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INTERCHANGEABLE USE OF AQUEOUS AND ORGANIC SOLVENTS IN A HYDROPHILIC POLY(VINYL ALCOHOL) GEL COLUMN

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SUMMARY

Water, methanol, chloroform and their mixtures were used as mobile phases and the elution behaviours of polyethylene glycols (PEG) and polystyrenes (PS) on a hydrophobic poly(vinyl alcohol) column were investigated. The column efficiency was not affected by variation of the mobile phase. Samples were eluted in order of decreasing molecular weight and separation was mainly achieved by size exclusion, a slight adsorption effect being superimposed. With water–methanol as the mobile phase, the minimum retention volume of PEG was obtained with a mobile phase composition of water–methanol (25:75, v/v) and a size-exclusion separation was mainly expected. With other compositions of this mobile phase an adsorption effect was superimposed on size exclusion. With chloroform–methanol as the mobile phase, the minimum retention volume of PS was obtained with a mobile phase composition of chloroform–methanol (80:20, v/v) and a slight adsorption effect was observed. Size-exclusion, adsorption and partition effects were evaluated from the variations of retention volumes, peak widths and solubility parameters.

INTRODUCTION

A number of applications of high-performance liquid chromatographic (HPLC) columns packed with polymer-based materials have been reported. Most of them involve porous polymer beads such as styrene–divinylbenzene copolymers which are hydrophobic in nature. These porous polystyrene (PS) gels have been used in both reversed-phase and normal-phase chromatography and also in size-exclusion chromatography (SEC) with various mobile phases¹. The first attempt to use PS gels as packing materials for aqueous SEC was reported by Chow², who separated dextrans having different molecular weights by using 0.1% sodium lauryl sulphate solution as the mobile phase.

However, because of the hydrophobic nature of PS gels, their use is limited to reversed-phase chromatography in addition to non-aqueous SEC. Polymer-based packing materials may have great stability with mobile phases over a wide range of pH and therefore they have attracted much attention. Polystyrene- and polyacrylamide-based packing materials have been developed for reversed-phase chromatography³ and applied to pharmaceutical analysis⁴. A solvent eluotropic scale for PS gels has

been developed⁵. To reduce their hydrophobicity, neutral hydrophilic groups were covalently bonded to the surface of porous PS gels⁶.

Several types of hydrophilic gels are now commercially available for aqueous SEC. These gels are used exclusively for water-soluble ionic and non-ionic polymers such as polysaccharides, proteins and sodium polystyrene sulphonates. However, these polymer gels undergo shrinkage with many organic solvents and the mobile phases available are limited to aqueous solutions plus small amounts of organic solvents.

Recently, it was found that some hydrophilic polymer gels containing a vinyl alcohol copolymer were compatible with polar and non-polar organic solvents⁷. Hence columns packed with these gels can be used interchangeably with both aqueous solutions and non-polar organic solvents as mobile phases without loss of column efficiency.

In this work three solvents, water, methanol and chloroform, were used as mobile phase components and the elution behaviour of polyethylene glycols (PEG), PS and related compounds were investigated on columns packed with these poly(vinyl alcohol) gels.

EXPERIMENTAL

The column used was Asahipak GS-310 (500 mm × 7.6 mm) (Asahi Chemical, Kawasaki, Japan) packed with a vinyl alcohol copolymer gel. The packing solvent was water-methanol (70:30, v/v). A Jasco (Japan Spectroscopic, Tokyo, Japan) Trirotar-V high-performance liquid chromatograph was used with two different types of detectors: a Jasco Uvidec-100 IV ultraviolet (UV) detector and a Shodex (Showa Denko, Tokyo, Japan) Model SE-11 refractive index (RI) detector. The UV detector was operated at 254 nm. Sample solutions were injected using a Model VL-611 variable-loop injector (Jasco).

The methanol phases were water, methanol, chloroform and mixtures of water and methanol and of chloroform and methanol. Elution was performed in the isocratic mode at a flow-rate of 0.5 ml/min. The concentration of the sample solutions was 0.5% and the volume of sample injected was 0.025 ml. Samples were dissolved in the solvent used as the mobile phase.

The samples were benzene, *n*-hexylbenzene, ethylene glycol, polyethylene glycols (PEG), polyethylene oxides (PEO) and polystyrenes (PS). These polymers were relatively monodisperse and their molecular weights were known.

When the mobile phase was changed, the flow rate was decreased to 0.3 ml/min and a pump was operated overnight to allow the new mobile phase to flow through the column in order to condition it. After this column conditioning the flow-rate was increased to 0.5 ml/min again.

RESULTS

Number of theoretical plates, N

When water and methanol were used as mobile phases, the values of *N* were 24 000 and 21 000 plates per column, respectively, on injecting 0.025 ml of 0.5% ethylene glycol solution. When the sample was benzene and the mobile phases were

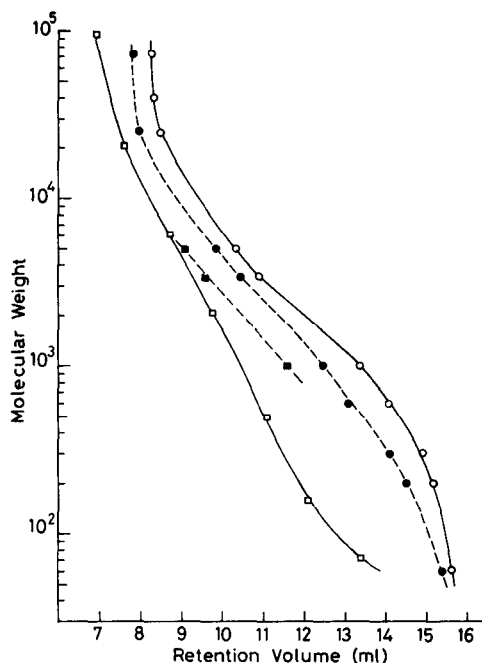


Fig. 1. Calibration graphs for PEG, PEO and PS in water, methanol and chloroform as mobile phases. Sample and mobile phase: (○) PEG, PEO in water; (●) PEG, PEO in methanol; (□) PS in chloroform; (■) PEG in chloroform.

chloroform and methanol, the values of N were 20 000 and 17 000 plates per column, respectively. Ethylene glycol was retained in the column when chloroform was used as the mobile phase.

Experiments were performed with mobile phases being changed in the order water, methanol, methanol–water, chloroform and chloroform–methanol. After a series of the experiments, the mobile phase was returned to water and the value of N was remeasured. It was unchanged (24 000 plates per column) and the retention volume of ethylene glycol was also stable. Only the column pressure increased from 25 kg/cm² initially to 35 kg/cm² after 7 months.

Calibration graphs

Calibration graphs of retention volume vs. log (molecular weight) for PEG and PEO in water and methanol as the mobile phases and those for PS and some PEG in chloroform are shown in Fig. 1. PEG and PEO in methanol eluted earlier from the column than when in water. Elution chromatograms of PEG 600, 1000 and 3400 in water and methanol are shown in Fig. 2 (the number after PEG represents the average molecular weight; henceforth the number given after each polymer's name also represents its average molecular weight).

Peak widths at half-height for these peaks were 0.75 ml (PEG 600), 0.725 ml (PEG 1000) and 0.45 ml (PEG 3400) in methanol and 0.825 ml (PEG 600), 0.85 ml (PEG 1000) and 0.59 ml (PEG 3400) in water. These results suggest that peak

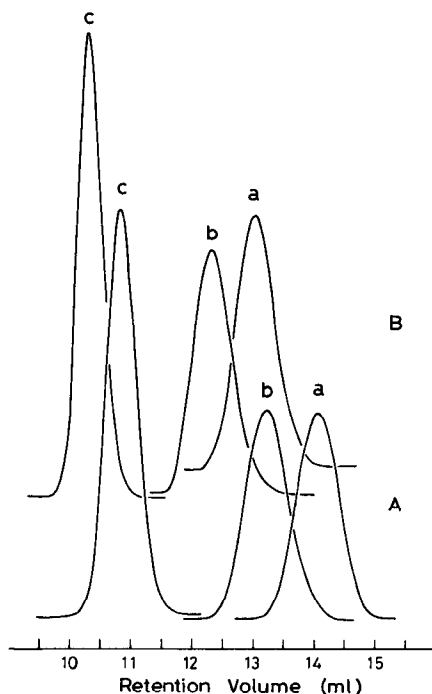


Fig. 2. Chromatograms of PEG in (A) water and (B) methanol as mobile phases. Samples: (a) PEG 600; (b) PEG 1000; (c) PEG 3400.

broadening and retardation of elution for PEG and PEO in the water mobile phase are due to the superimposition of an adsorption effect on the size-exclusion effect. However, viscosity differences among the mobile phases may also contribute to the variations in plate number.

When chloroform was used as the mobile phase, the peak shapes of PS were sharp for all samples and the adsorption effect seemed to be small. The peaks of PEG 600 and 1000 in chloroform were broad and PEG 200 was not eluted from the column. When the stationary phase was PS gel and the mobile phase was chloroform, PEG eluted earlier than PS of the same molecular weight⁸. Therefore, the results imply that an adsorption interaction between PEG and the surface of the poly(vinyl alcohol) gel occurred when chloroform was used as the mobile phase. The reason why PEGs in chloroform eluted earlier than those in methanol is probably due to the difference in the interstitial volumes of the column for the two mobile phases.

Mobile phase composition vs. retention volume

The changes in the retention volumes of benzene and ethylene glycol with variation in the mobile phase composition were determined and the results are shown in Fig. 3. The changes in the peak width at half-height of the solutes with variation in the mobile phase composition are also shown.

With chloroform-methanol as the mobile phase, the retention volume of benzene increased with increasing content of methanol in the mobile phase. The

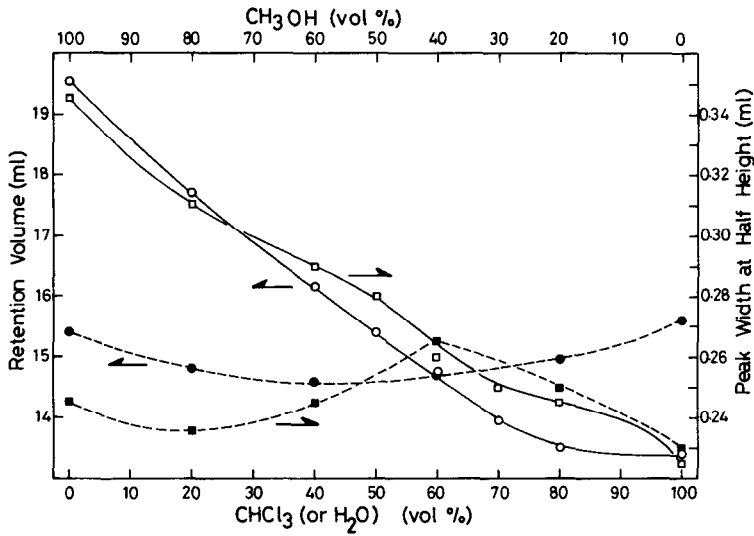


Fig. 3. Relationships between the mobile phase composition and retention volume (○, ●) or peak width at half-height (□, ■) of benzene and ethylene glycol. ○, □. Mobile phase, chloroform-methanol; solute, benzene. ●, ■. Mobile phase, water-methanol; solute, ethylene glycol.

peak-width at half-height of benzene also increased similarly. Therefore, the increase in retention volume of benzene with increasing methanol content in the mobile phase may be assumed to arise from the adsorption effect between benzene and the gel surface.

With water-methanol as the mobile phase, the minimum retention volume of

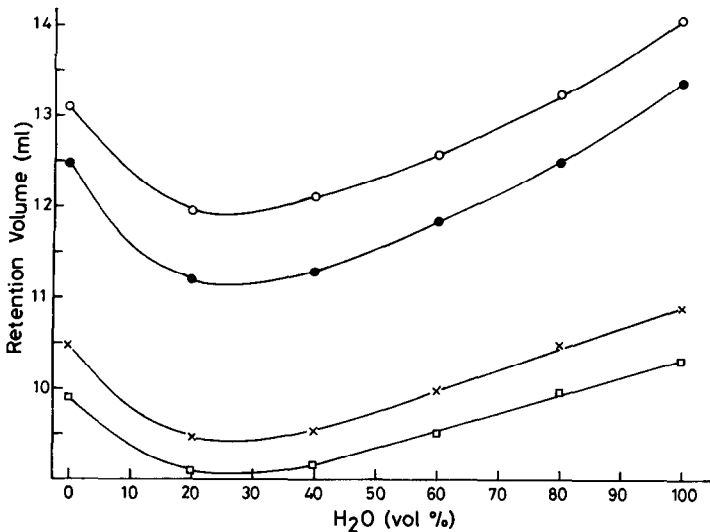


Fig. 4. Relationship between mobile phase composition and retention volume of PEG. (○) PEG 600; (●) PEG 1000; (×) PEG 3400; (□) PEG 5000.

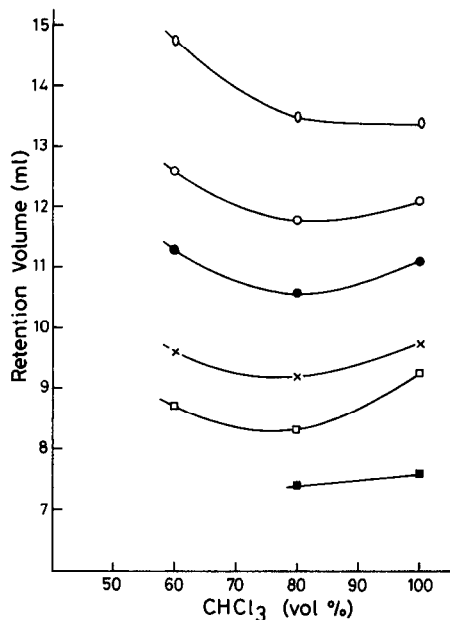


Fig. 5. Relationship between mobile phase composition and retention volume of PS and related compounds. (○) Benzene; (○) *n*-hexylbenzene; (●) PS 600; (×) PS 2100; (□) PS 6200; (■) PS 20400.

ethylene glycol was obtained at a mobile phase composition of water–methanol (40:60, v/v) and the maximum value with 100% water. The minimum peak width at half-height was obtained when 100% water was used as the mobile phase and the trend in the variation of peak width was not consistent, in contrast to the results with benzene. The next to the minimum peak width was obtained with water–methanol (20:80, v/v).

Fig. 4 shows the relationship for PEGs. The minimum retention volume was obtained at a mobile phase composition of water–methanol (25:75, v/v). This composition is similar to that for the next to the minimum peak width for ethylene glycol. The peak widths for each PEG with different compositions of the mobile phases were almost constant except with 100% water.

Fig. 5 shows the results for PS in chloroform and chloroform–methanol. The minimum retention volume was obtained at a composition of the mobile phase of chloroform–methanol (80:20, v/v). The peak widths for each PS with different compositions of the mobile phases were almost constant and the peaks were sharp.

DISCUSSION

The column efficiency for a given solute–solvent combination remained unchanged with the use of different solvents. The efficiency was checked by determining *N* values.

The samples used here were eluted from the column in order of decreasing molecular weight. Therefore, the separation mechanism is probably due mainly to size

exclusion. However, with the PEGs–water system, the peak broadening phenomena indicate that an adsorption effect is superimposed on the size-exclusion effect.

The minimum retention volume for ethylene glycol was obtained with water–methanol (40:60, v/v), for PEG with water–methanol (25:75, v/v) and for PS with chloroform–methanol (80:20, v/v). The concept of the solubility parameter may be applied to elucidate the elution mechanism⁹. Hildebrand solubility parameters for water, methanol, chloroform, ethylene glycol and poly(vinyl alcohol) are 47.9, 29.1, 18.8, 33.2 and 25.8 (J/cm³)^{1/2}, respectively¹⁰. According to this concept⁹, when the solubility parameter of the mobile phase is equal to that of the gel or a solute, then the separation occurs mainly by the size-exclusion effect.

The solubility parameter of the mobile phase water–methanol (25:75, v/v) is 33.8, which is nearly equal to that of ethylene glycol. If the solubility parameter of PEG has a similar value to that of ethylene glycol, then the separation in this system may be achieved by size exclusion. In the benzene–chloroform system, the solubility parameter of benzene is equal to that of chloroform and separation by size exclusion is expected. The solubility parameter of chloroform–methanol (80:20, v/v) is 24.8, which is nearly equal to that of the poly(vinyl alcohol) gel and the size-exclusion effect can be considered as the main separation mechanism in this system.

With water–methanol and chloroform–methanol mobile phases, separation of PEG and PS, respectively, by size exclusion alone may not be expected on a poly(vinyl alcohol) gel. We can say that the mobile phases water–methanol (25:75, v/v) and chloroform–methanol (80:20, v/v), which gave minimum retention volumes of PEG and PS, are effective for the SEC of PEG and PS because they minimize the adsorption effect.

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