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HIGH-SPEED GEL FILTRATION OF PROTEINS IN SODIUM DODECYL SULPHATE AQUEOUS SOLUTION ON TSK-GEL SW TYPE

YOSHIO KATO, KATSUO KOMIYA, HIROO SASAKI and TSUTOMU HASHIMOTO

Central Research Laboratory, Toyo Soda Manufacturing Co., Ltd., Tonda, Shinnanyo, Yamaguchi (Japan)

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SUMMARY

High-speed gel filtration of proteins was performed in sodium dodecyl sulphate aqueous solutions on TSK-GEL SW type columns. The separation ranges of G2000SW, G3000SW and G4000SW were, respectively, 15,000–25,000, 10,000–100,000 and 15,000–300,000. G3000SW showed the highest separation efficiency in the molecular weight range below *ca.* 60,000, while G4000SW showed the highest separation efficiency above molecular weight of *ca.* 60,000. The elution behaviour of proteins was greatly affected by sodium phosphate concentration in the eluent and the optimum sodium phosphate concentration was 0.05–0.2 *M*.

INTRODUCTION

Gel filtration in sodium dodecyl sulphate (SDS) aqueous solution is becoming increasingly popular as a means for estimating the molecular weight of protein constituent polypeptide chains or for the purification of proteins that do not dissolve in common buffer solutions such as membrane proteins. However, as proteins have gross conformations in SDS aqueous solution by forming protein-SDS complexes, column packings of large pore size must be employed. Therefore Sephadex, which has been used most often in gel filtration in common buffer solutions, is not suitable for this purpose.

Although agarose gel[1] and controlled pore glass[2,3] have been used, there are some problems such as low flow-rate or poor separation efficiency. In contrast, it has been reported that TSK-GEL SW type (Toyo Soda, Tokyo, Japan) showed very high separation efficiency at high speed[4]. In this paper, the separation range and separation efficiency are investigated in gel filtration of proteins in SDS aqueous solution on TSK-GEL SW type columns. Furthermore, as Imamura *et al.* reported that the sodium phosphate concentration in SDS aqueous solution greatly affects the elution behaviour of proteins[4], the effect of the sodium phosphate concentration is also investigated.

EXPERIMENTAL

Gel filtration was carried out at 25° on a commercial liquid chromatograph Model HLC-803 (Toyo Soda) equipped with UV detector at 280 nm. Column systems consisting of, respectively, two G2000SW, two G3000SW and two G4000SW columns (60 cm × 7.5 mm I.D.) were used. SDS aqueous solutions (0.1%) containing 0.02–0.5 *M* sodium phosphate (pH 7) were used as eluents. The flow-rate was 1 ml/min. The sample concentration of solution was 0.2% and the injection volume was 0.1 ml. The solutions were prepared by incubating proteins in 2% SDS aqueous solution containing 0.1 *M* sodium phosphate and 0.02 *M* dithiothreitol (pH 7) at 25° for one night. Subsequently, monoiodoacetamide was added in a concentration of 0.04 *M*, and the solutions were again incubated at 25° for more than 1 h.

The samples were commercial proteins, listed in Table I.

TABLE I

PROTEINS USED IN GEL FILTRATION

Protein	*MW of constituent polypeptide*	*Source**
Thyroglobulin	165,000	A
Bovine serum albumin	67,000	B
Ovalbumin	43,000	C
Myoglobin	16,900	A
Cytochrome *c*	12,400	D
Insulin	2900	A
γ-Globulin (human serum)	50,000 (heavy chain) 23,000 (light chain)	E
Ovalbumin (crude)		B

* A, Sigma (St. Louis, Mo., U.S.A.); B, Wako (Osaka, Japan); C, Seikagaku Kogyo (Tokyo, Japan); D, Miles Labs. (Elkhart, Ind., U.S.A.); E, Nakarai (Kyoto, Japan).

RESULTS AND DISCUSSION

Figs. 1–3 show the calibration curves of G2000SW, G3000SW and G4000SW in SDS aqueous solutions containing sodium phosphate in various concentrations. With an increase of the sodium phosphate concentration, the calibration curves shifted to higher elution volume, and the exclusion limits became slightly higher. At a sodium phosphate concentration of 0.5 *M*, however, the plots of long molecular weight *versus* elution volume for G3000SW and G4000SW were scattered and did not lie on smooth curves because of the adsorption. On the other hand, calibration curves of G2000SW were almost the same at sodium phosphate concentrations of 0.2 *M* and 0.5 *M*.

Figs. 4 and 5 show the elution curves of γ-globulin (human serum) obtained on G3000SW and on G4000SW at different concentrations of sodium phosphate. It can be seen from these figures that separation efficiency also depends on the sodium phosphate concentration. The resolution calculated for two main peaks is plotted against the sodium phosphate concentration in Fig. 6. The resolution increased up to 0.2 *M* and then decreased because of adsorption with increasing sodium phosphate

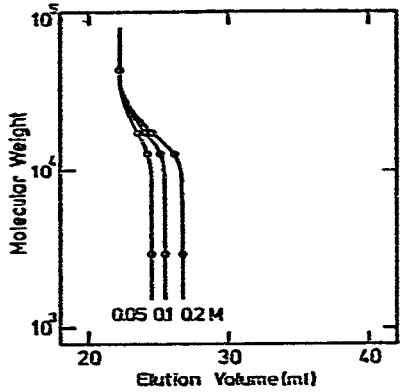
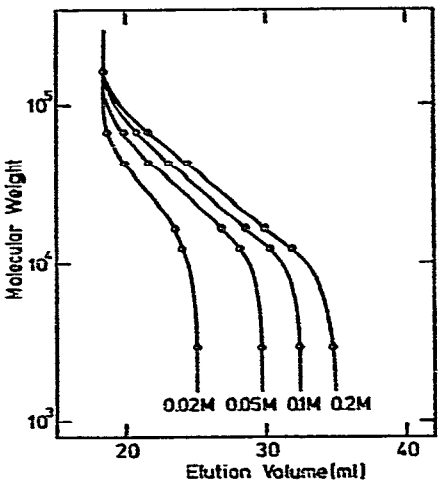


Fig. 1. Calibration curves of G2000SW for proteins in 0.1% SDS aqueous solutions containing 0.05, 0.1 and 0.2 *M* sodium phosphate (pH 7).



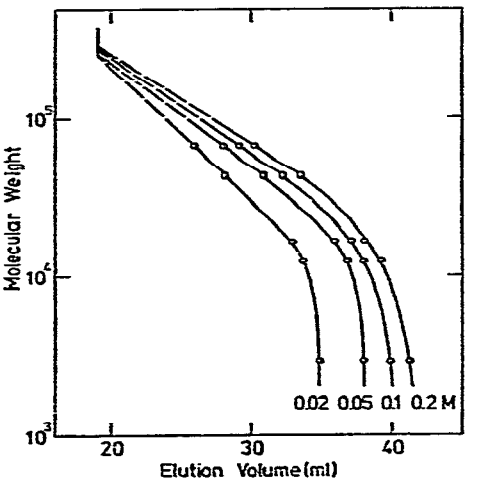


Fig. 2. Calibration curves of G3000SW for proteins in 0.1% SDS aqueous solutions containing 0.02, 0.05, 0.1 and 0.2 *M* sodium phosphate (pH 7).

Fig. 3. Calibration curves of G4000SW for proteins in 0.1% SDS aqueous solutions containing 0.02, 0.05, 0.1 and 0.2 *M* sodium phosphate (pH 7).

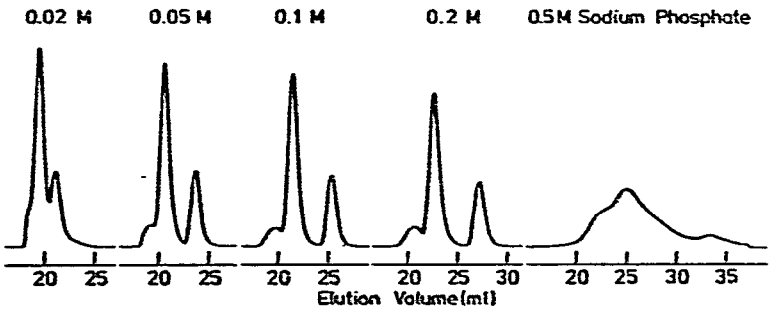


Fig. 4. Elution curves of γ-globulin (human serum) obtained by gel filtration on G3000SW in 0.1% SDS aqueous solutions containing 0.02, 0.05, 0.1, 0.2 and 0.5 *M* sodium phosphate (pH 7).

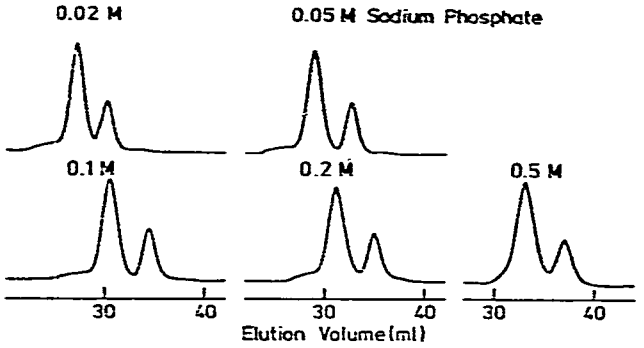


Fig. 5. Elution curves of γ-globulin (human serum) obtained by gel filtration on G4000SW in 0.1% SDS aqueous solutions containing 0.02, 0.05, 0.1, 0.2 and 0.5 *M* sodium phosphate (pH 7).

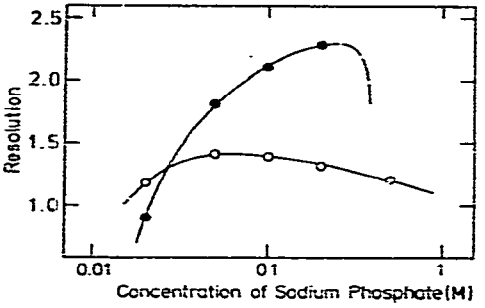


Fig. 6. Dependence of resolution for two main constituent polypeptide chains of γ-globulin (human serum) on the concentration of sodium phosphate in the eluent. ●, G3000SW; ○, G4000SW.

concentration in the case of G3000SW. G4000SW showed the highest resolution at sodium phosphate concentrations of 0.05–0.1 *M*, although the resolution changed only slightly with changing sodium phosphate concentration. However, the optimum concentration of sodium phosphate seems to vary depending on the sample, as can be understood from Figs. 7 and 8, which show the elution curves of a mixture of some proteins. In general, therefore, the best sodium phosphate concentration is considered to be *ca.* 0.1 *M*. It should be noted, however, that insulin began to adsorb on to the column packings at sodium phosphate concentration of 0.1 *M*, and severely tailing peaks were observed at 0.2 *M* on both G3000SW and G4000SW (Figs. 7 and 8). Imamura *et al.*[4] also have reported that aprotinin of molecular weight 6500 adsorbed considerably on to G3000SW column packings at a sodium phosphate concentration of 0.1 *M*.

Calibration curves and separation ranges at a sodium phosphate concentration of 0.1 *M* are shown in Fig. 9 and Table II, respectively, which indicate that polypeptide chains in the molecular weight (MW) range 10,000–300,000 can be separated on TSK-GEL SW type columns. The lower limit of 10,000 is approximately consistent with those observed in gel filtration in SDS aqueous solution on other column packings, although it has been reported by Imamura *et al.*[4] that the calibration curve was linear down to MW 3000 in the measurement on G3000SW. In our measure-

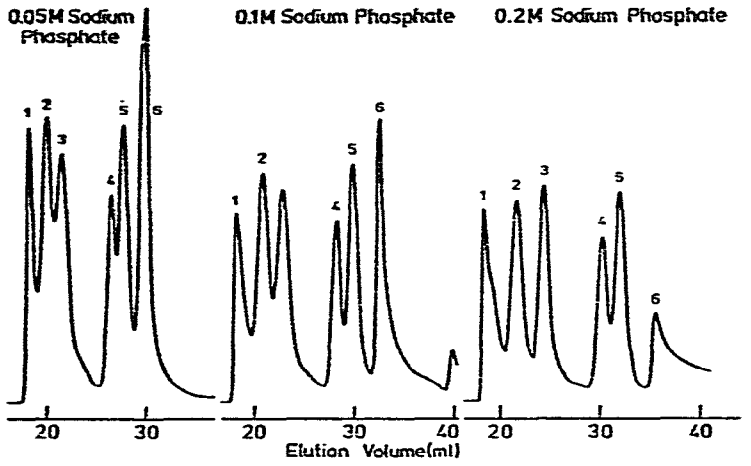


Fig. 7. Elution curves of a mixture of thyroglobulin (0.03%) (1), bovine serum albumin (0.06%) (2), ovalbumin (0.04%) (3), myoglobin (0.03%) (4), cytochrome *c* (0.02%) (5) and insulin (0.04%) (6) obtained by gel filtration on G3000SW in 0.1% SDS aqueous solutions containing 0.05, 0.1 and 0.2 *M* sodium phosphate (pH 7).

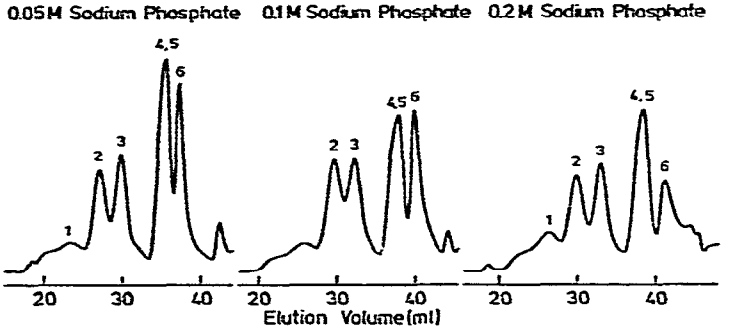


Fig. 8. Elution curves of the same mixture as in Fig. 7 obtained by gel filtration on G4000SW in 0.1% SDS aqueous solutions containing 0.05, 0.1 and 0.2 *M* sodium phosphate (pH 7). Peak numbers as in Fig. 7.

ments, the separation range of TSK-GEL SW type was not extended below MW 10,000 even by use of G2000SW, which has a smaller pore size than G3000SW.

In Fig. 10, specific resolutions for some pairs of proteins (bovine serum albumin–ovalbumin, ovalbumin–myoglobin, myoglobin–cytochrome *c*, cytochrome *c*–insulin) are plotted against average MWs of the two proteins. This figure indicates that G3000SW shows the highest separation efficiency in the MW range below *ca.* 60,000 and G4000SW shows the highest separation efficiency in the MW range above *ca.* 60,000. The separation efficiency of G2000SW was not so good as that of G3000SW; in addition, the separation range of G2000SW was fairly narrow compared with that of G3000SW. Therefore, G2000SW is not effective for gel filtration in SDS aqueous solution.

Commercial crude ovalbumin was measured on G3000SW as an example of the application of gel filtration in SDS aqueous solution on TSK-GEL SW type. The

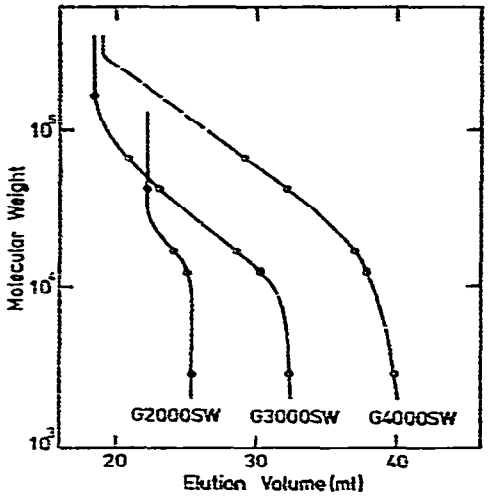


Fig. 9. Calibration curves of TSK-GEL SW types for protein in 0.1% SDS aqueous solution containing 0.1 *M* sodium phosphate (pH 7).

TABLE II

SEPARATION RANGE OF TSK-GEL SW TYPE FOR POLYPEPTIDE IN 0.1% SDS AQUEOUS SOLUTION CONTAINING 0.1 *M* SODIUM PHOSPHATE (pH 7)

Column	*Separation range (MW)*
G2000SW	15,000– 25,000
G3000SW	10,000–100,000
G4000SW	15,000–300,000

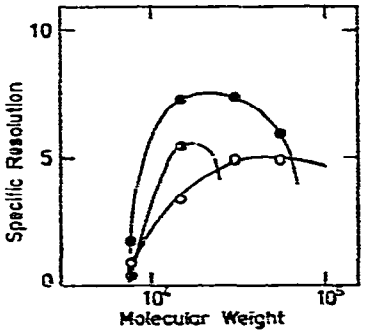


Fig. 10. Comparison of specific resolution for some pairs of proteins on TSK-GEL SW types. ◑, G2000SW; ●, G3000SW; ○, G4000SW.

sodium phosphate concentration was 0.1 *M*. The elution curve obtained is shown in Fig. 11. Four peaks were observed, besides the first peak corresponding to high-MW components which were totally excluded. The MWs of these four peaks were estimated from the calibration curve in Fig. 9 and are summarized in Table III. The main components of egg-white, other than ovalbumin (60%), are conalbumin

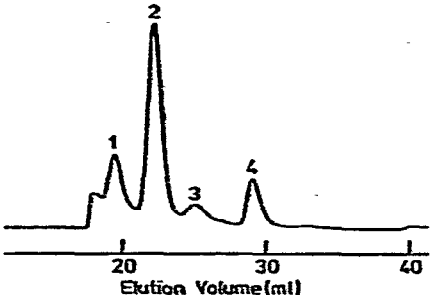


Fig. 11. Elution curve of crude ovalbumin obtained by gel filtration on G3000SW in 0.1% SDS aqueous solution containing 0.1 *M* sodium phosphate (pH 7).

TABLE III

MOLECULAR WEIGHTS OF POLYPEPTIDE CHAINS OF COMPOMENTS OF CRUDE OVALBUMIN DETERMINED BY GEL FILTRATION ON G3000SW IN 0.1% SDS AQUEOUS SOLUTION CONTAINING 0.1 *M* SODIUM PHOSPHATE (pH 7)

Component	*Molecular weight*
1	80,000
2	43,000
3	27,000
4	13,000

(14%, MW 70,000), ovomucoid (14%, MW 27,000–29,000) and ovoglobulin (12%, MW 14,000–17,000). Consequently, peaks 1, 2, 3 and 4 are presumed to correspond to conalbumin, ovalbumin, ovomucoid and ovoglobulin, respectively.

The MWs of the two main peaks appearing in the elution curves at a sodium phosphate concentration of 0.1 *M* in Figs. 4 and 5 were also estimated from respective calibration curves in Fig. 9 and are summarized in Table IV. A molecule of γ-globulin consists of two heavy chains (MW 50,000) and two light chains (MW 23,000), so the bigger peak and the smaller peak probably correspond to the heavy chain and the light chain, respectively, although the MWs estimated are a little larger than those generally accepted. Ratios of the MWs and peak areas of the two main peaks are also listed in Table IV. These ratios should be equal if the extinction coefficients of heavy and light chains at 280 nm are identical. However, the ratios obtained differ by

TABLE IV

GEL FILTRATION OF REDUCED γ-GLOBULIN (HUMAN SERUM) ON G3000SW AND G4000SW IN 0.1% SDS AQUEOUS SOLUTION CONTAINING 0.1 *M* SODIUM PHOSPHATE (pH 7)

	G3000SW	*G4000SW*
MW of bigger peak	54,000	55,000
MW of smaller peak	27,000	27,000
Ratio of MW of bigger peak to MW of smaller peak	2.00	2.04
Ratio of peak area of bigger peak to peak area of smaller peak	2.42	2.38

ca. 20%. Nevertheless, TSK-GEL SW type must be very effective for use in gel filtration in SDS aqueous solution as well as in common buffer solutions because the high separation efficiency is attainable at high speed also in SDS aqueous solution.

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