluent after the injection of air or a liquid that reduced the swelling of the gel considerably. In all experiments, descending elution was applied. A decrease in the gel bed volume was usually observed after injection of air or deswelling liquid and subsequent washing of the column. Therefore, the adjustable column end-pieces were re-set before efficiency testing with diluted solutions of benzene or *n*-heptane. A Model R-4 differential refractometer (Waters Assoc., Milford, Mass., U.S.A.) was used as the detector. The pressure applied was 0.05–0.5 MPa at an elution rate of 0.5–1.0 cm^3/min.

RESULTS AND DISCUSSION

Table I gives several typical examples that show the column efficiencies obtained after injection of air or deswelling liquid into gel bed. The results can be summarized as follows.

(1) Injection of small amounts of air or a liquid that reduces the swelling of the gel particles considerably into the column under controlled conditions may substantially increase the column efficiency. This effect is most pronounced in less efficient columns.

(2) The total filling of the gel bed with air does not necessarily destroy the column. This observation contradicts the statements that are often found in the literature.

TABLE I

COLUMN EFFICIENCIES AFTER INJECTION OF AIR OR LIQUID INTO THE GEL BED

Gel	*Eluent*	*Conditioning*	*Number of theoretical plates per metre*
Sephadex LH-20*	Methanol	—	1400
		2 cm^3 of air	1650
		2 × 2 cm^3 of air	1900
		20 × 2 cm^3 of air	3700
		Complete filling of the gel bed with air	3400
Sephadex LH-20*	Methanol	—	1600
		2 × 2 cm^3 of benzene–methanol (9:1, v/v)	1850
Sephadex LH-20*	Tetrahydrofuran	—	1700
		2 × 2 cm^3 of air	2000
Sephadex LH-20**	Benzene–methanol (77.8:22.2, v/v)	—	4900
		5 × 2 cm^3 of air	5200
Sephadex LH-20**	Methanol	—	5500
		20 × 2 cm^3 of air	6800
Hydroxypropylated Sephadex G-50***	Methanol	—	1500
		2 × 2 cm^3 of air	1750
Bio-Beads SX-3§	Tetrahydrofuran	—	2500
		5 × 2 cm^3 of air	3200
Bio-Beads SX-3§	Benzene–methanol (77.8:22.2, v/v)	—	4300
		2 × 2 cm^3 of air	1500

* Hydroxypropylated crosslinked dextran gel (Pharmacia Fine Chemicals, Uppsala, Sweden). Particle size, 25–125 μm (dry).

** Smallest and largest gel particles removed by sedimentation.

*** Irregular particles. Prepared by hydroxypropylation of Sephadex G-50.

§ Styrene–divinylbenzene gel (Bio-Rad Labs., Richmond, Calif., U.S.A.). Particle size, 37–74 μm (dry).

(3) Injection of air considerably reduces the column efficiency of styrene–divinylbenzene gel if benzene–methanol (77.8:22.2, v/v) is used as the eluent. This mixture is an extremely poor solvent for linear polystyrene. It swells the styrene–divinylbenzene gel only to a small extent and the gel particles tend to form clusters in the slurry. On the other hand, Sephadex LH-20 gel swells in the above mixture to virtually the same extent as in pure methanol and no cluster formation in the slurry was observed.

(4) No changes in the separation ranges according to the size of molecules of oligomers during gel chromatographic experiments after re-swelling of gels were observed. Hence the decreases in gel bed volumes and in the corresponding elution volumes are caused by decreases in the inter-particulate (dead) volumes only.

A possible explanation of these results involves local deswelling of the gel particles in the zone of eluent containing air or deswelling liquid and their subsequent tighter re-packing, which results in the decrease in the gel bed volume and an increase in the column efficiency. If the deswollen gel particles cannot move in the gel

bed sufficiently as the extent of deswelling is too small and/or their mutual adhesion is too great, channels appear in the gel bed and the column efficiency decreases. The gel particles may also be deswollen osmotically by relatively low concentrations of larger solute molecules injected into the column[3–6]. This could explain the frequently observed changes in the column separation efficiencies during gel chromatographic separations with soft gels.

The effects described have been routinely applied for several years in this laboratory for improving the separation efficiencies of low-pressure liquid chromatographic columns packed with soft organic gels. Small amounts of air are injected until no changes in the gel bed volume are observed. The use of air as a deswelling agent is generally most convenient as some adjustable column end-pieces are attacked by organic solvents that can be applied with hydrophilic gels.

In addition to the increase in column efficiency, an advantage of this column "conditioning" is that in the event of accidental penetration of air into the conditioned column its gel bed volume will not change further and the elution volumes of separated substances remain constant. Hence the column need not be re-calibrated.

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