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Note

Determination of some gel permeation chromatographic parameters of the gel Spheron P-1000

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The performance of gel permeation chromatographic (GPC) analysis depends on the properties of the gel packing and the choice of the experimental conditions. This work attempts to outline the chromatographic properties of the gel Spheron P-1000 for use in aqueous solvents.

EXPERIMENTAL

All measurements were made on a modular chromatograph consisting of the following main parts. MC 300 pump (Mikrotechna, Prague, Czechoslovakia); sample injector, six-way inlet valve with sample-injection loop of 0.271-ml capacity; supply lines, stainless-steel capillaries (diameter, 1 mm); detector, R-403 differential refractometer (Waters Assoc., Milford, Mass., U.S.A.); EZ 11 recorder (Laboratorní Přístroje, Prague, Czechoslovakia); flow-measuring device, siphon counter.

Spheron P-1000 is produced commercially (Lachema, Brno, Czechoslovakia) on the basis of a documentation obtainable from the Macromolecular Chemistry Institute of the Czechoslovak Academy of Sciences, Prague[1,2]. The gel is prepared by co-polymerization of 2-hydroxyethyl methacrylate with ethylenedimethacrylate in a non-polar dispersion medium. The gel particles are spherical; a maximum of 10% of the grains are of a different shape. The characteristics are summarized in Table I.

Standard fractions of Dextran T were obtained from Pharmacia (Uppsala, Sweden). 0.2% solutions in an eluent were prepared. The eluent was a 0.02% solution of sodium triazide in distilled water.

A solution of the proteins in Table II was prepared by the following procedure. 40 mg of haemoglobin, 40 mg of trypsin, 40 mg myoglobin and 20 mg of cytochrome *c* were dissolved in 10 ml of eluent with occasional stirring at 4°. After centrifugation, the clear fraction (*ca.* 8 ml) was filtered.

Column packing and separation efficiency

Five stainless-steel columns (1200 × 8 mm) were packed with Spheron P-1000 using a method described in ref. 3. Prior to packing, the gel was de-aerated by removing its aqueous suspension with a water vacuum pump (until air bubbles no longer escaped, *ca.* 0.5 h), and left to swell in the eluent for at least 24 h with occasional

TABLE I

CHARACTERISTICS OF THE GEL SPHERON P-1000

Swollen particle size (μm)	*Fractionation range (MW) of dextrans*	*Bed volume (ml per g of dry gel)*			
		water	*1 M acetic acid*	*tetrahydrofuran*	*benzene*
20–40	3000–10^6	4.50	4.60	4.80	3.80

TABLE II

THE PROTEINS USED

Compound	*MW*	*Supplier*
Haemoglobin	67 000	Serva, Heidelberg, G.F.R.
Trypsin	24 000	Davis, London, Great Britain
Myoglobin	17 600	Mann Labs., New York, N.Y., U.S.A.
Cytochrome *c*	12 400	I.B.F., Gennevilliers, France

TABLE III

CHARACTERISTICS OF THE PACKED COLUMNS

Column no.	*Flow-rate during packing (ml/h)*	*Final pressure (kp/cm²)*	*Flow-rate during tests (ml/h)*	$\bar{N} \cdot 10^{-3}$* (m^{-1})
1	200	22.0	20	2.63
2	140	16.3	20	2.69
3	110	15.6	20	3.29
4	110	16.0	21	3.75
5	80	14.0	20	2.10

* $\bar{N}$ = The mean number of theoretical plates per metre of gel bed obtained from two measurements.

decanting of suspended particles. The packing of one column required 24 h. During the final 2 h, the gel packing was pressurized by choking the valve at the end of the packing circuit. The number of theoretical plates for each column was determined using ethylene glycol. Data on the packing and separation efficiency of the columns are shown in Table III.

RESULTS AND DISCUSSION

Working pressure as a function of flow-rate

For a given experimental arrangement, the working pressure of the column depends on the flow-rate of the eluent and the resistance of the gel bed. When the latter is constant, the working pressure is affected only by the flow-rate. The results of varying the flow-rate of the eluent are shown in Fig. 1 for column 4 of Table III. The linear dependence of the working pressure on the eluent flow-rate is evident, indicating the high mechanical strength of the gel tested.

Separation efficiency as a function of flow-rate

It is well known that the eluent flow-rate is the factor that has the greatest influence on the separation efficiency of a packed column. Therefore, attention was paid to establishing the relation between the number of theoretical plates, $\bar{N}$, and the

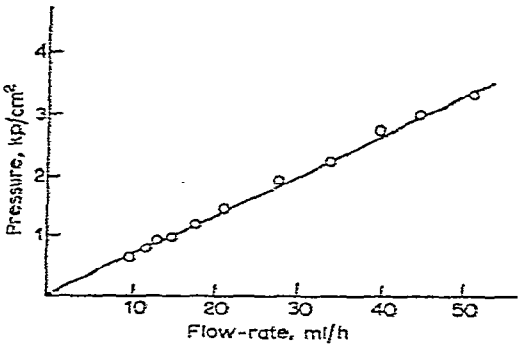

Fig. 1. Working pressure as a function of eluent flow-rate for column 4.

flow-rate. The results obtained from column 4 are summarized in Table IV and shown in Fig. 2.

Calibration graph

The dependence of the logarithm of the average molecular weight, log MW, of the standard fractions of Dextran T on the elution volume was determined for

TABLE IV

SEPARATION EFFICIENCY OF COLUMN 4

n = Number of theoretical plates per column; $\bar{n}$ = mean number of theoretical plates per column; $\bar{N}$ = mean number of theoretical plates per metre of the gel bed; and *HETP* = mean height equivalent to a theoretical plate.

Test no.	$n \cdot 10^{-3}$	$\bar{n} \cdot 10^{-3}$	$\bar{N} \cdot 10^{-3}$ (m^{-1})	$HETP \cdot 10^{2}$ (*cm*)	*Flow-rate (ml/h)*
1	7.26				
2	6.79	7.03	5.76	1.74	10.0
3	6.88				
4	6.76	6.82	5.59	1.79	12.3
5	6.27				
6	6.31	6.29	5.16	1.94	13.5
7	6.01				
8	5.89	5.95	4.88	2.05	15.4
9	5.47				
10	5.41	5.44	4.46	2.24	18.1
11	4.64				
12	4.50	4.57	3.75	2.67	21.0
13	4.12				
14	4.08	4.10	3.36	2.98	28.0
15	3.85				
16	4.09	3.97	3.25	3.07	34.0
17	4.12				
18	3.82	3.96	3.24	3.08	39.7
19	3.92				
20	3.92	3.92	3.20	3.11	45.0
21	3.70				
22	3.62	3.66	3.01	3.33	50.9

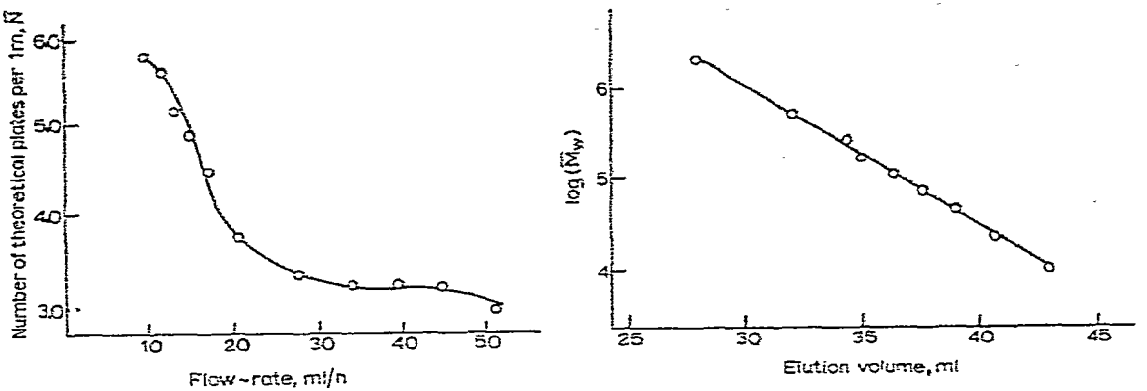

Fig. 2. Separation efficiency as a function of eluent flow-rate for column 4.

Fig. 3. Calibration plot for column 4.

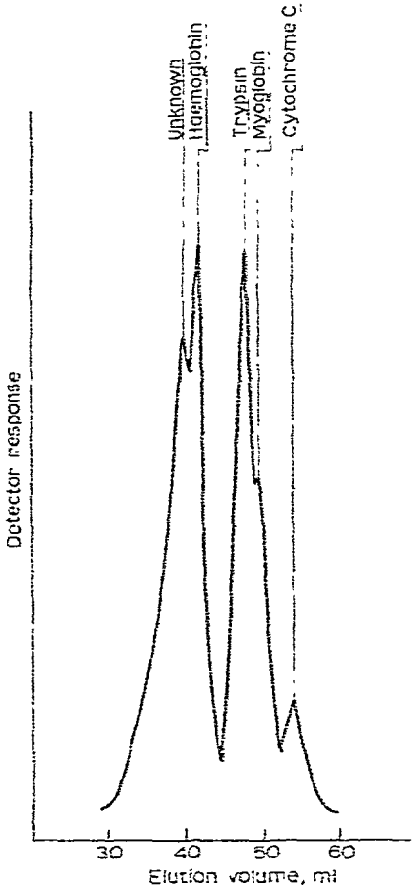

Fig. 4. Chromatogram of a mixture of proteins on column 4. Eluent flow-rate, 21.5 ml/h.

column 4 at an eluent flow-rate of 21 ml/h. From Fig. 3, it is apparent that the dependence is linear over the whole molecular-weight range.

Separation of a mixture of proteins

The separation efficiency of a gel can be determined by using a mixture of compounds which differ only slightly in their molecular weights in comparison with the very wide range of the gel. The chromatogram of a mixture of proteins on column 4 is shown in Fig. 4.

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