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Note

Column lifetime of a new agarose medium for high-performance gel filtration chromatography at basic pH

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The introduction of high-performance gel filtration chromatography (HPGFC) and its use for biopolymers has brought about a significant decrease in analysis time and an increase in resolving power compared to classical gel filtration. Packings commonly used for HPGFC are controlled-pore glasses[1,2], derivatized silica-based packings[1,3–5] and beads of organic polymer gels[5,6]. The silica-based supports are the most widely used[7]. However, a major limitation of the latter is the inherent instability of silica at neutral to alkaline pH[8]. This reduces the column lifetime when using physiological buffers as mobile phase[9].

Recently, a cross-linked agarose-based material for HPGFC has become commercially available under the trade-name of Superose®[10]. The purpose of this study was to determine the performance of a Superose 12 HR 10/30 column during 1000 repetitive injections of a serum sample at pH 8.4.

EXPERIMENTAL

Chemicals and apparatus

All inorganic reagents were of pro analys quality. The whey protein mixture was obtained from Arla (Uppsala, Sweden).

The serum sample was prepared from aliquots of a frozen normal human serum. The serum was diluted (1:4) in mobile phase buffer and filtered through a 0.22-μm bacteriological filter prior to injection. A 100-μl injection of a filtered whey protein mixture (5 mg/ml) also served as a sample. The mobile phase buffer was 0.05 *M* Tris (pH 8.4) with 0.15 *M* sodium chloride and 0.02% sodium azide. The eluent was filtered through a 0.45-μm filter.

The results were obtained with a Pharmacia prepacked Superose 12 HR 10/30 column. The column was tested on a Pharmacia FPLC system comprised of a P-5400 high-precision pump, a sample injector MV-7 with a 100-μl loop, a UV-monitor UV-1 (280 nm, HR 10 cell) and a recorder Rec-481. The column temperature was 22°C.

Procedure

Two injections of the serum sample were done at a flow-rate of 0.2 ml/min and 98 injections at 1.2 ml/min, respectively. This cycle was repeated, with freshly pre-

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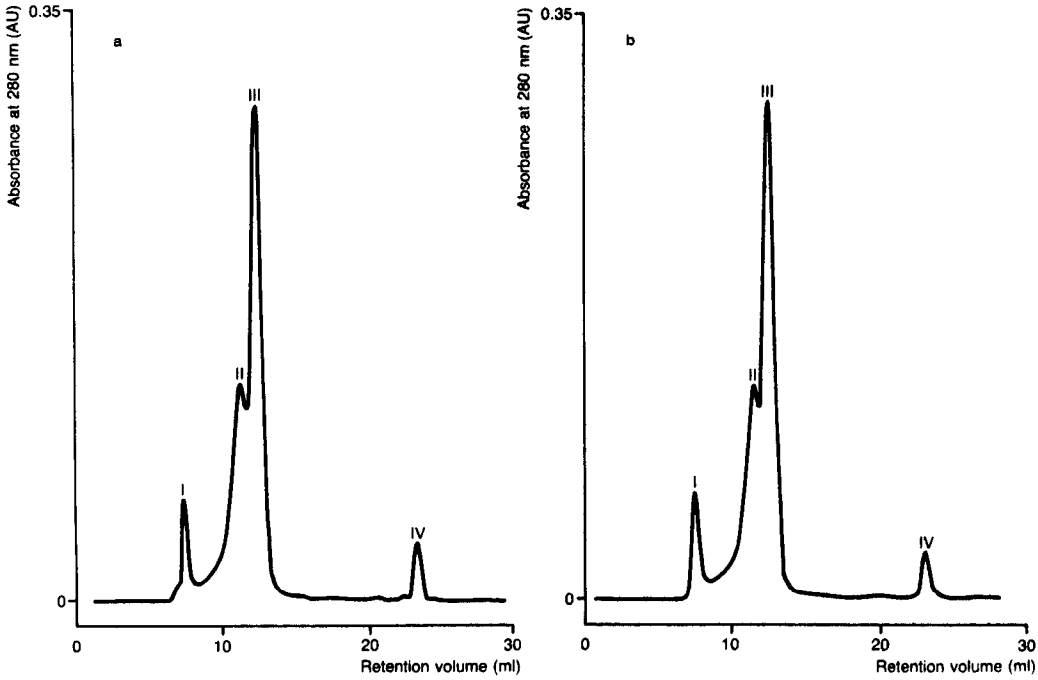

Fig. 1. Chromatograms from the 1st (a) and the 1000th (b) separation of normal human serum on the Superose 12 HR 10/30 column. Chromatographic conditions: sample, diluted serum (1:4); sample volume, 100 μl; eluent, 0.05 *M* Tris (pH 8.4) with 0.15 *M* sodium chloride and 0.02% sodium azide; flow-rate, 1.2 ml/min; column temperature, 22°C.

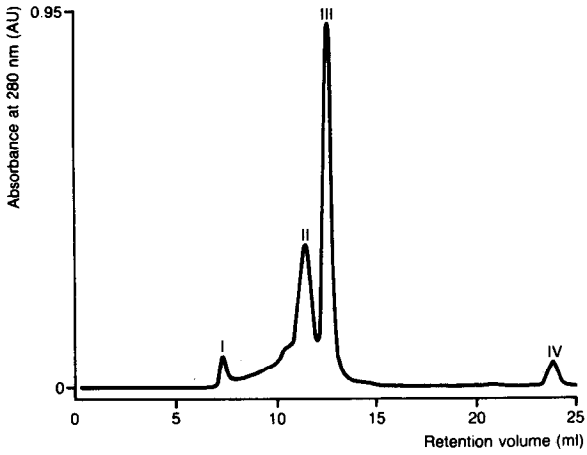

Fig. 2. Chromatogram from the 501st injection of normal human serum on the Superose 12 HR 10/30 column at a flow-rate of 0.2 ml/min; other conditions as in Fig. 1.

pared sample, every day, until 1000 injections had been completed. At the beginning and at the end of the test the whey protein sample was injected twice.

RESULTS AND DISCUSSION

The column lifetime of HPGFC columns is influenced by factors such as the chemical resistance of the packing, the flow-rate of the mobile phase and the purity of the injected sample[11]. In this study the resistance of a Superose 12 column to basic pH was investigated during 1000 repetitive injections. Moreover, a high flow-rate and a concentrated serum sample were used further to stress the column.

The chromatographic pattern from the serum sample at 1.2 ml/min was unchanged after 1000 injections (Fig. 1). The elution profile at a flow-rate of 0.2 ml/min (see procedure) was also unaltered by the test. A chromatogram obtained at a low flow-rate is depicted in Fig. 2. The relative standard deviation (R.S.D.) of the retention volumes, V_R, of the four main peaks (Fig. 1) was *ca.* 1% (Table I). These retention data obtained from 500 h of continuous use of the column indicate that Superose 12 columns are well suited for accurate molecular weight determination without daily

TABLE I

VARIATION OF THE RETENTION VOLUME, V_R, AND THE PEAK HEIGHT, h', OF THE FOUR MAIN PEAKS AND THE TOTAL ACCUMULATED AREA DURING 1000 INJECTIONS OF NORMAL HUMAN SERUM ON A SUPEROSE 12 HR 10/30 COLUMN AT pH 8.4 AND FLOW-RATE 1.2 ml/min

Injection number	*Peak 1*		*Peak 2*		*Peak 3*		*Peak 4*		*Total accumulated area above 0.015 AU (ml/AU)*
	V_R *(ml)*	h' *(AU)*	V_R *(ml)*	h' *(AU)*	V_R *(ml)*	h' *(AU)*	V_R *(ml)*	h' *(AU)*	
3	7.37	0.060	11.33	0.125	12.50	0.290	23.63	0.033	0.490
50	7.38	0.068	11.38	0.128	12.50	0.293	23.66	0.033	0.511
100	7.37	0.068	11.34	0.125	12.46	0.293	23.63	0.033	0.501
150	7.39	0.065	11.36	0.123	12.55	0.295	23.64	0.030	0.510
200	7.38	0.065	11.39	0.128	12.47	0.298	23.56	0.033	0.501
250	7.38	0.065	11.35	0.128	12.48	0.298	23.52	0.033	0.488
300	7.39	0.063	11.32	0.128	12.47	0.298	23.51	0.030	0.493
350	7.39	0.060	11.34	0.128	12.41	0.302	23.50	0.030	0.493
400	7.42	0.060	11.33	0.128	12.49	0.300	23.50	0.028	0.524
450	7.40	0.060	11.40	0.125	12.52	0.298	23.59	0.028	0.497
500	7.38	0.060	11.34	0.128	12.47	0.298	23.57	0.028	0.507
550	7.43	0.060	11.33	0.128	12.47	0.300	23.52	0.028	0.494
600	7.43	0.058	11.41	0.130	12.50	0.303	23.57	0.028	0.519
650	7.55	0.065	11.51	0.125	12.63	0.290	23.69	0.030	0.503
700	7.54	0.065	11.47	0.125	12.67	0.293	23.70	0.030	0.509
750	7.54	0.068	11.56	0.128	12.64	0.293	23.75	0.033	0.530
800	7.54	0.068	11.50	0.125	12.66	0.293	23.78	0.030	0.496
850	7.55	0.068	11.58	0.125	12.70	0.293	23.69	0.030	0.487
900	7.54	0.068	11.53	0.128	12.64	0.295	23.66	0.030	0.537
950	7.50	0.068	11.50	0.125	12.61	0.290	23.63	0.028	0.521
1000	7.55	0.068	11.58	0.128	12.72	0.295	23.75	0.028	0.520
Mean	7.45	0.064	11.42	0.127	12.55	0.296	23.62	0.030	0.506
R.S.D. (%)	1.00	5.65	0.81	1.44	0.74	1.31	0.37	6.67	2.85

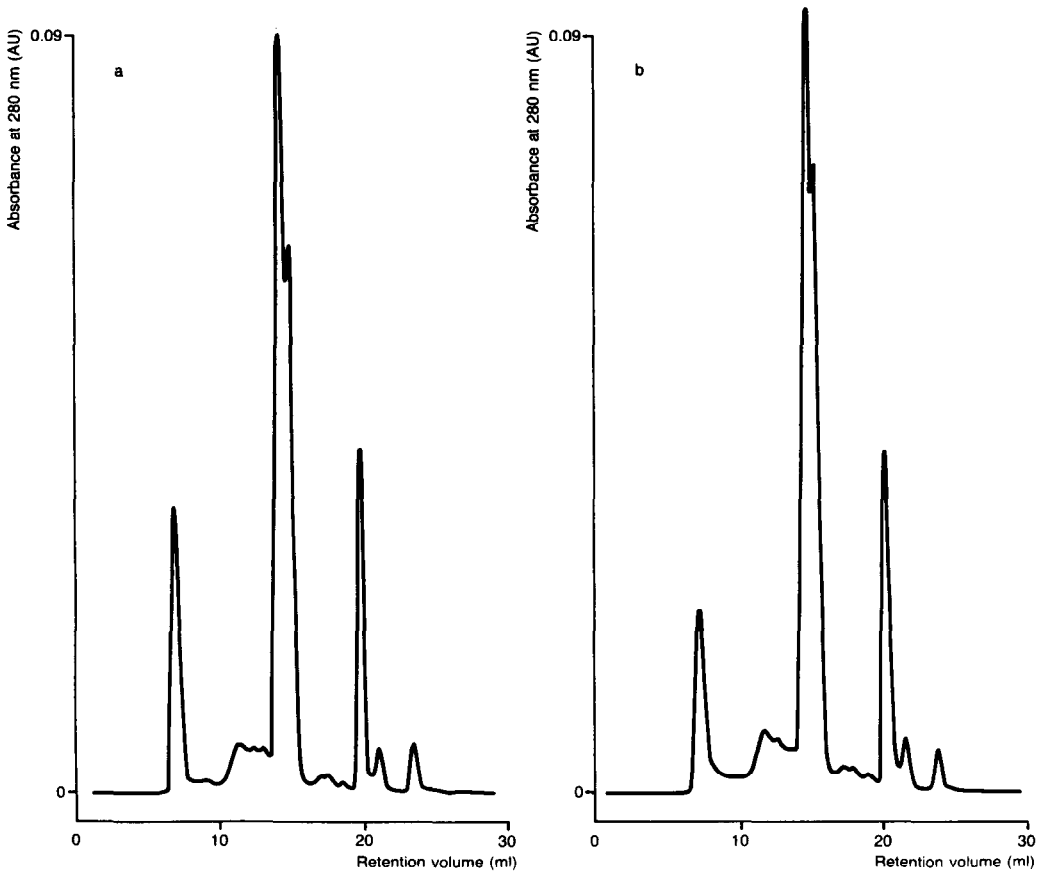

Fig. 3. Separation of the whey protein mixture (5 mg/ml) on the Superose 12 HR 10/30 column, tested after 50 (a) and 990 (b) injections of the serum sample. Chromatographic conditions as in Fig. 1.

calibrations. The Superose 12 column provides a good separation in the range $5 \cdot 10^2$–$5 \cdot 10^5$ daltons[10].

The peak height, h', of the four peaks, evaluated as the distance between the peak maximum and the baseline at the injection time, gives more precise data for peaks 2 and 3 (Table I). The spread of h' is largely explained by the uncertainty, 0.0025 a.u. at a monitor setting of 0.5 a.u., of the peak-height evaluation.

The back pressure was constant for up to 600 injections but between the 600th and 620th injection it increased from 1.7 to 2.5 MPa. However, changing the top filter after the 620th injection restored the back pressure to the initial value (1.7 MPa), which then remained throughout the test. Moreover, a slight misadjustment of the adaptor position up on this change increased the retention volumes of the four peaks by *ca.* 100 μl (Table I, compare for example V_R at the 600th and 650th injections).

In order moreclosely to observe changes in the gel structure or the pore-size distribution, the whey protein mixture was injected at the beginning and at the end of the test (Fig. 3). The resulting profiles together with the constant chromatographic

pattern of the serum sample (Fig. 1) show that the gel structure is stable under the test conditions.

It can be concluded that the end of the column life was not reached after 1000 repetitive injectiosns, as shown by the long-term reproducibility (Table I). The high stability of Superose 12 at basic pH shows that physiological buffers can be used with no restraints on the column lifetime.

REFERENCES

1 N. A. Parris, *Instrumental Liquid Chromatography (Journal of Chromatography Library*, Vol. 27), Elsevier, Amsterdam, New York, Oxford, Tokyo, 1984, pp. 264–266.
2 F. Regnier and R. Noel, *J. Chromatogr. Sci.*, 14 (1976) 316.
3 S. H. Chang, K. M. Gooding and F. E. Regnier, *J. Chromatogr.*, 125 (1976) 103.
4 O. Chiantore and M. Guaita, *J. Chromatogr.*, 260 (1983) 41.
5 Y. Kato, K. Komiya, H. Sasaki and T. Hashimoto, *J. Chromatogr.*, 193 (1980) 311.
6 J. Coupek, in T. C. J. Gribnau, J. Visser and R. J. F. Nivardy (Editors), *Affinity Chromatography and Related Techniques*, Elsevier, Amsterdam, 1982, pp. 165–179.
7 F. E. Regnier, *Science (Washington, D.C.)*, 222 (1983) 245.
8 J. G. Atwood, G. J. Schmidt and W. Slavin, *J. Chromatogr.*, 171 (1979) 109.
9 T. W. Hearn, F. E. Regnier and C. T. Wehr, *Int. Laboratory*, January (1983) 16–35.
10 T. Andersson, M. Carlsson, L. Hagel, J.-C. Janson and P.-Å. Pernemalm, *J. Chromatogr.*, in press.
11 K. Unger, *Methods Enzymol.*, 104C (1984) 167.