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# Note

# Gel permeation chromatography for the determination of the molecular mass distribution of some industrial proteins

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Gel permeation chromatography (GPC) is an important method of purification and is used in the analysis of proteins, nucleic acids and other bioligands and biopolymers with gels of different pore sizes as the stationary phase[1–3].

## EXPERIMENTAL

### *Gel preparation*

Sephadex gels which are dextran polymers cross-linked by epichlorohydrin, were purified by decantation and swollen in the solvent used for elution. Homogeneity of the gel formed in the column limits the column resolution. Therefore, the swollen gel is introduced into the column in the form of a suspension.

### *Gel filtration*

Table I gives the properties of model proteins used for preparation of the calibration graphs. The proteins were filtered in a series of glass columns (45 × 1.5 cm, 55 × 1.5 cm and 55 × 2.0 cm I.D.) filled with Sephadex G-100 grains of size 40–120 μm (Pharmacia, Uppsala, Sweden). Degassed phosphate buffer ($Na_2HPO_4$ + $KH_2PO_4$) of concentration 0.066 mol/l and pH 6.6 was used as the eluent. Elution was carried out without a pump at a rate of about 12 ml/h and at an appropriately selected

TABLE I

PROPERTIES OF MODEL PROTEINS USED IN THE CALIBRATION OF THE GEL FILTRATION MOLECULAR MASS DISTRIBUTION ANALYSIS

| *Model protein* | *Molecular mass* | *Isoelectric point* | *UV–VIS diagnostic band (nm)* |
|---|---|---|---|
| γ-Globulin | 180 000 | 5.6–8.2 | 280 |
| Serum albumin | 69 000 | 4.7–4.9 | 280 |
| α-Amylase | 50 000 | 7.0 | 280 |
| Egg albumin | 42 000 | 4.6 | 280 |
| Trypsin | 23 000 | 10.9 | 280 |
| Cytochrome *c* | 13 000 | 9.8–10.1 | 412 |

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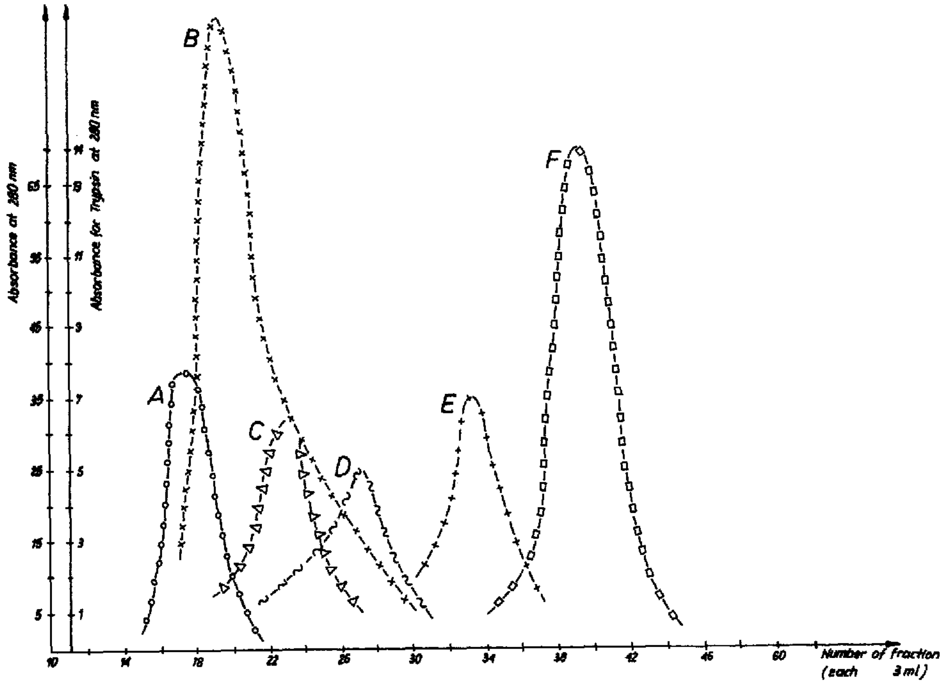


Fig. 1. Elution curves for several model proteins and blue dextran obtained from a 55 × 1.5 cm I.D. Sephadex G-100 column. A = Blue dextran; B = $\gamma$-globulin; C = serum albumin; D = egg-albumin; E = trypsin; F = Cytochrome *c*.

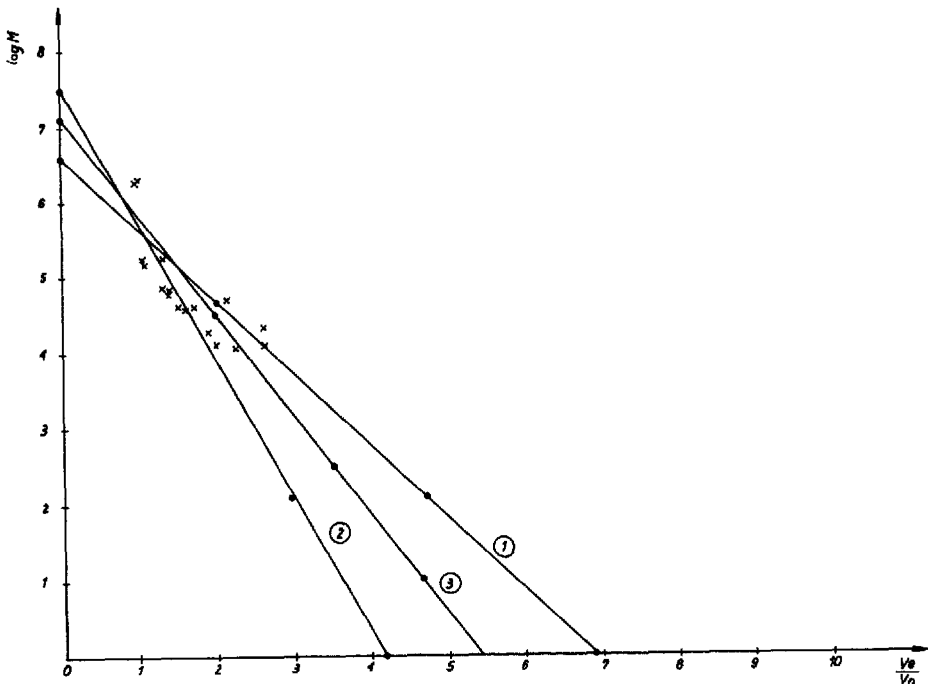


Fig. 2. Calibration graphs of log *M* *vs.* $V_e/V_0$ for gel filtration of proteins using Sephadex G-100 columns: 1 = 45 × 1.5 cm I.D.; 2 = 55 × 1.5 cm I.D.;3 = 55 × 2.0 cm I.D.

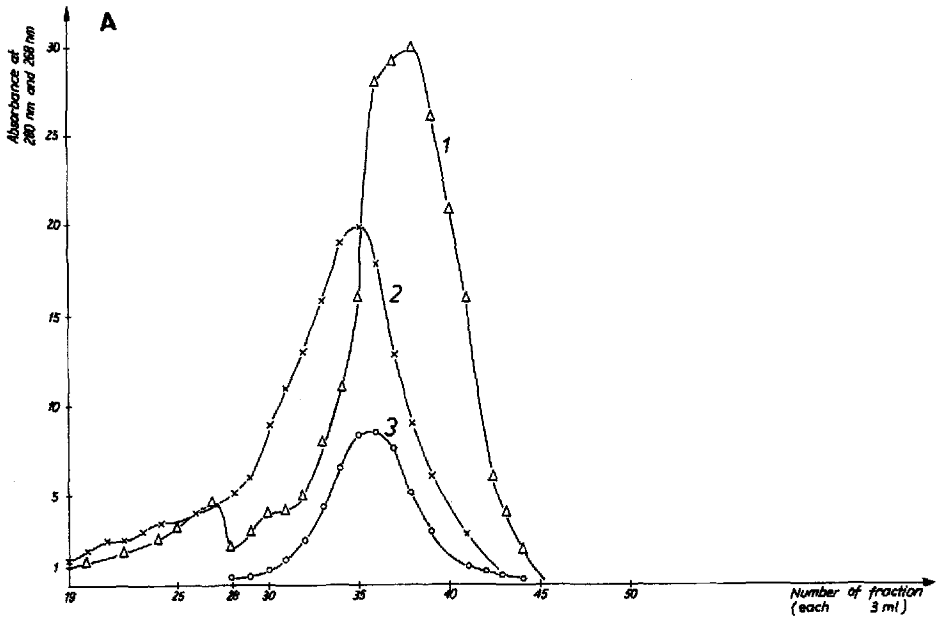


Fig. 3. (A) Elution curves of raw, α-amylase sample and ultrafiltration retentate and filtrate. 1 = α-Amylase sample; 2 = retentate; 3 = filtrate. Column: 55 × 1.5 cm I.D. (B) Elution curves of raw pectase sample and ultrafiltration retentate. 1 = Pectase sample; 2 = retentate. Column: 55 × 1.5 cm I.D.

hydrostatic pressure. The void volume of the column ($V_0$) was determined by means of blue dextran ($M = 2 \cdot 10^6$). The course of the separation was monitored by recording a UV–VIS spectrum for every fraction (3 ml) using a Specord UV–VIS spectrometer (Carl Zeiss, Jena, G.D.R.). Elution curves of several model proteins and blue dextran are shown in Fig. 1.

*Determination of calibration graph*

Determination of a calibration graph of log $M$ *vs.* $V_e/V_0$ is essential for calculating the molecular mass distribution of biopolymers. Directional coefficients ($a$ and $b$) are calculated from the log $M = a(V_e/V_0) + b$ relationship using the least-squares method. The elution volume ($V_e$) for reference proteins was determined on the basis of the results obtained during gel permeation on Sephadex G-100. Fig. 2 shows the calibration graphs for the gel permeation of proteins.

## RESULTS

Gel permeation was performed for a series of samples of α-amylase, several pectinases (industrial samples of bacterial origin) and streams of their filtrates and concentrates obtained from ultrafiltration on polyacrylonitrile ad poly(vinyl chloride) membranes. The elution curves are shown in Fig. 3. A knowledge of the molecular mass distribution for the filtrate and concentrate makes it possible to characterize the ultrafiltration process compared with the molecular mass distribution of the original sample of the industrial active protein, *e.g.*, α-amylase or pectinase. Molecular mass distributions of these samples obtained by gel chromatography are given in Figs. 4 and 5.

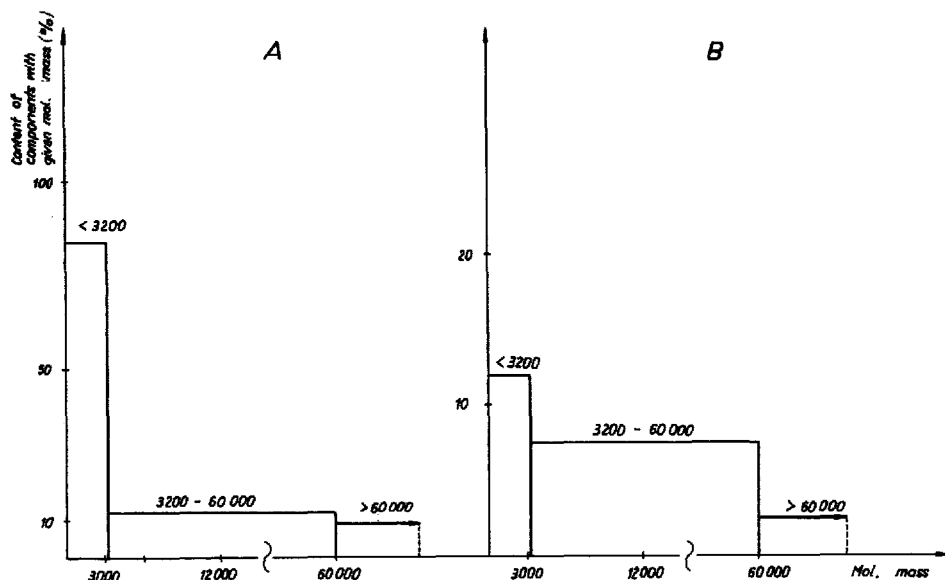


Fig. 4. Gel chromatographic molecular mass distributions of (A) raw pectase sample and (B) ultrafiltration retentate.

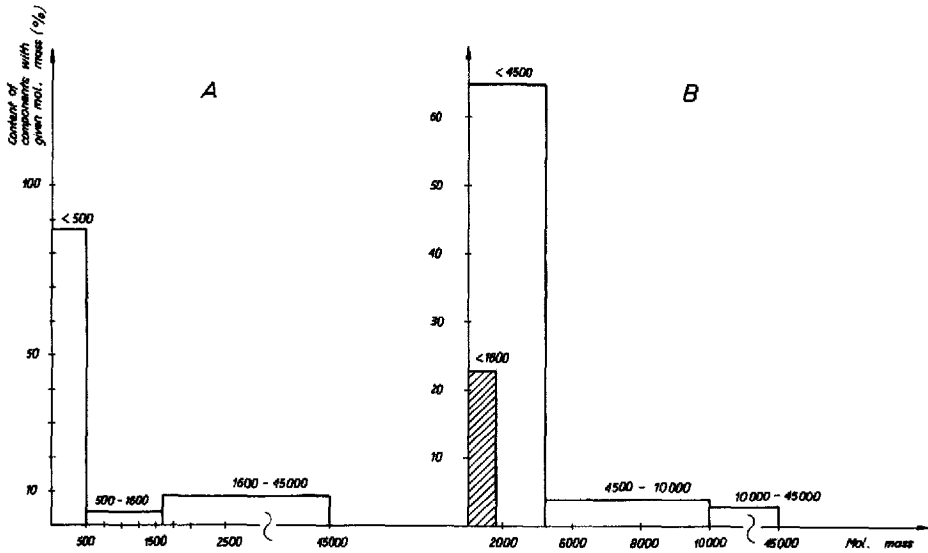


Fig. 5. Gel chromatographic molecular mass distributions of (A) raw α-amylase sample and (B) ultrafiltration retentate (open boxes) and filtrate (hatched box).

## CONCLUSION

The proposed method allows the determination of the molecular mass distribution of protein mixtures in the streams emanating from ultrafiltration of technical proteins using polyacrylonitrile and poly(vinyl chloride) membranes.

## REFERENCES

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