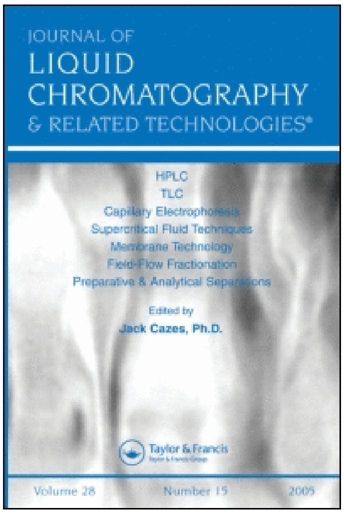

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Sadao Mori[a]; Takayo Mori[a]; Yoshiyuki Mukoyama[b]
[a] Department of Industrial Chemistry Faculty of Engineering, Mie University Tsu, Mie, Japan [b] Research and Development Department Kashima Works, Hitachi Chemical Company, Ibaraki, Japan

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ELUTION BEHAVIOR OF POLYETHYLENE GLYCOLS ON A HYDROPHILIC POLYMER GEL COLUMN USED FOR SIZE EXCLUSION CHROMATOGRAPHY

SADAO MORI*[1], TAKAYO MORI[1], AND YOSHIYUKI MUKOYAMA[2]
[1]*Department of Industrial Chemistry*
Faculty of Engineering
Mie University
Tsu, Mie 514, Japan
[2]*Research and Development Department*
Kashima Works
Hitachi Chemical Company
Hasaki-cho, Kashima-gun, Ibaraki 314-02, Japan

ABSTRACT

Water, methanol, tetrahydrofuran and their mixtures were used as mobile phases and the relationship between retention volumes of polyethylene glycol (PEG) and the composition of the mobile phases on a hydrophilic polymethacrylate gel column was investigated. The column packed with this polymer gel could be used with both polar and non-polar organic solvents as well as aqueous solvents. Retention volume of PEG having the same molecular weight changed with changing the composition of the mobile phase. The change of molecular size of PEG with changing the composition of the mobile phase was small compared to the difference in retention volume, and PEG samples were considered to be separated mainly by size exclusion, secondary effects being superimposed. In order to calculate molecular weight averages of PEG, the adequate selection of the mobile phase which minimizes the peak width of PEG is important and the recommended combination of PEG and the mobile phase with the column used here for PEG are PEG - water, PEG higher than 1000 molecular weight - THF/methanol (50/50) or THF.

INTRODUCTION

Hydrophilic polymer gels such as polyhydroxyethyl methacrylate gels and polyvinyl alcohol gels are now commercially available for aqueous size-exclusion chromatography (ASEC). Most of these polymer gels undergo shrinkage with many organic solvents and, therefore, the mobile phases available to ASEC are limited to aqueous solutions plus a small amount of organic solvents. Fortunately, some of these hydrophilic polymer gels were found to be compatible with both polar and non-polar organic solvents (1,2) and the elution behavior of several types of oligomers were investigated on columns packed with these hydrophilic polymer gels using water, methanol, tetrahydrofuran (THF), chloroform, n-hexane and the mixtures of two of these solvents (2-4).

It is commonly recognized that packing materials used for ASEC should be different from those for non-aqueous SEC: ASEC with hydrophilic polymer gels and non-aqueous SEC with hydrophobic polymer gels. It must be very economical if one can use the same column for non-aqueous SEC with non-polar solvents and ASEC with aqueous solvents. In SEC, polymers which have the same molecular size are supposed to elute at the same retention volume independently of any types of polymers and from this fact, one can estimate molelcular weight (MW) or MW averages of one type of polymers using a calibration curve constructed with the other type of polymers. If any secondary effects such as the adsorption between the solutes and the stationary phase exist,then it becomes difficult to measure MW and MW averages of polymers precisely and accurately. Because, the magnitude of the secondary effects in SEC depends on both MW and chemical structures of the polymers, especially of oligomers and, therefore, the linear relation between log MW and retention volume cannot be expected among different polymers and oligomers.

In the present work, retention volumes of polyethylene glycols (PEG) on a column packed with the hydrophilic gels which can be used with both hydrophilic and hydrophobic solvents were measured using several solvents as the mobile phase and were compared with molecular sizes of PEG. The selection of the adequate solvent for the mobile phase is important for SEC and the procedure is proposed.

EXPERIMENTAL

A Gelpak GL-W550 column (300 mm x 10.7 mm i.d.) (Hitachi Chemical Co., Shinjuku-ku, Tokyo 160, Japan) packed with hydrophilic polymethacryalte gels having hydrophilic pendant groups was used in this experiment. The gels packed in this column have wide pores and are exclusively used for polymer analysis. The number of theoretical plates (N) per column was 13000 plates on injecting 0.1 ml of a 0.1% ethylene glycol (EG) solution with water as the mobile phase. The decrease of the column efficiency was not observed by replacing the solvent in the column to another solvents.

A high-performance liquid chromatograph Model TRIROTAR (Jasco Inc., Hachioji, Tokyo 192, Japan) was used with a refractive index detector (RI) Model R401 (Waters, Milford, Mass., USA). Mobile phases were water, methanol,THF and mixtures of water and methanol and of THF and methanol. The flow rate was 1.0 ml/min and the injection volume of sample solutions was 0.1 ml. Samples were dissolved in the solvent used as the mobile phase in the concentration of 0.1%.

The samples used in this experiment were EG, PEGs 200, 300, 400, 600, 1000, 2000, 6000, 2.5×10^4 and 7.3×10^4 (the figures represent the nominal MW). The intrinsic viscosity of PEG in several solvents was measured by using a Ubbelohde-type viscometer in a constant-temperature water bath at 35 °C.

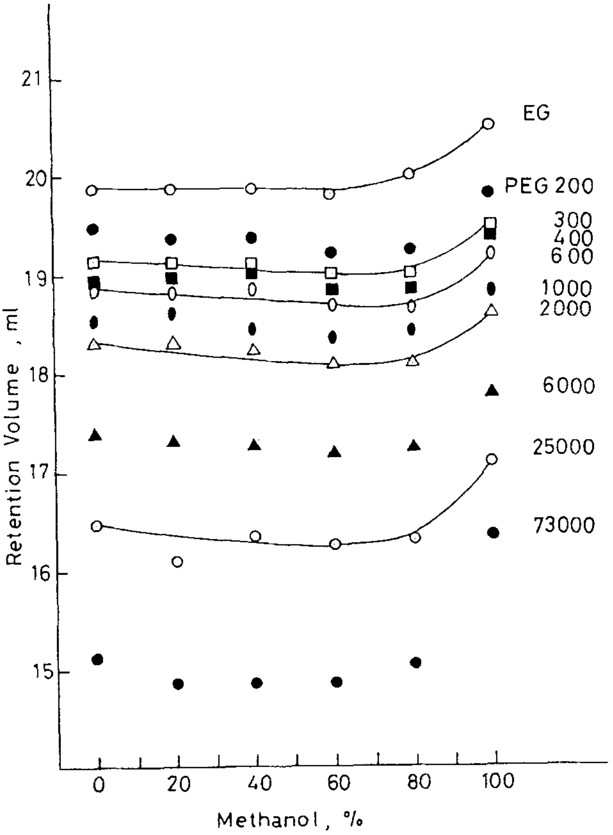

FIGURE 1. Relationship between retention volume of PEG and mobile phase composition. Mobile phase: water, methanol, and water-methanol (80/20, 60/40, 40/60, 20/80).

RESULTS AND DISCUSSION

Retention Volume versus Mobile Phase Composition.

The changes in the retention volumes of EG and PEGs with variation in the mobile phase composition were determined and the results are shown in FIGURES 1 and 2. The results in water,

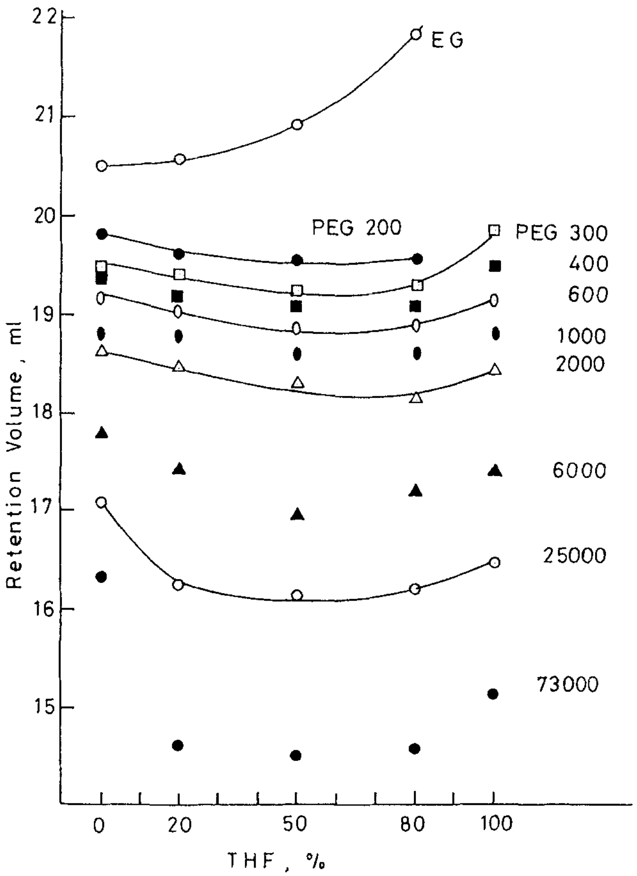

FIGURE 2. Relationship between retention volume of PEG and mobile phase composition. Mobile phase: methanol, THF and methanol-THF (80/20, 50/50, 20/80).

methanol and mixtures of water and methanol are shown in FIGURE 1. Retention volumes of the samples obtained at the mobile phase of water were smaller than those obtained at the mobile phase of methanol. The PEG samples eluted from the column with the mobile phases of mixtures of water and methanol earlier than they did with water or methanol alone.

The elution behavior of PEGs shown in FIGURE 1 is somewhat different from that obtained in the column of polyvinyl alcohol (PVA) gels (3). In the PVA column, retention volumes of the samples obtained with the mobile phase of water were larger than those obtained with the mobile phase of methanol.

Retention volumes of EG and PEGs obtained with the mobile phases of methanol, THF and the mixtures of methanol and THF are shown in FIGURE 2. The retention volumes of PEGs obtained with the mobile phases of the mixtures of methanol and THF were smaller than those obtained with the mobile phase of methanol or THF alone. These results are similar to those in FIGURE 1. On the contrary, the retention volume of EG increased with increasing the content of THF in methanol.

Retention volumes of PEGs obtained with the mobile phase of THF were lower than those obtained with the mobile phase of methanol if MW of PEG was higher than 1000. The situation was reversed when MW of PEG was lower than 1000. The retention volume of PEG 1000 with the mobile phase of THF was similar to that with the mobile phase of methanol. The minimum retention volume was obtained at a mobile phase composition of methanol - THF (50/50).

When methanol, THF and mixtures of THF - methanol (the compositions of THF/methanol were 20/80, 50/50 and 80/20) were used as mobile phases, the values of N were 14000, 7200, 16000, 19000, and 9200 plates per column, respectively in that order. The experiments were performed with mobile phases being changed in the order water, water - methanol, methanol, THF and THF - methanol. After a series of the experiments, the mobile phase wasd returned t water and the value of N was remeasured. It was unchanged and the retention volume of EG was also stable. The maximum value of N was obtained at a mobile phase composition of THF - methanol (50/50) and at this mobile phase, the retention volume and the peak width at half-height of EG were 20.91 ml and 0.36 ml, respectively. The minimum value of N at a THF mobile phase resulted from the large value of the peak width at half-height of EG (=0.65

ml), eventhough the retention volume of EG at THF mobile phase increased to 26.90 ml. However, this increase in the peak width was not due to the degradation of the column by changing mobile phases,but resulted in the peak broadening dur to the interactions between EG and the stationary phase. Peak width of EG with mobile phases of water, methanol and their mixtures was unchanged and the increase in the value of N with the methanol mobile phase was due to the increase in retention volume.

Molecular Size of PEG.

Two reasons can be considered for the difference in retention volumes of PEGs such as as shown in FIGURES 1 and 2: the change of the molecular size of PEG in different solvents and the difference in the magnitude of the secondary effects between PEGs and the stationary phase.

The hydrodynamic volume of polymers in a solvent which is proportional to the molecular size is defined as the intrinsic viscosity times MW. The intrinsic viscosities of PEGs in water, methanol, THF and their mixtures are plotted against the composition of the solvents and the results are shown in FIGURE 3. The values of the intrinsic viscosity in water were larger than those in methanol and those in the mixtures of water and methanol decreased with increasing the content of methanol in water. Similarly, the values of the intrinsic viscosity in THF was larger than those in methanol and those in the mixtures of THF and methanol decreased with increasing the content of methanol in THF.

The results shown in FIGURE 3 indicate that the difference in the molecular sizes of PEGs in water, methanol, and THF affected the values of retention volumes to some extent and that the smaller retention volumes of PEGs in the mixed solvents than those in pure solvents cannot be explained only by the difference in the molecular sizes. The magnitude of the secondary effects must be considered.

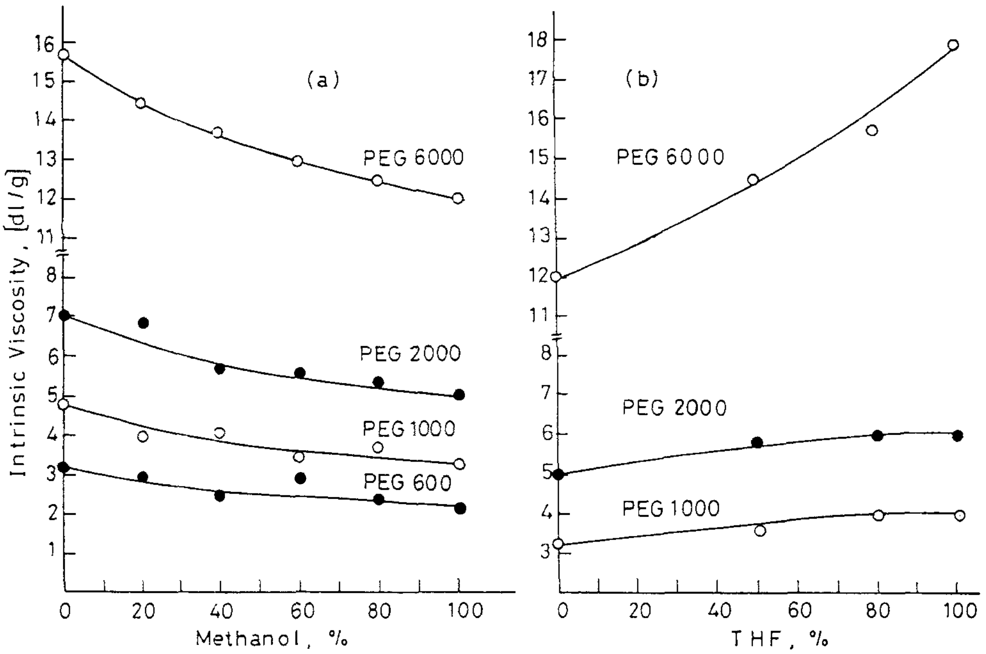

FIGURE 3. Intrinsic viscosity of PEG in several solvents. Solvent: (a) water, methanol and their mixtures; (b) methanol, THF and their mixtures.

Secondary Interactions.

The concept of the solubility parameter defined by Hildebrand can be applied to elucidate the elution mechanism that the secondary effects are superimposed on the size exclusion effect. According to the theory proposed by one of the authors (S.M.)(5), when the solubility parameter of the mobile phase is equal or nearly equal to that of the gel or a solute, then the separation occurs mainly by the size exclusion effect. Hildebrand solubility parameters for water, methanol and THF are 47.9, 29.1 and 20.5 $(J/cm^3)^{1/2}$, respectively. The parameters for the mixtures are as follows: water/methanol 80/20 - 43.8; 60/40 - 40.2; 40/60 - 36.3; 20/80 - 32.7; methanol/THF 80/20 - 27.3; 50/50 - 24.8; 20/80 -

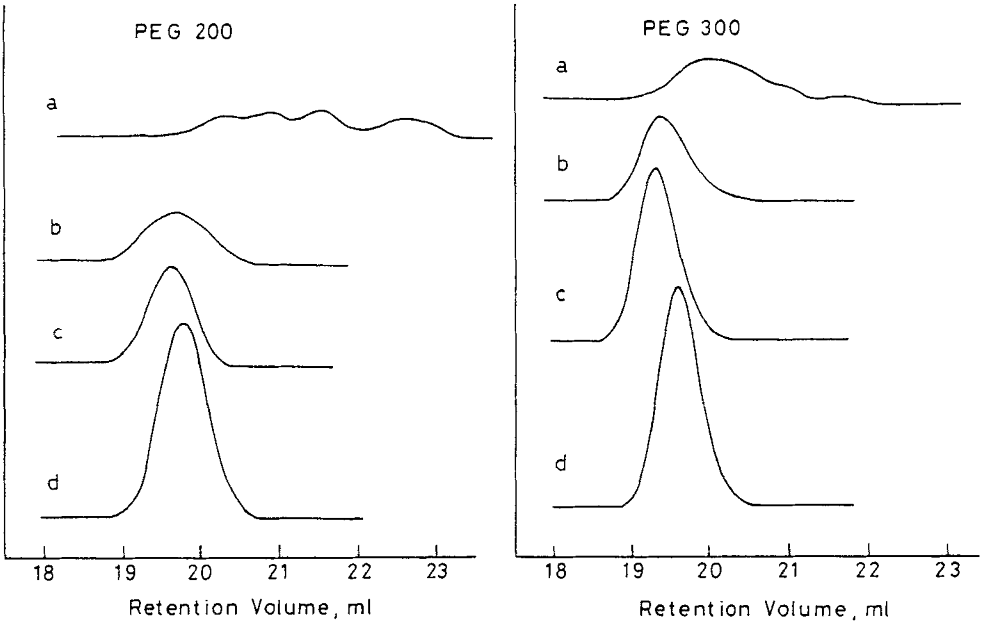

FIGURE 4. Chromatograms of PEG 200 and PEG 300 in THF and mixed solvents of THF and methanol. Mobile phase: (a) THF; (b) THF - methanol 80/20; (c) THF - methanol 50/50; (d) THF - methanol 20/80.

22.3. The parameters for EG is 33.2 and those for PEGs are between 21.4 and 32.7, depending on their MW. Therefore, the mobile phases water-methanol (20/80 - 40/60), which gave minimum retention volumes of PEG, have similar solubility parameters to PEGs and minimize the secondary effects. The solubility parameters of hydrophilic polymethacrylate gels are not known in the literature, but from FIGURE 2, the parameter of the hydrophilic polymer gels used in this experiment was estimated to be around 22.3 - 24.8.

Secondary interactions such as the adsorption effect and the partition effect are superimposed on the size exclusion effect in the SEC system used in this work and the extent of these secondary effects is dependent on the composition of the mobile phases. In SEC for PEG, molecular weight averages can be calculated using a calibration curve of log MW of PEG vs. retention volume, and therefore, any mobile phase seems to be used as far as PEGs are eluted in order of decreasing MW. In the system of the mobile phases water-methanol (FIGURE 1), peaks of PEGs were all symmetrical, but peak widths obtained with the mobile phase of methanol were 10% wider than those with the mobile phase of water.

With THF mobile phase, PEG 200 and PEG 300 were separated into several peaks. The results are shown in FIGURE 4. PEG 400 and PEG 600 with THF showed only one peak but their peaks were broad and 25 - 50% wider than those with water. PEGs larger than MW 1000 were all sharp and symmetrical in the THF mobile phase. When THF and mixtures of THF and methanol were used as mobile phases, peak widths at half-height of PEGs having MW larger than 1000 were smaller than those obtained with the mobile phase of water, and those of PEGs having MW smaller than 600 were larger than those with water. The pea widths obtained with the mobile phase of methanol were larger than those with water.

The peak broadening phenomena originated in the secondary effects which are superimposed on the size exclusion effect allows to increase in the calculated values of the polydispersity of polymers ($=M_w/M_n$). Therefore, when one determines MW and MW averages of PEG using a column W-550, the combination of the mobile phase and PEG which minimizes peak width of PEG should be selected. In this point, the combination of PEG lower than 1000 MW - THF on a W-550 column is not adequate to SEC. The recommended combinations of PEG and the mobile phase with a W-550 column are as follows: PEG - water, PEG higher than 1000 MW - THF/methanol (50/50) or THF.

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