

Direction of temperature gradient for normal-phase temperature gradient interaction chromatography in polystyrene fractionation

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Abstract

Temperature gradient interaction chromatography (TGIC) is a powerful technique for molecular weight fractionation of polymers, in which the interaction strength is controlled by varying the column temperature. In the present paper, the effects of the sign of the temperature dependence of the retention and the direction of the temperature gradient (raising or lowering) on TGIC in the normal-phase mode were studied for the molecular weight fractionation of polystyrene samples in organic mobile phases. It was found that a positive temperature gradient was effective in the system consisting of amino-modified silica (NH₂) column and the eluent mixture of tetrahydrofuran and *n*-hexane where retention decreased with increasing temperature. A negative temperature gradient was effective for the systems consisting of a bare-silica column//chloroform/*n*-hexane and NH₂-column//chloroform/*n*-hexane, where retention increased with increasing temperature. Increasing retention with increasing temperature has been found, so far, only for a water-soluble polymer (PEO) in an aqueous mobile phase in RP-TGIC.

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1. Introduction

Temperature gradient interaction chromatography (TGIC) is a powerful technique, recently developed, for molecular characterization of polymers [1,2]. TGIC separates polymer components in the order of molecular weight utilizing mainly the enthalpic interaction between solutes and the stationary phase, such as adsorption, unlike size exclusion chromatography (SEC) in which the entropic effect plays the key role. In TGIC, the strength of interaction is controlled by varying the column temperature to offset the effect of the molecular weight on the adsorption strength. The resolution of TGIC is superior to that of SEC for molecular weight fractionation [3,4], and TGIC has been applied not only for the molecular-weight fractionation of linear polymers but also for the characterization of polymers with different chain architectures, such as star, ring and H-shapes [5,6].

Since the study of TGIC started from systematic research on the effect of temperature on the retention of polymers in

reversed-phase high performance liquid chromatography (RP-HPLC) [1], many studies of TGIC were performed in the RP mode. In almost all cases of RP-TGIC, the column temperatures were raised according to the respective protocols, which is appropriate in case of a negative temperature dependence of the retention $(\partial V_r/\partial T)_P < 0$, where V_r is the retention volume. However, Lochmüller et al. [1] reported that the retention of poly(ethylene oxide) (PEO) increased as the temperature increased in RPLC using C₁₈ bonded silica and a mixture of acetonitrile (ACN) and water. A positive temperature dependence of the retention $(\partial V_r/\partial T)_P > 0$, was observed. Andersen et al. [7] and Cho et al. [8] reported also that PEO samples were separated using negative temperature gradients in similar systems, which is in accordance with $(\partial V_r/\partial T)_P > 0$. In RPLC, there are both cases of negative and positive temperature dependencies of the retention: $(\partial V_r/\partial T)_P < 0$ for the common organic polymers in organic mobile phases and $(\partial V_r/\partial T)_P > 0$ for PEO in the aqueous mobile phase, respectively.

On the other hand, only a few results in the normal-phase (NP) mode TGIC were reported by Lee et al. [9] and Cho et al. [8] as far as we know. In the former study [9], polystyrene (PS) and polyisoprene (PI) were fractionated by molecular weight, effectively, using bare silica and diol bonded silica as the column packing

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materials and isocratic mixtures of tetrahydrofuran (THF) and isooctane (*i*-Oct) with the respective compositions as the eluents for the respective polymers. In the latter [8], PEO samples were separated using NH₂-bonded silica as the adsorbent and a mixture of water and ACN as the eluent. In these cases, negative temperature dependencies $(\partial V_r/\partial T)_P < 0$, were observed and the column temperatures were raised for TGIC as in almost cases of RP-TGIC. However, these observations appear to be incompatible with the papers previously published, where higher temperature yielded prolonged retention time, that is $(\partial V_r/\partial T)_P > 0$ in the NP adsorption mode for PS [10] and poly(styrene-co-methyl methacrylate) [11], respectively. These phenomena suggest that lowering the temperature may be effective in NP-TCIC.

In the present work, we studied the sign of the temperature dependence of the retention in NP-TGIC for PS samples using common organic mobile phases. We found both cases of negative and positive temperature dependencies of the retention in NP-mode. Remarkably, a positive temperature dependence of the retention was found even in the NP-mode by using common organic mobile phases. This has been found, so far, only for an amphiphilic water-soluble PEO polymer in an aqueous mobile phase in the RP-mode [1,7,8]. In the present paper, the preliminary results are reported. The study is continued for the other combinations.

2. Experimental

The apparatus for TGIC was a typical HPLC system except for a modification to control the temperature of a separation column. A schematic diagram of the TGIC apparatus is shown in Fig. 1. The temperature of the separation column was controlled by a temperature program bath (EYELA, NCB 3200) in which the separation column was immersed. In order to ensure that the temperatures of the column and that the eluent are the same, the eluent was also passed through a long stainless tubing in the bath before the injector (Rheodyne, 7725).

The mobile phase was a mixture of chloroform (CHCl₃)/*n*-hexane (*n*Hx) or tetrahydrofuran (THF)/*n*Hx. CHCl₃ (Taisei Chemical, Premium grade) was purified by distillation after passing through molecular sieves 4 Å to remove the stabilizer. The other reagents (Taisei Chemical, Premium grade) were used after distillation. Two types of separation columns were used; a bare silica column (Chemco, Nucleosil 100 Å 150 mm × 4.0 mm I.D.) and a NH₂-modified silica column (Waters, μ-bondasphere 100 Å 150 mm × 3.9 mm I.D.). The flow rate was 0.5 ml/min.

Table 1
Polystyrene standard samples^a

Sample number	M_w	M_w/M_n
1	2.63×10^3	1.05
2	1.02×10^4	1.02
3	3.79×10^4	1.01
4	9.64×10^4	1.01
5	1.90×10^5	1.04
6	1.09×10^6	1.08
7	4.48×10^6	1.14

^a From Tosoh Co.

Seven standard polystyrene (PS) samples obtained from Tosoh Co. (Japan) were used. The sample numbers, the weight-average molecular weights and polydispersity indices (M_w/M_n) were summarized in Table 1. Sample solutions were made in a solvent of approximately the critical composition or the pure solvent. The sample solution was made at the concentration of about 0.25 mg/ml for each standard, i.e. totally 1.75 mg/ml. The sample solutions were filtered with a 0.50 μm membrane filter (Advantec, DISMIC-3) and then injected through a 7725 Rheodyne injector with a 20 μl sample loop. Chromatograms were recorded with a UV absorption detector (Agilent, VWD detector 1100 series) at the wavelength of 254 nm and with an evaporative light scattering (ELS) detector (Polymer laboratories, PL-ELS 1000).

3. Results and discussion

The TGIC separation of seven PS standards was carried out by using a column packed with bare silica and a mixture of CHCl₃ and *n*Hx (System I; Si/CHCl₃/*n*Hx). Since the TGIC condition is, in general, similar to the critical condition between SEC and adsorption conditions, the isocratic elutions at the various compositions of the eluents were tested at a constant temperature, 30 °C, to find the critical condition. In Fig. 2, eight chromatograms of a mixture of seven standards obtained by the eluents with the respective CHCl₃ contents are shown. Small peaks appeared around 3.5 min in the chromatograms except those obtained by eluents of high CHCl₃-content suggested so-called breakthrough peak [12], since the sample solutions injected were prepared not with the respective eluents but with pure CHCl₃. However, the peaks may be solvent peaks, since it was made sure by ELS that these peaks did not contain polymeric substances. Certainly, pure CHCl₃ does not absorb

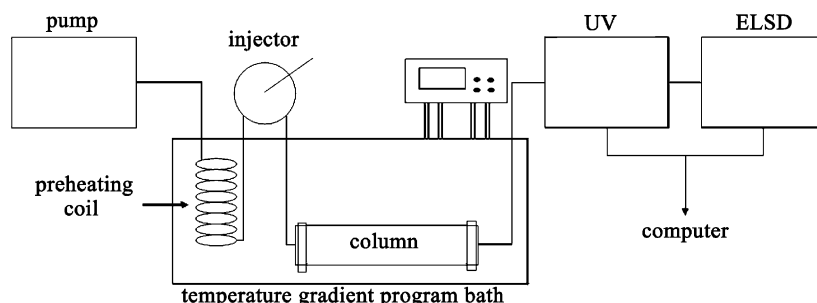


Fig. 1. Schematic presentation of the TGIC apparatus.

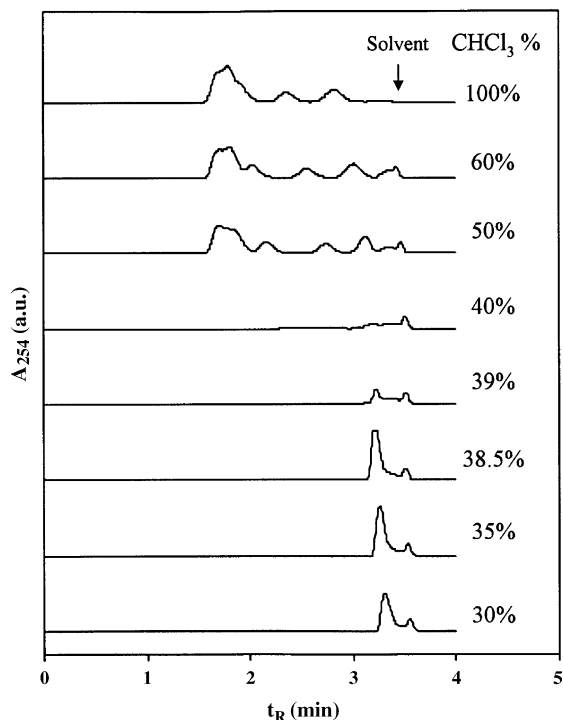


Fig. 2. Solvent composition effect on the chromatograms of 7PS standards. The isocratic elutions of $\text{CHCl}_3/n\text{Hx}$ at different compositions as labeled in the figure were tested at a constant temperature, 30°C , to find the critical condition. A bare silica column (Chemco, Nucleosil 100 \AA $150\text{ mm} \times 4.0\text{ mm}$ I.D.) was used. Injection sample concentration was 0.3 mg/ml in total. The flow rate was 0.5 ml/min .

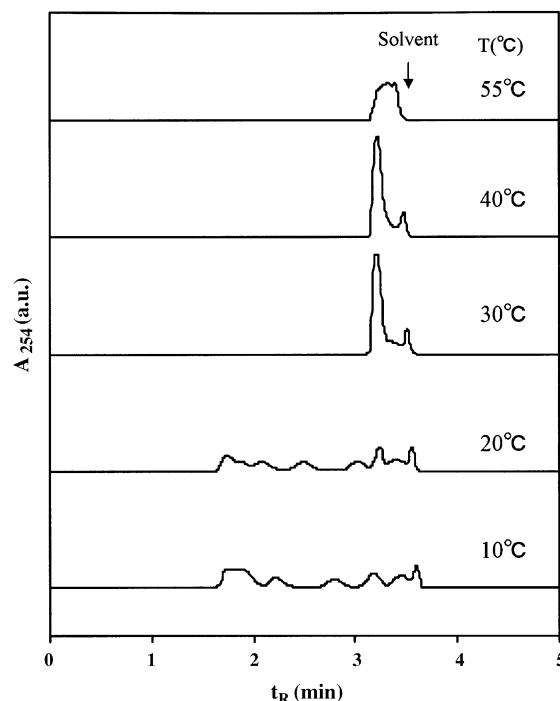


Fig. 3. Temperature effect on the isothermal chromatograms. The chromatograms obtained by the isocratic ($\text{CHCl}_3/n\text{Hx} = 38.5/61.5, \text{ v/v}$) and isothermal elutions at different temperatures as labelled in the figure. Otherwise, separation conditions are the same as in Fig. 2.

UV ray at 254 nm . Pure CHCl_3 may concentrate some impurities with UV absorbance in the column. In the eluents with higher CHCl_3 -contents, the samples were separated in the SEC-mode, whereas the samples were retained strongly in the eluents with lower CHCl_3 -contents. The mixture containing $38.5\text{ vol.}\%$ of CHCl_3 appeared to be around the critical composition. In Fig. 3, the chromatograms obtained using the mixture (CHCl_3 ; $38.5\text{ vol.}\%$) as the eluent at different temperatures are displayed. At lower temperatures, all the samples eluted at smaller elution volume than the solvent peak, so that the main separation mechanism appeared to be size exclusion. At higher temperatures, the adsorption appeared predominant, since the samples were retained strongly. These results suggest that the temperature dependence of the retention is positive ($\partial V_r/\partial T)_P > 0$) and that a negative temperature gradient is effective for the molecular weight fractionation, in contrast with the results of Lee et al. [9] where the temperature was raised.

In Fig. 4, the TGIC chromatograms obtained by the isocratic elution ($\text{CHCl}_3/n\text{Hx} = 38.5/61.5, \text{ v/v}$) and with a negative temperature gradient from 40 to 0°C (shown in the right-hand-side vertical scale) are shown. All samples were separated effectively by the lowering temperature gradient. The sample numbers in the figure were identified by single-standard injections. By using NH_2 -modified silica as the packing material and the same eluent (System II; $\text{NH}_2//\text{CHCl}_3/n\text{Hx}$), similar experiments were carried out. It was found that the eluent containing $33.5\text{ vol.}\%$ of CHCl_3 yielded the critical condition. The chromatograms

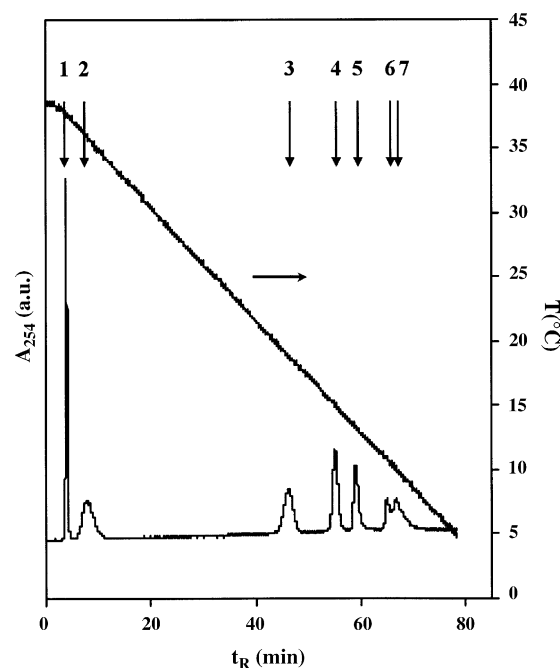


Fig. 4. NP-TGIC Chromatogram of a mixture of 7PS standards by system I. The TGIC chromatogram was obtained by the isocratic elution ($\text{CHCl}_3/n\text{Hx} = 38.5/61.5, \text{ v/v}$). A bare silica column (Chemco, Nucleosil 100 \AA $150\text{ mm} \times 4.0\text{ mm}$ I.D.) was used. Temperature gradient is shown in the figure. The sample numbers are listed in Table 1. The sample solution was made at the concentration of about 0.25 mg/ml for each standard. The flow rate was 0.5 ml/min .

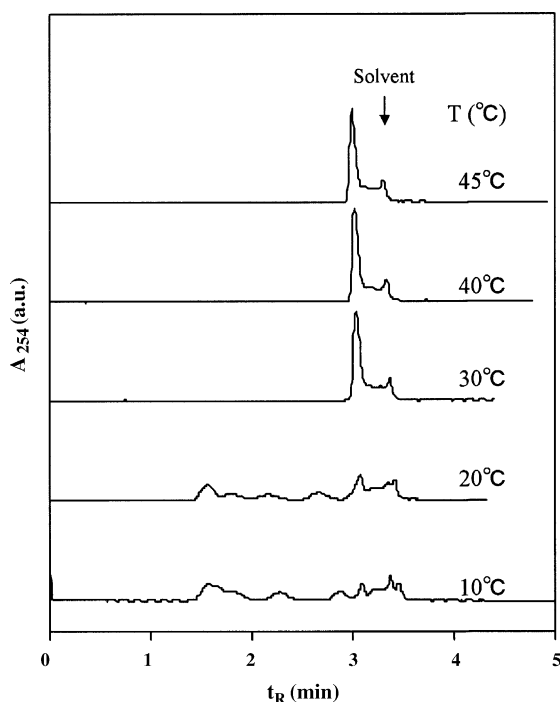


Fig. 5. Temperature effect on the chromatograms from the NH_2 -column. TGIC chromatograms obtained by the isocratic ($\text{CHCl}_3/n\text{Hx} = 33.5/66.5$, v/v) and isothermal elution at different temperatures as labelled in the figure. A NH_2 column (Chemco, Nucleosil 100 Å 150 mm \times 4.0 mm I.D.) was used. Otherwise, separation conditions are the same as in Fig. 2.

obtained by using this eluent at different temperatures are shown in Fig. 5. At higher temperatures, the samples were retained strongly, whereas at lower temperatures, all samples were eluted in the SEC-mode. This means a positive temperature dependence of the retention. As shown in Fig. 6, the samples were separated with a negative temperature gradient as expected from the positive temperature dependence. Although the peaks of standards 6 and 7 overlapped, they might be separated clearly, if the slope of the temperature gradient were lowered in the lower temperature region, as was shown by Lee et al. [9].

It may be important that a positive temperature dependence of the retention was found for PS in ordinary organic mobile phases on the two different adsorbents in NPLC. The positive dependency has been found, so far, only for PEO in aqueous mobile phases in the RP-mode.

By using the same column and a mixture of THF and $n\text{Hx}$ (System III; $\text{NH}_2/\text{THF}/n\text{Hx}$), similar experiments were carried out. It was found that 47.5 vol.% of THF was the critical composition at 30 °C in this system. From the chromatograms obtained at different temperatures using this eluent (THF; 47.5 vol.%), a negative temperature dependence was suggested. The sample mixture was separated with a positive temperature gradient, in accordance with the negative temperature dependence, as shown in Fig. 7.

In Table 2, the results obtained by the NP-TGIC, including those from the literature [8,9], were summarized classified by the directions of temperature gradient, that is, the sign of the temperature dependence of the retention. It is clear that both signs of the temperature dependence of the retention have been

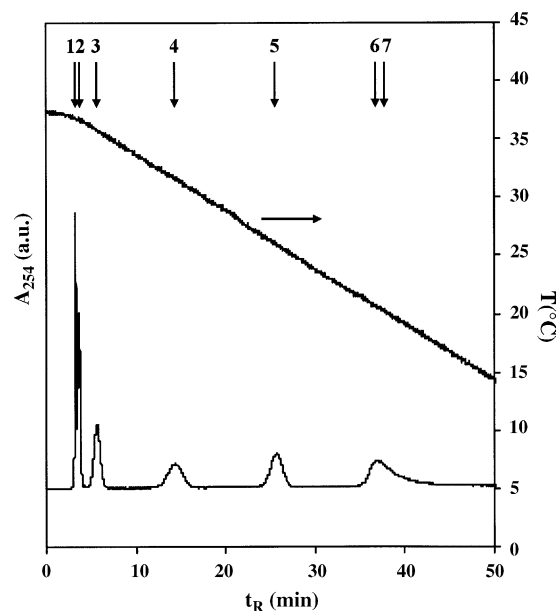


Fig. 6. NP-TGIC Chromatogram of 7PS standards using system II. The TGIC chromatogram was obtained by the isocratic elution ($\text{CHCl}_3/n\text{Hx} = 33.5/66.5$, v/v). A NH_2 column (Chemco, Nucleosil 100 Å 150 mm \times 4.0 mm I.D.) was used. Temperature gradient is shown in the figure. The sample numbers are listed in Table 1. The sample solution was made at the concentration of about 0.25 mg/ml for each sample. The flow rate was 0.5 ml/min.

observed in NP-mode LC. Therefore, either direction (positive or negative) of temperature gradient may be effective for NP-TGIC depending on the systems used.

In general, the distribution constant for adsorption chromatography, K_{ad} , which is equal to the ratio of the sample (polymer) concentration in the stationary phase and that in the

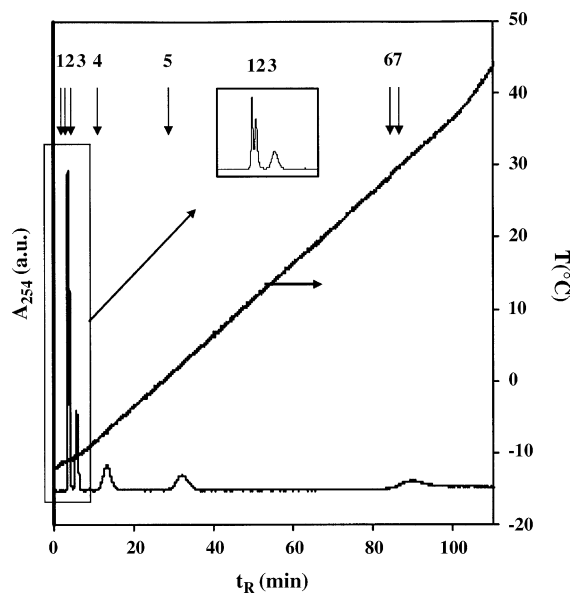


Fig. 7. NP-TGIC Chromatogram of 7PS standards using system III. The TGIC chromatogram was obtained by isocratic elution ($\text{THF}/n\text{Hx} = 47.5/52.5$, v/v). A NH_2 column (Chemco, Nucleosil 100 Å 150 mm \times 4.0 mm I.D.) was used. Temperature gradient is shown in the figure. The sample numbers are listed in Table 1. The sample solution was made at the concentration of about 0.25 mg/ml for each sample. The flow rate was 0.5 ml/min.

Table 2
The NP-TGIC systems classified according to positive and negative temperature gradients

Class	Sample	Adsorbent ^a	Eluent	Reference
Positive gradient	PS	Bare Si	THF/ <i>i</i> -Oct.	Lee et al. [9]
	PS	NH ₂ -	THF/ <i>n</i> Hx	Present work (III)
	PI	(OH) ₂ -	THF/ <i>i</i> -Oct.	Lee et al. [9]
	PEO	NH ₂ -	H ₂ O/ACN	Cho et al. [8]
Negative gradient	PS	Bare Si	CHCl ₃ / <i>n</i> Hx	Present work (I)
	PS	NH ₂ -	CHCl ₃ / <i>n</i> Hx	Present work (II)

^a Si: Silica; NH₂-: NH₂-modified silica; (OH)₂-: diol-modified silica.

mobile phase, is related to the changes of Gibbs energy $\Delta G_{\text{ad}}^{\circ}$, enthalpy $\Delta H_{\text{ad}}^{\circ}$ and entropy $\Delta S_{\text{ad}}^{\circ}$ for adsorption:

$$\Delta G_{\text{ad}}^{\circ} = \Delta H_{\text{ad}}^{\circ} - T\Delta S_{\text{ad}}^{\circ} = -RT \ln K_{\text{ad}} \quad (1)$$

The capacity factor k' is related to K_{ad} as follows:

$$k' = \frac{V_r - V_m}{V_m} = K_{\text{ad}}\phi \quad \phi = \frac{V_s}{V_m} \quad (2)$$

where V_m and V_s are the mobile-phase and stationary-phase volumes, respectively. From both equations, the variation of the capacity factor with temperature $(\partial \ln k' / \partial T)_p$, which has the same sign as $(\partial V_r / \partial T)_p$, is expressed as follows:

$$\left(\frac{\partial \ln k'}{\partial T} \right)_p = - \left\{ \frac{\partial (\Delta G_{\text{ad}}^{\circ} / T)}{\partial T} \right\} \frac{1}{R} + \left(\frac{\partial \ln \phi}{\partial T} \right)_p \quad (3)$$

If the term of $(\partial \ln \phi / \partial T)_p$ is negligible, from Gibbs–Helmholtz equation $\{(\partial(\Delta G/T)/\partial T)\}_p = -\Delta H/RT^2$, $(\partial \ln k' / \partial T)_p$ and $(\partial V_r / \partial T)_p$ have the same sign as $\Delta H_{\text{ad}}^{\circ}$. There is no discussion on the exothermic adsorption systems ($\Delta H_{\text{ad}}^{\circ} < 0$) where a negative temperature dependence is observed and a positive temperature gradient is effective in TGIC, as in almost cases of RP-TGIC reported, and also in some cases of the NP-TGIC including our present system III (see Table 2).

In the RP-mode, a few cases of positive temperature dependencies were reported for PEO in the aqueous eluents, where $\Delta H_{\text{ad}}^{\circ} > 0$ and $\Delta S_{\text{ad}}^{\circ} > 0$ were observed [1] and the samples

were separated with a negative temperature gradient [7,8]. Cho et al. explained this phenomenon by the entropy gain accompanying the release of the water molecules surrounding the amphiphilic chains (PEO) where the water molecules may adopt a more organized structure (in low entropic state) [8].

In the NP-mode, a positive temperature dependence was found in the present work for PS in a mobile phase of CHCl₃ and *n*Hx (systems I and II). This implies that in this case $\Delta H_{\text{ad}}^{\circ}$ is positive. $\Delta S_{\text{ad}}^{\circ}$ in the present cases may be positive as in the above cases of the RP-mode, though there is no direct evidence observed. Further studies are needed to make the molecular mechanism for the temperature dependence clear.

4. Conclusion

In the NP-mode, both cases of negative and positive temperature dependencies of the retention were found and then PS standards were fractionated by both directions of temperature gradient in the NP-TGIC. It is remarkable that a positive temperature dependence, which implies positive $\Delta H_{\text{ad}}^{\circ}$, was found even in the NP-mode for PS in common organic eluents.

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