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Note

Column lifetime in fast protein liquid chromatography

BO-LENNART JOHANSSON* and NILS STAFSTRÖM
Pharmacia Fine Chemicals AB, P.O.Box 175, S-751 04 Uppsala (Sweden)
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The lifetime of high-performance liquid chromatographic columns is influenced by many factors. It is well known that the choice of solvent, salts and buffer components is important[1–3]. Further, mechanical damage caused by high back-pressure, pressure surge at the injection and high flow-rates has also been observed[4,5]. For the prolongation of column life it is also essential that the samples are cleaned of particulate and other extraneous material[1,6,7]. In the present study one anion- and one cation-exchange column for fast protein liquid chromatography (FPLC) were investigated. The performance of the columns was tested and evaluated during 1000 repetitive injections of a protein mixture.

EXPERIMENTAL

Chemicals and apparatus

Cytochrome *c* and transferrin were purchased from Sigma (St. Louis, MO, U.S.A.). Ribonuclease-A and ovalbumin were obtained from Pharmacia (Uppsala, Sweden). Inorganic reagents were all p.a. purity, and the buffers N-2-hydroxyethylpiperazine-N′-2-ethanesulphonic acid (HEPES) and piperazine were of purum quality.

The results were obtained with a Pharmacia strong cation-exchange (Mono S) HR 5/5 column and a strong anion-exchange (Mono Q) HR 5/5 column on a Pharmacia FPLC system.

Procedure

In the gradient elution of the Mono Q and the Mono S columns, solvent A contained 0.020 *M* piperazine (pH 6.00) and 0.010 *M* HEPES (pH 8.00), respectively. Solvent B contained solvent A with the addition of 0.30 *M* sodium chloride in case of Mono Q and 0.40 *M* lithium chloride for Mono S. All eluents were filtered through a 0.22-μm bacteriological filter, and sodium azide (0.020%) was added to the Mono S eluents as a bacteriostat. A 100-μl injection of ovalbumin (1.0 mg/ml) and transferrin (0.50 mg/ml) served as a test sample for the anion-exchange column, and 100 μl of ribonuclease (0.50 mg/ml) and cytochrome *c* (0.25 mg/ml) were used for the cation-exchange column. In both cases the sample substances were dissolved in solvent A and filtered through 0.22-μm bacteriological filters. Each sample solution was frozen in ten aliquots, until used.

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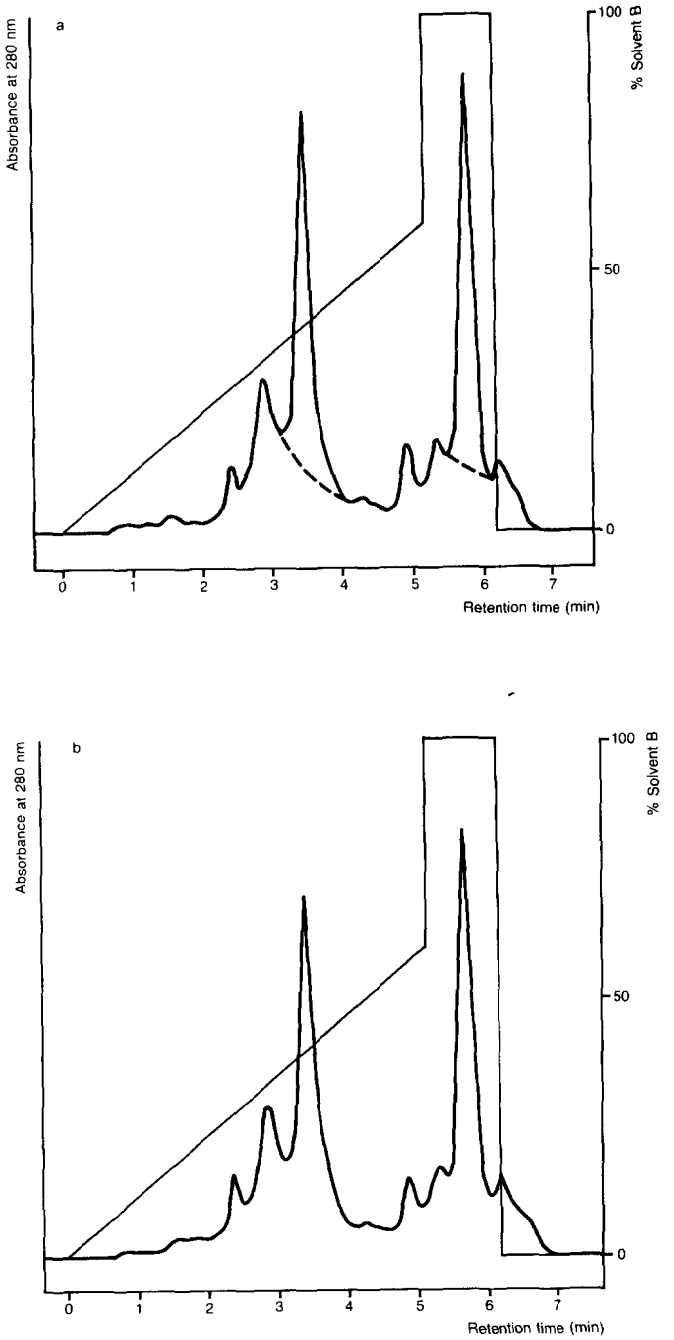

Fig. 1. Chromatograms from the first (a) and the thousandth (b) run of the Mono Q separation. Chromatographic conditions: sample, ovalbumin (1 mg/ml) and transferrin (0.5 mg/ml); sample volume, 100 μl; solvent A, 0.020 *M* piperazine (pH 6.00); solvent B, 0.30 *M* sodium chloride in solvent A; flow-rate, 1.00 ml/min. The dashed line represents the baseline at area calculations.

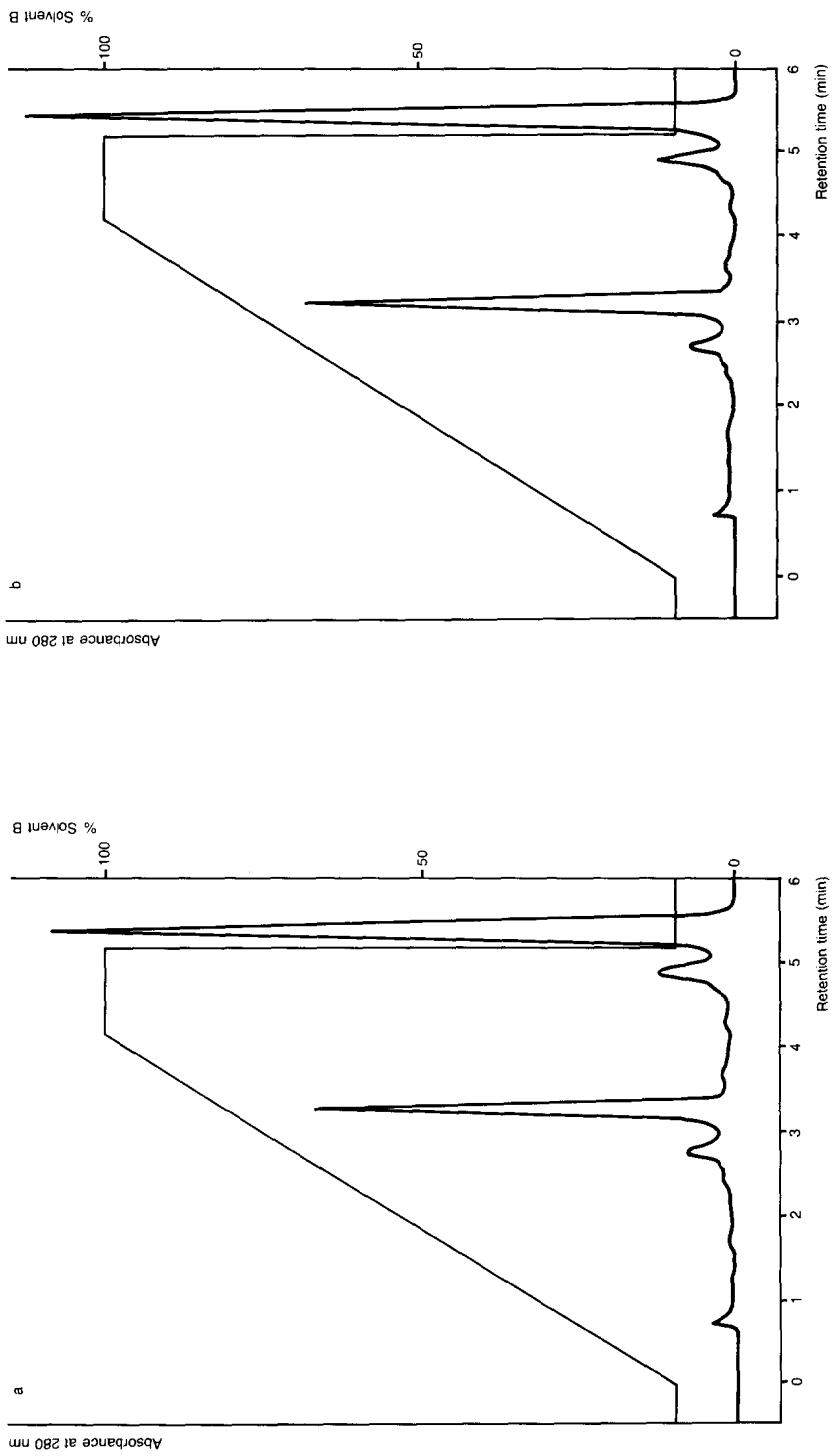

Fig. 2. Chromatograms from the first (a) and the thousandth (b) run of the Mono S separation. Chromatographic conditions: sample, ribonuclease (0.50 mg/ml) and cytochrome *c* (0.25 mg/ml); sample volume, 100 μl; solvent A, 0.01 *M* HEPES (pH 8.00); solvent B, 0.40 *M* lithium chloride in solvent A; flow-rate, 1.00 ml/min.

TABLE I

VARIATION OF THE RETENTION VOLUME OF THE MAIN PROTEIN PEAKS DURING 1000 INJECTIONS ON THE MONO Q AND THE MONO S COLUMNS

Injection number	*Retention volume (ml)*			
	Mono Q		*Mono S*	
	Transferrin	*Ovalbumin*	*Ribonuclease-A*	*Cytochrome c*
1	3.51	5.75	3.18	5.35
100	3.46	5.74	3.18	5.36
200	3.48	5.74	3.16	5.35
300	3.47	5.75	3.21	5.30
400	3.45	5.76	3.19	5.38
500	3.47	5.73	3.19	5.38
600	3.52	5.79	3.19	5.36
700	3.48	5.76	3.18	5.38
800	3.45	5.74	3.19	5.34
900	3.45	5.73	3.21	5.35
1000	3.46	5.70	3.18	5.35
Mean	3.47	5.74	3.19	5.35
S.D. (%)	0.68	0.39	0.45	0.43

Column equilibration and sample elution was affected in 10 min both for the Mono Q and the Mono S separations. The gradients used in the separations are depicted in Figs. 1 and 2 for the anion-exchange and the cation-exchange columns, respectively.

RESULTS AND DISCUSSION

The lifetimes of the Mono S and Mono Q columns were investigated by making 1000 repetitive injections of protein mixtures. The chromatographic pattern from the gradient separation was retained throughout the series for both columns (Figs. 1 and 2). A closer interpretation of the retention data of the two largest peaks in each chromatogram shows that the relative standard deviation was less than 1% (Table I). The maximum spread, calculated from the highest and lowest V_R obtained during the 1000 injections, was less than 3% for the main peaks in the chromatograms of the Mono Q and the Mono S separations. These retention data indicate that the Mono Q and the Mono S columns are well suited for sample characterization using V_R values[8].

The peak height, evaluated as the distance between the peak maximum and the baseline at the injection time, decreased by *ca.* 10% for the main peaks of transferrin and ovalbumin during the Mono Q study (Fig. 3). This decrease is not a function of sample accumulation on the column, because the total area for all peaks between 0.5 and 7 min (Fig. 1) remained constant. Therefore the reduction of peak height reflects a decrease in the column plate count (N). Normally, a change in N should not influence the peak area. However, the chromatographic pattern (Fig. 1)

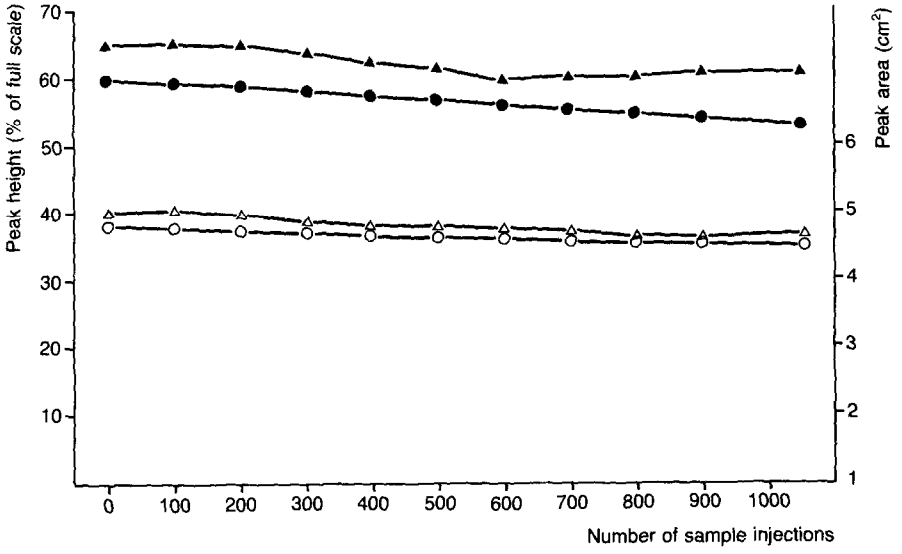

Fig. 3. Changes in peak height and peak area for the main peaks of transferrin (● and ○) and of ovalbumin (▲ and △) during 1000 injections on the Mono Q column.

makes it difficult to measure the areas of the main peaks accurately. This explains why the peak area values decreased during the study (Fig. 3).

The peak heights and the peak areas of the main peaks of ribonuclease and cytochrome *c* (Fig. 2) remained at the same level throughout the 1000 injections on the Mono S column (Fig. 4). The higher spread for the peak of cytochrome *c* reflects the instability of this protein, even though the sample solution was protected from air by helium gas and from light.

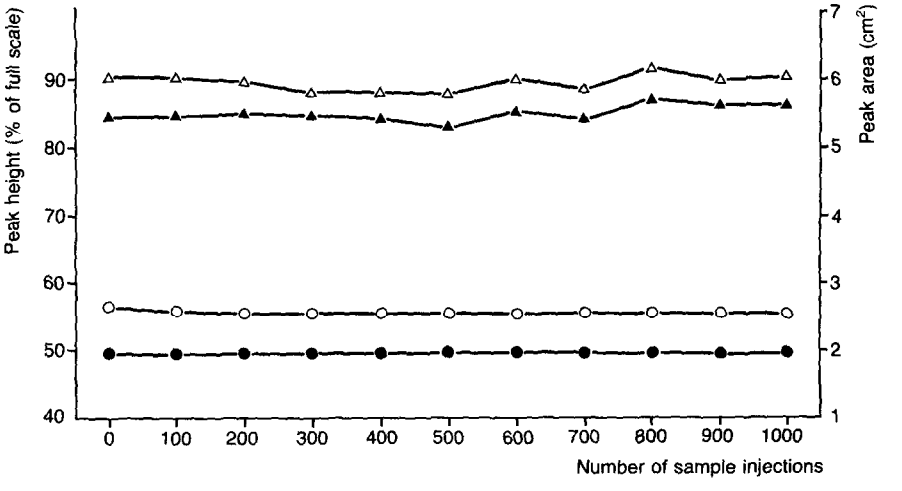

Fig. 4. Changes in peak height and peak area for the main peak of ribonuclease (● and ○) and of cytochrome *c* (▲ and △) during 1000 injections on the Mono S column.

During the test of the Mono Q column the back-pressure was steadily increased from 1 MPa to 5 MPa, whereas the pressure was held constant at 1 MPa in the Mono S column. The trends in Fig. 3 therefore seem to be related to the column pressure. Increasing column pressure results in a decreasing peak height/N value. Bacterial growth was probably responsible for the pressure enhancement, because the addition of sodium azide to the mobile phase resulted in a stable pressure when testing the Mono S column.

It can be concluded that the end of column life was not reached in these tests, as the resolution of the proteins was maintained after 1000 successive injections (Figs. 1 and 2). However, maximum column performance was retained only when the column pressure was kept at 1 MPa throughout.

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