No.035
SEPARATION REPORT

TOSOH

GFC Analysis of Water-Soluble Polymers with TSKgel PW-type Columns

— Contents —

1. Introduction

After Tosoh developed GPC packing materials for organic solvent systems in 1971, we also developed and marketed various packing materials for HPLC, as shown in Table 1. Several years ago, in response to the demands of the time, we developed and began selling TSKgel SW and PW-type columns as GFC packing materials for water-soluble systems.

Examples of GFC analysis of water-soluble polymers using TSKgel PW-type columns are introduced in this report.

2. Separation ranges for each grade of TSKgel PW-type column

Table 2 shows separation ranges for TSKgel PW-type columns. The G6000PW has the largest pore size and the G3000PW has the smallest.

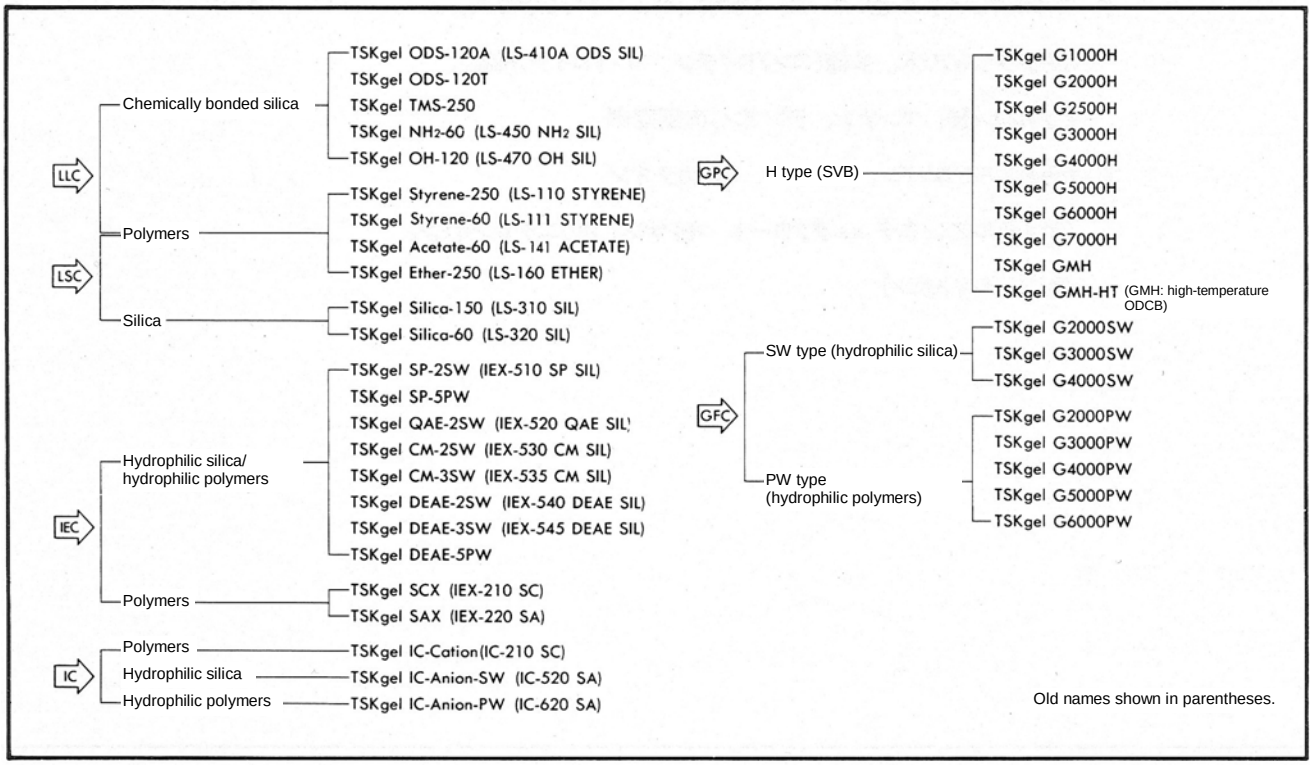
By combining the G6000PW and G3000PW columns, the calibration curves will provide good linearity over a wide range of molecular masses, suitable for analyzing the molecular mass of water-soluble polymers.

Table 2 Separation ranges of TSKgel PW-type columns

Product name	Molecular mass exclusion limit*	Separation range
TSKgel G6000PW		$\geq 2 \times 10^4$
TSKgel G5000PW	10^6	10^4 - 7×10^5
TSKgel G4000PW	3×10^5	3×10^3 - 2×10^5
TSKgel G3000PW	10^5	2×10^2 - 8×10^4

*Calculated by polyethylene oxide

Table 1 TSKgel columns listed by separation mode



3. GFC apparatus for water-soluble systems

For GFC analysis of water-soluble polymers, a good system is one in which a conventional GFC device is connected to a low-angle laser light-scattering photometer (LALLS) LS-8000 with a built-in differential refractometer (RI-8011), as shown in Figure 1.

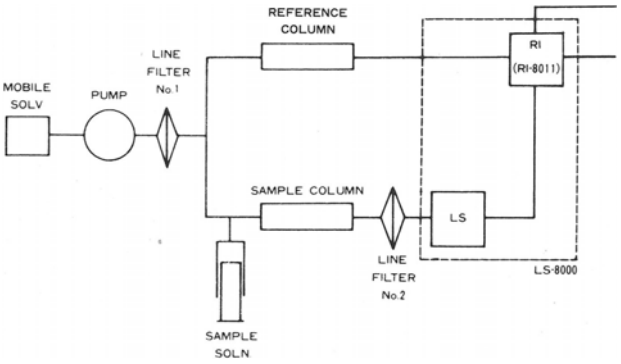


Figure 1 Flow diagram

Figure 2 shows the principle differences between conventional GFC and a GFC-LALLS system in analyzing molecular mass and molecular mass distribution. In conventional GFC, the elution curve of the differential refractometer (RI) is converted to the molecular mass distribution curve using the calibration curve.

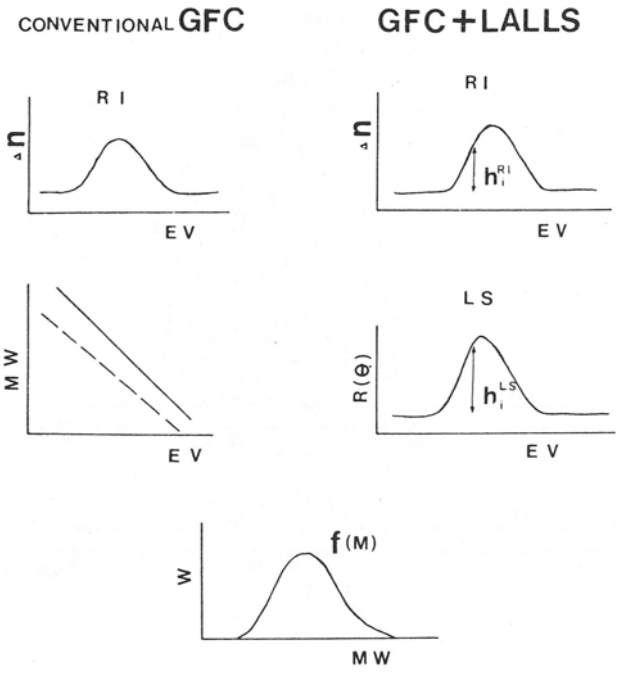


Figure 2 Comparison of conventional GFC and GFC-LALLS

In a GFC-LALLS system, because the RI response is proportional to the concentration C, and the LALLS (LS) response is proportional to the product of the concentration C and the molecular mass M, by dividing the LS response by the RI response, the molecular mass at each elution position can be determined, and a calibration curve becomes unnecessary.

In GFC for water-soluble systems, unlike organic solvent systems, in addition to the molecular sieving effect, ionic interactions (Fig. 3) and partition/adsorption interactions (Fig. 4) tend to occur between the sample and the gel matrix. Consequently, the GFC chromatogram frequently does not accurately reflect the molecular mass distribution. As a result, addition of an inorganic salt or organic solvent of around 10-20% acetonitrile or methanol to deionized water becomes necessary to prepare the solvent. Selection of the pH of the solvent is also necessary. However, with a GFC-LALLS system, because the molecular mass Mi can be measured at each elution position without the use of a calibration curve, solvent preparation does not have to be such a sensitive and stressful operation.

When analyzing water-soluble polymers, because lowering the viscosity of the solvent increases separation, analysis is usually performed at a temperature above room temperature (40-50°C).

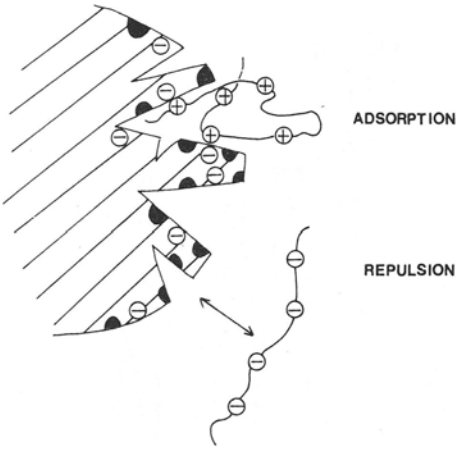


Figure 3 Ionic interaction

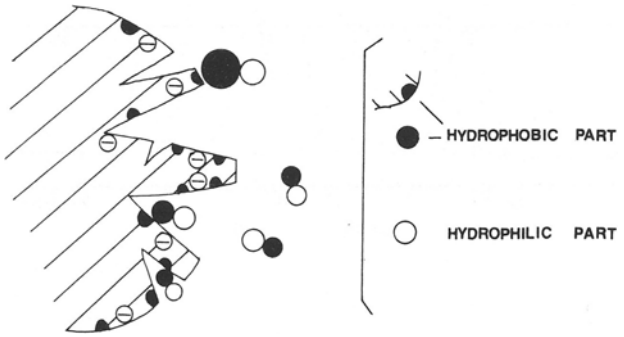


Figure 4 Partition/adsorption interaction

4. Application of various standard samples

4-1. Standard polyethylene oxide

Figure 5 shows LS and RI chromatograms of a group of polyethylene oxides of narrow molecular mass distribution that were synthesized by anionic polymerization.

Note): As polyethylene oxide tends to deteriorate in aqueous solutions, a small amount of ethanol must be added to the solution.

4-2 Pullulan

Pullulans with a narrow molecular mass distribution are sold for reference standard of molecular mass measurement. Figure 6 shows LS and RI chromatograms of a group of pullulans.

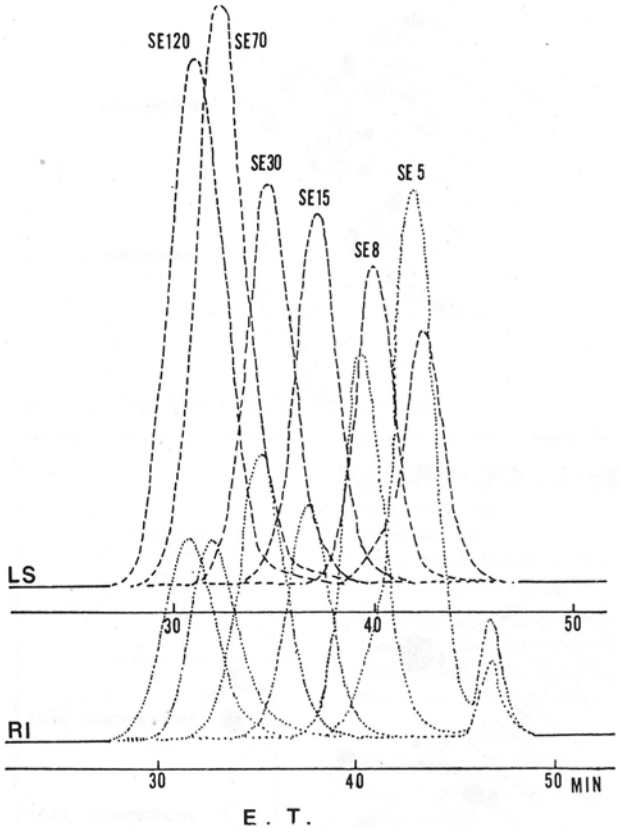


Figure 5 Separation of standard polyethylene oxide

Columns: TSKgel G6000PW + G4000PW
7.5 mm I.D. × 60 cm × 2
Solvent: 0.2 mol/L phosphate buffer. (pH 6.9)
Flow rate: 0.9 mL/min
Temperature: 40°C

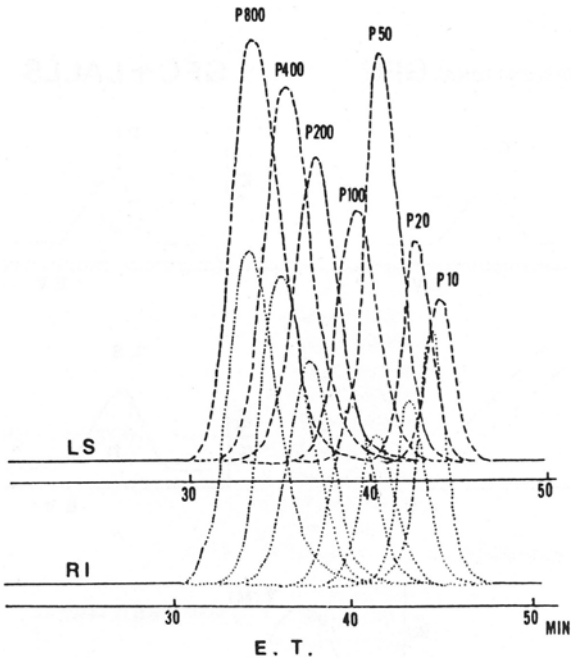


Figure 6 Separation of pullulans

Columns: TSKgel G6000PW + G4000PW
7.5 mm I.D. × 60 cm × 2
Solvent: 0.2 mol/L phosphate buffer. (pH 6.9)
Flow rate: 0.9 mL/min
Temperature: 40°C

4-3. Standard sodium polystyrene sulfonate

As a hydrophobic interaction will occur between the sample and the packing material because the sample is aromatic, standard sodium polystyrene sulfonate with a narrow molecular mass distribution were analyzed using a solvent containing 10% added acetonitrile. Figure 7 shows LS and RI chromatograms.

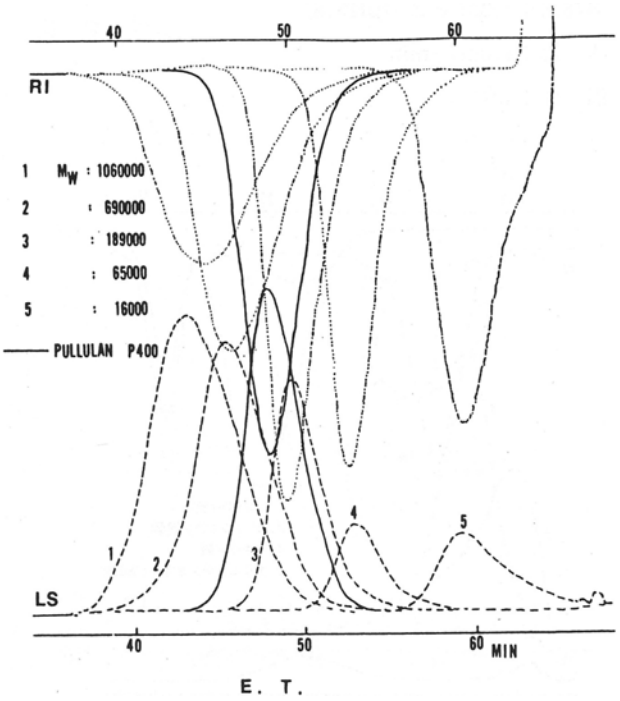


Figure 7 Separation of standard sodium polystyrene sulfonate

Columns: TSKgel G6000PW + G3000PW
7.5 mm I.D. × 60 cm × 2
Solvent: 10% CH_3CN/0.2 mol/L phosphate buffer. (pH 6.9)
Flow rate: 0.6 mL/min
Temperature: 40°C

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

Chromatograms are shown in Figures 8 through 15. Phosphate buffer (0.1-0.2 mol/L) (pH 7.0) was primarily used for the solvent.

These solvent conditions can also be used for analyzing polyanions in polyelectrolytes.

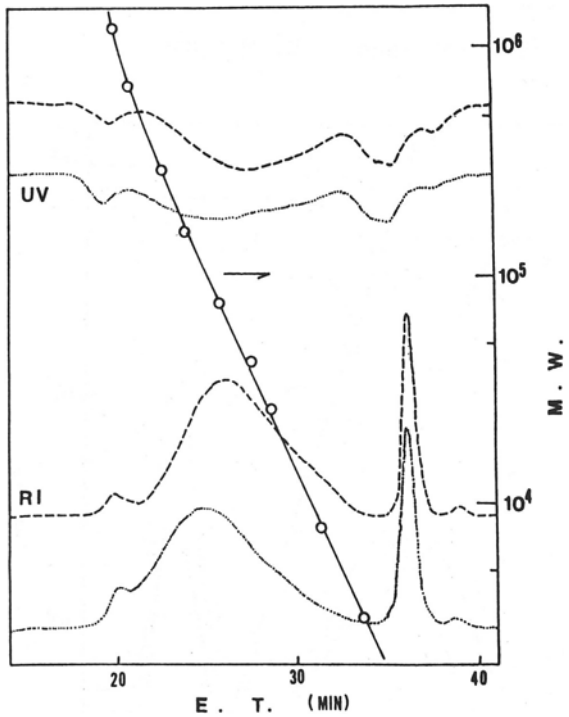


Figure 8 Separation of carboxymethyl cellulose

Columns: TSKgel G5000PW + G3000PW
7.5 mm I.D. × 60 cm × 2
Solvent: 0.1 M P.B. (pH 6.8)
Flow rate: 1.0 mL/min
Temperature: 40°C

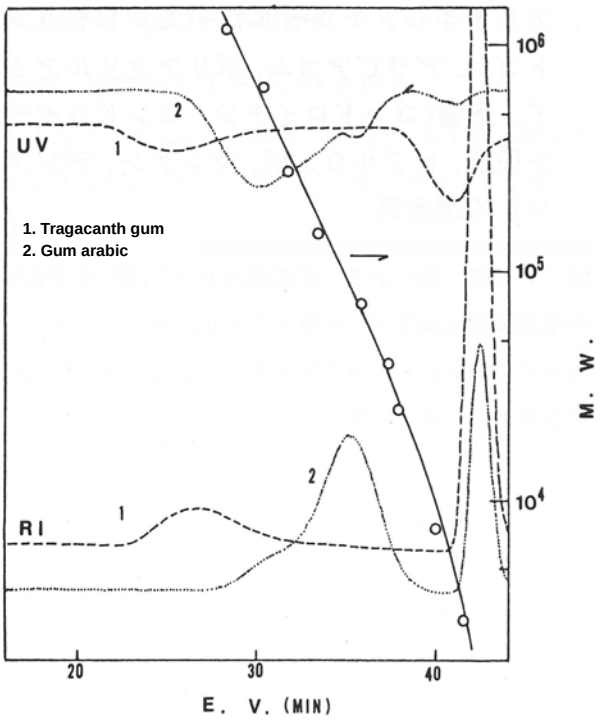


Figure 9 Separation of tragacanth gum and gum arabic

Columns: TSKgel G5000PW + G3000PW
7.5 mm I.D. × 60 cm × 2
Solvent: 0.1 mol/L phosphate buffer (pH 6.8)
Flow rate: 1.0 mL/min
Temperature: 40°C

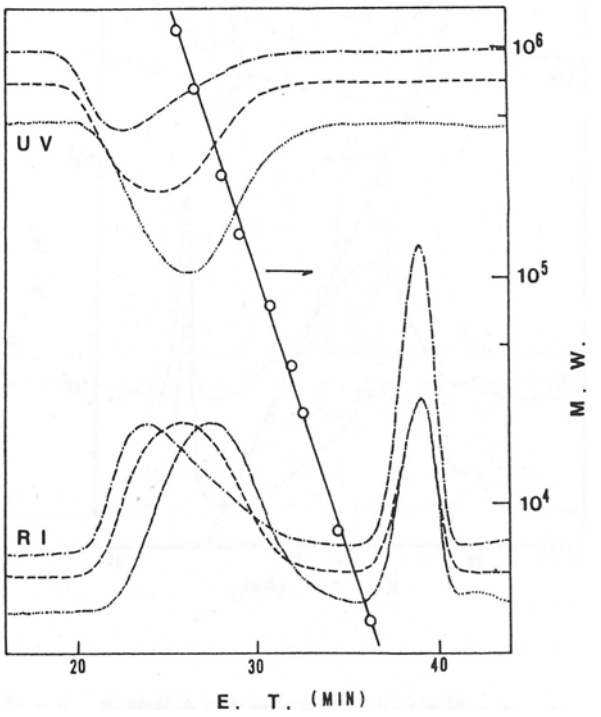


Figure 10 Separation of polyacrylamides

Columns: TSKgel G6000PW + G3000PW
7.5 mm I.D. × 60 cm × 2
Solvent: 0.1 mol/L phosphate buffer (pH 6.8)
Flow rate: 1.0 mL/min
Temperature: 40°C

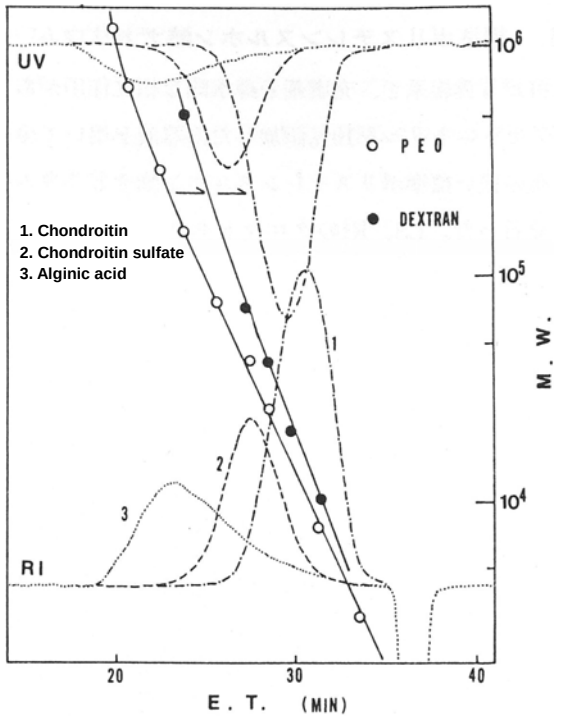


Figure 11 Separation of polysaccharides

Columns: TSKgel G5000PW + G3000PW
7.5 mm I.D. × 60 cm × 2
Solvent: 0.2 mol/L phosphate buffer. (pH 6.8)
Flow rate: 1.0 mL/min
Temperature: 40°C

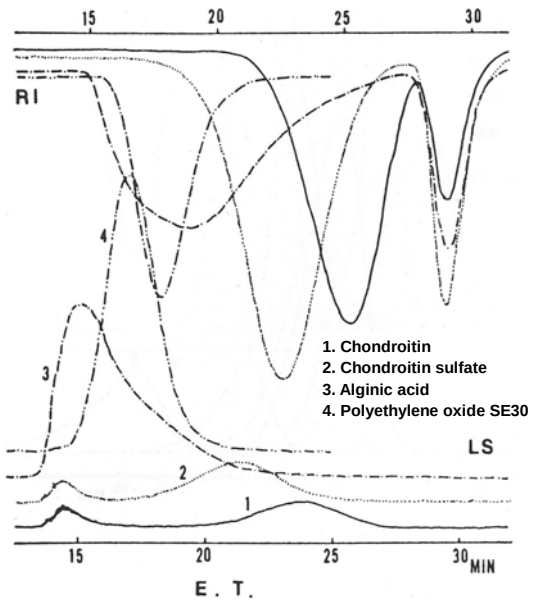


Figure 12 Separation of polysaccharides

Column: TSKgel G4000PW
7.5 mm I.D. × 60 cm
Solvent: 0.2 mol/L phosphate buffer. (pH 6.8)
Flow rate: 0.7 mL/min
Temperature: 40°C

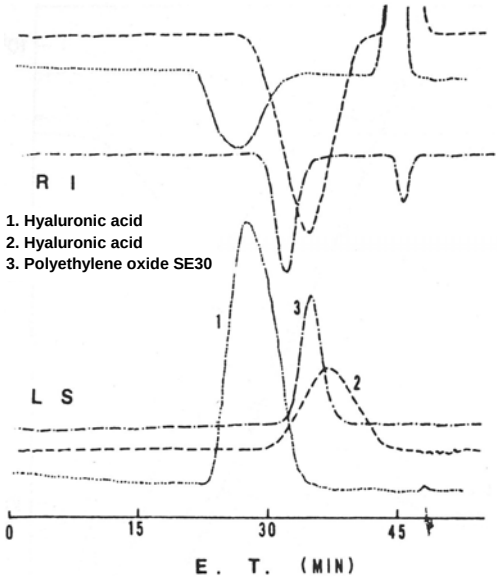


Figure 13 Separation of hyaluronic acid

Columns: TSKgel G6000PW + G4000PW
7.5 mm I.D. × 60 cm × 2
Solvent: 0.2 mol/L NaCl
Flow rate: 0.9 mL/min
Temperature: 40°C

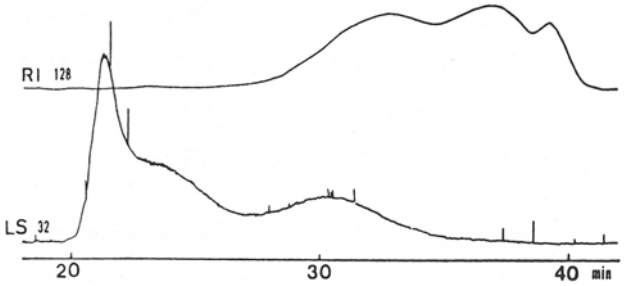


Figure 15 Separation of starch

Columns: TSKgel G5000PW + G3000PW
7.5 mm I.D. × 60 cm × 2
Solvent: 0.2 mol/L phosphate buffer. (pH 6.9)
Flow rate: 0.8 mL/min
Temperature: 40°C

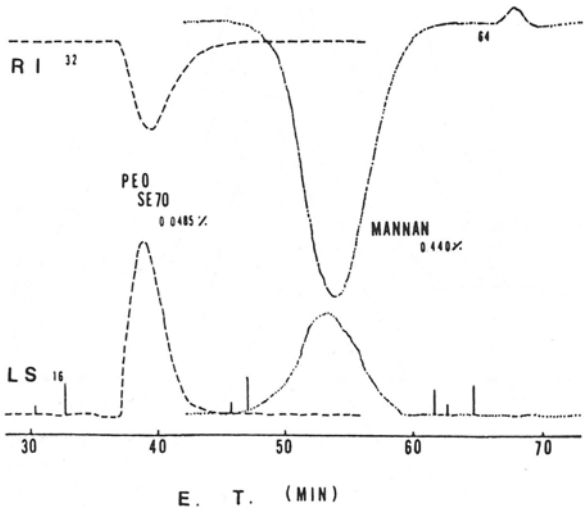


Figure 14 Separation of mannan

Columns: TSKgel G5000PW + G3000PW
7.5 mm I.D. × 60 cm × 2
Solvent: 0.2 mol/L phosphate buffer. (pH 6.8)
Flow rate: 0.5 mL/min
Temperature: 40°C

6. Application of polyvinylpyrrolidone, gelatin, polycations (dimethylamino ethyl methacrylate, chitosan, polyethyleneimine, glycol chitosan)

For the above water-soluble polymers, the composition of the solvent must be rigorously tested, both experimentally and theoretically.

For polyvinylpyrrolidone, 20% methanol must be added to an aqueous solution with a certain salt concentration. Caution is required with gelatin, as the elution behavior varies depending on sample and pH.

As trace amounts of carboxyl groups are present in the gel matrix, when analyzing polycations, the solvent must be acidic and to increase its salt concentration in order to prevent disassociation.

Application are shown in Figures 16-23.

Be aware that adsorption is very likely to occur with polyanionic samples if a column used to analyze polycationic samples is then used to analyze polyanionic samples.

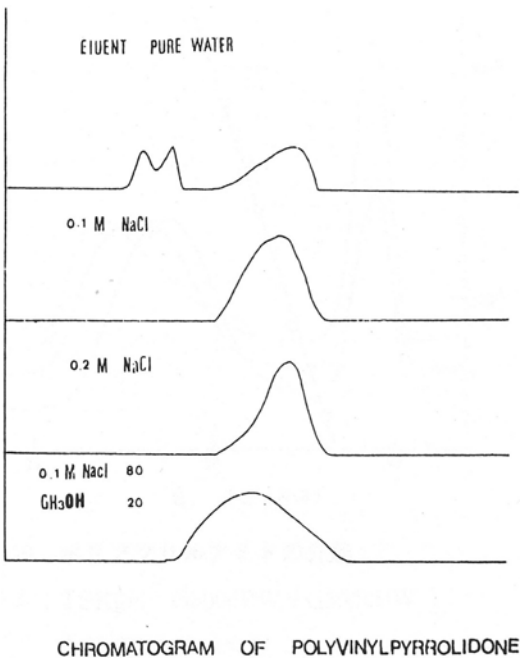


Figure 16 Separation of polyvinylpyrrolidone

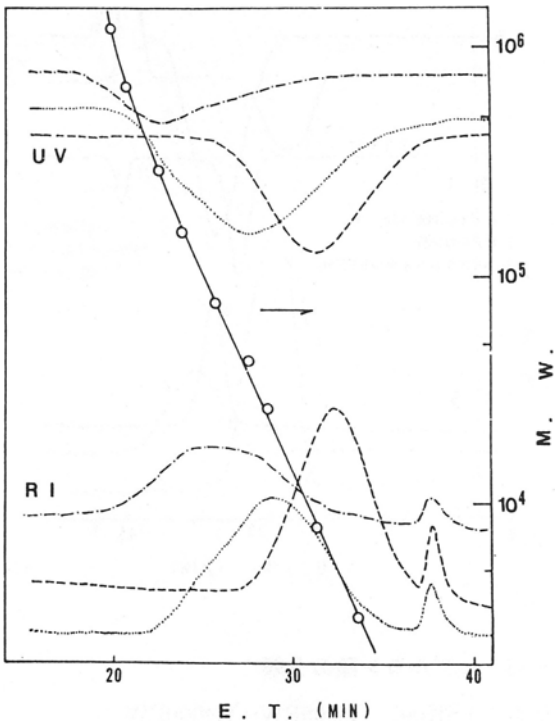


Figure 17 Separation of polyvinylpyrrolidone

Columns: TSKgel G5000PW + G3000PW
7.5 mm I.D. × 60 cm × 2
Solvent: 20% CH_3OH/0.1 mol/L CH_3COONa
Flow rate: 1.0 mL/min
Temperature: 40°C

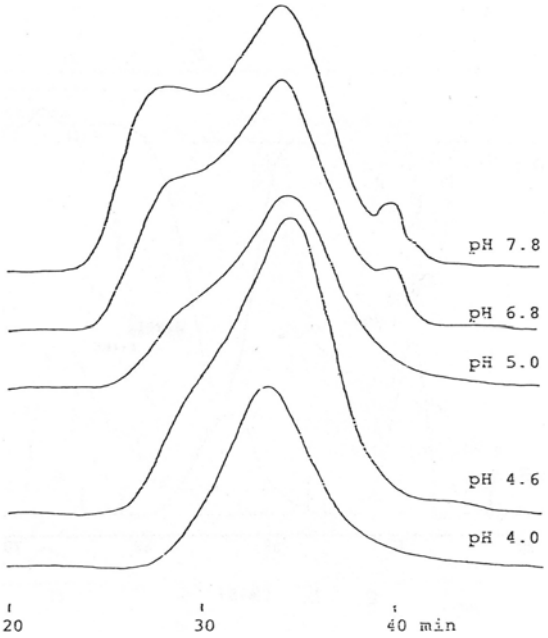


Figure 18 Separation of gelatin

Columns: TSKgel G6000PW + G4000PW
7.5 mm I.D. × 60 cm × 2
Solvent: 0.2 mol/L phosphate buffer.
Flow rate: 1.0 mL/min
Temperature: R.T.

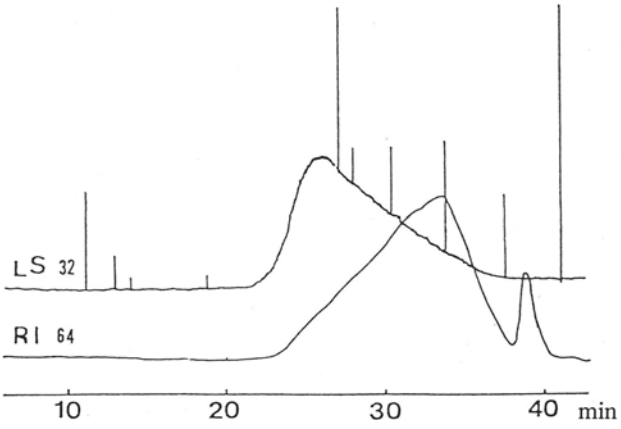


Figure 19 Separation of gelatin

Columns: TSKgel G6000PW + G3000PW
7.5 mm I.D. × 60 cm × 2
Solvent: 0.2 mol/L phosphate buffer. (pH 6.9)
Flow rate: 1.0 mL/min
Temperature: 40°C

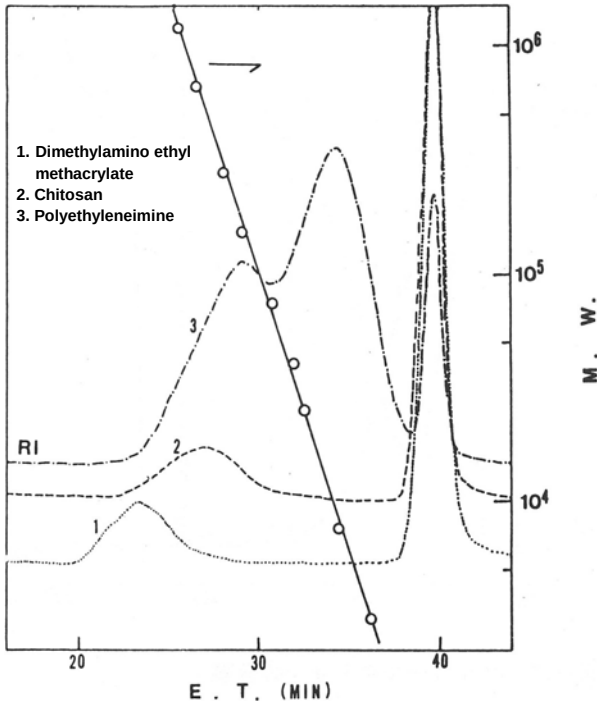


Figure 21 Separation of polycations

Columns: TSKgel G6000PW + G3000PW
7.5 mm I.D. × 60 cm × 2
Solvent: 0.5 mol/L CH_3COOH + 0.5 mol/L CH_3COONa
Flow rate: 1.0 mL/min
Temperature: 40°C

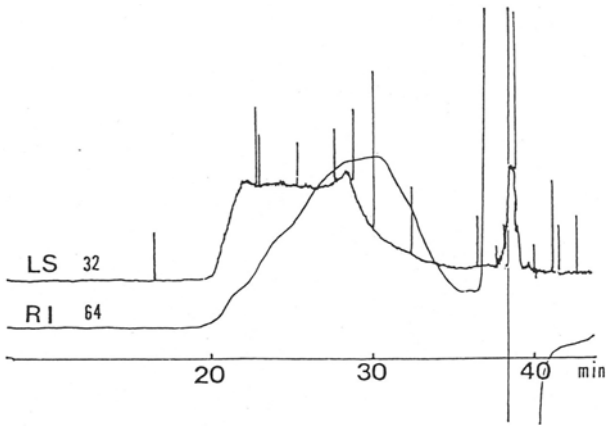


Figure 20 Separation of gelatin

Columns: TSKgel G6000PW + G3000PW
7.5 mm I.D. × 60 cm × 2
Solvent: 0.2 mol/L phosphate buffer. (pH 4.5)
Flow rate: 1.0 mL/min
Temperature: 40°C

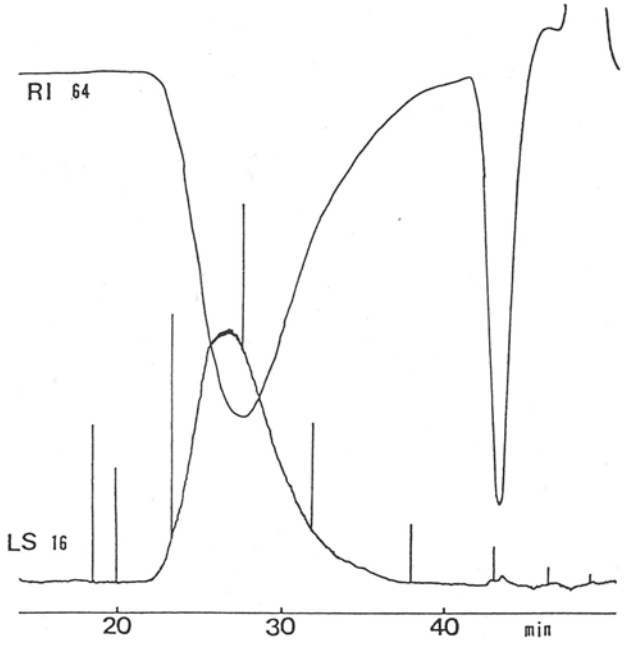


Figure 22 Separation of glycol chitosan

Columns: TSKgel G5000PW + G3000PW
7.5 mm I.D. × 60 cm × 2
Solvent: 0.1 mol/L TEA* + Conc. H_3PO_4 (pH 2.9)
Flow rate: 0.7 mL/min
Temperature: 40°C (TEA*: triethanolamine)

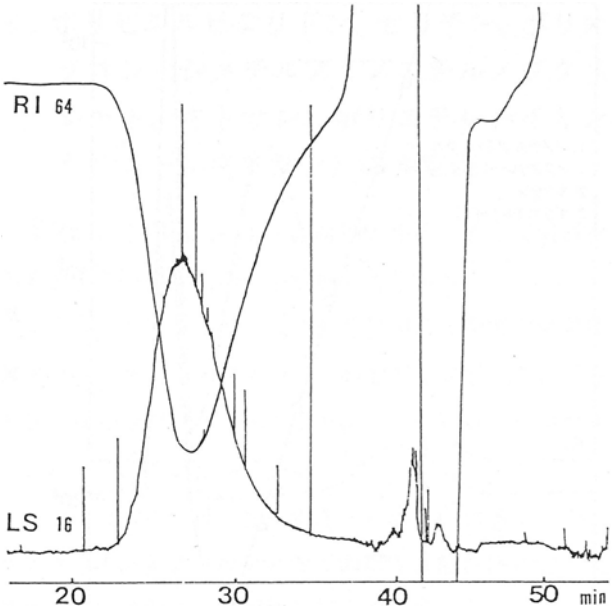


Figure 23 Separation of glycol chitosan

Columns: TSKgel G5000PW + G3000PW
7.5 mm I.D. × 60 cm × 2
Solvent: 0.5 mol/L CH_3COOH + 0.3 mol/L Na_2SO_4
Flow rate: 0.7 mL/min
Temperature: 40°C