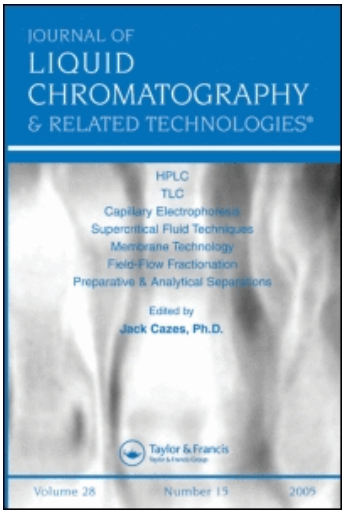

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INFLUENCE OF TEMPERATURE ON THE RESPONSE OF DIFFERENTIAL REFRACTOMETER FOR QUANTITATIVE ANALYSIS IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Some of commercially available RI detectors are thermostated, so as to keep the base-line drift minimum. However, for quantitative analysis, care must be taken to control the difference of temperature (Δt) of a liquid in a sample cell (t_s) and that in a reference cell (t_r), because the variation of the response of a RI detector is a function of Δt. Even t_s is equal to t_r, or Δt is constant through the period of the experiments, t_s at determination (t_d) of samples must be the same to that at calibration (t_c) with standards, because the variation of the RI response is also a function of $\Delta t'$ ($=t_d - t_c$). The smaller is the difference of the refractive indeces of a solute and the mobile phase and the larger is the difference of the temperature coefficients of a solute and the mobile phase, the more serious is the influence of the temperature differences. Calculated and observed results for the influence of temperature are demonstrated.

INTRODUCTION

The differential refractometer (RI) is one of the most widely used detector in high-performance liquid chromatography(HPLC),

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especially in size-exclusion chromatography(SEC). This device monitors the difference in refractive index between the mobile phase in the reference cell and the column effluent in the sample cell. This difference in refractive index between liquids in both cells causes a deflection in the location of the light beam, passing through the prism-cell assembly, on the surface of the possition-sensitive photodetector, and the deflection of the light beam is proportional to the concentration of the solute in the mobile phase in the sample cell (the deflection-type RI detector).

It is known that the refractive index of a solvent is a function of temperature and decreases with increasing temperature as shown

$$n_t = n_o + k(t - t_o) \qquad (1)$$

where n_t is the refractive index at temperature t , n_o is that at the reference temperature t_o , and k is the temperature coefficient. The magnitude of k is around $1x10^{-4}$ deg^{-1} and corresponds to the determination range in SEC, so that it is seemed to require to maintain the temperature of the refractometer cells with the precision as much as $\pm$ 0.001 ^{o}C to obtain optimum sensitivity and stability with the RI detector. Since the RI detector employs a differential mode, fortunately, fluctuations caused by temperature change are generally minimized, though total compensation is not obtained even in the differential measurement. Therefore, special regulation of temperature is not considered to some commercial RI detectors.

Because of a slight difference between the temperature coefficients of components composing the column effluent, the response of the RI detector is not entirely independent of temperature [1]. Maintaining the temperatures of both liquids in the referece and sample cells constant may be the most important requisition to obtain good baseline stability and minimized baseline drifts. At the same time, the cells should be at exactly the same temperature or the temperature difference between both liquids in two cells also have to be regulated in order to obtain reproducible results.

In this report, how much influence there will be observed is discussed when the temperatures of both liquids in two cells are different or when the temperature differences in the liquids are not the same at calibration and at determination.

CALCULATION

The refractive index n of a solution composed of two solvents A and B is expressed empirically as

$$\begin{aligned} n &= n_A F_A + n_B F_B \\ &= n_A + (n_B - n_A) F_B \end{aligned} \tag{2}$$

where n_A and n_B are the refractive indices of solvents A and B, and F_A and F_B are weight, volume, or mole fraction of solvents A and B [2]. For a dilute solution, the refractive index n is also expressed as a function of a solute concentration C

$$n = n_A + KC \tag{3}$$

where K is a constant. The deflection of the light beam in the deflection-type RI detector is a function of n/n_A and it will be proportional to $(n - n_A)$ in a small range of concentration.

(i) When a liquid temperature in the sample cell t_s and that in the reference cell t_r is different ($t_s \neq t_r$), the response in the RI detector will be proportional to

$$\begin{aligned} n - n_A &= n_A + k_A\Delta t + (n_B + k_B\Delta t - n_A + k_A\Delta t)F_B - n_A \\ &= k_A\Delta t + \{(n_B - n_A) + (k_B - k_A)\Delta t\} F_B \end{aligned} \tag{4}$$

where $\Delta t = t_s - t_r$

n_A , n_B : the refractive indices of a solvent A and a solute B at temperature t_r

Since the response in the RI detector in this case is a function

of the temperature difference Δt, the value Δt must be constant or zero, i.e., the temperature difference between both liquids is constant or both liquid temperatures are the same during the period for the quantitative analysis.

The variation of the response of a RI detector with changing the value of Δt is expressed in terms of the percent increase in the response compared with that at $\Delta t = 0$.

$$\frac{(n - n_A)_{\Delta t} - (n - n_A)_{\Delta t=0}}{(n - n_A)_{\Delta t=0}} \times 100$$

$$= \frac{k_A \Delta t + (k_B - k_A)\Delta t F_B}{(n_B - n_A)F_B} \times 100 \ (\%) \qquad (5)$$

The smaller is the value of $n_B - n_A$, i.e., the smaller is the difference of the refractive indices between the mobile phase and a solute, the more serious is the influence of the temperature difference.

(ii) When t_s is equal to t_r or the difference of t_s and t_r ($= \Delta t$) is constant but the temperature at determination t_d is different from that at calibration t_c, the percent increase in the response at t_d compared with that at t_c is

$$\frac{(n - n_A)_{t_d} - (n - n_A)_{t_c}}{(n - n_A)_{t_c}} \times 100$$

$$= \frac{\{(n_B - n_A)_{t_c} + (k_B - k_A)\Delta t'\}F_B - (n_B - n_A)_{t_c} F_B}{(n_B - n_A)_{t_c}} \times 100$$

$$= \frac{k_B - k_A}{(n_B - n_A)_{t_c}} \Delta t' \times 100 \ (\%) \qquad (6)$$

where $\Delta t' = t_d - t_c$

$(n_B - n_A)_{t_c}$: the difference of the refractive indices of A, B components at t_c °C

The equation (6) implies that the influences of temperature increase with increasing the difference of k_A and k_B and with decreasing that of n_A and n_B, and that, when k_A equals k_B, the influences disappear at all.

EXPERIMENTAL

Determination of Refractive Index

The Abbé refractometer manufactured from Atago Optics Co., Tokyo was used at 20°, 25°, 35°, 42°, and 50°C, respectively. The prism casing was thermostated at specified temperatures to an accuracy of 0.02 °C by passing warm water from a thermo-bath unit. Commercially available samples (reagent grade) of oligoethylene glycols, phthalate esters, alkylbenzenes, and several typical solvents which are generally used in HPLC were used without further purification for the measurements of their refractive indices.

Determination of Temperature Dependence of the RI Responses

The cell casing of the RI detector Model SE-11 (Showa Denko Co.) was thermostated at 25° and 45 °C to an accuracy of 0.01 °C by circulating constant-temperature water from a large-scale thermo-bath unit. A solution (0.1% concentration) and its solvent were thermostated in the thermo-bath unit, and they were injected alternately into the sample cell of the RI detector. After five minutes of injection, the responses were traced on a recorder chart and the difference of the responses of the solvent (the same to the solvent in the reference cell) and the solution was measured from the trace on the chart. The value divided the response difference by 20 represents the variation of the response per 1 °C.

Reproducibility Test in HPLC

A system used in this experiment was a Jasco TRIROTAR high-performance liquid chromatograph (Japan Spectroscopic Co., Ltd.,

Hachioji, Tokyo 192, Japan) with a differential refractometer Model SE-11 and a column 50 cm x 2.1 mm i.d. packed with a porous polystyrene gel. The RI unit and the column were thermostated at 25 °C. A portion of 0.1 ml of each solution (0.5%) of DEG, TeEG, DMP, and DBP (see Table 1 for abbreviations) in chloroform was injected. The mobile phase was chloroform at 0.5 ml/min and attenuation of the RI unit was x64.

Reproducibility Test in SEC

The above HPLC system was also used in this experiment except two SEC columns 50 cm x 8 mm i.d. Shodex A80M being used. The RI unit and the columns were not thermostated. A portion of 0.25 ml of 0.2% polystyrene (NBS706) solution in tetrahydrofuran (THF) was injected. The mobile phase was THF at 1.0 ml/min of flow rate and attenuation of the RI unit was x8. The measurement was performed at several different days and molecular weight averages were calculated by the usual manner.

RESULTS AND DISCUSSION

Figure 1 shows the relationship between temperature and refractive index for several samples. At the intersection of two straight lines for a solute and a solvent, no solute-response is observed (e.g., for diethylene glycol and chloroform at 19 °C and for dibutyl phthalate and toluene at 40 °C) and lowering or heightening the temperature would result in the inversion of the peak position.

The refractive indices of these samples at the reference temperature t_o (here 0 °C) were estimated by extrapolation from these linear plots and their temperature coefficients were calculated as slopes. Values obtained are listed in Table 1.

As can be seen in eqs. (5) and (6), the influence of temperature varies with the refractive indices and/or temperature coeffi-

TABLE 1

Refractive Index at 0 °C and Temperature Coefficient

Sample	Refractive Index at 0 °C n_D^o	Temperature Coefficient $k \times 10^4$ (deg^{-1})
Ethylene glycol (EG)	1.4370	-2.815
Diethylene glycol (DEG)	1.4505	-2.702
Triethylene glycol (TEG)	1.4598	-2.879
Tetraethylene glycol (TeEG)	1.4632	-2.929
Dimethylphthalate (DMP)	1.5218	-3.732
Diethylphthalate (DEP)	1.5087	-3.730
Di-n-butylphthalate (DBP)	1.4993	-3.659
Dioctylphthalate (DOP)	1.4940	-3.654
Di-n-nonylphthalate (DNP)	1.4904	-3.530
Ethylbenzene (EB)	1.5032	-4.603
n-Propylbenzene (PB)	1.5015	-4.981
iso-Propylbenzene (iPB)	1.5014	-4.918
n-Butylbenzene (BB)	1.5026	-5.018
n-Hexylbenzene (HB)	1.4951	-4.453
n-Octylbenzene (OB)	1.4937	-4.407
n-Dodecylbenzene (DB)	1.4959	-4.179
Water (W)	1.3368	-4.161
Methanol (M)	1.3386	-1.304
n-Hexane (H)	1.3857	-5.017
Tetrahydrofuran (THF)	1.4172	-5.056
Chloroform (C)	1.4557	-5.504
Toluene (T)	1.5058	-5.156

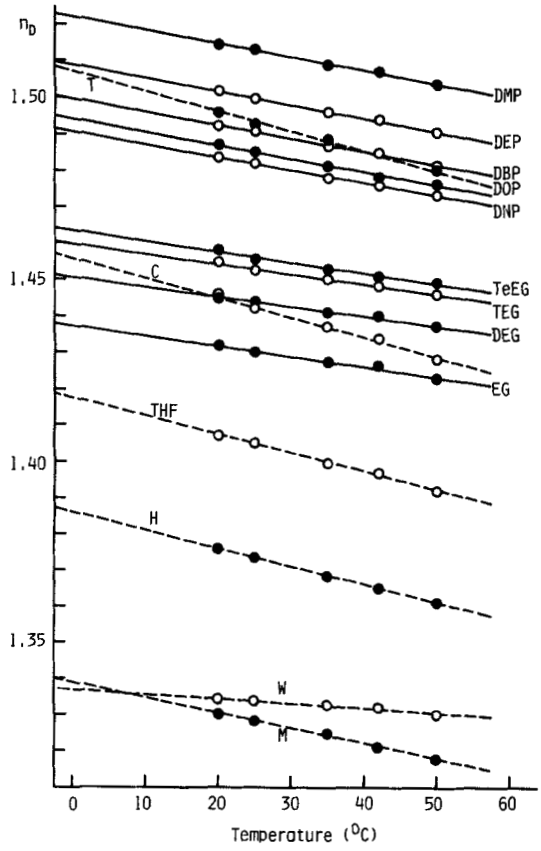

FIGURE 1. The Temperature-Refractive Index Relationship for Several Solutes and Solvents. Abbreviations are the same to those in Table 1.

cients of both solvent and solute. Four combinations of a solvent and a solute were chosen for the estimation of temperature dependence of the RI response.

(i) The combination of a solvent and a solute having larger difference of the refractive indices. M + DMP, H + DBP.

(ii) The combination of a solvent and a solute having small difference of the refractive indices. T + DMP, C + DEG, C + TeEG.

(iii) The combination of a solvent and a solute having large difference of the temperature coefficients. C + DMP, C + DBP.

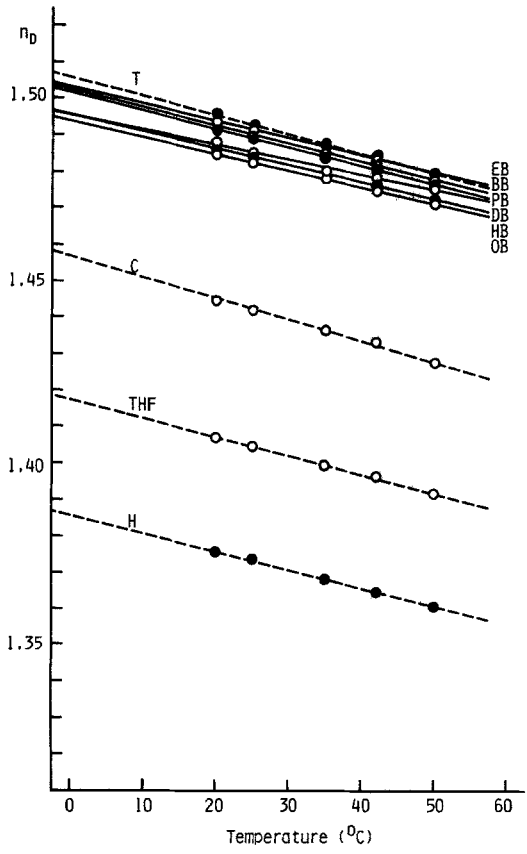

FIGURE 1 (continued)

(iv) The combination of a solvent and a solute having small difference of temperature coefficients. H + BB.

(Abbreviations are the same to those in Table 1.)

When t_s is not equal to t_r, the influence of temperature to the RI response can be estimated by using eqs. (4) or (5). Table 2 shows the calculated values of the percent increase in the response when t_s (26 ^oC) is 1 ^oC higher than t_r (25 ^oC) (Δt= 1^oC). The eq. (5) was used for calculation and the solute concentration was postulated to be 0.1%. The influence of temperature is significant in the case of the combination (ii) (and (iii)), impling

TABLE 2

The Calculated Percent Increase in the RI Response at $\Delta t = 1\ ^{\circ}C$

	Combination (Solvent + Solute)	Calculated Value Eq. (5)
(i)	Methanol + DMP	-2.3 %
	n-Hexane + DBP	4.6
(ii)	Toluene + DMP	-31.2
	Chloroform + DEG	111
	Chloroform + TeEG	70.2
(iii)	Chloroform + DMP	-8.1
	Chloroform + DBP	-12.2
(iv)	n-Hexane + BB	4.2

the importance of being Δt to be zero or to remain constant throughout the experimental period for quantitative analysis.

Though t_s equals t_r or Δt (= t_s - t_r) is constant, if t_d is different from t_c , the influence of temperature might be observed as suggested from eq. (6). Calculated and observed results when t_d (26 $^{\circ}C$) is 1 $^{\circ}C$ higher than t_c (25 $^{\circ}C$) ($\Delta t' = 1^{\circ}C$) at $\Delta t = 0$ are listed in Table 3. This variation is significant when n_A approaches n_B or k_A is apart far from k_B .

In conclusion, for quantitative analysis, Δt must be zero or constant, i.e., the value Δt at determination of samples must be the same to that at calibration. Similarly, t_d must be identical to t_c. By careful regulation of temperature of the RI unit, the relative standard deviations of the RI responses for the samples of the combinations (ii) and (iii) can be kept between 0.5 and 1.5 % (the numbers of determination were 10). Some of commercially available RI detectors are now thermostated, but its purpose is to keep the base-line drift minimum. However, care must be taken to

TABLE 3

The Calculated and Observed Percent Increase in the RI Response at $\Delta t' = 1\ ^{\circ}C$

	Combination (Solvent + Solute)	Calculated, Eq. (6)	Observed,
(i)	Methanol + DMP	0.022 %	0.23 %
	n-Hexane + DBP	0.12	0.72
(ii)	Toluene + DMP	0.73	2.0
	Chloroform + DEG	15.8	8.0
	Chloroform + TeEG	1.85	2.27
(iii)	Chloroform + DMP	0.25	1.16
	Chloroform + DBP	0.38	2.0
(iv)	n-Hexane + BB	0.00	0.84

control the difference of t_s and t_r and the regulation of t_d and t_c.

In SEC, fortunately, calculation of molecular-weight averages is based on the normalized SEC chromatogram, so that, as far as base-line is smooth and stable during determination of the chromatogram, precise values of molecular-weight averages can be expected even in different days or without accurate control of temperatures. For example, in SEC of polystyrene NBS 706, the relative standard deviation of the areas of the chromatograpms was 4.2% for ten determination, but that of the molecular-weight averages was 2.0%. Similarly, the maximum area in ten chromatograms was 17% higher than the minimum area, but the maximum molecular-weight averages was only 5% higher than the minimum one, and the latter two values were obtained from different chromatograms from the former two. For SEC conditions, see the section of EXPERIMENTAL.

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