

Note

Dependences of the Distribution Coefficients of Hydrophobic Solutes on Porous Methyl Methacrylate Resin on the Temperature and Methanol Content of the Eluent

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The elution curves of caffeine, vanillin, coumarin, and methyl paraben, which have different hydrophobicities, in a bed packed with porous methyl methacrylate resin, were measured by the pulse response technique using methanol-water mixtures with various methanol contents as the eluent in the temperature range of 30°C to 200°C. The more hydrophobic solutes eluted at a slower rate under all conditions. All solutes eluted faster with eluents with higher methanol contents and at higher temperatures. The distribution coefficients of the solutes to the resin under various conditions were evaluated by moment analysis of elution curves. The adsorption enthalpy changes, ΔH , of the solutes were estimated from the plots of the distribution coefficients versus the reciprocal of absolute temperature. For the eluents with methanol contents higher than 75%, the ΔH values of all solutes were practically zero. This indicates that the solutes scarcely adsorbed on the resin. For eluents with lower methanol contents, solutes were eluted at a slower rate and their ΔH values were more negative. When water was used as an eluent, elution behavior showed the opposite tendency.

Keywords: distribution coefficient, subcritical water, reversed-phase liquid chromatography, eluent composition

Introduction

Reversed-phase liquid chromatography (RPLC) is widely used because of its high separation efficiency and simplicity in use. In an RPLC system, an organic solvent, such as methanol, acetonitrile or their mixture with water, is usually used to elute hydrophobic compounds. However, most organic solvents are costly and toxic to human health and the environment. Therefore, reduction of their use is desired.

Water that maintains its liquid state from 100°C to 374°C under a pressurized condition is called subcritical water. The relative dielectric constant of subcritical water is much lower than that of water at room temperature and is similar to that of a polar organic solvent, such as methanol or acetonitrile. Therefore, it has recently been reported that subcritical water can be used as an eluent for high-performance liquid chromatography (HPLC) (Sminth and Burgess, 1997; Greibrokk and Andersen, 2003; Tiihonen *et al.*, 2005). We reported that the relative dielectric constant is not the sole factor govern-

ing the elution behavior of a solute for an RPLC system (Mori and Adachi, 2006). It is also known that changing the temperature causes the separation efficiency and selectivity to improve in an RPLC system (Dolan, 2002; Teutenberg *et al.*, 2006).

In this study, the distribution coefficients of four solutes having different hydrophobicities on porous methacrylate resin were measured using water or methanol-water mixtures as the eluent at various temperatures. We discuss the dependence of the distribution coefficients of the solutes on the resin on the temperature and methanol content of the eluent and show that the elution behavior can be regulated both by changing the operating temperature and the methanol content of the eluent from the viewpoint of adsorption enthalpy.

Materials and Methods

Materials Porous methyl methacrylate resin (CHP2MG), the mean diameter of which was 10 μm , was supplied by Mitsubishi Chemical Corporation (Tokyo, Japan) and was packed into a stainless steel column (CE-410, 150 \times 4.6 I.D. mm, Chemco Scientific Co., Osaka, Japan). Analytical-

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grade caffeine, vanillin, coumarin and methyl paraben were purchased from Wako Pure Chemical Industries (Osaka, Japan) and used as solutes. The $\log P$ values, where P is the partition coefficient between 1-octanol and water phases, of the solutes at 25°C were calculated using Advanced Chemistry Development (ACD/Labs, Toronto, Ontario, Canada) Software V8.14 (for caffeine, vanillin, and methyl paraben) or V9.04 (for coumarin) using Solaris (ACD/Labs) software. The $\log P$ values of caffeine, vanillin, coumarin and methyl paraben were -0.131 , 1.19 , 1.39 and 1.87 , respectively.

Apparatus A column was installed in an oven (DO-300FA, As One, Osaka, Japan). An eluent was fed to the column with an LC-10AT_{VP} pump (Shimadzu, Kyoto, Japan). To prevent vaporization of the eluent, the pressure of the system was maintained at 5.3 MPa using a backpressure regulator (Upchurch Scientific, Oak Harbor, WA, USA). The concentrations of caffeine, vanillin, coumarin and methyl paraben in the column effluent were monitored at 273, 279, 330 and 282 nm, respectively, using an SPD10A_{VP} UV-vis detector (Shimadzu, Kyoto, Japan). The chromatogram was recorded every 0.5 s using Chromatopac C-R8A (Shimadzu, Kyoto, Japan).

Pulse response experiment Caffeine, vanillin, coumarin, and methyl paraben were separately dissolved in each eluent at the concentration of 0.2 g/L. A 10- μ L sample was applied to the column through a sample injector and eluted at a flow rate of 0.15, 0.20 or 0.30 mL/min. The flow rates were the values at room temperature. Column temperature was regulated at a temperature in the range of 30 to 200°C.

The normalized first-order statistical moment, μ_1' , which is defined by Eq. (1a), can be related to the distribution coefficient, K_c by Eq. (1b) (Kucera, 1965; Nakanishi *et al.*, 1977):

$$\mu_1' = \int_0^\infty tCdt / \int_0^\infty Cdt \quad (1a)$$

$$= (L/u_0)[\varepsilon_b + (1-\varepsilon_b)K_c] \quad (1b)$$

where C is the concentration of the solute, t is the time, u_0 is the superficial velocity, L is the column length, and ε_b is the bed voidage. The integrations of Eq. (1a) were numerically performed. The μ_1' values observed at various flow rates were plotted versus the superficial residence times, L/u_0 . The plots produced a straight line passing through the origin, and the slope indicated the $\varepsilon_b + (1-\varepsilon_b)K_c$ value. The ε_b value is usually estimated from the elution behavior of a macromolecule, such as dextran, which is too large to penetrate into the pores (Hashimoto, 2005). However, the pore size of the resin used in this study is very large (25 nm), and it was impracticable to find an adequate macromolecule for estimating the bed voidage. Since the ε_b value could not be estimated, the $\varepsilon_b + (1-\varepsilon_b)K_c$ value was used instead of the K_c value to assess the affinity of each solute for the resin.

Results and Discussion

Figure 1 shows the elution curves of the solutes using 15% (v/v) methanol in water as an eluent at 0.20 mL/min and at various temperatures. The solute having a larger $\log P$ value was eluted at a slower rate. However, at higher temperatures, all solutes eluted faster and had markedly sharper

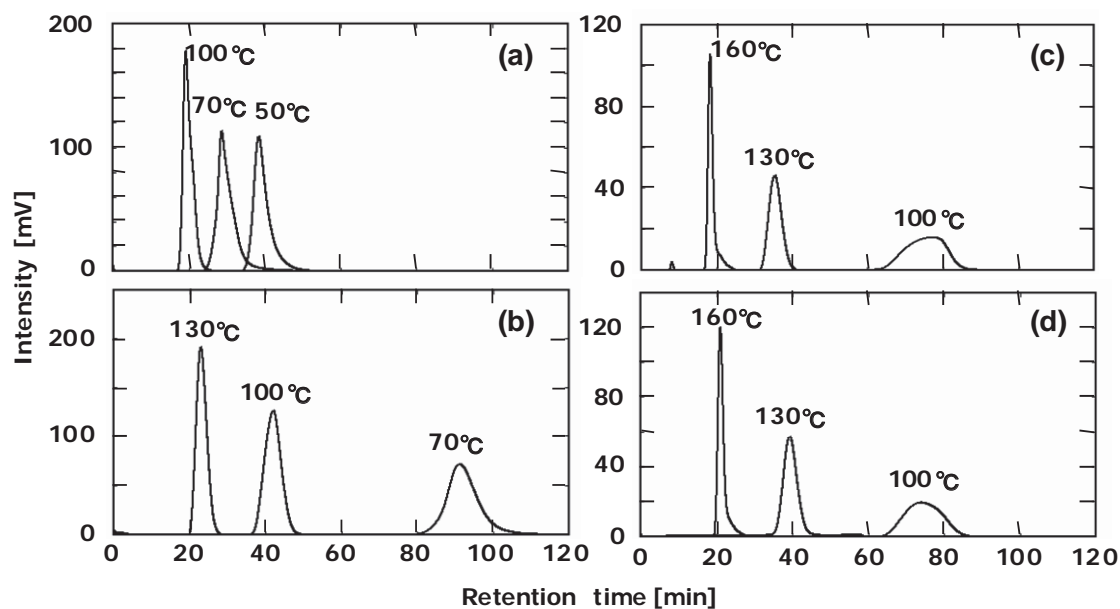


Fig. 1. Elution curve of (a) caffeine, (b) vanillin, (c) coumarin, and (d) methyl paraben using 15% methanol as an eluent at 0.20 mL/min and at various temperatures.

peaks.

The normalized first-order statistical moment, μ_1' , was calculated from elution curves of the solutes at each flow rate according to Eq. (1a). The μ_1' values for vanillin under some conditions are plotted versus the superficial residence time, L/u_0 (Insert of Fig. 2). The plots for a specific condition produced a straight line passing through the origin. The $\varepsilon_b + (1-\varepsilon_b) K_c$ value was evaluated from the slope of the line. The plots of the μ_1' values versus L/u_0 values for vanillin under other conditions also produced straight lines and the $\varepsilon_b + (1-\varepsilon_b) K_c$ values were evaluated.

For the other solutes, the plots of the μ_1' values versus L/u_0 values under any condition also produced a straight line, the slope of which gave the $\varepsilon_b + (1-\varepsilon_b) K_c$ value.

Figure 2 shows the temperature dependence of the $\varepsilon_b + (1-\varepsilon_b) K_c$ values obtained for vanillin using eluents with different methanol contents. At higher temperatures, $\varepsilon_b + (1-\varepsilon_b) K_c$ values were lower. For eluents with methanol content lower than 60%, the elution behavior of vanillin was strongly dependent on both the methanol content and the temperature.

Similar dependences of the $\varepsilon_b + (1-\varepsilon_b) K_c$ value on temperature and methanol content of the eluent were also found for the other solutes. These results suggested that changing the temperature causes the separation efficiency to improve greatly for the eluents with low methanol contents.

The ε_b value could not be estimated because of the very large pore size of the resin. Therefore, we assumed that the

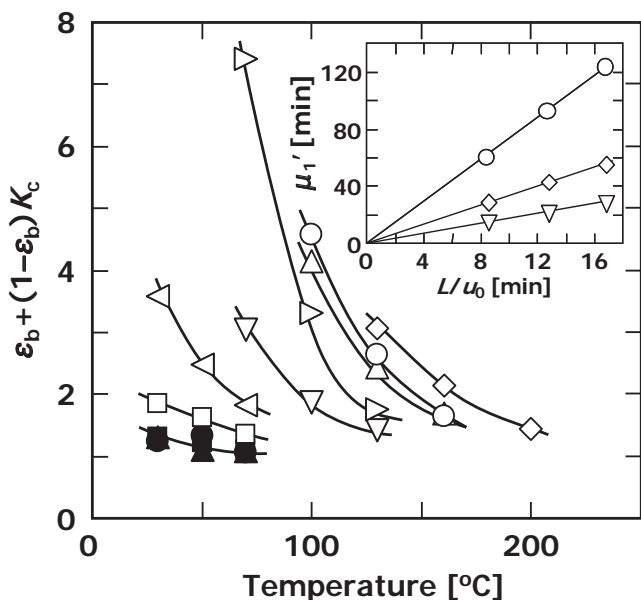


Fig. 2. Dependence of the $\varepsilon_b + (1 - \varepsilon_b) K_c$ values of vanillin on the temperature of the eluents. The eluents were (●) 100% (v/v), (▲) 90% (v/v), (■) 75%, (□) 60%, (◁) 45%, (▽) 30%, (▷) 15%, (△) 10%, (○) 7% methanol, and (◇) water. Insert: Dependences of the μ_1' values of vanillin on superficial residence time. The eluent used was 15% methanol at (○) 70, (◇) 100, and (▽) 130°C.

ε_b value was 0.36 according to the manufacturer of the resin. Based on this value, the distribution coefficients, K_c , of the solutes under all the elution conditions were estimated.

The adsorption enthalpy change, ΔH , can be estimated based on the following equation:

$$\frac{d \ln K_c}{d(1/T)} = -\frac{\Delta H}{R} \quad (2)$$

where T is the absolute temperature, and R is the gas constant. Figure 3a shows the plots of $\ln K_c$ versus $1/T$ for vanillin. Eluents having different methanol contents were used. The plots for the eluent with any methanol content produced a straight line, and the ΔH value was evaluated from the slope of the line. The slope of a line for any eluent was positive, and hence the ΔH value was negative, indicating that the adsorption of vanillin on the resin was exothermic. There was no significant difference in the slope among the lines for the eluents having methanol contents higher than 75%.

Figure 3b also shows the plots of $\ln K_c$ versus $1/T$ for all solutes, the K_c values of which were obtained using the eluent with 15% methanol content. The plots for the respective solute produced a straight line to enable us to evaluate the

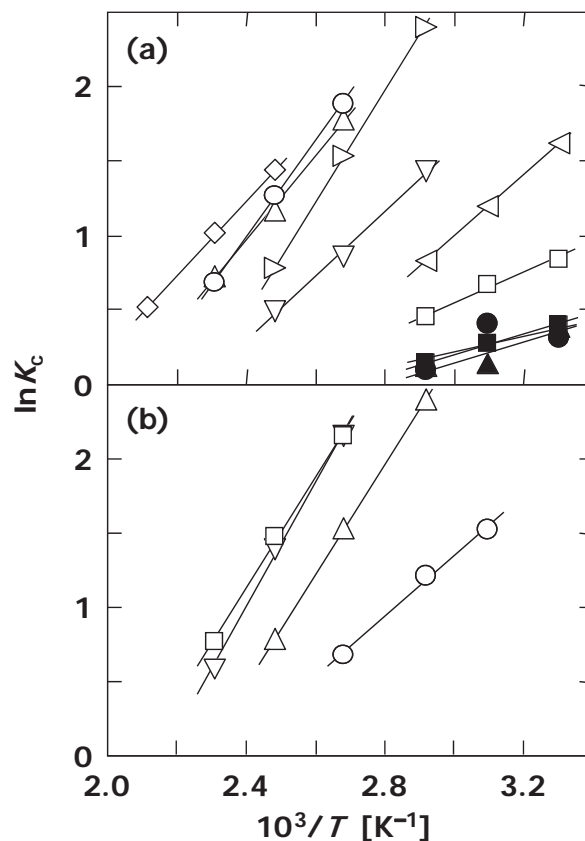


Fig. 3. (a) Plots of $\ln K_c$ versus $1/T$ for vanillin. The symbols for the eluent are the same as in Fig. 2. (b) Plots of $\ln K_c$ versus $1/T$ for (○) caffeine, (△) vanillin, (□) coumarin, and (▽) methyl paraben. The eluent was 15% methanol.

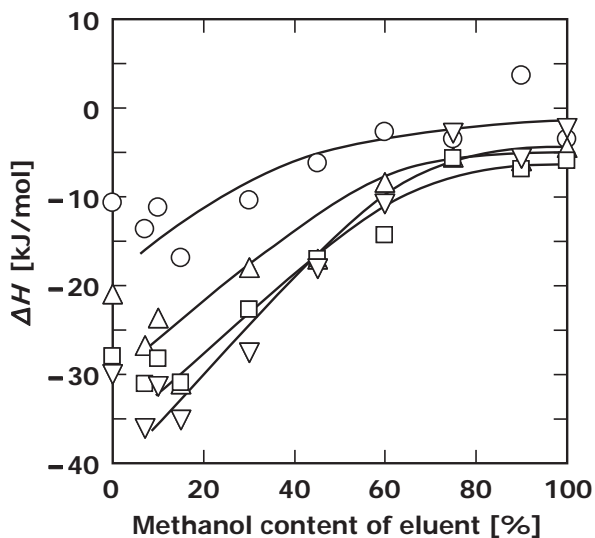


Fig. 4. Relationships between adsorption enthalpy of solutes and methanol content of the eluent. The solutes were (○) caffeine, (△) vanillin, (□) coumarin, and (▽) methyl paraben.

ΔH value. The ΔH values for vanillin, coumarin and methyl paraben were almost the same, and their absolute values were larger than that for caffeine, which is the least hydrophobic among the solutes, based on their $\log P$ values.

The plots of $\ln K_c$ versus $1/T$ for the other eluents also produced straight lines, and the ΔH values were evaluated.

Figure 4 shows the relationships between the ΔH values and the methanol content of the eluent for all solutes. For any solute, the ΔH value was almost constant at methanol contents higher than 75%, but decreased (its absolute value became larger) as the methanol content decreased. There was a tendency for the dependence of the ΔH value on the methanol content for the eluents having methanol contents lower than 75% to be greater for a more hydrophobic solute.

For all solutes, the ΔH values estimated for water as the eluent were greater than those which were estimated by extrapolating the ΔH -methanol content curves, although the

reason remains unclear.

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