A Simplified Method for Predicting the Effect of Temperature on the Separation Performance by Chromatography

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A simplified method for predicting the effect of temperature on the separation performance by chromatography was developed. As a model separation system, two polyphenol component separation by polymer–resin chromatography with ethanol–water mobile phase was chosen. HETP values as a function of mobile phase velocity for a wide range of temperatures 15–45°C were well correlated by a single universal curve in the dimensionless HETP and the dimensionless velocity plots. A simplified equation that can predict the resolution was derived. The equation includes the temperature dependence of the distribution coefficients and the molecular diffusion coefficients. The relative separation performance (resolution) calculated by this equation showed that the existence of the maximum resolution depends on the temperature dependence of the distribution coefficients. Even when the resolution decreases with temperature, higher temperature operations may be advantageous as both the separation time and the separation volume decrease.

Keywords: chromatography, distribution coefficient, resolution, diffusion coefficient, activation energy, temperature dependence

1. Introduction

Temperature is an important parameter affecting the performance of many separation unit operations. Although chromatography is a well-known separation method, the effect of temperature on the separation performance has not been investigated fully especially for preparative/process separations of food- and bio–products [1–4]. For sugar separations by simulated moving bed (SMB) chromatography, temperatures higher than room temperature are often employed, and found to be more efficient due to the higher mass transfer (diffusion) rates [5]. Recently, HPLC (high performance liquid chromatography) of intact antibodies at very high temperatures such as 80°C has been proposed as a high resolution separation method [6].

In this paper, the effect of temperature on the separation performance by polymer–resin chromatography was investigated. Polyphenol separation by isocratic elution with ethanol–water mobile phases was chosen as a model system [7–9]. HETP (height equivalent to a theoretical plate) values within the temperature range 15–45°C were examined based on the dimensionless variables containing particle diameter, mobile phase velocity and molecular diffusion coefficient. Simplified equations were derived, which can describe the resolution as a function of temperature with HETP and the distribution coefficient as a function of temperature.

2. Experimental

The data of two polyphenols, catechin and epigallocatechin gallate (EGCG) by polymer–resin chromatography were obtained by the method described in our previous studies [7–9]. The resin used was Diaion HP20SS from Mitsubishi Chemical (Tokyo, Japan), which is used for reversed phase chromatography [i].

3. Results and discussion

3.1 HETP

For linear isocratic elution (distribution coefficient $K = \text{constant}$), HETP is a commonly employed measure for the column efficiency based on the peak width and the retention time [1–3,10–12].

\[
\text{HETP} = \frac{Z}{N} = \frac{Z(\sigma / t_0)^2}{Z(\sigma / V_0)^2} = \frac{Z(\sigma / V_0)^2}{Z(\sigma / t_0)^2} (1)
\]

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Here $Z=$ column bed length, $N=$ plate number (the number of plates), $\sigma=$ standard deviation of the elution curve, $t_k=$ peak retention time, $\sigma_v=$ $\sigma$ in volume unit and $V_v=$ retention volume.

Although the peak retention time $t_k$ and the variance of the curve $\sigma^2$ can be determined by the first moment $\mu_1$, and the second moment $\mu_2$ by numerical integration of the elution curve, $\mu_2$ is markedly influenced by the peak tailing [1]. Therefore, HETP is often better to be directly calculated simply by measuring the peak width at half of the elution curve, which contains two terms, the first (axial dispersion) and the third (stationary phase diffusion coefficient) is usually given by the following equation.

$$\sigma = \frac{\tau}{2}$$

$\tau$ is the maximum retention time [1]. Namely, $\mu_2 = \tau_0$ and the second moment $\mu_1 = \sigma^2$.

$V_k$ or $t_k$ for linear isocratic elution is given by the following equation.

$$V_k = t_k = V_0 + (V_0 - V_0)K = V_0 (1 + HK)$$

$F$ is the volumetric flow rate, $V_o$ is the column void volume and $V_k$ is the total column volume. $H = (V_k - V_0)/V_0 = (1 - \epsilon)/\epsilon$ is the volumetric phase ratio, and $\epsilon = V_0/V_k$ is the void fraction (interstitial volume of the bed).

When the HETP is plotted against the flow velocity (linear mobile phase velocity) $u$, a so-called van Deemter curve is obtained.

$$ \text{HETP} = \frac{Z}{N} = 2 \left( \frac{D_s}{H} \right) + \frac{d^2}{50D_s(1+HK)^2}u = A^0 + C_u^0$$

Here $d_p=$ particle diameter, $D_s=$ stationary phase diffusion coefficient (in most cases, pore diffusion coefficient), $D_h=$ axial dispersion coefficient, $u = F/ (\epsilon \pi d^2/4)$, and $d=$ column diameter.

Although the complete van Deemter equation contains four terms, the first (axial dispersion) and the third (stationary phase diffusion) terms shown in Eq. (3) are dominant for preparative separations, which contains two zone spreading parameters, $D_h$ and $D_s$ [1,2,7,8,10,13].

The data on the effect of temperature on $D_1$ are rather limited. We have shown that $(2D_h/u)$ or $A^0$ values are constant, and approximated to 2-10 $d_p$, which do not change with temperature significantly [1, 7, 8, 13].

The stationary phase (pore) diffusion coefficient $D_s$ increases with temperature. The temperature dependence of molecular diffusion coefficient $D_m$ (free diffusion coefficient in solution) is usually given by the following relation [14].

$$D_m \eta/T = f \text{(molecular shape, molecular radius, } \eta_m \text{ or molecular weight, } M_0)$$

Here $\eta$ is the viscosity of the solution and $T$ is the absolute temperature. $D_m(T)$ can be also described with the activation energy $E_D$ as

$$D_m = D_m(0) \exp \left( - \frac{E_D}{R_o T} \right) = D_{HETP} \exp \left( - \frac{E_D}{R_o (1 - \frac{1}{T_{ref}})} \right)$$

$D_{HETP}$ is the pre-exponential factor, $R_o$ is the gas constant, $D_{HETP}$ is $D_m$ at $T = T_{ref}$ and $T_{ref}$ is the reference temperature. $D_m$ can be calculated by Eq. (5) by assuming that $E_D$ can be approximated to the activation energy for the viscosity of the solution ($=20$ kJ/mol for water). If we assume that $\gamma = D_s/D_m$ does not change with $T$, $D_s$ is expressed as

$$D_s = D_m \exp \left( - \frac{E_D}{R_o T} \right) = D_{HETP} \exp \left( - \frac{E_D}{R_o (1 - \frac{1}{T_{ref}})} \right)$$

$D_{HETP}$ is the pre-exponential factor and $D_{HETP}$ is $D_s$ at $T = T_{ref}$.

It is convenient to use a dimensionless form of the HETP equation as the data over a wide range of chromatography conditions such as $d_p$ and $u$ can be compared in the same scale [1, 10, 13]. With the dimensionless (reduced) HETP, $h$ and the dimensionless (reduced) velocity $\nu$$=$, the HETP equation (3) can be rewritten as

$$h = A^* + C^* \nu$$

$$h = \text{HETP}/d_p$$

$$\nu = ud_p/D_m$$

$$A^* = 2(D_s/u)/d_p$$

$$C^* = HK/[30 \gamma(1 + HK)^2]$$

$$\gamma = D_s/D_m$$

Another empirical expression for $h$ is given by

$$h = E^* \nu^n$$

where $E^*$ and $n$ are fitting parameters.

This equation can describe the experimental data well within a certain range of velocity.

Typical elution curves are shown in Fig.1 for three different ethanol concentrations, and two different temperatures. As is clear from the figure, the retention volume (and hence $R$) decreases with an increase in ethanol concentration or temperature.

Figure 2A shows HETP--$u$ curves for the temperature range 15--45$^\circ$C. The slope of HETP--$u$ became shallower with temperature, which means increasing $D_s$ with temperature according to Eq. (3). When the HETP--$u$ data were converted to $h$--$\nu$, the data were well correlated by a single curve over a wide range of conditions (temperature in Celsius $T_c = 15$--45$^\circ$C, ethanol concentration $I = 20$ and 30%) as shown in Figs. 2B and 2C. Moreover, the data were well fitted by a single curve $h = 1.08 \nu^{0.71}$. These results show that the effect of temperature on the axial dispersion term is negligible and the temperature dependence of $D_s$ can be approximately described by that for $D_m$. 

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3.2 Resolution $R_s$

In addition to HETP, another measure for the separation efficiency $R_s$ defined by the following equation is widely employed. The subscripts 1 and 2 imply for the first and second elution curve component, respectively.

$$ R_s = \frac{t_{R,2} - t_{R,1}}{(W_1' + W_2')/2} \tag{14} $$

$W=4 \sigma$ is the peak width at the base line.

When the two peaks are similar in terms of the peak height and the peak area, the complete resolution or the baseline separation is achieved at $R_s > 1.3$.

After inserting Eqs. (1)–(3) the above equation is rearranged as

$$ R_s = \frac{\sqrt{Z}}{2} \frac{V_{R,2} - V_{R,1}}{V_R \sqrt{\text{HETP}_0 \cdot \text{HETP}_2}} \tag{15} $$

$$ = \frac{\sqrt{Z}}{2} \frac{H(K_2 - K_1)}{(1 + HK_2)(1 + HK_2) \sqrt{\text{HETP}_0 \cdot \text{HETP}_2}} $$

Although $R_s$ can be calculated by HETP equation (3)

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**Fig. 1** Typical calculated and experimental elution curves at different ethanol concentrations and temperatures. $V_F$: sample volume, $C_0$: sample concentration. A. Temperature $T_c = 25^\circ C$, Ethanol concentration $I = 20\%$, $Z = 15.5$ cm, $\varepsilon = 0.44$, $u = 7.8$ cm/min; B. $T_c = 25^\circ C$, $I = 27\%$, $Z = 17.5$ cm, $\varepsilon = 0.41$, $u = 0.75$ cm/min; C. $T_c = 34^\circ C$, $I = 25\%$, $Z = 15.5$ cm, $\varepsilon = 0.38$, $u = 1.98$ cm/min. The calculation method is shown in Refs. 7,8.

**Fig. 2** Relationships between HETP and mobile phase velocity (A) HETP-$u$ for $I = 30\%$, (B) $h-v$ for $I = 30\%$ and (C) $h-v$ for $I = 20\%$. The curves shown in (A) were determined by the least square fitting with the fixed intercept value for $T_c = 25^\circ C$, which is taken as the reference temperature in Fig. 3. $D_m$ values were calculated according to the method in Refs. 7,8.
together with $K_1$ and $K_2$, it is more convenient to use a simple equation. By assuming that $W_{V,1}=W_{V,2}=4\sigma_{V,1}$\cite{1,2,12} and inserting Eqs. (2) and (3) into the above $R_s$ equation we obtain

$$ R_s=\left[\frac{H(K_2-K_1)}{4(1+HK_{av})}\right]^{\frac{1}{\sqrt{V}}} = \left[\frac{H(K_2-K_1)}{4(1+HK_{av})}\right]^{\frac{1}{\sqrt{N}}} \quad (16) $$

Here, $K_{av}=(K_1+K_2)/2$

Further, when Eq. (13) is used for the HETP equation, the following equation is obtained.

$$ R_s=\left[\frac{H(K_2-K_1)}{4(1+HK_{av})}\right]^{\frac{1}{2}} \left\{Z^{1/2}u^{-n/2}d_p^{(1-n)/2}D_m^{n/2}\right\} \quad (17) $$

Equation (17) is given as the product of the two terms. The first term contains the distribution coefficients whereas the second term is consisted of mass transfer related properties.

When $K$ does not depend on temperature such as size-exclusion chromatography (SEC), the temperature dependence can be simply described by the temperature dependence of $D_m$.

The relative $R_s$ values, $R_s/R_{s,ref}$ for SEC calculated by Eq. (17) as a function of temperature $T_c$ are shown in Fig. 3. $R_s/R_{s,ref}$ increases with $T_c$. This means that $R_s$ can be improved simply by increasing the column temperature provided that both the column and the sample are stable at high temperatures.

When $K$ is a function of temperature, $K(T)$, the resulting $R_s$ equation becomes complicated. For most adsorption chromatography $K$ decreases with $T$ (exothermic reaction or negative enthalpy), and the difference of the two distribution coefficients $\Delta K=(K_2-K_1)$ also decreases with $T$\cite{9}. The relative $R_s$, $R_s/R_{s,ref}$ values were calculated by considering $K(T)$ and $D_m(T)$ by Eq. (17) where $R_{s,ref}$ is the $R_s$ value at $T_{ref}=298K$ ($25\,^{\circ}\text{C}$). The results are shown in Fig.3 for $I=20\%$ and $30\%$. For $I=20\%$ (Fig. 3B), $R_s/R_{s,ref}$ slightly increases with $T_c$ until $T_c=33\,^{\circ}\text{C}$ although $\Delta K$ decreased with $T_c$. A further increase in $T_c$ resulted in a slight decrease in $R_s/R_{s,ref}$. However, since the volume needed for the separation $(1+HK_{av})$ also decreases with $T_c$, shorter separation time with smaller separation volume can be achieved.

The same trend was observed for $I=30\%$ where both $R_s/R_{s,ref}$ and $(1+HK_{av})$ decreased with $T_c$ monotonously.

For process or preparative separations, both separation time and separation volume should be reduced in order to increase the process efficiency or economics. Rigorous calculations are needed for such process efficiency evaluations. Nevertheless, equations proposed in this study are useful for a quick assessment/evaluation of the separation performance when the temperature is increased.

Fig. 3 Dependencies of $\Delta K$, $(1+HK_{av})$ and $R_s/R_{s,ref}$ on $T_c$.

$R_{s,ref}$ is the value at $T_c=25\,^{\circ}\text{C}$. $(R_s/R_{s,ref})_{max}$ for $I=20\%$ has the maximum at $T_c=33\,^{\circ}\text{C}$. $K_1$ and $K_2$ were calculated by the data shown Ref. 9.
4. Conclusion

A simplified method was developed for predicting the effect of temperature on the separation performance by isocratic elution chromatography of polyphenols with ethanol-water mobile phase. HETP values for a wide range of temperatures 15–45°C were well correlated by a single universal curve in the dimensionless HETP and the dimensionless velocity plots. A simplified resolution equation was developed, which includes the temperature dependence of the distribution coefficients and the molecular diffusion coefficients. By using this equation, it was possible to examine the maximum separation performance as a function of temperature. Even when the resolution decreases with temperature, higher temperature operations may be advantageous as both the separation time and the separation volume decrease.

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クロマトグラフィー分離の温度依存性の簡単な推算方法

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クロマトグラフィー分離の温度依存性の簡単な推算方法を考案した。エタノール水移動相を用いたポリマー粒子充填クロマトグラフィーによる2種類のポリフェノール分離をモデル系として選択した。15-45℃におけるHETPと線速度の関係は無次元化することにより、一本の曲線で表現することができた。分配係数と拡散係数の温度依存性を考慮した簡単な分離度の式を導いた。この式を用いて、解析したところ、分離度の最大値を与える温度がある場合とない場合があることが明らかとなった。温度を高くすることにより分離度が多少低下しても、分離時間と分離溶媒量が減少することを考慮すると、好ましい条件になることも考えられる。

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クロマトグラフィー分離の温度依存性の簡単な推算方法を考案した。エタノール水移動相を用いたポリマー粒子充填クロマトグラフィーによる2種類のポリフェノール分離をモデル系として選択した。15-45℃におけるHETPと線速度の関係は無次元化することにより、一本の曲線で表現することができた。分配係数と拡散係数の温度依存性を考慮した簡単な分離度の式を導いた。この式を用いて、解析したところ、分離度の最大値を与える温度がある場合とない場合があることが明らかとなった。温度を高くすることにより分離度が多少低下しても、分離時間と分離溶媒量が減少することを考慮すると、好ましい条件になることも考えられる。