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Note

Column lifetime of Superose 6 at 37°C and basic pH

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We are currently involved in a large-scale study of the lifetime of liquid chromatography columns for the separation of biomolecules. A recent study has shown that the lifetime of cation- and anion-exchange columns (Mono Q and Mono S) for fast protein liquid chromatography (FPLC) is well over 1000 repetitive injections[1]. Furthermore, it has been shown that the agarose-based high-performance gel filtration columns (Superose® 12) are unaffected by 1000 repetitive serum injections at basic pH[2].

Yet another newly launched agarose-based gel filtration column, Superose 6 (ref. 3), has been investigated in this work. This agarose matrix is cross-linked to a lesser extent than Superose 12, and is therefore a gel designed for larger biomolecules[3]. The lifetime of a Superose 6 column has been investigated under physiological conditions, *i.e.*, with a column temperature of 37°C and a mobile phase of basic pH (8.4).

EXPERIMENTAL

Chemicals and apparatus

All inorganic reagents were of p.a. quality. The serum sample was prepared daily from aliquots of frozen, normal, human serum. The serum was diluted (1:8) with mobile phase buffer and filtered through a 0.22-μm bacteriological filter prior to injection. The mobile phase buffer was 0.05 *M* Tris (pH 8.4) with addition of 0.15 *M* sodium chloride and 0.02% sodium azide. The eluent was filtered through a 0.45-μm filter.

The Pharmacia-prepacked Superose 6 HR 10/30 column was tested on a Pharmacia FPLC system comprising an LCC-500 control unit, a P-500 high-precision pump, a UV-1 UV monitor (280 nm, HR 10 cell), an MV-7 sample injector with a 100-μl loop, and an REC-481 recorder. To control the temperature at 37°C (±0.1°C), the column was water-jacketed.

Procedure

The serum was injected every 44 min, at a flow-rate of 0.6 ml/min, until 1000 injections had been completed.

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RESULTS AND DISCUSSION

The lifetime of a Superose 6 HR 10/30 column was investigated by making 1000 repetitive injections. The choice of eluent (phosphate buffer, pH 8.4) and column temperature (37°C) was done to make it possible to draw conclusions about the stability of Superose 6 under physiological conditions. In addition, the use of a serum sample makes the test more realistic.

The chromatograms from the 1st and the 1000th serum injections (Fig. 1) show that the Superose 6 column can withstand 1000 repetitive injections. In addition, the retention volume (V_R) stability was excellent for the three main peaks, as shown in Fig. 1. The relative standard deviation (R.S.D.) was lower than 0.2% (Table I).

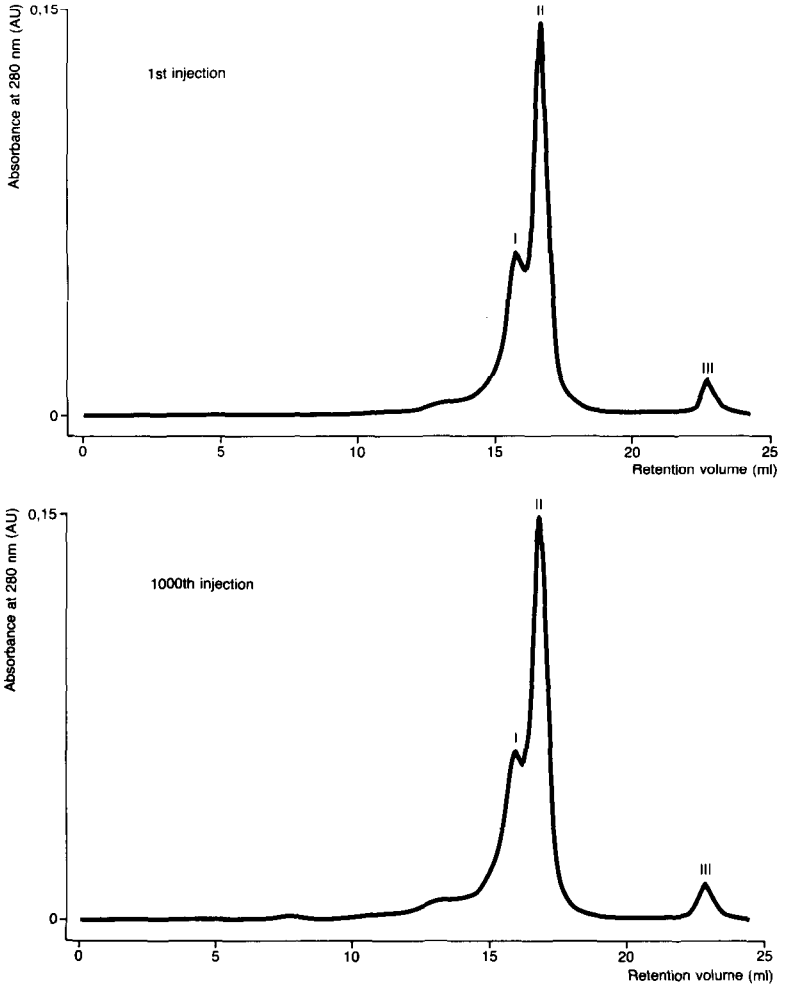

Fig. 1. Chromatograms from the 1st and the 1000th separation of normal human serum on Superose 6 HR 10/30. Chromatographic conditions: sample volume, 100 μl; eluent, 0.05 *M* Tris in 0.15 *M* sodium chloride adjusted to pH 8.4; flow-rate, 0.6 ml/min; column temperature, 37°C.

TABLE I

VARIATION OF RETENTION VOLUME (V_R) AND PEAK HEIGHT (h') OF THE THREE MAIN PEAKS AND THE TOTAL ACCUMULATED AREA DURING 1000 INJECTIONS OF NORMAL HUMAN SERUM ON A SUPEROSE 6 HR 10/30 COLUMN AT pH 8.4 AND COLUMN TEMPERATURE 37°C

Injection number	*Peak 1*		*Peak 2*		*Peak 3*		*Total accumulated area above 0.006 AU (ml AU)*
	V_R *(ml)*	h' *(AU)*	V_R *(ml)*	h' *(AU)*	V_R *(ml)*	h' *(AU)*	
1	16.07	0.060	16.97	0.146	22.91	0.014	0.208
50	16.03	0.060	16.95	0.147	22.92	0.013	0.205
100	16.04	0.061	16.94	0.149	23.00	0.014	0.207
150	16.03	0.063	16.94	0.150	22.98	0.013	0.208
200	16.07	0.062	16.95	0.149	23.01	0.014	0.206
250	16.08	0.062	16.96	0.151	23.01	0.014	0.203
300	16.04	0.061	16.93	0.148	22.94	0.013	0.209
350	16.07	0.062	16.98	0.151	22.99	0.014	0.213
400	16.04	0.063	16.94	0.152	22.96	0.013	0.209
450	16.04	0.060	16.91	0.148	23.00	0.014	0.207
500	16.06	0.059	16.95	0.147	22.96	0.014	0.207
550	16.05	0.059	16.96	0.148	22.97	0.013	0.206
600	16.09	0.059	16.94	0.148	22.98	0.013	0.206
650	16.07	0.059	16.95	0.148	23.00	0.013	0.208
700	16.04	0.058	16.93	0.146	22.97	0.013	0.203
750	16.03	0.059	16.94	0.147	23.01	0.013	0.214
800	16.09	0.058	16.95	0.150	22.97	0.012	0.205
850	16.01	0.058	16.93	0.148	22.94	0.013	0.210
900	16.07	0.058	16.94	0.148	22.96	0.013	0.210
950	16.07	0.058	16.95	0.144	22.99	0.012	0.203
1000	16.02	0.062	16.92	0.149	22.91	0.014	0.205
Mean	16.05	0.060	16.94	0.148	22.97	0.013	0.207
R.S.D. (%)	0.14	2.9	0.09	1.26	0.14	4.8	1.43

Furthermore, the maximum spread, calculated from the highest and lowest V_R obtained during the test, was less than 150 μl for all three peaks. The detector response (peak height, h') of the three main peaks was constant throughout the test (Table I). Furthermore, no significant baseline drift was observed and the total accumulated area showed no trends during the test (Table I). These results indicate that the sample did not accumulate in the gel matrix. However, the back-pressure rose from 0.6 MPa to 0.8 MPa during the test. This is probably caused by clogging of the column top filter, which was also observed by Johansson and Ellström[2].

CONCLUSION

It has been demonstrated that a Superose 6 column can manage 1000 repetitive injections of a serum sample under physiological conditions, with no significant degradation in column performance. The high precision of the retention data (Table I) shows that Superose 6 columns are well suited for accurate molecular-weight determinations during long-term use.

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