Journal of Chromatography, 161 (1978) 319–323

CHROM. 11,178

Note

Comparison of the gel chromatographic properties of Sephacryl S-200 Superfine and Sephadex G-150

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(Received May 22nd, 1978)

Sephacryl S-200 Superfine® (S-200) is a gel chromatography matrix which has recently been introduced by Pharmacia (London, Great Britain), and is claimed to have superior properties to the more familiar Sephadex matrices[1]. The resolving power of S-200 is said to be greater than that of Sephadex G-150, although the molecular weight ranges of the two matrices are similar. In view of these claims, a series of investigations were carried out to compare the performance of S-200 with Sephadex G-150 in two situations: (a) in an accurately calibrated column for molecular weight determinations, and (b) in a small-scale preparative column used for fractionation of serum proteins. The results of these investigations are described below.

MATERIALS AND METHODS

Analytical chromatography was carried out in a glass column (125 × 0.6 cm I.D.), with temperature accurately controlled at 4° ± 0.1°. S-200 and G-150 (Pharmacia) were consecutively packed in the same glass column at the manufacturers recommended flow-rates, 40 ml/cm²·h and 18 ml/cm²·h, respectively. Elution was performed using 0.01 *M* sodium phosphate buffer pH 7.4, at a flow-rate of 2.5 ml/h, the eluate being continuously monitored at 254 nm and UV absorption recorded directly. Columns were calibrated using a series of proteins of known molecular weight: human serum albumin fraction V, fetuin, ovalbumin, myoglobin (from Sigma, Kingston-upon-Thames, Great Britain) and cytochrome *c* (from BDH, Poole, Great Britain). Blue Dextran 2000 (Pharmacia) and potassium iodide or benzyl alcohol were used to determine column void and total volumes, respectively. Combinations of the above proteins (2–5 mg) contained in a 0.2-ml sample were chromatographed. To overcome possible effects of the presence of polar groups in the matrix, several repeat runs were carried out using an elution buffer with added sodium chloride at an initial concentration of 0.02 *M*. This concentration was increased after each series of separations by increments of 0.02 *M* until a concentration of 0.1 *M* sodium chloride was attained.

Small-scale preparative fractionation of human serum was carried out using

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a 85 × 2.5 cm I.D. column. The elution buffer was 0.008 *M* tris-(hydroxymethyl)-methylamine, containing 0.55 *M* sodium chloride, and brought to pH 8.6 with citric acid. Again, S-200 and G-150 were compared after packing according to the manufacturers' instructions. Fresh serum (8 ml) obtained from a single donor was dialysed against elution buffer and applied to the column. A flow-rate of 12 ml/h was employed for both columns. The eluate was again monitored at 254 nm and 6.5-ml fractions were collected. All operations were carried out at 4°. The fractions obtained were analysed by mono-rocket immunoelectrophoresis using mono-specific antisera to the following proteins: albumin, immunoglobulin G, immunoglobulin M, Factor B and Complement Component C_4. (Antisera were kindly donated by Dr. A. R. Bradwell of the Immunodiagnostic Laboratory, University of Birmingham.)

RESULTS

The results of the first series of experiments are shown in Fig. 1 where K_{av} is

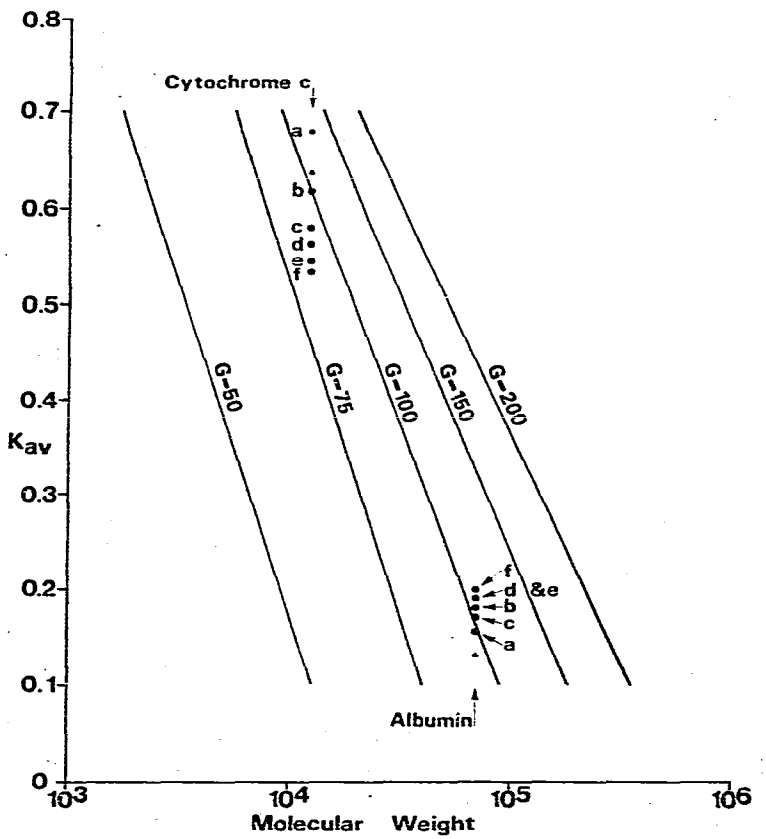

Fig. 1. Performance of a 125 × 0.6 cm I.D. S-200 column. Elution of albumin and cytochrome *c* with 0.01 *M* sodium phosphate buffer pH 7.4 in the presence of increasing salt concentrations: a = zero salt; b = 0.02 *M*; c = 0.04 *M*; d = 0.06 *M*; e = 0.08 *M* and f = 0.10 *M* NaCl. A second batch of S-200 (▲) tested under identical conditions to the first batch, at zero salt concentration.

defined as:

$$K_{av} = \frac{\text{elution volume} - \text{void volume}}{\text{total volume} - \text{void volume}}$$

and is plotted against molecular weight on a logarithmic scale. In the region shown, the idealised behaviour of a number of Sephadex gels produces a series of curvilinear traces[2]. K_{av} values obtained from the G-150 column fell on the G-150 curve, however, K_{av} values obtained from the S-200 column approximated more nearly to the G-100 curve. In addition, the presence of salt in the elution buffer significantly changed the K_{av} values obtained for both basic and acidic proteins, as illustrated by cytochrome *c* and albumin. In the case of the former, increasing salt concentrations displaced the points towards the G-75 curve, reducing separation from the void volume marker, whilst in the case of the latter, increasing salt concentrations shifted the points towards the G-150 curve, thus improving separation of the protein from the void volume marker. A different batch of S-200 also produced similar results.

The results of fractionation of human serum using S-200 and G-150 are shown in Figs. 2 and 3, respectively. The resolution of selected serum proteins, calculated

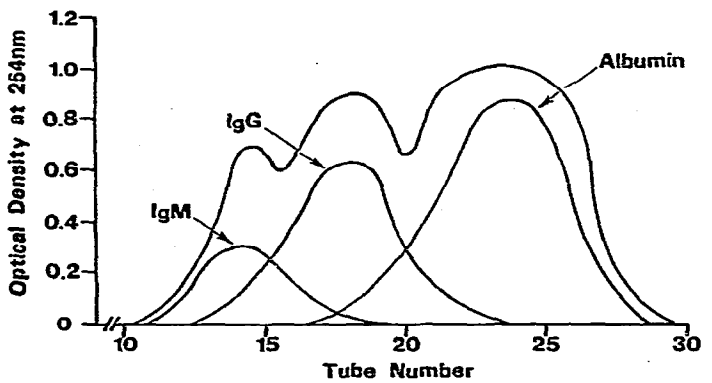

Fig. 2. Elution profile of human serum on an 85 × 2.5 cm I.D. S-200 column. Elution buffer 0.008 *M* tris–citrate pH 8.6 containing 0.55 *M* sodium chloride. Flow-rate 12 ml/h. 6.5 ml fractions collected. Immunoglobulin M, immunoglobulin G and albumin identified by immunoelectrophoresis.

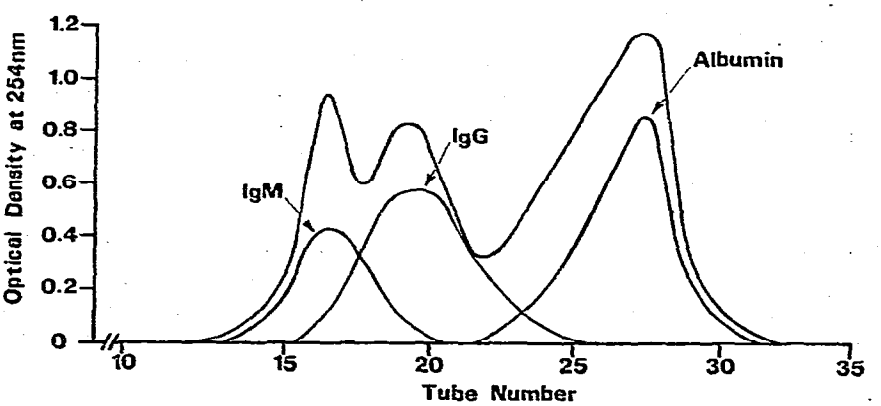

Fig. 3. Elution profile of human serum on an 85 × 2.5 cm I.D. G-150 column. Elution buffer 0.008 *M* tris–citrate pH 8.6 containing 0.55 *M* sodium chloride. Flow-rate 12 ml/h. 6.5-ml fractions collected. Immunoglobulin M, immunoglobulin G and albumin identified by immunoelectrophoresis.

by the expression:

$$\text{Resolution} = \frac{\text{peak centre (1)} - \text{peak centre (2)}}{\text{standard deviation peak (1)} + \text{standard deviation peak (2)}}$$

is shown in Table I.

TABLE I

RESOLUTION OF SOME SERUM PROTEINS

Ig = Immunoglobulin.

Proteins compared	*Respective molecular weights* ($\cdot 10^{-3}$)	*Resolution on G-150*	*Resolution on S-200*
IgM/C_4	950/205	1.26	1.14
IgM/IgG	950/160	1.49	1.12
IgM/Factor B	950/ 93	2.77	1.95
IgM/albumin	950/ 68	4.58	2.54
IgG/albumin	160/ 68	2.56	1.43
Albumin/C_4	68/205	3.27	1.94
Albumin/Factor B	68/ 93	1.42	0.59

DISCUSSION

It can be seen from Fig. 1 that S-200 behaves like Sephadex G-100, rather than G-150 as anticipated. The poor performance of S-200 could not be attributed to defects in construction of the column since the same glass column packed with G-150 produced points which lie exactly on the G-150 curve. That the S-200 tested was part of a faulty batch was ruled out by repacking the column with a different batch of S-200 (kindly donated by Pharmacia), and which produced similar results to the first batch.

Fig. 1 also illustrates the polar nature of S-200 and that at pH 7.4 the resin carries a net negative surface charge, and behaves as a cation exchanger[3]. Increasing the salt concentration reduces the adsorption of basic proteins as demonstrated by cytochrome *c*. Further evidence for the presence of charged groups in the matrix is afforded by the persistent "tailing" of iodide used as the total volume marker. This problem was circumvented by use of benzyl alcohol in place of potassium iodide. The existence of charged groups in the matrix may therefore prevent the application of S-200 to electrofocusing, a technique in which neutral Sephadex may be employed.

In an attempt to minimise protein-matrix charge interactions in S-200 during fractionation of human serum, the ionic strength of the elution buffer was increased to 0.55 *M* using sodium chloride, so that separation should be achieved by passive molecular sieving alone. The results of fractionating serum proteins on S-200 and G-150 are shown in Figs. 2 and 3, respectively. At first glance there appears to be little difference between the two graphs, however, mathematical analysis of the resolution of certain selected proteins reveals that the performance of G-150 is consistently superior to that of S-200 for every pair of proteins considered (Table I).

CONCLUSION

Under analytical and small-scale preparative conditions the performance of S-200 was found to be inferior to that of G-150 and for proteins within the effective

fractionation range of 5000–250,000, its behaviour approximated to that of G-100. At pH 7.4 the matrix is polar and behaves as a cation exchanger. The presence of up to 0.55 *M* sodium chloride reduces protein-matrix charge interactions. However, resolution remains inferior to that of G-150.

S-200 may, however, be of use for separation of certain basic proteins, for separations where a fast flow rate is required, or for large-scale and industrial purposes. In the latter case, the greater mechanical strength of S-200 compared to the softer large pore Sephadex gels may allow for better packing of very large columns, thus reducing the void volume where remixing can occur, and thereby more than compensating for its lesser chromatographic performance under normal laboratory conditions.

REFERENCES

1 *Sephacryl S-200 Superfine*, Pharmacia Fine Chemicals, Uppsala, Sweden ,1976.
2 *Sephadex gel filtration in theory and practice*, Pharmacia Fine Chemicals, Uppsala, Sweden, 1974.
3 M. Belew, J. Porath, J. Fohlman and J.-C. Janson, *J. Chromatogr.*, 147 (1978) 205.