Aqueous Size Exclusion Chromatography for Strong Anionic Polyelectrolytes on Hydrophilic Polymer Gel

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ABSTRACT

Elution behavior of sodium polystyrenesulfonates (NaPSS) on a TSK-GMPW column was discussed. Mobile phases were aqueous solutions of a mixture of Na_2HPO_4 and NaH_2PO_4 at pH 8.0 (A), NaH_2PO_4 (B), and Na_2CO_3 (C) at various concentrations. At low concentration of monomeric electrolytes in the mobile phase, peaks of NaPSS were sharp and Gaussian and the early elution of NaPSS relative to nonionic polymers was observed. Elution of NaPSS compounds retarded with increasing the concentration of electrolytes in the mobile phase and retained in the column over some value of the concentration. Larger molecular weight NaPSS compounds were apt to retain in the column at lower concentration of electrolytes in the mobile phase. In comparison with chromatograms of NaPSS 6,500 (molecular weight was 6,500) in three different mobile phases, A, B, and C, a hypothesis has been proposed that a factor influences retention volume of NaPSS is not the total ionic strength but the partial ionic strength of Na^+. However, the increase the ionic strength to decrease the electrostatic interactions resulted in the increase in the hydrophobic interactions and ideal SEC cannot be achieved in this system. For a low molecular weight compound, sodium-p-toluenesulfonate, a concentration of

0.01 M of a buffer solution was adequate because both electrosta-
tic and hydrophobic interactions were minimized and ideal SEC was
achieved.

INTRODUCTION

    Hydrophilic polymer gels are currently available as packing
materials for aqueous size exclusion chromatography (ASEC) of
proteins and ionic and nonionic water-soluble synthetic polymers.
Hydrophilic polymers gels are apparently nonionic neutral macro-
molelcules, but they bear some ionizable surface sites which
influence the elution of charged macromolecules. The cation-ex-
change capacity of Sephadex G-25 (dextran gel) was reported to be
2.2 ± 0.2 μeq/g of dry gel and elution of humic acids was influ-
enced by the ionic strength of the mobile phase[1].   TSK-PW gel
(Tosoh Co., Japan), which is supposed to be a cross-linked hydro-
philic polyether, also contains small amount of COOH groups[2].
    The early elution of humic acids, which are a kind of anionic
polyelectrolytes, on Sephadex was suppressed by the addition of
some monomeric electrolytes to the mobile phase (mostly deionized
water), but excess addition resulted in retaining humic acids[1].
However, the electrostatic effects between porous glass used as
the packing material and anionic polyelectrolytes[2] or proteins
[3] were suppressed at high ionic strength in the mobile phase.
Therefore, the mechanisms of interactions between column packing
materials made of polymer gels and solutes may be different from
those obtaied with porous glass.   Pfannkoch et al.[4] studied
elution behavior of several ionic compounds in several commercial-
ly available SEC columns and found that under certain conditions
they exhibited ion exclusion, ion exchange, and hydrophobic inter-
actions.
    This paper is concerned with the observation of the retention
behavior of sodium polystyrenesulfonates (NaPSS) (strong anioic

polyelectrolytes) on TSK-GMPW by changing the ionic strength in the mobile phase.   Linear polysaccharides (pullulan) and poly-ethylene oxides were used for comparison purposes.

EXPERIMENTAL

The column used in this experiment was a TSK-GMPW column which is supposed to contain a mixture of hydrophilic polyether gels of various pore sizes.   The column size is 7.5-mm-i.d. x 60-cm-length. The number of theoretical plates was 13,100 plates per 60 cm obtained by injecting 0.025 ml of a 20% D_2O solution. Deionized water was used as the mobile phase for the measurement of the column efficiency.

ASEC measurements were performed on a high-performance liquid chromatograph Model TRIROTAR-V (Japan Spectroscopic Co., Tokyo, Japan) with an ultraviolet absorption detector (UV) Model UVIDEC-100 V at a wavelength of 254 nm.   A differential refractive index detector(RI) Model SE-11 was also used for non-UV absorbing sub-stances.   Sample solutions were injected with a loop injector having a 0.025-ml-capacity-loop.

Samples were strong anionic polyelectrolytes and nonionic water-soluble polymers.   Strong polyelectrolytes were sodium polystyrenesulfonates (NaPSS) (Pressure Chemical Co., USA) (their molecular weights (MWs) were 4,000, 6,500, 16,000, 31,000, 88,000, 177,000, 690,000, and 10^6).   As nonionic water-soluble polymers, pullulan standards (linear polysaccharides) (Showa Denko, Japan) (MWs 5,800, 12,200, 23,700, 48,000, 100,000, 186,000, 380,000, and 853,000) and polyethylene oxide standards (Tosoh Co.) (MWs 25,000, 73,000, 280,000, and 1.2×10^6) were used for comparison purposes.

Mobile phases used in this study were made up from sodium monohydrogen phosphate Na_2HPO_4 and sodium dihydrogen phosphate NaH_2PO_4 to the desired ionic strength at pH 8.0.   Sodium dihydrogen phosphate solutions and sodium carbonate solutions at

various concentrations were also prepared.   A flow rate was 1.0
ml/min in all experiments.

RESULTS

Size exclusion chromatograms of nonionic water soluble poly-
mers are shown in Figure 1.   The mobile phase was deionized
water.   All peaks were Gaussian and they eluted between the
exclusion limit and the total permeation limit which was estimated
from retention volume of D_2O, 21.9 ml, or that of ethylene glycol,
20.8 ml.   Therefore, it may be said that nonionic water-soluble
polymers can be separated by SEC with this column system.   The
relationship of log molecular weight (MW) and retention volume for
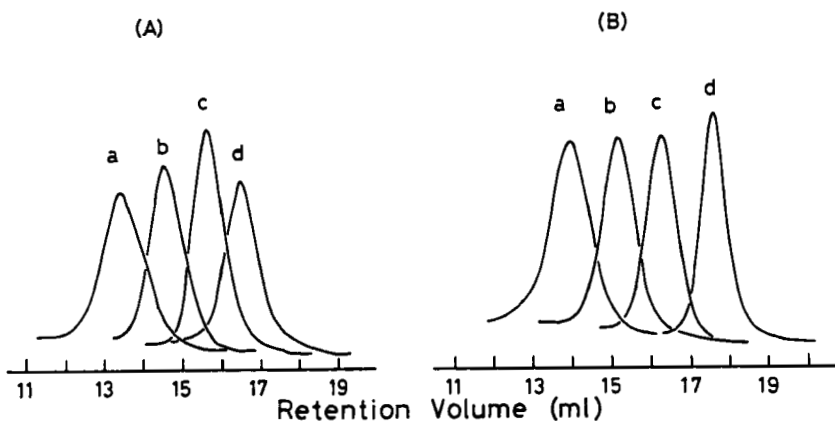pullulan is discussed later section.

FIGURE 1.   Size exclusion chromatograms of polyethylene oxide (A)
and pullulan (B) standards.   Mobile phase: water; sample injec-
tion: 0.2%, 0.025 ml; detector: RI, x4; molecular weight: (A)(a)
1.2×10^6, (b) 280,000, (c) 73,000, (d) 25,000, (B)(a) 853,000, (b)
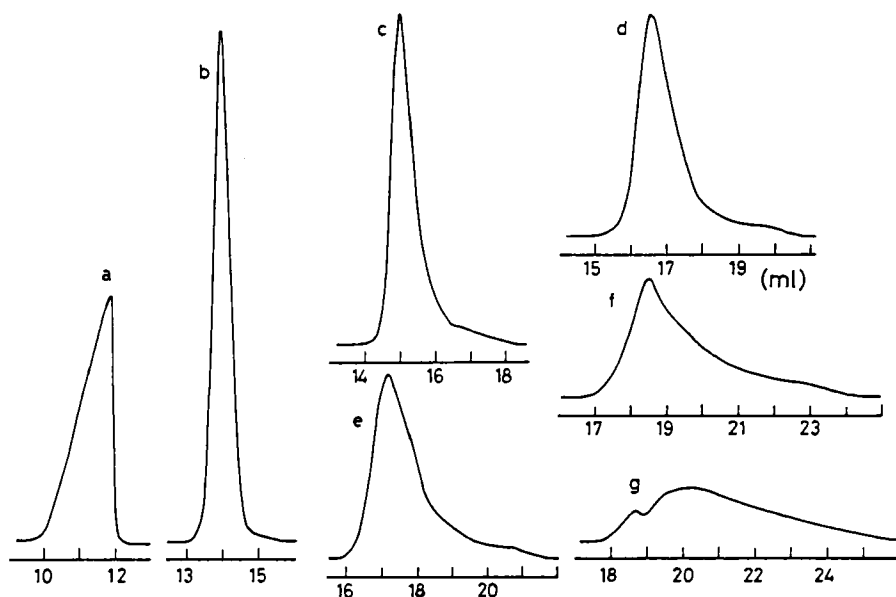186,000, (c) 48,000, (d) 5,800.

FIGURE 2.   Size exclusion chromatograms of NaPSS 6,500 in water and in a mixture of Na_2HPO_4 and NaH_2PO_4 as the mobile phase at pH 8.0.   Mobile phase: (a) deionized water, (b) - (g) a mixture of Na_2HPO_4 and NaH_2PO_4; concentration (M): (b) $5x10^{-4}$, (c) $1.15x10^{-3}$, (d) $5x10^{-3}$, (e) $1x10^{-2}$, (f) $2.76x10^{-2}$, (g) $5x10^{-2}$; sample injection: 0.1%, 0.025 ml; detector: UV, x0.08.

When NaPSS was sample and deionized water was the mobile phase, NaPSS having MW less than 31,000 could elute from the column, though their peaks were broad and showed leading.   NaPSS having larger MW than MW 31,000 retained in the column.   After several experiments with the mobile phases of buffer solutions or various salt solutions, the mobile phase was again changed to deionized water, then NaPSS employed in this work could elute from the column with Gaussian peaks.   However, after several sample injections, their peaks became broad and showed leading, though even NaPSS 10^6 (this figure means molecular weight---the same shall apply hereafter) could elute from the columns.   Some monomeric electrolyte molecules in the mobile phase might block some portion of the surface of polymer gels.
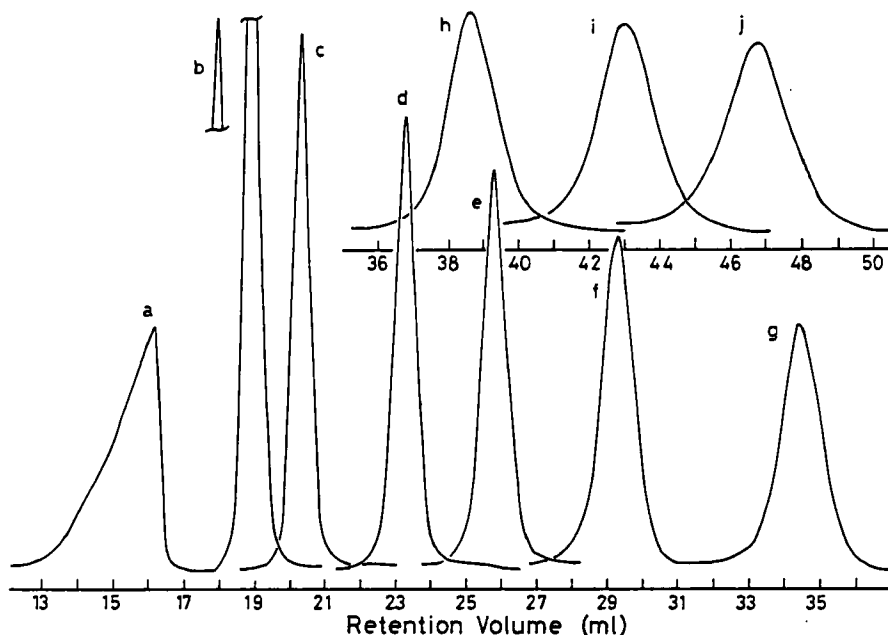
FIGURE 3.   Size exclusion chromatograms of sodium-p-toluenesul-
fonate in water and in a mixture of Na_2HPO_4 and NaH_2PO_4 as the
mobile phase at pH 8.0.   Mobile phase: (a) deionized water, (b) -
(g) a mixture of Na_2HPO_4 and NaH_2PO_4; concentration (M): (b)
5.0×10^{-3}, (c) 1×10^{-2}, (d) 2.76×10^{-2}, (e) 5.0×10^{-2}, (f) 0.1, (g)
0.2, (h) 0.3, (i) 0.4, (j) 0.5; sample injection: 0.1%, 0.025 ml;
detector: UV, x0.08.

Buffer solutions made up from Na_2HPO_4 and NaH_2PO_4 at pH 8.0

were used as the mobile phase at various concentrations.   Exam-

ples for chromatograms of NaPSS 6,500 are shown in Figure 2.   A

tailing peak was observed when deionized water was the mobile

phase as explained above (peak a).   A sharp peak was obtained

when the concentration of the buffer solution was the lowest as

5×10^{-4} M (peak b), and the peak became broader and retention

volume increased much more with increasing the concentration of

the mobile phase (peaks c to e) .   NaPSS 4,000 and 6,500 could

elute with these conditions of the mobile phase, but NaPSS 31,000

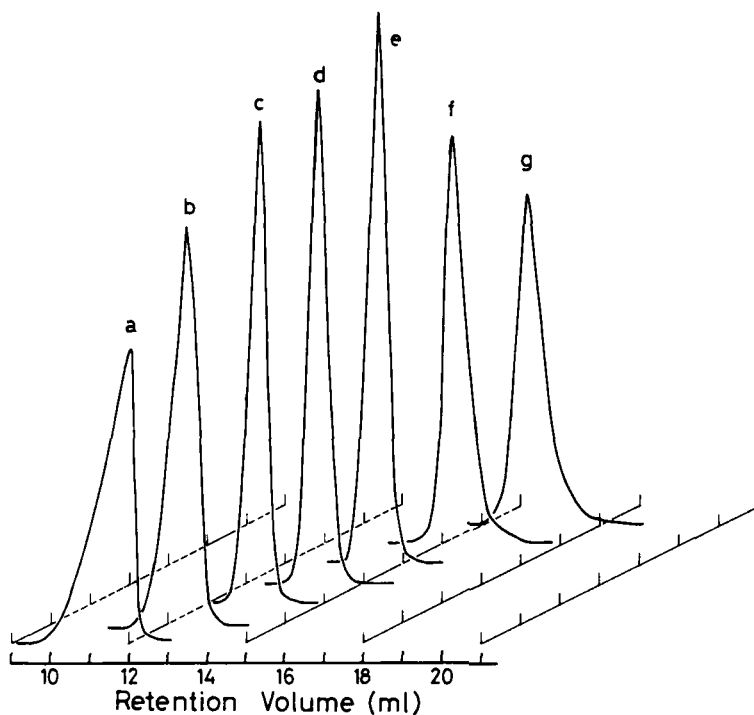eluted only when the concentration of the mobile phase was below

FIGURE 4.   Size exclusion chromatograms of NaPSS 6,500 in the mobile phase of Na_2CO_3 solutions.   Concentration (M) and pH:(a) 0 (deionized water), 7.0, (b) $1x10^{-4}$, 8.6, (c) $1x10^{-3}$, 9.9, (d) $1.2x10^{-3}$, 9.92, (e) $2.0x10^{-3}$, 10.35, (f) $5.0x10^{-3}$, 10.55, (g) $1.0x10^{-2}$, 10.7.

$1.15x10^{-3}$ M.   Other NaPSS compounds having MW larger than 31,000 used in this experiment were retained in the column at any concentrations.

Sodium-p-toluenesulfonate is a model compound of a monomer of NaPSS.   Chromatograms of the monomer in a buffer solution made up from Na_2HPO_4 and NaH_2PO_4 at various concentrations are shown in Figure 3.   When deionized water was the mobile phase, the peak showed leading as in the cases of NaPSS 6,500 in Figure 2.   At the concentration of the mobile phase $5x10^{-3}$ M, the peak was sharp
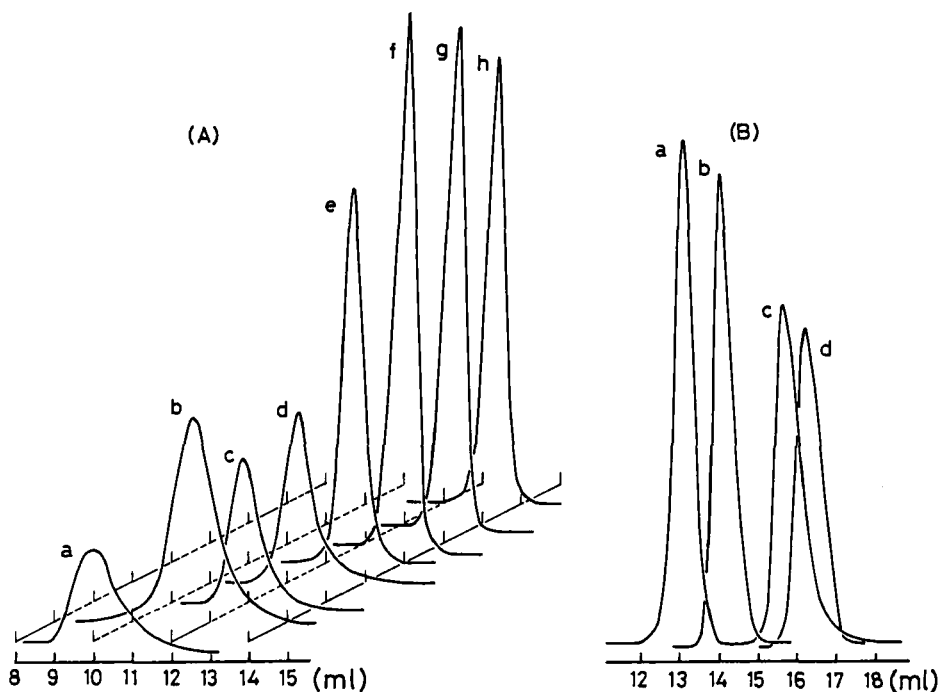
FIGURE 5.   Size exclusion chromatograms of NaPSS in the mobile phase of NaH_2PO_4 solutions.   (A) Concentration (M) and pH of the mobile phase: 5×10^{-4}, 5.8; molecular weight: (a) 1×10^6, (b) 690,000, (c) 177,000, (d) 88,000, (e) 31,000, (f)16,000, (g) 6,500, (h) 4,000.   (B) NaPSS 6,500; concentration (M) and pH of the mobile phase: (a) 5.0×10^{-4}, 5.8, (b) 1.0×10^{-3}, 5.5, (c) 5.0×10^{-3}, 5.05, (d) 1.0×10^{-2}, 4.8.

and eluted near the total permeation limit.   With increasing the concentration of the mobile phase, the peak became broad and the retention volume of the peak increased.   By plotting the peak retention volume aganinst the concentration of the mobile phase, the concentration at which the retention volume of the peak was at the total permeation limit (21.9 ml by D_2O) was calculated as to be 1.9×10^{-2} M.   Over this concentration, the monomer appeared after the total permeation limit, and it means that adsorption
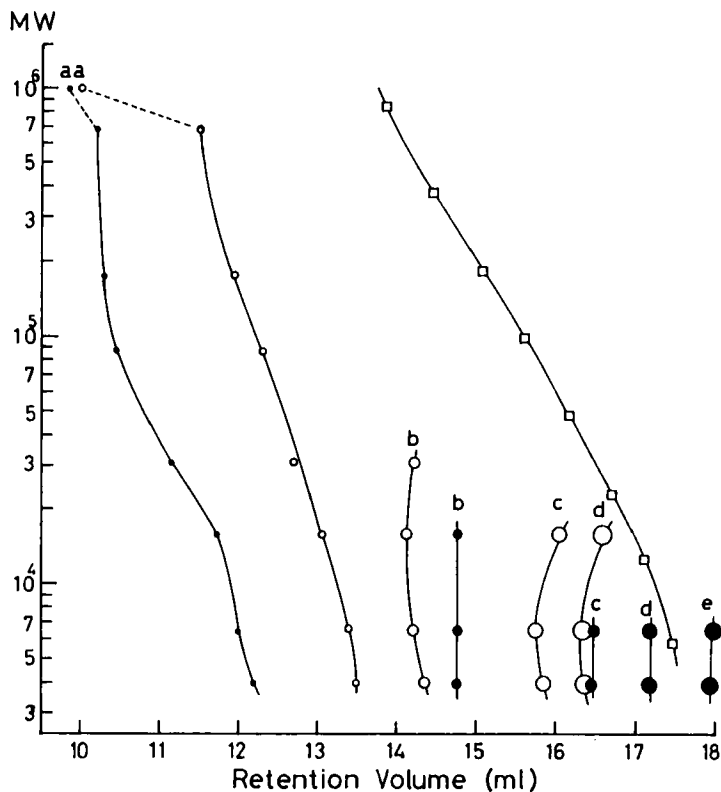
FIGURE 6.   Relationship of log M vs retention volume for NaPSS in phosphate buffer solutions.   Electrolyte: (●) a mixture of Na_2HPO_4 and NaH_2PO_4 at pH 8.0, (o) NaH_2PO_4; concentration (M) of electrolyte: (●)(a) 0 (deionized water), see text, (b) 1.15×10^{-3}, (c) 5.0×10^{-3}, (d) 1.0×10^{-2}, (e) 2.0×10^{-2}; (o)(a) 5×10^{-4} (pH 5.8), (b) 1.0×10^{-3} (pH 5.5), (c) 5.0×10^{-3} (pH 5.05), (d) 1.0×10^{-2} (pH 4.8); (□) pullulan.

effect or hydrophobic interactions are superimposed on size exclusion effect for the separation of the monomer.

        Figure 4 shows chromatograms of NaPSS 6,500 in Na_2CO_3 solutions used as the mobile phase.   The pH value of the mobile phase increases with increasing the concentration of Na_2CO_3, and at the concentration of 1×10^{-2} M (pH 10.7), the peak was Gaussian and sharp, but the retention volume of the peak increased as much.
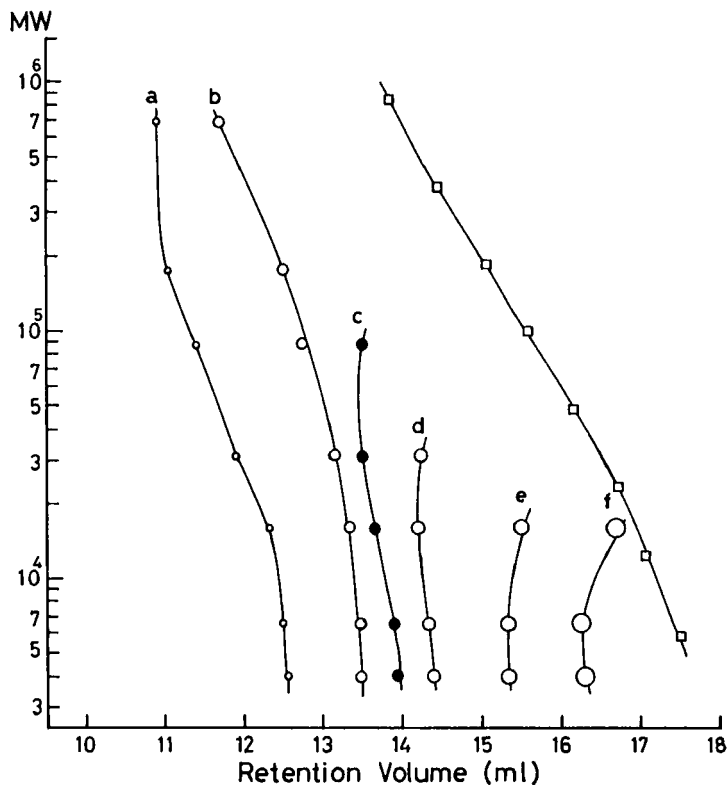
MW

FIGURE 7.   Relationship of log M   vs retention volume for NaPSS
in Na_2CO_3 solutions.   Concentration (M) and pH: (a) $1x10^{-4}$, 8.6,
(b) $1.0x10^{-3}$, 9.9, (c) $1.2x10^{-3}$, 9.95, (d) $2.0x10^{-3}$, 10.35, (e)
$5.0x10^{-3}$, 10.55, (f) $1.0x10^{-2}$, 10.70; (□) pullulan.

        When NaH_2PO_4 alone was used as an electrolyte in the mobile

phase and the concentration was $5x10^{-4}$ M (pH 5.8), peaks for NaPSS

were all sharp and even NaPSS 10^6 eluted from the column.   These

results are shown in Figure 5 (A).   Figure 5 (B) is chromatograms

of NaPSS 6,500 at different concentrations of NaH_2PO_4 in the

mobile phase.   The peak was sharp even at the concentration of

$1x10^{-2}$ M of the electrolyte in the mobile phase, though the reten-

tion volume of the peaks increased with increasing the concentra-

tion.

Relationships of log MW vs retention volume for NaPSS in the
mobile phases of a mixture of Na_2HPO_4 and NaH_2PO_4 and of NaH_2PO_4
alone at various concentrations were shown in Figure 6 and those
in the mobile phase of Na_2CO_3 in Figure 7.   A plot of MW vs
retention volume for pullulan is shown in Figures 6 and 7 for
comparison purposes.   The relation of log MW vs retention volume
for NaPSS in the mobile phase of deionized water is plotted in
Figure 6 (●) a.   The data were obtained after several experiments
with the mobile phases of buffer solutions.

DISCUSSION

Although nonionic water soluble polymers eluted according to
their sizes by size exclusion using deionized water as the mobile
phase (Figure 1), anionic polymers NaPSS eluted at the exclusion
limit because of ion exclusion by the electrostatic interactions
between substrates and NaPSS (peak a in Figures 2 and 4).   At low
concentration of monomeric electrolytes in the mobile phase, peaks
for NaPSS became sharp and Gaussian, but still eluted at the lower
retention volume relative to nonionic polymers of the some molecu-
lar sizes (Figures 2, 4, 6, and 7).   This is the evidence of
intramolecular expansion and substrate repulsion [5].   With in-
creasing the concentration of electrolytes in the mobile phase,
elution of NaPSS compounds retarded, and over some values of the
concentration, NaPSS retained in the column.   Larger molecular
weight NaPSS were apt to retain in the column even at lower con-
centration of electrolytes in the mobile phase (Figures 6 and 7).

A peak of p-toluenesulfonate was sharp and eluted near the
total permeation limit below the concentration of $1x10^{-2}$ M for a
buffer solution of a mixture of Na_2HPO_4 and NaH_2PO_4 (peaks b and c
in Figure 2).   The peak became broad and eluted across the total
permeation limit over this concentration (peaks d to j in Figure
2).   This may be the evidence that the ion exclusion effect

minimizes at this concentration and adsorption or hydrophobic
interactions start to increase over this concentration.

In comparison with chromatograms of NaPSS 6,500 in three
different types of mobile phases, a mixture of Na_2HPO_4 and
NaH_2PO_4, NaH_2PO_4, and Na_2CO_3, the peak in a solution of a mixture
of Na_2HPO_4 and NaH_2PO_4 (pH 8.0) was sharp below 1.15×10^{-3} M
concentration and broad at 1×10^{-2} M, but the peaks in other types
of the mobile phase were still sharp at 1×10^{-2} M (peaks e in
Figure 2, g in Figure 4, and d in Figure 5(B)). The pH values of
the latter two mobile phases were 4.8 in a NaH_2PO_4 solution at
1×10^{-2} M and 10.7 in a Na_2CO_3 solution at 1×10^{-2} M. It is said
that the elution of ionic compounds is controlled by the ionic
strength and pH value of the mobile phase. The ionization of
COOH groups on the surface of polymer gel may be suppressed in the
NaH_2PO_4 solution and thus ion exclusion is considered to be mini-
mized. Therefore, retention volume of NaPSS 6,500 with the
mobile phase of a NaH_2PO_4 solution should be higher and the peak
should be broader than those of a solution of a mixture of Na_2HPO_4
and NaH_2PO_4. However, the results were reversed. A different
hypothesis must be concidered.

In Figure 6 (plots b, c, and d), the early elution of NaPSS
in a NaH_2PO_4 solution was observed than the elution of NaPSS in a
mixture of Na_2HPO_4 and NaH_2PO_4 of the same concentration (the same
ionic strength). A proposed hypothesis is that a factor influ-
ences the elution is not the total ionic strength including both
cations and anions, but the partial ionic strength of Na^+. This
hypothesis is understandable, because the surface of both NaPSS
and polymer gels are surrounded with fixed anions (negative
charge) and free cations (positive charge) will gather around the
surface, thus free cations influence the elution much more than
free anions. The proof of this hypothesis is now under conside-
ration.

In Figure 7, on the other hand, the mobile phase was basic
and COOH groups on the surface of polymer gels were completely
ionized and adsorption or hydrophobic interactions might be sup-

pressed, and therefore, NaPSS compounds of higher molecular weight could elute even at the concentration of 1×10^{-3} M in the mobile phase. However, similarly to the results in Figure 6, the elution of NaPSS retarded with increasing the concentration of Na_2CO_3 in the mobile phase, meaning the existence of hydrophobic interactions.

In any cases, ideal SEC cannot be achieved in this system for ionic polymers, eventhough ion exclusion and hydrophobic interactions are tried to be suppressed.

It is said that the addition of organic solvent in the mobile phase minimizes the adsorption effect on the polymer gels. In our experiment, a 10% methanol aqueous solution was used as the mobile phase. Up to NaPSS 10^6 could elute but their peaks were eluted earlier than expected and showed broad and leading shapes. They eluted between 10 and 12.6 ml and sodium-p-toluenesulfonate elute at 14.8 ml.

ACKNOWLEDGMENT

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