Aqueous Gel Permeation Chromatography: The Effect of Solvent Ionic Strength*

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Synopsis

Porous glass packing materials have been used for gel permeation chromatography using an aqueous phosphate buffer. Elution volumes were determined for polystyrene sulfonates, dextrans, and small neutral and charged molecules at three different ionic strengths, viz., 0.01*M*, 0.1*M*, and 1.0*M* phosphate, pH 7.0. The pore diameters of the glasses studied were 75, 240, 700, and 2000 Å. Elution volumes of nonionic species were unaffected by changing the solvent ionic strength. Elution volumes of charged species were markedly affected by the ionic strength of the solvent. This was attributed to a combination of decreased polymer dimensions and decreased ionic exclusion with increasing buffer concentration. The use of low ionic strength solvents may be exploited to tailor the separating range for polyelectrolytes with porous glass packings. This is particularly useful in the low molecular weight range where the lowest pore size available is 75 Å.

INTRODUCTION

Porous glass column packings have become very popular in gel permeation chromatography (GPC) because of their chemical, mechanical, and temperature stability in a wide variety of solvents. A detailed investigation of the characterization and chromatographic properties of Corning controlled pore glass (CPG) has been published.[1] Elimination of adsorption in organic solvents has also been reported.[2] Porous glasses with narrow particle and pore size distributions are commercially available, allowing good efficiency and reproducibility in analytic GPC applications.

A review of available packing materials for aqueous GPC has recently appeared.[3] Successful characterizations of the molecular weight distribution (MWD) of polyacrylamide in water using porous glass and silica,[4] and poly(styrenesulfonates) (NaPSS) and dextrans using 0.2*M* Na_2SO_4 with CPG packings[5] have been reported. Dextran has been characterized in aqueous solution using CPG[6,7] and porous silica[8,9] supports. Poly(vinyl alcohol) has also been characterized by GPC using a porous silica which had undergone a proprietary deactivation process.

Due to the surface composition of controlled pore glasses, some difficulties have been encountered in their application to aqueous GPC. Porous glasses are formed by leaching two-phase systems of borosilicate glass, a B_2O_3–Na_2O phase within a SiO_2 matrix, with dilute acid to form a porous bead consisting of mostly silica traces of B_2O_3 and Na_2O. Boron impurities are thought to provide Lewis-acid sites for chemisorption of amine-containing molecules and other species with unshared electron pairs.[11] Metal ions may also be present and form coordination complexes. The silica matrix itself presents a surface consisting

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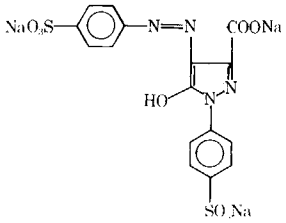
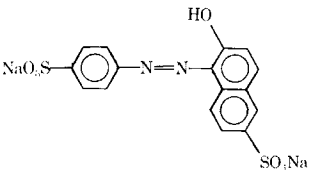
TABLE I
Solutes Used in Aqueous GPC

Solute	Molecular weight
Polyacrylamide[a]	5×10^6
Dextran[b]	
T2000	2×10^6
T500	500,000
T70	70,000
T40	40,000
T10	10,000
Polystyrene sulfonate, sodium salt[c]	
SY-9	208,000
SY-8	70,100
SY-7	38,600
SY-6	18,900
SY-11	6,700
SY-5	3,980
SY-4	1,140
Sodium adipate	190
Sodium propionate	119
Sodium formate	68
Raffinose[d]	594
Sucrose	342
Glucose	180
Tartrazine 	534
Sunset Yellow 	452

[a] Polysciences.

[b] Dextrans: commercially available samples from Pharmacia Fine Chemicals.

[c] Polystyrene sulfonate: These were prepared from narrow MWD polystyrene standards (Pressure Chemical Co.) by the method of Carroll and Eisenberg.[19] The molecular weight reported is that of the sodium form, assuming 100% monosulfonation.

[d] Raffinose was obtained from Supelco, Inc.

of silanols and siloxanes which exhibit a slight negative charge at neutral pH. Positively charged molecules can bind ionically to such a surface. Both of these effects are detrimental to a GPC application in which a steric exclusion mechanism is desired.

Adsorption problems have been eliminated by coating the glass surface with carbowax[12,13] or the addition of urea to the solvent to prevent adsorption of protein–SDS complexes.[14] The addition of amino acids to aqueous buffers[15,16] has also been reported to reduce adsorption of proteins to glass surfaces. Other

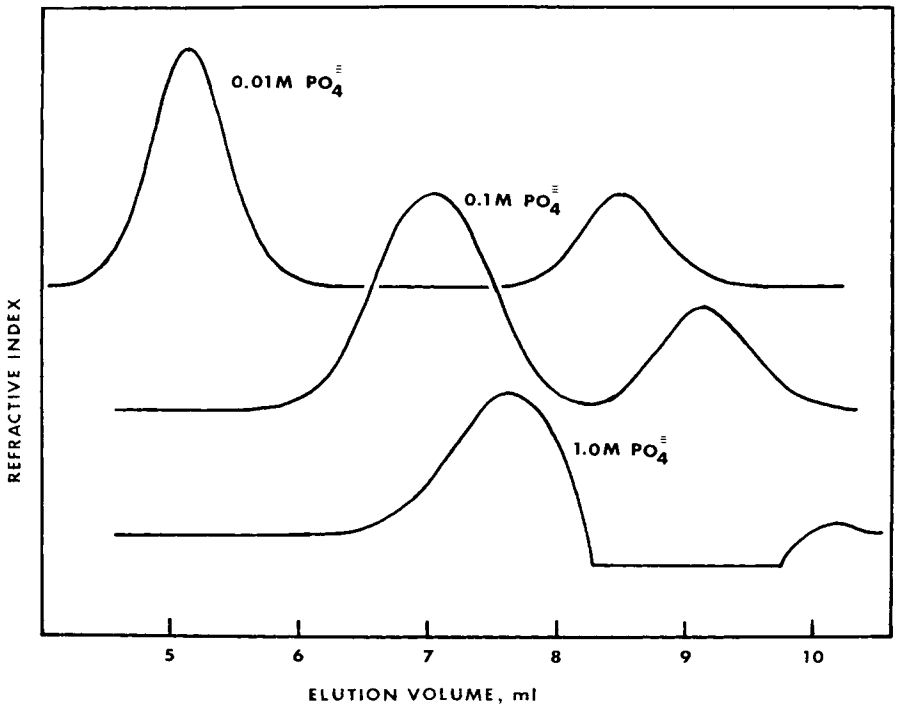


Fig. 1. GPC traces of 18,900 MW polystyrene sulfonate for the CPG 240-Å column.

methods employed to reduce protein adsorption include reacting the glass surface with glycerolpropylsilane.[17,18] For a neutral polymer, poly(vinyl alcohol) eluted with water, the uncoated CPG glass was found to be preferable.

In this work, we have investigated the effect of ionic strength of aqueous eluants on the elution volumes of negatively charged polymeric and small molecules for CPG packings of different pore sizes. The results have been interpreted in terms of the polymer coil size dependence on ionic strength and the interaction of charges on the solute and the support surface.

EXPERIMENTAL

Solutes

The solutes used in this study are listed in Table I.

Solvent

Phosphate buffer solutions were prepared from analytical reagent-grade mono- and dibasic sodium phosphate and adjusted, if necessary, to pH 7.0 with NaOH.

Gel Permeation Chromatography

A Milton Roy Mini-Pump (5000 psi rating) was used to maintain a flow rate of 1.0 ml/min through the GPC columns. Columns were constructed using 0.25-in.-O.D. stainless steel tubing; each column was 2 ft long and had an I.D.

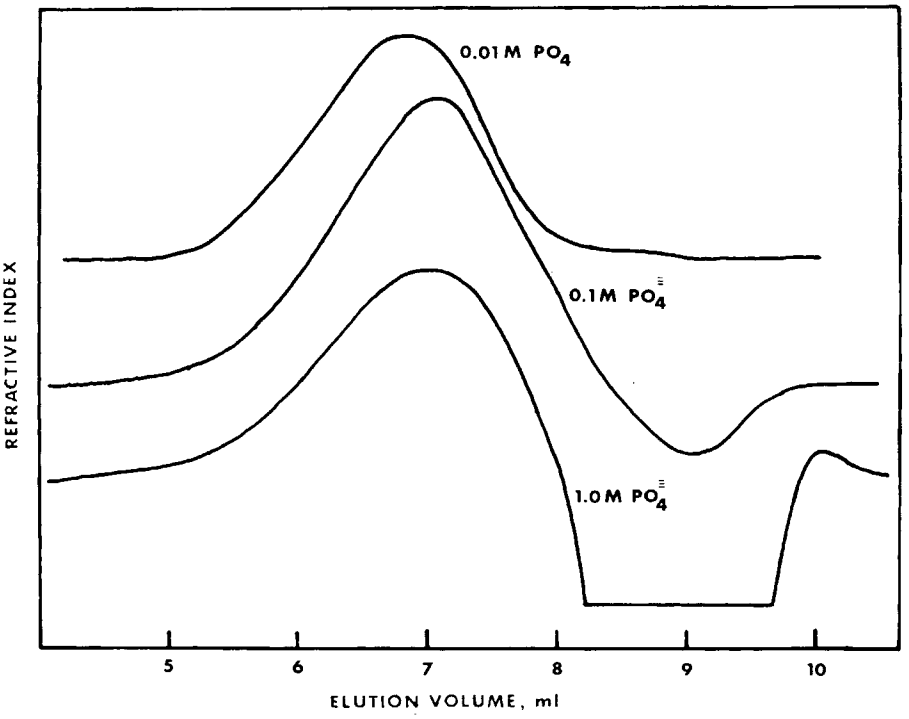


Fig. 2. GPC traces of 40,000 MW dextran for the CPG 240-Å column.

of 0.18 in. The column packing material was Corning CPG-HS with a narrow particle size distribution, 37–44μ. The pore diameters of the glasses used were 75, 240, 700, and 2000Å. Solutes were introduced into the column by injection of a 1 mg/ml solution using a 0.2-ml injection loop with a Chromatronix injection valve (HPSV-20). Detection was performed with a Waters Associates differential refractometer Model No. R403. Elution volumes were recorded with a syphon which was calibrated at each solvent ionic strength employed. Buffer used in preparing solute samples was taken directly from the solvent reservoir in order to eliminate spurious solvent peaks.

Each column was calibrated at each of the three phosphate buffer concentrations.

RESULTS AND DISCUSSION

Figure 1 shows the GPC elution profiles of a polystyrene sulfonate having a molecular weight of 18,900, using various ionic strength solvents with the CPG 240-Å column. The first peak eluted is the polymer, and the second may be either low molecular weight contaminants or an artifact of the ionic system or both. Secondary negative and positive peaks have been reported in the literature for GPC systems involving the elution of polyelectrolytes with dilute electrolyte solutions.[20] However, as yet we have no explanation for the trend of this behavior in our system.

It is obvious that increasing the phosphate concentration of the buffer dramatically increases the elution volumes of the polyelectrolyte. This phenomenon was qualitatively the same for all charged molecules eluting within the resolution

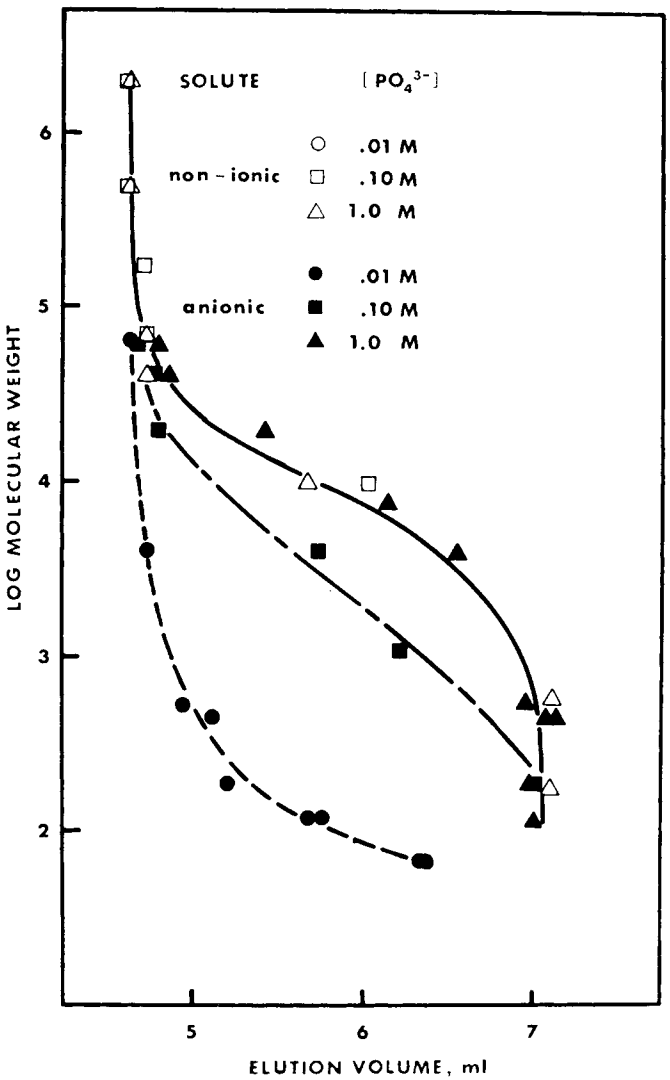


Fig. 3. GPC calibration curves for the CPG 75-Å column.

range of each glass. Undoubtedly, this effect is partly due to the decrease of site–site repulsion on the polyelectrolyte with increased ionic strength and hence smaller molecular dimensions.

A similar comparison of the elution of 40,000 MW dextran on the 240-Å column (Fig. 2) shows peak elution volumes which are essentially independent of phosphate concentration. Again secondary negative total liquid volume peaks were observed which became larger with increasing ionic strength. This is particularly interesting since, while a Donnan equilibrium might be invoked to explain secondary peaks with ionic solutes,[20] an uncharged polymer should not be able to participate in such a mechanism.

Figure 3 shows the calibration of the 75-Å column at the three solvent ionic strengths employed. The first observation which can be made is that the calibration curve for dextrans at both 0.1*M* and 1.0*M* phosphate are coincident with that of the ionic solutes at 1.0*M*. Lower phosphate concentrations produce

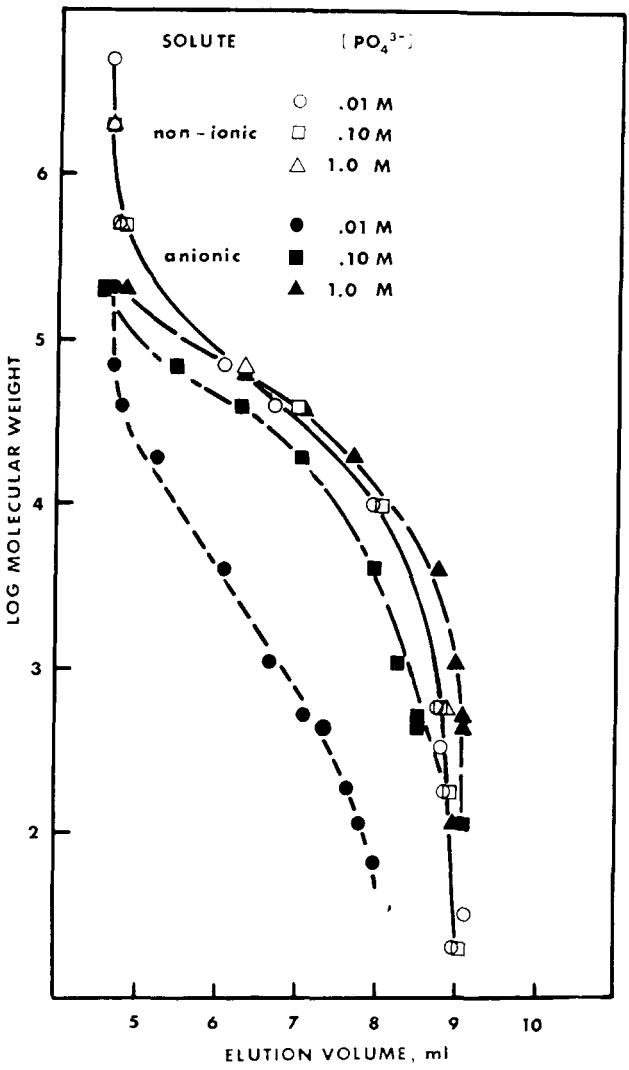


Fig. 4. GPC calibration curves for the CPG 240-Å column.

successively lower elution values for all anionic solutes which elute after the exclusion limit. For 0.01*M* phosphate, the curve does not even exhibit the normal sigmoidal shape defining an exclusion limit and a total liquid volume.

Phosphate concentrations higher than 1.0*M* were not investigated, since the calibration curve for charged molecules coincided with that for uncharged molecules.

The calibration curves for the 240-Å column, shown in Figure 4, are similar to those for the 75-Å column. In this case, a sigmoidal curve is observed for the lowest phosphate concentration. However, a smaller apparent total liquid volume is observed than at the higher phosphate concentrations, which are fairly well in agreement. The probable explanation for this is that the charged molecules are partially excluded from the pores in the low ionic strength environment. They experience an effective pore volume which is smaller than that available to uncharged molecules or to charged molecules at higher solvent ionic strengths. This is because larger electrolyte concentrations more effectively

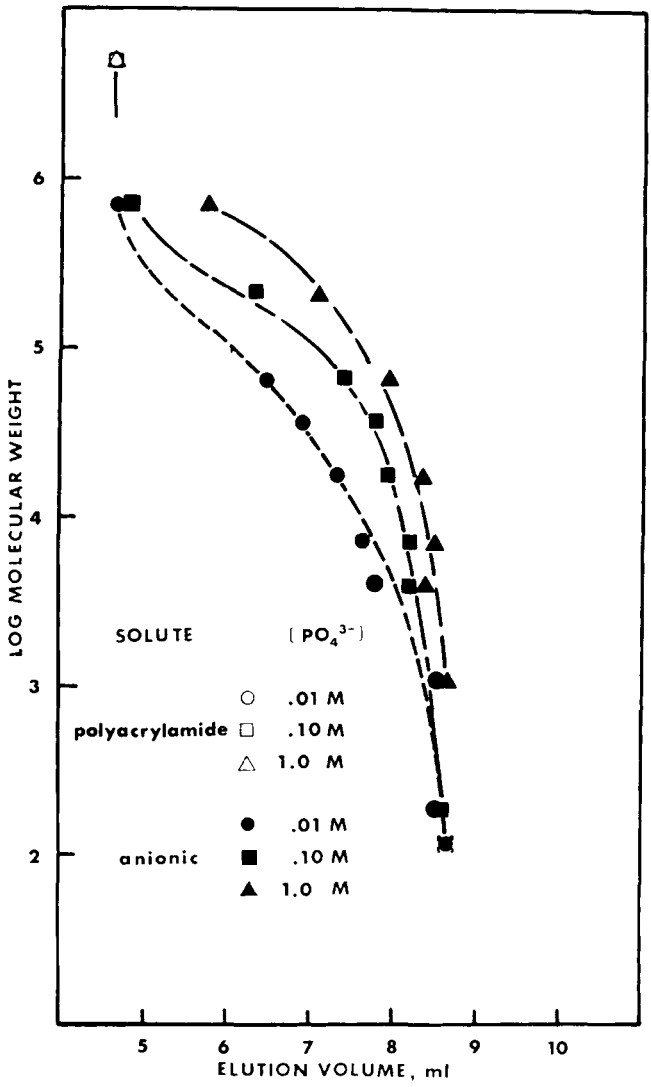


Fig. 5. GPC calibration curves for the CPG 700-Å column.

screen charge–charge interactions between the charged solute and the charged glass surface.

Spatorico and Beyer[5] reported the intrinsic viscosities of dextrans and NaPSS in 0.2*M* and 0.8*M* sodium sulfate. They found a threefold reduction in the intrinsic viscosity of NaPSS at the higher ionic strength, whereas the intrinsic viscosity of dextran changed less than 10%. The GPC universal calibration plot was found to be valid for these polymers and solvents. In terms of ionic strength, these two sodium sulfate concentrations fall between our experimental phosphate concentrations of 1.0*M* ($I = 2.2$) and 0.1*M* ($I = 0.22$). At lower ionic strengths, e.g., 0.01*M* phosphate, other factors are important. A further decrease in phosphate concentration will increase the hydrodynamic volumes of the various NaPSS fractions, causing shifts to higher elution volumes. However, the elution volume of sodium formate on the 240-Å column with 0.01*M* phosphate solvent was equivalent to the 10,000 MW dextran, and on the 75-Å column was even

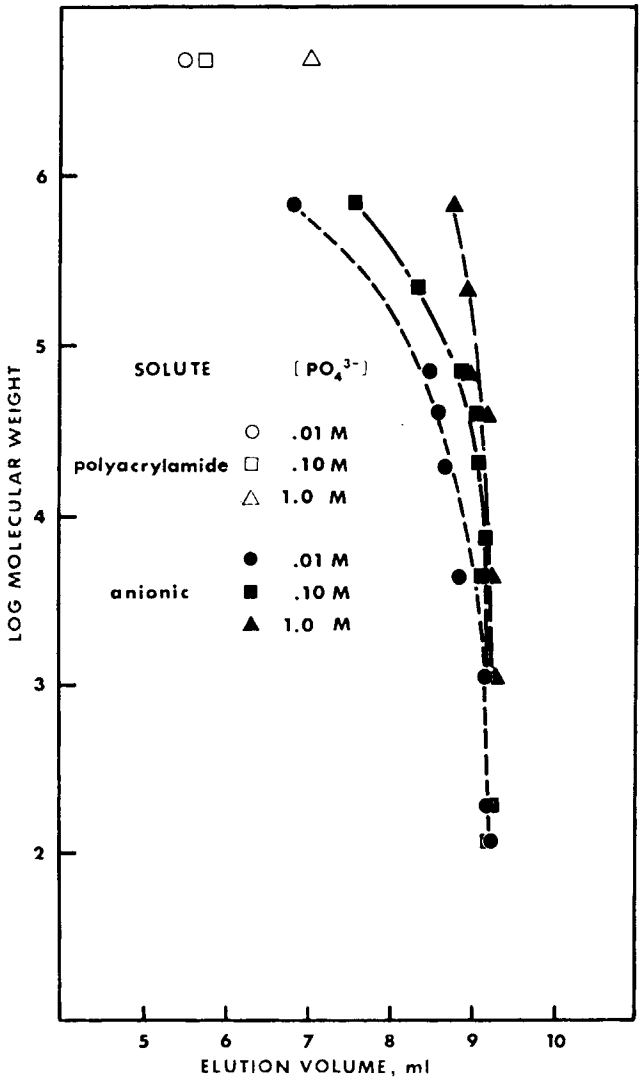


Fig. 6. GPC calibration curves for the CPG 2000-Å column.

smaller than that of the 10,000 MW dextran. This cannot be accounted for by chain expansion; it must be caused by an interaction of the charged molecules with the glass surface, which restricts accessibility of the charged species to the pore volume. It is impossible, therefore, for the GPC universal calibration to be valid for these systems at low solvent ionic strength.

Charge exclusion on controlled pore glass has been observed previously for negatively charged proteins at low ionic strength.[21] It has also been proposed as a method for the separation of small ionic compounds. A roughly linear relationship between net negative charge and elution volumes of amino acids on 80 Å CPG has been reported.[22]

The calibration curves for the 700-Å and 2000-Å CPG columns are shown in Figures 5 and 6, respectively. Here, on each figure the calibration curves coincide at low molecular weight to give a common total liquid volume. The same trend of charged molecule elution was again observed, but no NaPSS of sufficiently

high molecular weight was available as an exclusion limit marker. Polyacrylamide, MW 5×10^6, was eluted and showed a constant elution volume at all three ionic strengths for the 700-Å CPG column but eluted later with increasing ionic strength for the 2000-Å CPG column.

On both the 700- and 2000-Å columns dextrans gave broad elution profiles with ill-defined maxima which did not exceed the total liquid volume of the column. Leaching of pores in the production of porous glasses is a chemical process. The surface and bulk composition of such glasses can be quite different in terms of boron sites.[11] It is quite possible that the adsorptive properties of the individual glasses are quite different. However, several reports of successful GPC with dextrans and CPG packings using water as the solvent[6,7] or sodium sulfate solution[5] indicate that the effect we have found may also be due to the phosphate buffer components.

CONCLUSIONS

Controlled pore glass (CPG) packing materials are capable of exhibiting an ion exclusion mechanism in low ionic strength phosphate buffer solvents. Calibration curves for polyelectrolytes were found to be highly dependent on solvent ionic strength. This effect may be exploited to tailor the separating range for a particular CPG material. This is particularly useful for increased resolution in the low molecular weight range because 75 Å is the lowest CPG pore size available. Ion exclusion can also be completely eliminated by increasing the solvent ionic strength, allowing the universal calibration approach to be used.

References

1. A. R. Cooper, A. R. Bruzzone, J. H. Cain, and E. M. Barrall II, *J. Appl. Polym. Sci.*, **15**, 571 (1971).
2. A. R. Cooper and J. F. Johnson, *J. Appl. Polym. Sci.*, **13**, 1487 (1969).
3. A. R. Cooper and D. P. Matzinger, *Am. Lab.*, 13 (Jan. 1977).
4. A. H. Abdel-Alim and A. E. Hamielec, *J. Appl. Polym. Sci.*, **18**, 297 (1974).
5. A. L. Spatorico and G. L. Beyer, *J. Appl. Polym. Sci.*, **19**, 2933 (1975).
6. A. M. Basedow and K. M. Ebert, *Makromol. Chem.*, **176**, 745 (1975).
7. A. M. Basedow, K. H. Ebert, H. Ederer, and H. Hunger, *Makromol. Chem.*, **177**, 1501 (1976).
8. S. A. Barker, B. W. Hatt, and P. J. Somers, *Carbohydr. Res.*, **11**, 355 (1969).
9. S. A. Barker, B. W. Hatt, J. B. Marsters, and P. J. Somers, *Carbohydr. Res.*, **9**, 373 (1969).
10. K. J. Bombaugh, W. A. Dark, and J. N. Little, *Anal. Chem.*, **41**, 1337 (1969).
11. M. L. Hair and A. M. Filbert, *Research/Development*, **20**, 34 (1969).
12. C. W. Hiatt, A. Shelokov, E. J. Rosenthal, and J. M. Galimore, *J. Chromatogr.*, **56**, 362 (1971).
13. G. L. Hawk, J. A. Cameron, and L. B. DuFault, *Prep. Biochem.*, **2**, 193 (1972).
14. M. J. Frenkel and R. J. Blagrove, *J. Chromatogr.*, **111**, 397 (1975).
15. T. Mizutani and A. Mizutani, *J. Chromatogr.*, **111**, 214 (1975).
16. T. Mizutani and A. Mizutani, *J. Chromatogr.*, **120**, 206 (1976).
17. F. E. Regnier and R. Noel, *J. Chromatogr. Sci.*, **14**, 316 (1976).
18. C. Persiani, P. Cukor, and K. French, *J. Chromatogr. Sci.*, **14**, 417 (1976).
19. W. Carrol and H. Eisenberg, *J. Polym. Sci. A2*, **4**, 599 (1966).
20. P. Neddermeyer and L. Rogers, *Anal. Chem.*, **41**, 94 (1969).
21. H. D. Crone, R. M. Dawson, and E. M. Smith, *J. Chromatogr.*, **103**, 71 (1975).
22. H. D. Crone, *J. Chromatogr.*, **107**, 25 (1975).

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