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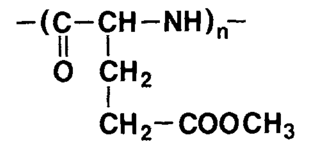
Selective Retention for Structural Isomers in Aqueous Liquid Chromatography Using Poly(γ-Methyl L-Glutamate) Spherical Particles

Chuichi HIRAYAMA, Hirotaka IHARA, Shoji NAGAOKA, and Tadayoshi SYONO
Department of Applied Chemistry, Faculty of Engineering, Kumamoto University, Kumamoto 860

Poly(γ-methyl L-glutamate) spherical particles having micropores showed higher retention capacity for *n*-isomers of alcohols (with remarkable temperature dependencies) than for *tert*-isomers in the aqueous liquid chromatography. No similar temperature dependence was observed for either spherical particles having macropores or alkyl-bonded silica gels.

Synthetic polypeptides from α-amino acids are well-known as typical examples of synthetic polymers which can produce secondary structures such as α-helix and β-structure spontaneously, and have been developed as model compounds of proteins. Therefore, some functional membrane systems[1-3] and stationary phases[4-6] utilizing unique properties of polypeptides have been reported. In this communication, we wish to show the possibility of molecular shape-selective separation using polypeptide spherical particles in which the main chain forms α-helices.

The spherical particles (PMG-0, PMG-1 and PMG-2) were prepared from poly(γ-methyl L-glutamate) (PMG F-8000, with a degree of polymerization of 1000, manufactured by Ajinomoto Co., Ltd.) without diluent and with 12.5 wt% (for PMG) of dihexadecyldimethylammonium bromide or cetyltrimethyl-ammonium bromide as surfactants, according to the *suspension evaporation* method reported previously.[7] The diluent was removed by washing with methanol. Various particle diameters were obtained by adjusting the stirring speed in the sphering process. It was confirmed by FT-IR spectroscopy that the main chains in the particles form α-helices.[8] Particles with average diameters of 22 - 44 µm were packed into a stainless steel column and their liquid chromatographic behavior was examined in water.



$-(\underset{\underset{O}{\|}}{C}-\underset{\underset{CH_2-COOCH_3}{\underset{|}{\underset{CH_2}{|}}}}{CH}-NH)_n-$

poly(γ-methyl L-glutamate)

Hydrophilic compounds such as D_2O, methanol, ethylene glycol and polysaccharides were eluted in order of their molecular sizes in both the PMG-0 and -1 columns. This shows that these solutes were eluted in a size exclusion chromatographic separation mode and that PMG particles do not interact with these hydrophilic solutes. On the other hand, these columns provided higher retention capacity[9] for hydrophobic compounds (such as propyl alcohol, butyl alcohol (Fig. 1-a and -b) and pentyl alcohol) than for hydrophilic compounds. These substances were eluted in order of their alkyl lengths, which were the same as those of reversed-phase liquid chromatography (RPLC) packings such as butylated silica gels (C_4-silica).[10] Therefore, the long retention for *n*-alcohols includes hydrophobic interaction between the PMG and the solutes.

Figure 1 also shows temperature dependencies with respect to retention capacity for structural isomers of butyl alcohol. Their elution orders also obeyed an RPLC mode. However, their retention behaviors are very dependent on the particles. For example, the C_4-silica column shows much higher retention capacity for all isomers (Fig. 1-d) than the PMG columns. This is caused by the fact that larger hydrophobicity was derived from butyl groups of C_4-silica than from residual groups of PMG particles, since C_4-silica has no other interaction with the solutes. The PMG-0 and -1 columns showed remarkable temperature dependencies with respect to the retention capacity, especially for butyl alcohol (Fig. 1-a and -b). In addition, the temperature dependencies include those of separation factor (α)[11] (Fig. 2) and were reversible at temperatures ranging between 30 and 70 °C (Fig. 3). No similar temperature dependence was observed in either the PMG-2 or C_4-silica columns (Fig. 1-c and -d). Therefore, the PMG-0 and -1 particles have a specific retention mechanism different from the PMG-2 and C_4-silica particles.

The capacity factor k'[11] is related to thermodynamics parameters as follows:[12]

$$\log k' = \log f + \frac{\Delta H_{s\text{-}m}}{2.3RT} + \frac{\Delta S^{\circ}_{s\text{-}m}}{2.3R}$$

where f is the ratio of the volumes of stationary to mobile phases, $\Delta H_{s\text{-}m}$ is the heat of transfer from the stationary to mobile phases, $\Delta S_{s\text{-}m}$ is the standard entropy of transfer assuming that the standard state is unit molar concentration in either phase. Fig. 4 shows plots of log k' against 1/T. The C_4-silica column provided a positive slope. Similar positive slopes are commonly observed in RPLC packings such as octadecylated silica gels[12] and carbon beads.[13] These can be explained by the increase of the solubility of the solutes from stationary to mobile phase with increase of temperature.[12,13] On the contrary, the PMG particles provided negative slopes in Fig. 4 and especially the PMG-0 and -1 particles had critical bending points in their plots. In order to understand these

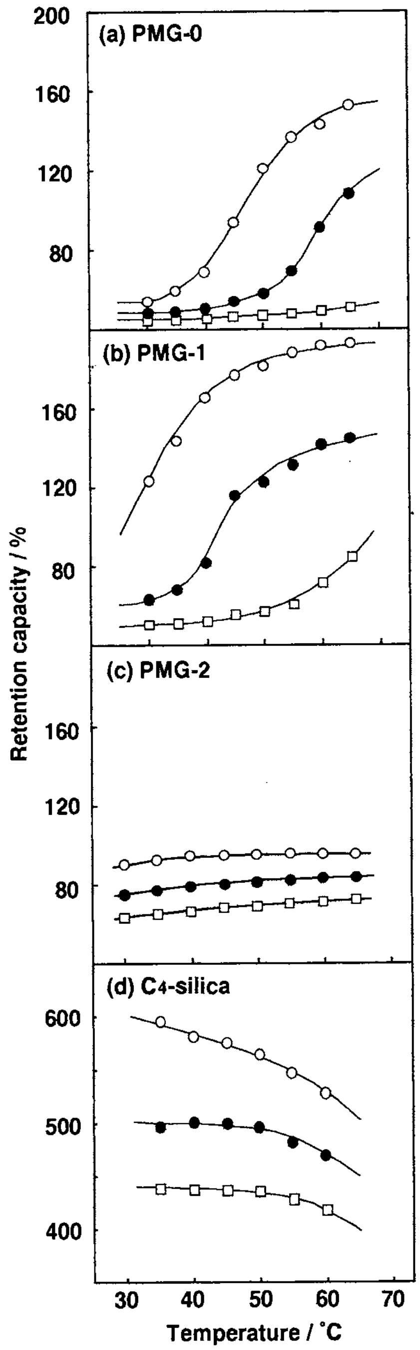


Fig. 1. Temperature dependencies on retention capacity (%) for butanol.
○ *n*-BuOH, ● *s*-BuOH, □ *t*-BuOH

unusual phenomena, it is assumed that the PMG-0 and -1 particles possess specific molecular pockets (or slits) which recognize the chemical structure of solutes and which have temperature-dependent diameters or shapes. Figure 1 includes significant information to support these assumptions. The PMG-0 and -1 columns showed values of 45 - 50% for the retention capacity (V_{30})[9] of *t*-butyl alcohol at 30 °C. The V_{30} value of butyl alcohol also approached these values with decrease of temperature. These values are nearly equal to the void volume: this indicates that *tert*-butyl alcohol does not only interact with the PMG particles, but also can not permeate into the particle interior at lower temperatures such as 30 °C. On the contrary, the PMG-2 column provided a V_{30} value of 70%. This value indicates that *tert*-butyl alcohol can permeate into the particle interior. The pore-size distribution of the PMG particles was estimated by using hydrophilic standard molecular markers, as reported previously.[14] The PMG-2 particles possess larger pores (into which pullulan molecules with approximate molecular weights of 1 x 10^4 are permeable) than do the PMG-0 and -1 particles (below 10^3). These results indicate that the large pores allow penetration of the solutes into the particle interiors but do not

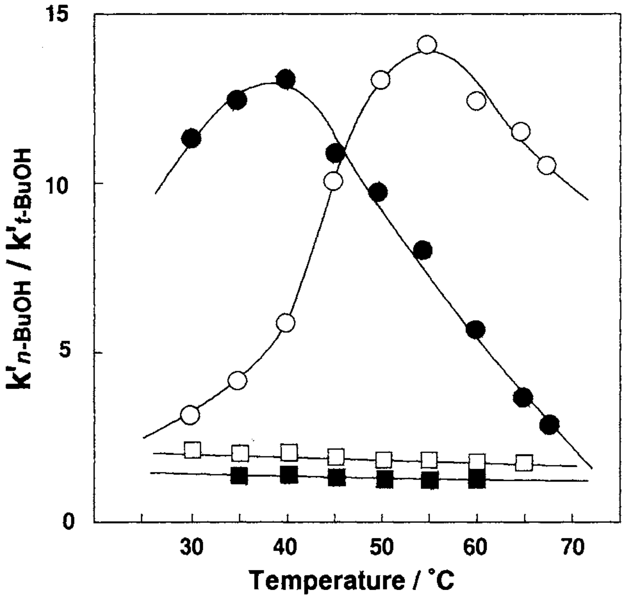


Fig. 2. Temperature dependencies on the separation factor (k'$_{n\text{-BuOH}}$ / k'$_{t\text{-BuOH}}$).
○ PMG-0, ● PMG-1, □ PMG-2, ■ C4-silica

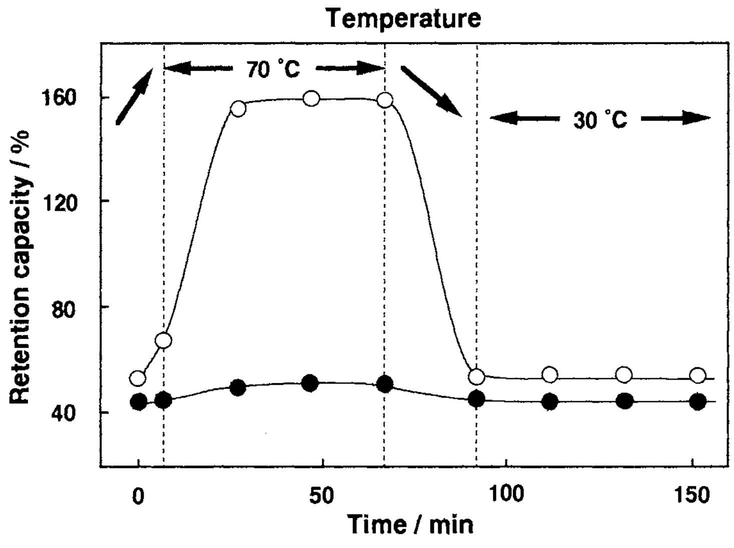


Fig. 3. Reversible temperature dependencies on the retention capacity in the PMG-0 column. ○ *n*-BuOH, ● *t*-BuOH

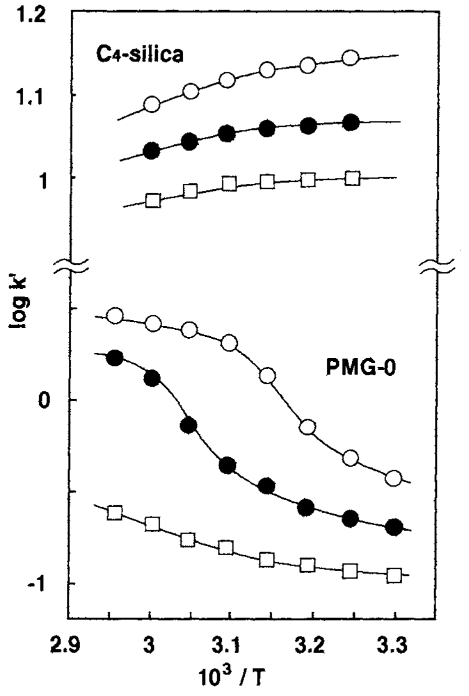


Fig. 4. Van't Hoff plots of log k' against 1/T for the elution of *n*-BuOH (○), *s*-BuOH (●), and *t*-BuOH (□).

contribute to the retention of the solutes, and that the small pores induce the unusual retention behavior. In addition, the bell-shaped temperature dependencies on the separation factor in Fig. 2 strongly suggest a close relation between the molecular size of solutes and the diameter (or slit width) of the small pores in PMG particles.

In conclusion, the PMG particles having small pores showed selective retention for structural isomers of alcohols. Similar selectivity was also observed in the mixture of planner and non-planner substances (for example, *p*-xylylene glycol and cyclohexane dimethanol[15]). Presumably, the rigidity of the PMG main chain derived from α-helices is related to the formation of specific molecular pockets (slits) and the temperature change does work as an "on-off" switch.

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8) The FT-IR spectra using diffuse reflection method provided peaks in 1652 cm^{-1} (amide I) and 620 cm^{-1} (amide V) due to α-helical conformation.
9) The retention capacity was determined by 100 x V_e/V_t (%), where V_e and V_t are the elution volume of solutes and the total column volume, respectively. V_{30} shows retention capacity at 30 °C.
10) C_4-silica (YMC-Packed Column A-802) was purchased from Yamamura Chemical Laboratories Co., Ltd.
11) The capacity factor (k') was calculated by $(V_e-V_0)/V_0$, where V_o is a void volume. The separation factor (α) was estimated by the ratio of the capacity factors $(k'_{n\text{-BuOH}}/k'_{tert\text{-BuOH}})$.
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15) The separation factors between xylylene glycol to cyclohexane dimethanol were 1.13, 1.24, 1.38, 1.78, and 1.65 at 30, 40, 50, 60, and 70 °C, respectively.

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