TOSOH

SEPARATION REPORT NO. 35

GFC ANALYSIS OF WATER-SOLUBLE POLYMERS WITH TSKgel PW COLUMNS

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1. Introduction

After Tosoh developed packing materials for gel permeation chromatography (GPC) in 1971, various packing materials for HPLC were developed and marketed, as shown in Table 1. Since then, Tosoh scientists have introduced many new products for the analysis of polymers in various mobile phases, i.e. organic solvents, aqueous eluents and buffers, and mixtures of aqueous and polar organic solvents. In addition, Tosoh has developed several gel filtration column lines for the analysis of proteins.

In this report, we discuss the benefits and features of TSKgel PW columns for the analysis of water-soluble polymers by gel filtration chromatography (GFC).

2. Separation ranges for each grade of TSKgel PW column

Table 2 shows separation ranges for TSKgel PW columns. The TSKgel G6000PW column has the largest pore size, while the TSKgel G3000PW column has the smallest average pore size.

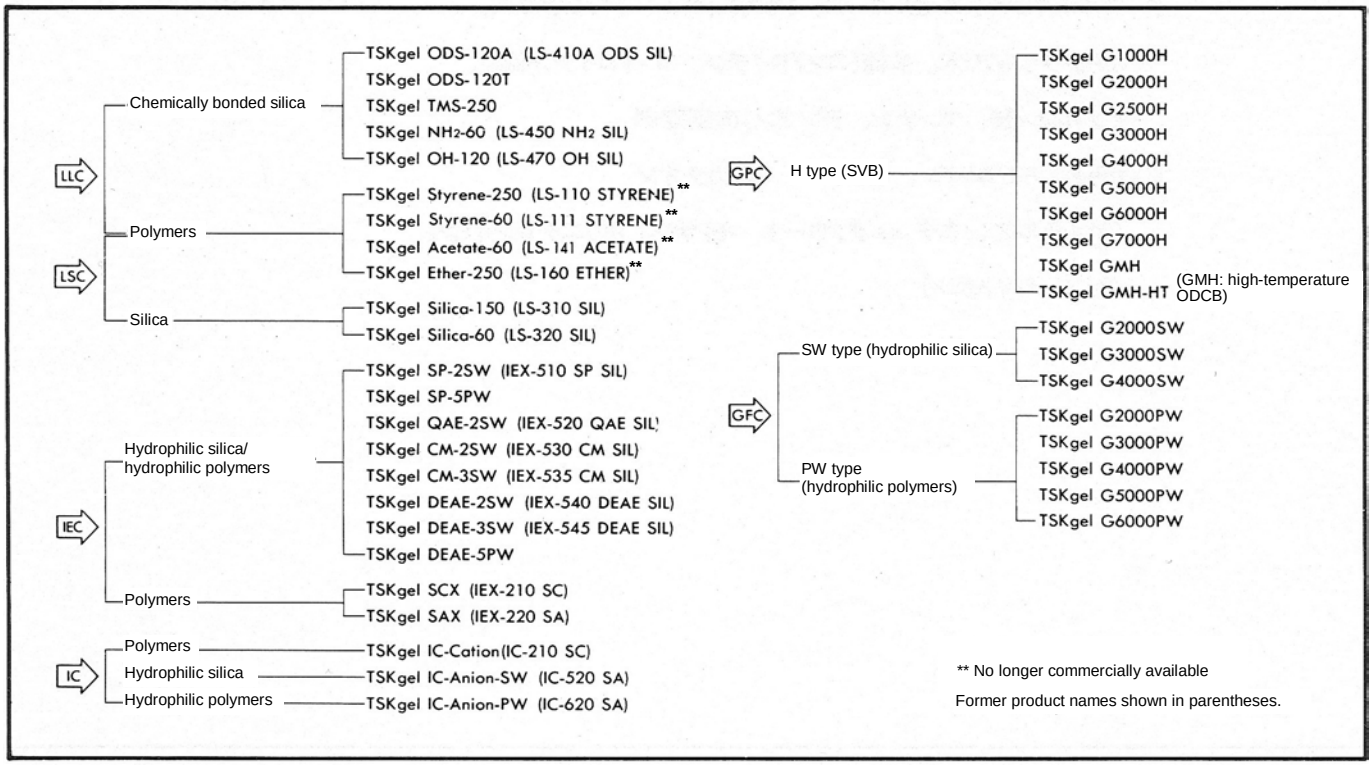
The calibration curve obtained by combining the TSKgel G6000PW and G3000PW columns provides good linearity over a wide range of molar masses and is suitable for analyzing water-soluble polymers.

Table 2 Separation ranges of TSKgel PW columns

Product name	Exclusion limit* (Da)	Separation range
TSKgel G6000PW	Up to 8 x10^6	≥ 2 x 10^4
TSKgel G5000PW	1 x 10^6	1 x 10^4 - 7 x 10^5
TSKgel G4000PW	3 x 10^5	3 x 10^3 - 2 x 10^5
TSKgel G3000PW	5 x 10^4	2 x 10^2 - 4 x10^4

*Based on polyethylene oxide standards.

Table 1 TSKgel columns listed by separation mode



3. HPLC system for GFC analysis of water-soluble polymers

An example of an HPLC system that is suitable for GFC analysis of water-soluble polymers is shown in Figure 1. In this figure, the column is connected to a light-scattering (LS) photometer with a built-in differential refractometer.

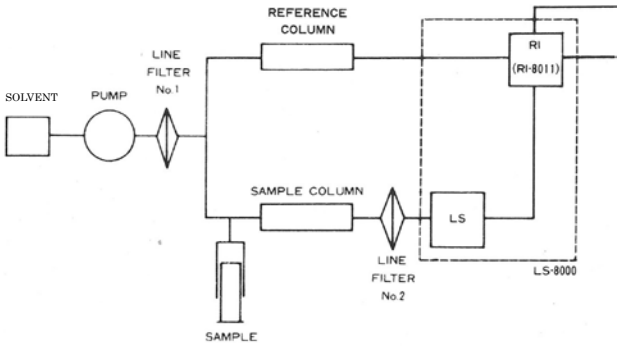


Figure 1 Flow diagram

Figure 2 shows the main differences between using a conventional GFC (with RI detector) and a GFC-LS system in analyzing the molar mass averages and molar mass distributions of an analyte. In conventional GFC, the elution curve of the differential refractometer (RI) is converted to the molar mass distribution curve using a calibration curve.

In a GFC-LS system the molar mass of an analyte is determined independent of a calibration curve. In general, the RI detector response is proportional to the concentration, c_i, of the analyte at each slice (i), and the LS detector response is proportional to the product of the concentration, c_i, and the molar mass, M_i, of the analyte at each elution slide, thus the molar mass of the analyte at each elution slice is determined by dividing the LS detector response by the RI detector response.

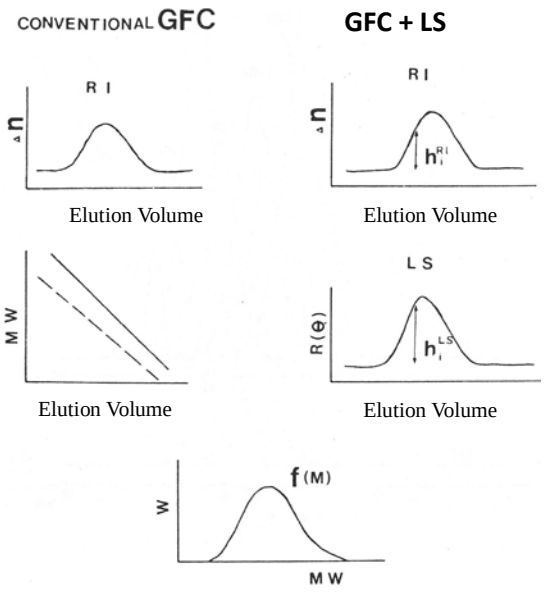


Figure 2 Comparison of conventional GFC and GFC-LS

When analyzing water-soluble polymers by GFC, ionic interactions (Fig. 3) and partition/adsorption interactions (Fig. 4) may occur between the sample and the gel matrix *in addition to the molecular sieving effect*. Consequently, if these enthalpic interactions occur, the GFC chromatogram may not reflect an accurate molar mass distribution. To eliminate these enthalpic interactions it may be necessary to add an inorganic salt or ~10-20% acetonitrile or methanol to the aqueous mobile phase. In some cases it may also be necessary to buffer the mobile phase. However, solvent preparation becomes less critical when using an HPLC system that contains a light scattering detector, as the molar mass, M_i, can be measured at each elution slice without a calibration curve.

When analyzing water-soluble polymers, it is often advantageous to use a temperature above room temperature (40-50°C) as this lowers the viscosity of the solvent, which results in higher column efficiency and improved resolution.

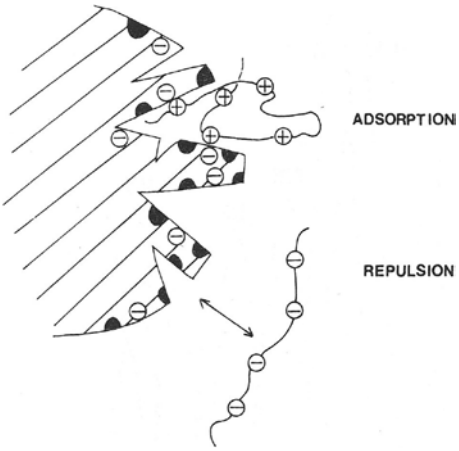


Figure 3 Ionic interaction

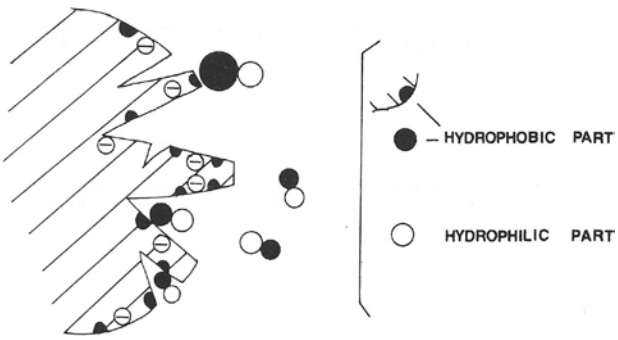


Figure 4 Partition/adsorption interactions

4. Application of various standard samples

4-1. Polyethylene oxide standards

Figure 5 shows LS and RI chromatograms of a series of narrow polydisperse polyethylene oxide standards that were synthesized by anionic polymerization.

Note: Since polyethylene oxide slowly breaks down in aqueous solutions, it is suggested to add a small amount of ethanol to the sample solution.

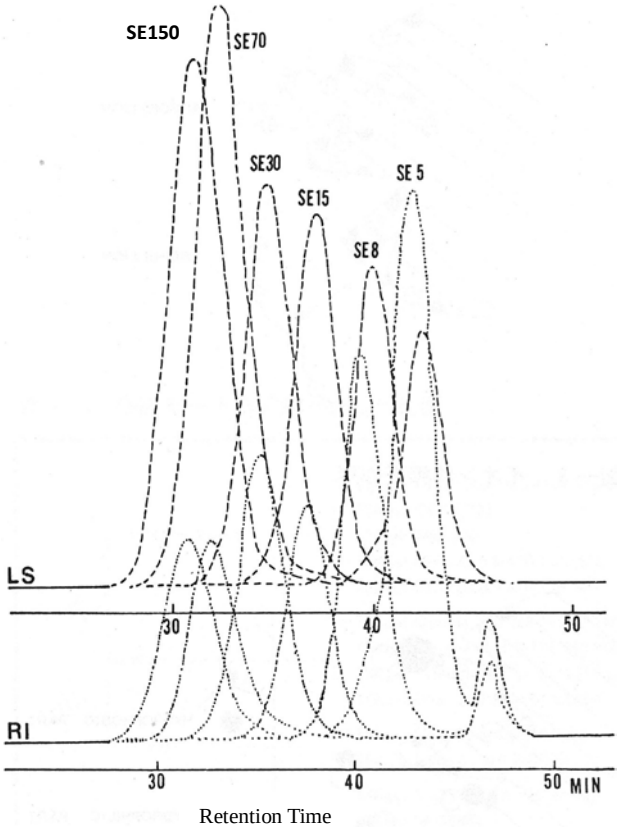


Figure 5 Separation of polyethylene oxide standards

Columns:	TSKgel G6000PW + G4000PW, 7.5mm ID × 60cm × 2
Mobile phase:	0.2mol/L phosphate buffer, pH 6.9
Flow rate:	0.9mL/min
Temperature:	40°C
Samples:	SE-150 ($1x10^6$ Da) SE-70 ($5.9x10^5$Da) SE-30 ($2.5x10^5$Da) SE-15 ($1.5x10^5$Da) SE-8 ($8.6x10^4$Da) SE-5 ($3.9x10^4$Da)

4-2. Pullulan

Figure 6 shows LS and RI chromatograms of a series of narrow polydisperse pullulan standards.

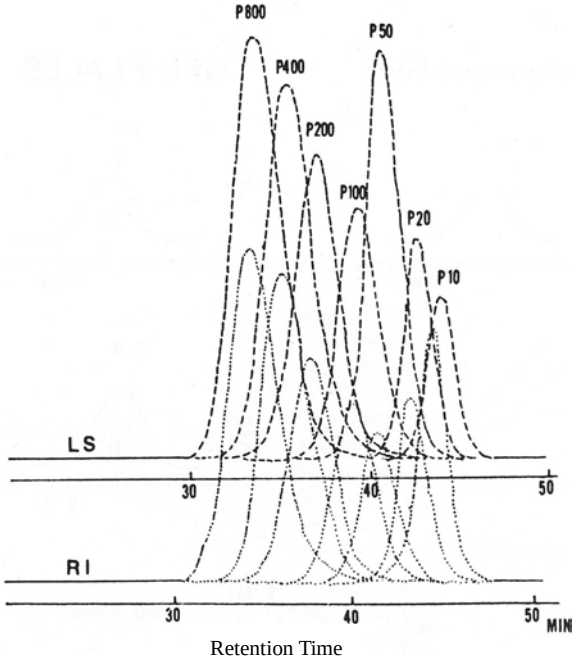


Figure 6 Separation of pullulan standards

Columns:	TSKgel G6000PW + G4000PW, 7.5 mm ID × 60cm × 2
Mobile phase:	0.2mol/L phosphate buffer, pH 6.9
Flow rate:	0.9mL/min
Temperature:	40°C
Samples:	P-800 (788,000Da) P-400 (404,000Da) P-200 (212,000Da) P-100 (112,000Da) P-50 (47,300Da) P-20 (22,800Da) P-10 (11,800Da)

4-3. Sodium polystyrene sulfonate standards

When a water-soluble polymer contains aromatic or alkyl groups, the addition of a low percentage of a water-miscible organic solvent to the mobile phase may be needed to eliminate the hydrophobic interaction between the sample and the packing material. Figure 7 shows LS and RI chromatograms of sodium polystyrene sulfonate standards when analyzed using a pH 6.9 phosphate buffer containing 10% acetonitrile.

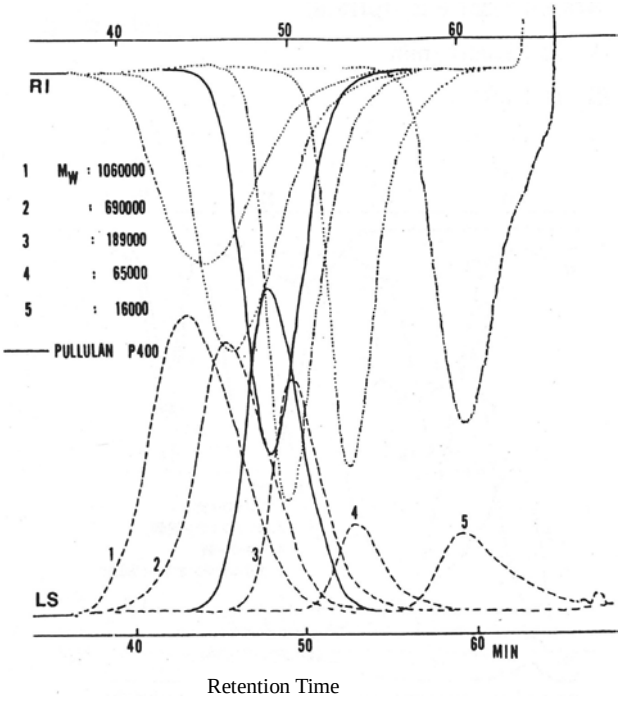


Figure 7 Separation of sodium polystyrene sulfonate standards

Columns: TSKgel G6000PW + G3000PW, 7.5mm ID × 60cm × 2

Mobile phase: 10% CH_3CN/0.2mol/L phosphate buffer, pH 6.9
Flow rate: 0.6mL/min
Temperature: 40°C

5. Applications: carboxy methylcellulose, tragacanth gum, gum arabic, polyacrylamide, and polysaccharides (chondroitin, chondroitin sulfate, hyaluronic acid, mannan, and starch)

Chromatograms of various water-soluble polymers are shown in Figures 8 through 15. The solvent conditions for these polymers were primarily phosphate buffer (0.1-0.2mol/L) at pH 7.0. Note these solvent conditions are also ideal for analyzing polyanions in polyelectrolytes.

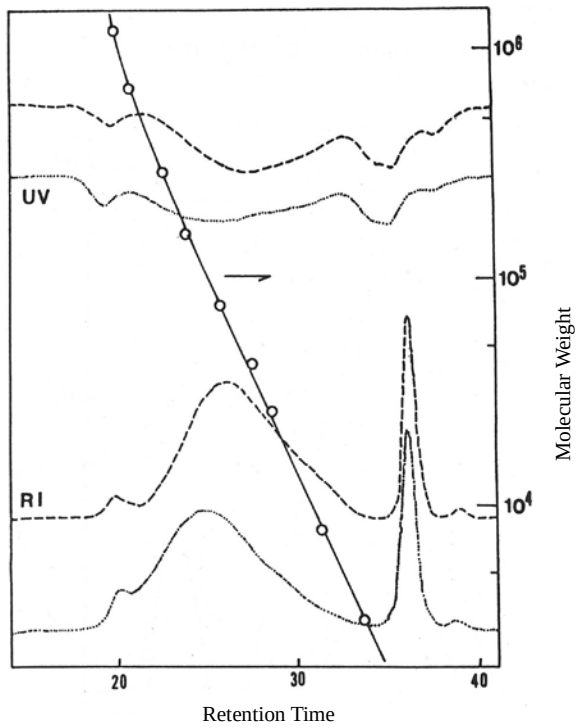


Figure 8 Separation of carboxymethyl cellulose

Columns: TSKgel G5000PW + G3000PW, 7.5mm ID × 60cm × 2

Mobile phase: 0.1mol/L phosphate buffer, pH 6.8
Flow rate: 1.0mL/min
Temperature: 40°C

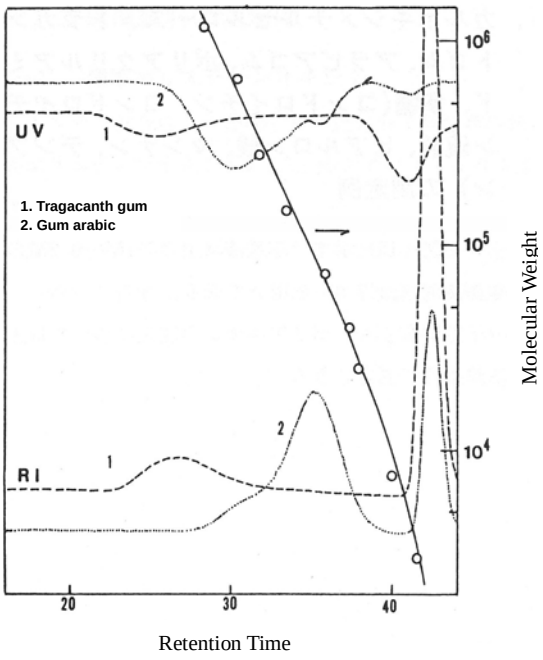


Figure 9 Separation of tragacanth gum and gum arabic

Columns: TSKgel G5000PW + G3000PW, 7.5mm ID × 60cm × 2

Mobile phase: 0.1mol/L phosphate buffer, pH 6.8
Flow rate: 1.0mL/min
Temperature: 40°C

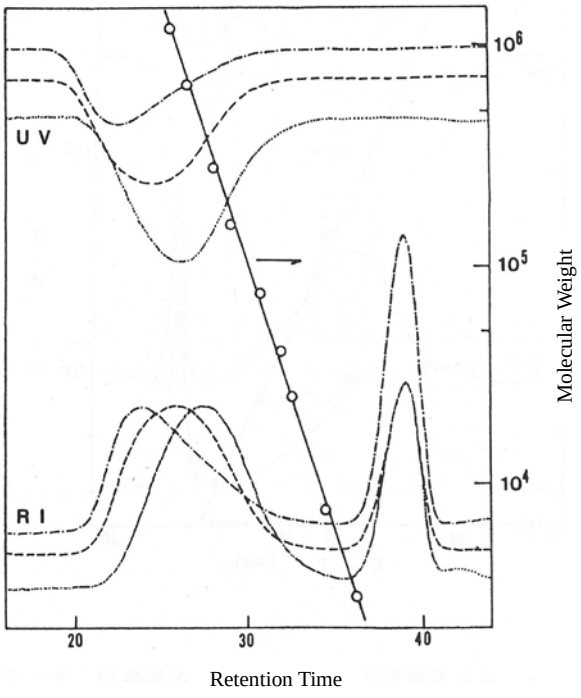


Figure 10 Separation of polyacrylamides

Columns: TSKgel G6000PW + G3000PW, 7.5mm ID × 60cm × 2

Mobile phase: 0.1mol/L phosphate buffer, pH 6.8
Flow rate: 1.0mL/min
Temperature: 40°C

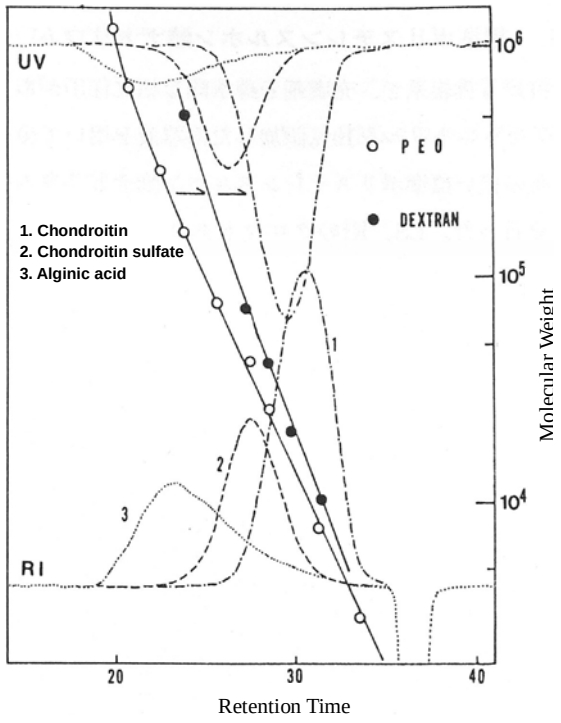


Figure 11 Separation of polysaccharides

Columns: TSKgel G5000PW + G3000PW, 7.5mm ID × 60cm × 2

Mobile phase: 0.2mol/L phosphate buffer, pH 6.8
Flow rate: 1.0mL/min
Temperature: 40°C

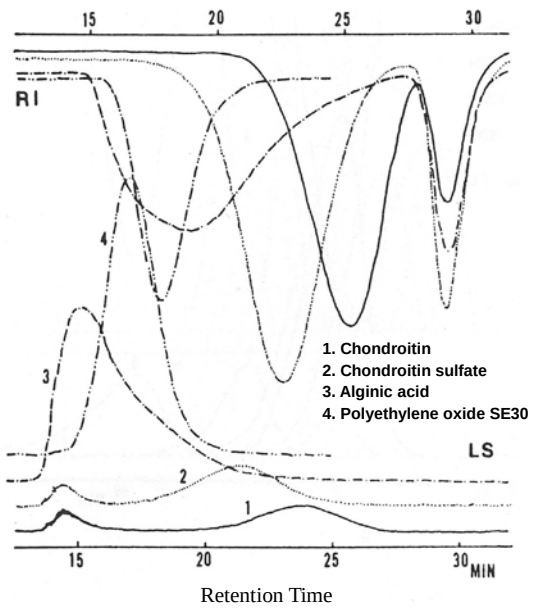


Figure 12 Separation of polysaccharides

Column: TSKgel G4000PW, 7.5mm ID × 60cm

Mobile phase: 0.2mol/L phosphate buffer, pH 6.8
Flow rate: 0.7mL/min
Temperature: 40°C

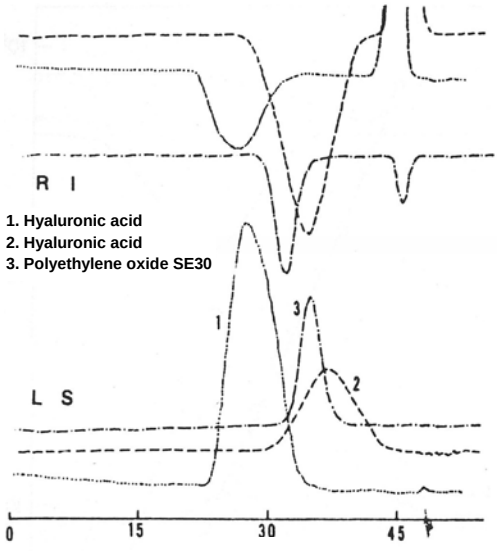


Figure 13 Separation of hyaluronic acid

Columns: TSKgel G6000PW + G4000PW,
7.5mm ID × 60cm × 2

Mobile phase: 0.2mol/L NaCl
Flow rate: 0.9mL/min
Temperature: 40°C

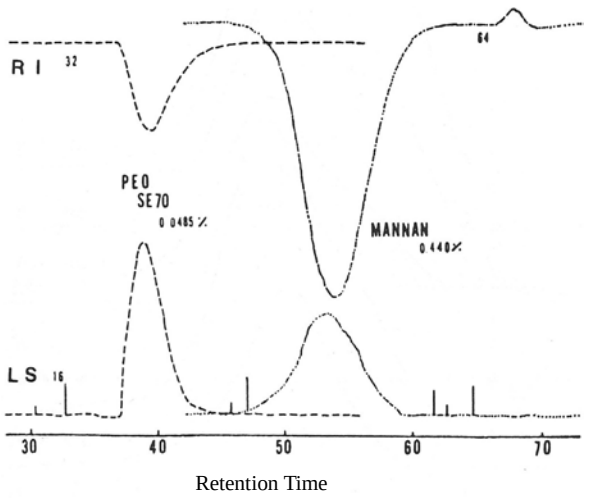


Figure 14 Separation of mannan

Columns: TSKgel G5000PW + G3000PW,
7.5mm ID × 60cm × 2

Mobile phase: 0.2mol/L phosphate buffer, pH 6.8
Flow rate: 0.5mL/min
Temperature: 40°C

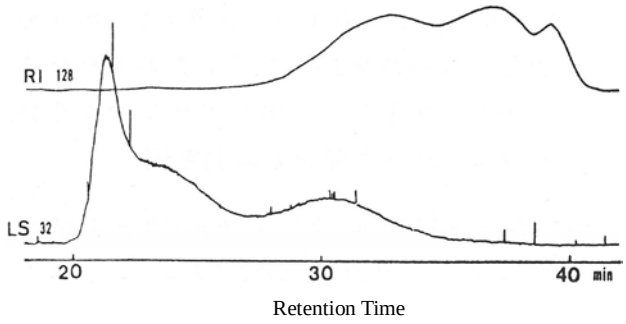


Figure 15 Separation of starch

Columns: TSKgel G5000PW + G3000PW,
7.5mm ID × 60cm × 2

Mobile phase: 0.2mol/L phosphate buffer, pH 6.9
Flow rate: 0.8mL/min
Temperature: 40°C

6. Additional Applications: polyvinylpyrrolidone, gelatin, polycations (dimethylamino ethyl methacrylate, chitosan, polyethyleneimine, glycol chitosan)

Chromatograms for water-soluble polymers such as polyvinylpyrrolidone, gelatin, and polycations, as well as other applications are shown in Figures 16-23The analysis of these specific water-soluble polymers requires careful attention to be paid to mobile phase composition, as the elution profile and retention volume may vary significantly as a result of mobile phase composition. For example, the analysis of polyvinylpyrrolidone, requires the addition of 20% methanol to an aqueous salt solution, whereas the elution profile of gelatin varies significantly with the sample and mobile phase pH. Additionally, when analyzing polycations a mobile phase with an acidic pH is necessary, as well as an increase in salt concentration, in order to protonate the trace amounts of (negatively charged) carboxyl groups found on the matrix of the TSKgel PW columns and to prevent dissociation.
Note: adsorption is more likely to occur with polyanionic samples if a column was first used to analyze polycationic samples and then used to analyze polyanionic samples.

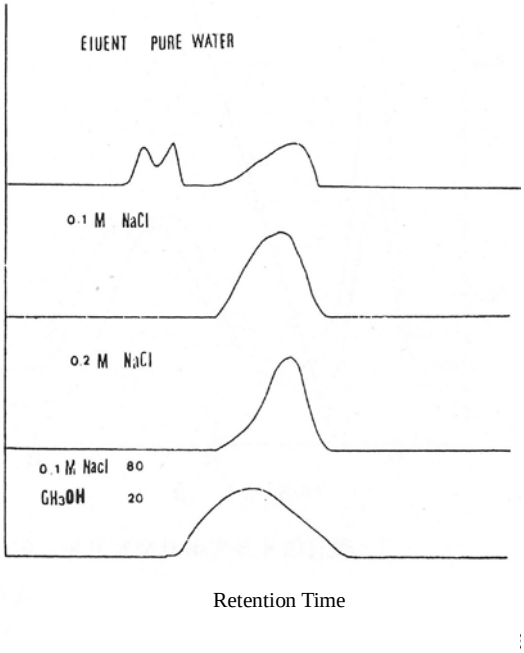


Figure 16 Separation of polyvinylpyrrolidone

Columns: TSKgel G5000PW + G3000PW, 7.5mm ID × 60cm × 2

Mobile phase: See Figure
Flow rate: 1.0mL/min
Temperature: 40°C

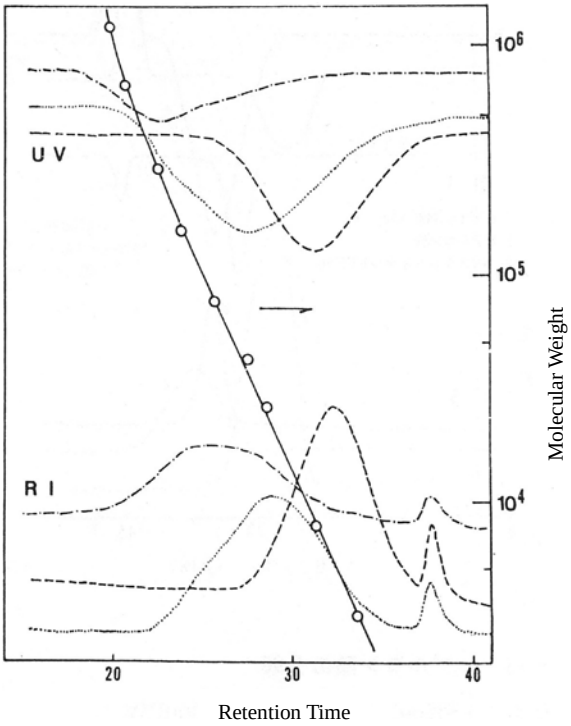


Figure 17 Separation of polyvinylpyrrolidone

Columns: TSKgel G5000PW + G3000PW, 7.5mm ID × 60cm × 2

Mobile phase: 20% CH_3OH/0.1mol/L CH_3COONa
Flow rate: 1.0mL/min
Temperature: 40°C

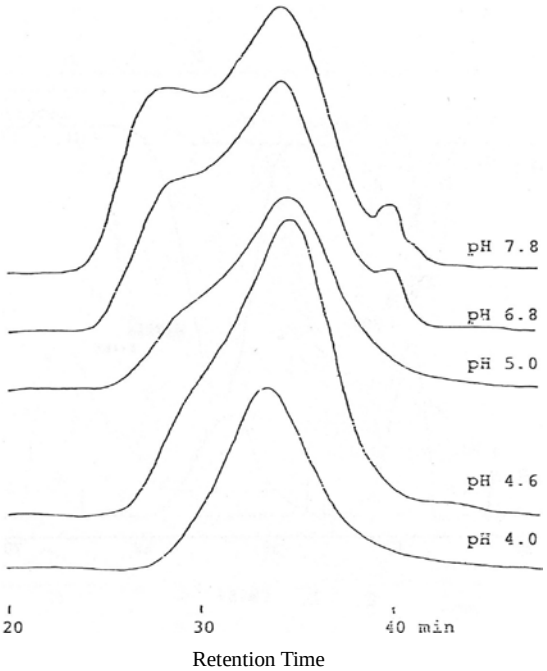


Figure 18 Separation of gelatin

Columns: TSKgel G6000PW + G4000PW, 7.5mm ID × 60cm × 2

Mobile phase: 0.2mol/L phosphate buffer
Flow rate: 1.0 mL/min
Temperature: R.T.

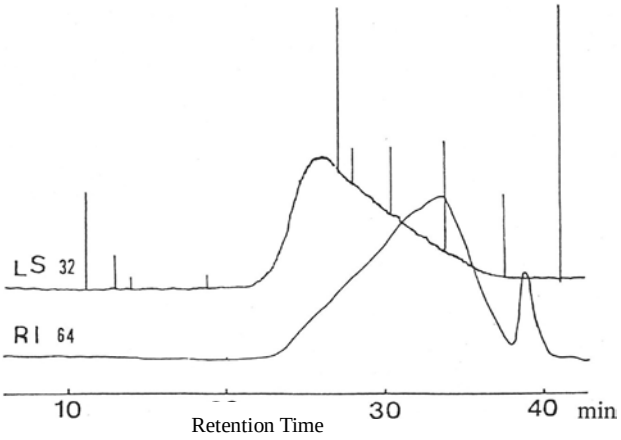


Figure 19 Separation of gelatin

Columns: TSKgel G6000PW + G3000PW, 7.5mm ID × 60cm × 2

Mobile phase: 0.2mol/L phosphate buffer, pH 6.9
Flow rate: 1.0mL/min
Temperature: 40°C

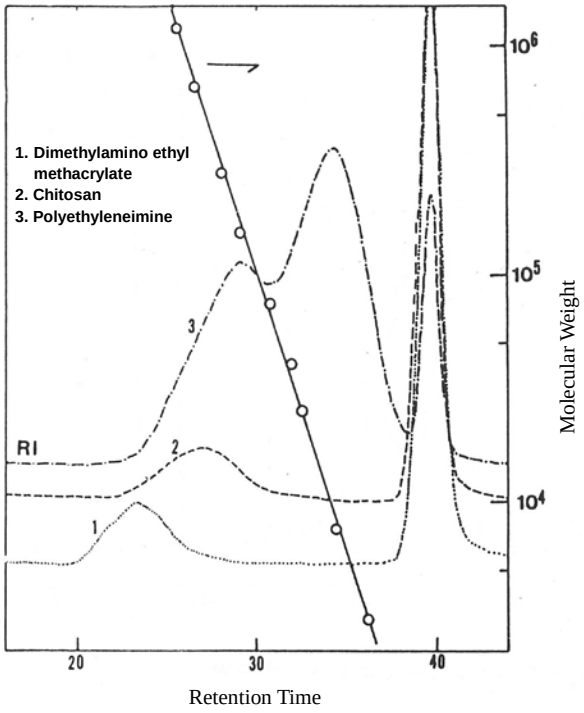


Figure 21 Separation of polycations

Columns: TSKgel G6000PW + G3000PW, 7.5mm ID × 60cm × 2

Mobile phase: 0.5mol/L CH_3COOH + 0.5mol/L CH_3COONa
Flow rate: 1.0mL/min
Temperature: 40°C

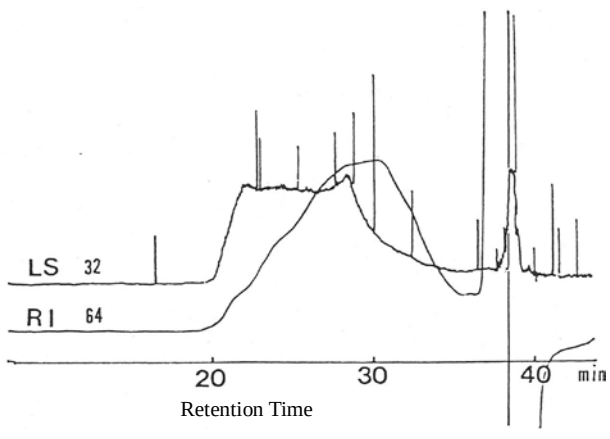


Figure 20 Separation of gelatin

Columns: TSKgel G6000PW + G3000PW, 7.5mm ID × 60cm × 2

Mobile phase: 0.2mol/L phosphate buffer, pH 4.5
Flow rate: 1.0mL/min
Temperature: 40°C

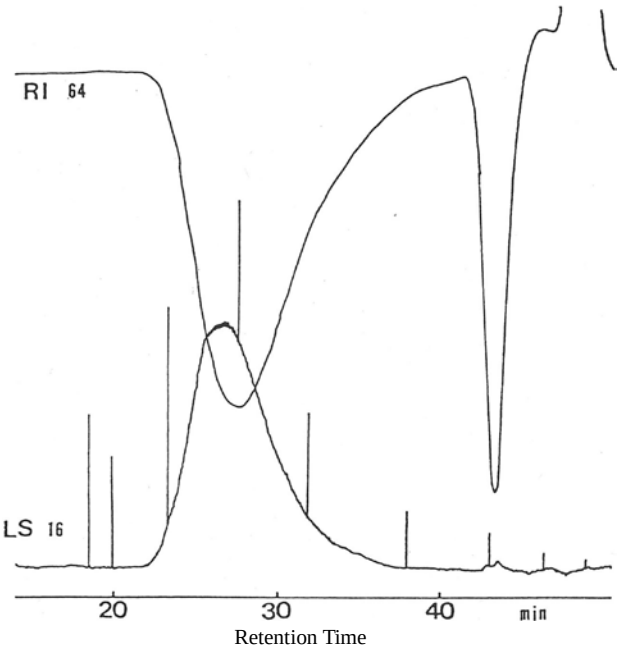


Figure 22 Separation of glycol chitosan

Columns: TSKgel G5000PW + G3000PW, 7.5mm ID × 60cm × 2

Mobile phase: 0.1mol/L TEA* + Conc. H_3PO_4, pH 2.9
Flow rate: 0.7mL/min
Temperature: 40°C
TEA*: triethanolamine

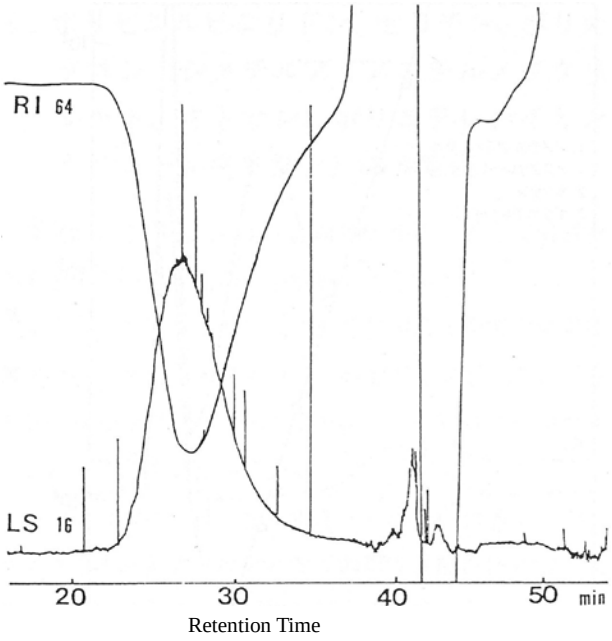


Figure 23 Separation of glycol chitosan

Columns:	TSKgel G5000PW + G3000PW, 7.5mm ID × 60cm × 2
Mobile phase:	0.5mol/L CH_3COOH + 0.3mol/L Na_2SO_4
Flow rate:	0.7mL/min
Temperature:	40°C