EFFECT OF TEMPERATURE ON PRECISION OF RETENTION MEASUREMENTS IN LIQUID CHROMATOGRAPHY

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SUMMARY

The effect of temperature on precision of retention measurements in liquid chromatography for a number of chromatographic systems has been examined. These have included both reversed-phase systems used in combination with aqueous–organic mobile phases, as well as bonded phases used in combination with a completely aqueous mobile phase. The effect of varying degrees of surface coverage (amount of bonded carbon) on these measurements also has been evaluated. In addition to these systems, several normal-phase systems have been studied. These experiments have been carried out by varying surface polarity, as well as mobile phase composition.

INTRODUCTION

With the continued improvement in the performance of liquid chromatographic hardware solvent delivery increasingly is becoming more reliable. Thus, the ability to set, reset, or maintain a given flow-rate is less of a problem in making precise retention measurements. However, due to other experimental parameters significant variations in solute retention may occur; one of these is temperature. In a number of systems changes in the capacity factors (k'), to a large part, can be attributed to instability of the column temperature at ambient conditions. When other experimental systems are employed thermal changes produce minimal effects on solute retention.

In liquid chromatography there has been a tendency to overlook the influence of temperature on chromatographic measurements. This totally has not been true and several investigators have examined the effect of temperature on solute behavior[1–24]. Reported variations in the magnitude of changes in solute retention as a function of thermal changes have been shown to be dependent upon the solute–surface–mobile phase combinations used. Generally, the emphasis on the importance of temperature has paralleled the types of systems studied.

Two types of systems which have been reported to be affected most dramati-

cally by temperature are: (1) normal-phase systems where very polar surfaces and modifiers are used in conjunction with non-polar solvents. Examples of these are hexane containing ethanol[3] and hexane saturated with water[24] used in combination with silica; (2) bonded-phase systems which apparently undergo transitions in surface orientation[25]. In a number of cases, variations in k' greater than 25% have been observed by a change in temperature of 1°C or less.

Other solvent and surface combinations produce much smaller changes in retention as a function of thermal fluctuations in column conditions. Reversed-phase systems such as octadecyl surfaces used with a mobile phase of water or an aqueous buffer, and methanol or acetonitrile are influenced only moderately by variation in ambient conditions. Similarly minimal effects also have been reported recently for pyrocarbon surfaces used with combinations of aqueous–organic solvents[23].

Besides affecting the magnitude of change in k' an additional consideration is that in some cases, temperature may cause a change in the direction of solute retention. This was initially reported by Chang[2] and reinvestigated by Maggs[3], who found that additions of different polar modifiers to a non-polar mobile phase (n-hexane) could be used to either increase or decrease retention with increasing temperature. These experiments were carried out on a silica column using dinonyl phthalate as the test solute. Not only has the relative polarity of the modifier been found to be important, but the surface polarity also has been shown to be significant in controlling magnitude and direction of changes in solute retention[24]. As a result of these effects, the degree of error in making retention measurements for a given system may not be constant as a function of changes in temperature.

In view of the above, a systematic investigation of the influence of temperature on the retention for several representative chromatographic systems has been carried out. The systems examined represent extremes in terms of these effects. These have included both long chainlength surfaces used in combination with aqueous–organic mobile phases, as well as in conjunction with a completely aqueous mobile phase. The effect of varying degrees of surface coverage (amount of bonded material) on these measurements also has been evaluated. In addition to these reversed-phase systems, several normal-phase systems have been studied. Under normal-phase conditions experiments have been carried out by varying surface polarity, as well as water content of the mobile phase for a saturated system.

EXPERIMENTAL

Column preparation

Empty 25-cm columns were prepared from 2.4 and 4.6 mm I.D. stainless-steel tubing. Before packing, they were cleaned thoroughly with non-polar and polar solvents, and rinsed with acetone. After cleaning each column, one of the following procedures was employed. (1) Columns were packed and used unmodified or after *in situ* modification. (2) Packing materials were prepared using batch modification and then packed. The exact details of each of these procedures are outlined below. In all experiments, LiChrosorb Si 60 (average particle diameter 10 μm; EM Labs., Elmsford, NY, U.S.A.) was used.

After preparation, all columns were conditioned with at least 100 ml of acetonitrile followed by 100 ml of the test mobile phase. The particular reaction

condition and packing procedure for each column examined along with micro-carbon data are summarized in Table I. The amounts of bound carbon were determined by Huffman Labs. (Wheatridge, CO, U.S.A.).

TABLE I

SUMMARY OF COLUMN PREPARATION PROCEDURES

Column	Packing procedure	Modification procedure	Carbon loading (%)
Silica	Static	n.a.	n.a.
n-Butyl	Static	in situ	—
2-Carbomethoxyethyl	Static	in situ	—
3-Cyanopropyl	Static	in situ	—
Decyl (C_{10}I)	Dynamic	batch	10.4
Decyl (C_{10}II)	Dynamic	batch	12.7
Octadecyl (C_{18}I)	Static	batch	7.7
Octadecyl (C_{18}II)	Static	batch	10.7
Octadecyl (C_{18}III)	Static	batch	15.8

In situ modification
Following packing and evaluation, columns were modified in situ as previously described[26,27]. Pre-reaction conditions were established using 100 ml water-saturated toluene followed by 200 ml dry toluene. Reactions were carried out using 30 ml of a 50% solution of trichlorosilane monomer in dry toluene.

Batch modification
After slurrying the silica with water, excess water was removed and the material dried at 110°C for 2 h. The degree of pre-reaction hydration was established further by equilibration of the dried silica for at least 4 h with varying amounts of water-saturated toluene. Following this procedure, the silica was refluxed with 50 ml of a 10% reaction mixture of the trichlorosilane monomer in dry toluene. After reaction, the modified silica was washed five times with 50-ml portions of dry toluene and twice each with 50-ml portions of water-saturated toluene and diethyl ether, respectively. The material was dried to remove solvent.

Static reservoir packing
For filling the 2.4 mm I.D. columns a slurry was prepared by placing 1 g of dried packing in 10 ml 1,2-dibromoethane (3 g and 30 ml for the 4.6 mm I.D. columns, respectively) and shaking the mixture for a minimum of 45 min prior to use. The 1,2-dibromoethane was purified by passing it through Silic AR CC-7 silica from Mallinckrodt (St. Louis, MO, U.S.A.). Columns were packed using a Haskel Model DST-126-32 pump and hardware assembly[27]. The slurry reservoir was modified slightly to contain a total volume of 30 ml for packing the larger diameter (4.6 mm I.D.) columns.

Dynamic reservoir packing
Approximately 3 g of packing material were placed in a dynamic reservoir system which contained 30 ml of isopropanol and the mixture stirred for at least

30 min. After sealing the reservoir, an empty column was attached and the system was pressurized to between 7000 and 8000 p.s.i. using a Haskel Model DST-126-52 pump. Using methanol as the follow-up delivery solvent, the pressure was maintained until a minimum of 200 ml of methanol had passed through the column. The inlet pressure to the Haskel pump was shut-off and the system was allowed to depressurize slowly.

Equipment

The high-performance liquid chromatographic (HPLC) experiments were carried out with either a Hewlett-Packard Model 1084A microprocessor controlled liquid chromatograph equipped with an automatic injection system and heated solvent reservoirs and oven compartment or an LDC Model GLC 401 gradient liquid chromatograph with dual 6000 p.s.i. pumps, digital display, gradient control master and dynamic mixer system. The temperature was controlled in a water bath equipped with a Tempunit Model TU-14 zero cross-over proportional controller and a FTS systems Model LC-40 liquid cooler. The injection system was a Rheodyne Model 70-10 valve and 70-11 loop filler port which was immersed in the water bath and maintained at the temperature studied.

Reagents

All chromatographic solvents were distilled-in-glass grade from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.). The toluene used was analytical reagent grade (Mallinckrodt). It was dried by refluxing over calcium hydride (Fisher Scientific, Pittsburgh, PA, U.S.A.) for at least 4 h and maintained in this condition over calcium hydride until used. The water-saturated toluene was prepared at ambient conditions (26°C). The *n*-hexane was saturated at pre-set temperatures as previously described[24]. All trichlorosilane reagents were obtained from Petrarch Systems (Levittown, PA, U.S.A.) and were used in the condition received.

RESULTS AND DISCUSSION

The influence of temperature on the precision of capacity factor measurements has been studied. Experiments have been run using chromatographic conditions chosen to reflect major differences of the influence of thermal changes on solute retention. Both reversed-phase systems (influenced least) and normal-phase systems (more dramatically effected) have been examined. Within each of these major catagories, a cross section of parameters have been evaluated. For the reversed-phase mode, the amount of bonded material, hydrocarbon chainlength and solvent composition have been studied. In the normal-phase mode, the effect of surface polarity of the bonded functionality has been investigated. The surface types used have included unmodified silica and three modified materials (*n*-butyl, 2-carbomethoxyethyl and 3-cyanopropyl). Selectivity of these surfaces under ambient[27] and elevated[24] temperature conditions previously have been reported.

Reversed-phase systems

Shown in Figs. 1–3 are ln k' *vs.* $1/T$ (T = temperature) plots for selected test solutes. These results were obtained in the reversed-phase mode under conditions

typical of the types investigated in this study. All data points are averages obtained from a minimum of three to five determinations. From the slope of the linear least squares analysis of the data shown in Figs. 1–3 and similar plots, the percentage change in k' as a function of temperature was calculated. This was done for each combination of test solute, column type and mobile phase composition examined. These results are listed in Table II.
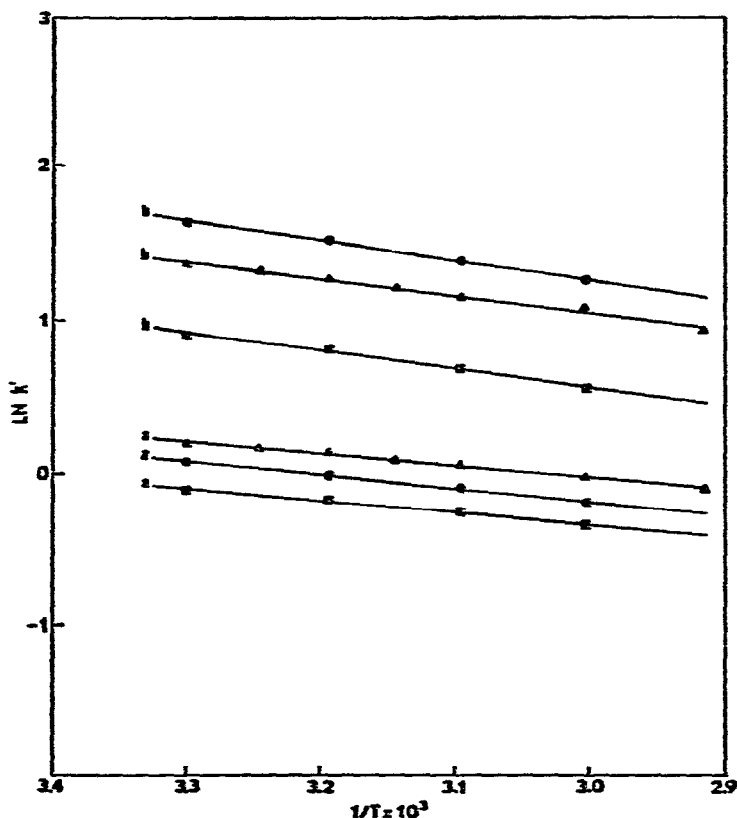

Fig. 1. Representative plots as a function of percent surface modification. Columns: ■ = $C_{18}I$; ▲ = $C_{18}II$; ● = $C_{18}III$. Test solutes: a = triphenylmethanol; b = 1,3,5-triphenylbenzene. Mobile phase: acetonitrile–water (80:20) for ■ and ● and acetonitrile–water (70:30) for ▲.

The data in Figs. 1–3 were obtained on three octadecyl- and two decyl-modified surfaces. Percent surface coverages of (a) 7.7, 10.7 and 15.8 for the C_{18} and (b) 10.4 and 12.7 for C_{10} columns were studied. As expected for a given mobile phase composition overall plots were shifted to longer retention as both the amount of bonded material and hydrocarbon chainlength were increased[28–32]. In addition, these plots were nearly parallel only exhibiting minor differences in selectivity as a function of operating temperature. The data for the decyl columns are shown only up to 60°C. Above this temperature and under certain experimental conditions these surfaces have been found to exhibit a non-linear relationship between solute retention and further increases in column temperature. The non-linear retention behavior of
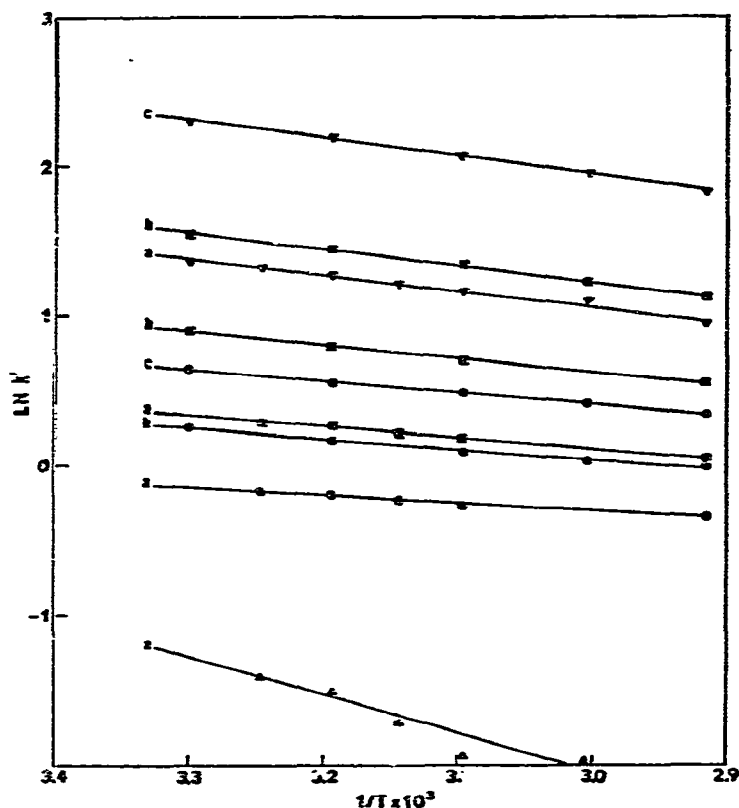
Fig. 2. Representative plots as a function of mobile phase composition. Column: C_{18}II. Test solutes: ● = benzene; ▲ = p-hydroxybenzoic acid; ■ = biphenyl; ▼ = 1,3,5-triphenylbenzene. Mobile phase: acetonitrile–water; a = 70:30; b = 60:40; c = 50:50.

these and other similar bonded midrange hydrocarbon phases have been explained in terms of a transition in surface orientation of the bonded moiety[25].

Data as a function of mobile phase modification are illustrated in Figs. 2 and 3. These results were obtained on the C_{18} surface of medium coverage (10.7%) for solvent compositions ranging from total aqueous (Fig. 3) to acetonitrile–water (70:30, v/v) (Fig. 2). Again only small changes in selectivity between runs were found with increasing temperature. For combination mobile phase systems of water and either acetonitrile or methanol approximately a 1–2% change in k' resulted from ± 1°C fluctuation in temperature. These data are consistent with other reported reversed-phase results[4–6], as well as data recently obtained on pyrocarbon containing adsorbents[23]. In the case of the totally aqueous systems, typical changes in retention in the range of 4–5% per degree centigrade were noted. The observed increase in using water as the mobile phase is consistent with increases in ΔH with increasing water content of aqueous–methanol mobile phases for the pyrocarbon systems[23].

The amount of error made in determining k' due to a variation in column temperature of ± 1°C for all combinations of conditions studied is summarized in Table II. In all cases reported variations in solute retention were small to moderate,
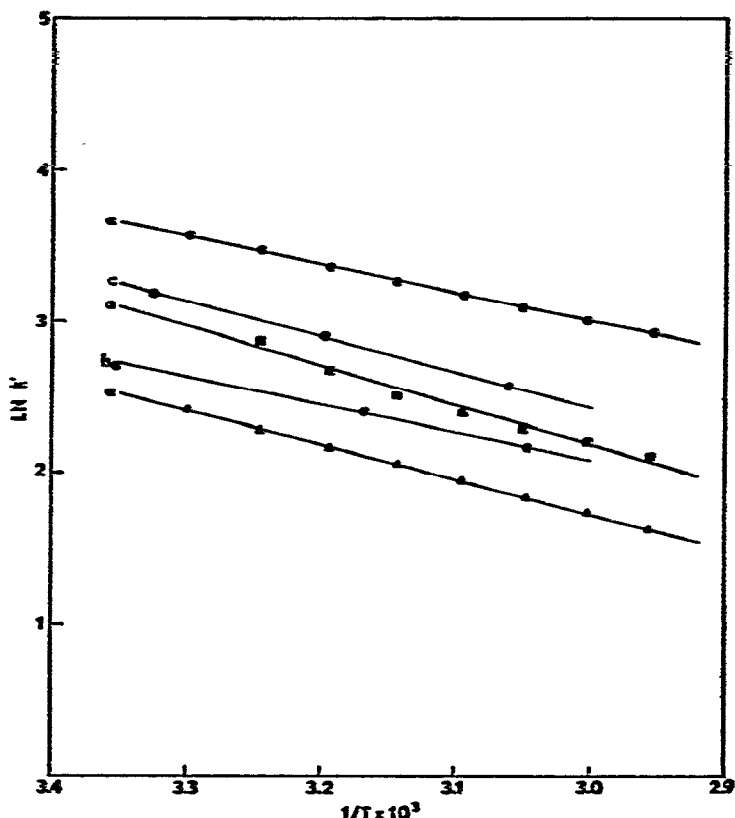
Fig. 3. Representative plots for a totally aqueous mobile phase. Columns: a = C_{18}II; b = C_{10}I; c = C_{10}II. Test solutes: ▲ = resorcinol; ■ = catechol; ● = phenol.

and the calculated error constant over the range examined. However, exceptions to this trend have been seen[25]. The data summarized in Table II were obtained using conditions where temperature affects chromatographic behavior least. In these systems, a temperature control of between 0.2 and 0.5 °C seems adequate to insure a precision of 1% in k' measurements.

Normal-phase systems

The effect of temperature on solute retention and selectivity for a series of four surfaces (silica, n-butyl, 2-carbomethoxyethyl and 3-cyanopropyl) using a non-polar mobile phase and a partially soluble polar modifier previously were reported[24]. Representative plots of data obtained on the four surface types for two selected test solutes (o-chloroaniline and N,N-dimethylaniline) are shown in Fig. 4. From these curves, the magnitude and the direction of change in k' were observed to be dependent both on the temperature range and polarity of the bonded moiety. These results were explained in terms of changes in modifier concentration in the mobile phase and on the surface as a function of temperature[24].

From the above data the percent error in measuring k' values per degree increment in temperature vs. operating temperature range has been calculated. These

TABLE II

ERROR IN k' DUE TO ± 1° C TEMPERATURE FLUCTUATION FOR REVERSED-PHASE SYSTEMS

Column: A = $C_{18}I$; B = $C_{18}II$; C = $C_{18}III$; D = $C_{10}I$; E = $C_{10}II$. Mobile phase: Composition and components (v/v): a = 100; b = 50:50; c = 60:40; d = 70:30; e = 80:20; f = 85:15. X = Water; Y = acetonitrile–water; Z = methanol–water. Compounds: 1 = Benzene; 2 = biphenyl; 3 = p-hydroxybenzoic acid; 4 = methyl-p-hydroxybenzoate; 5 = ethyl-p-hydroxybenzoate; 6 = propyl-p-hydroxybenzoate; 7 = butyl-p-hydroxybenzoate; 8 = triphenylmethanol; 9 = triphenylmethane; 10 = triphenylsilane; 11 = 1,3,5-triphenylbenzene; 12 = resorcinol; 13 = catechol; 14 = phenol.

| No.	Run	Error (%)	No.	Run	Error (%)	No.	Run	Error (%)	No.	Run	Error (%)
1	AeY1	1.1	15	BdY6	1.2	29	BcY10	2.2	43	CeY11	2.3
2	AeY2	1.7	16	BdY7	1.4	30	BcY11	2.4	44	BfZ1	0.7
3	AeY3	6.5	17	BdY8	1.5	31	BbY1	1.6	45	BfZ2	1.3
4	AeY4	1.1	18	BdY9	1.7	32	BbY2	2.2	46	BfZ3	4.7
5	AeY5	1.7	19	BdY10	1.9	33	BbY4	1.4	47	BfZ8	1.1
6	AeY6	1.6	20	BdY11	2.3	34	BbY5	1.5	48	BfZ9	1.4
7	AeY7	1.8	21	BcY1	1.4	35	BbY6	1.7	49	BfZ10	1.7
8	AeY8	1.7	22	BcY2	1.9	36	BbY7	1.9	50	BfZ11	2.9
9	AeY9	1.9	23	BcY4	1.2	37	CeY1	1.5	51	BaX12	4.5
10	AeY10	2.2	24	BcY5	1.3	38	CeY2	1.8	52	BaX13	4.8
11	AeY11	2.5	25	BcY6	1.5	39	CeY7	1.1	53	BaX14	3.8
12	BdY1	1.1	26	BcY7	1.6	40	CeY8	1.4	54	DaX12	4.3
13	BdY2	1.5	27	BcY8	1.7	41	CeY9	1.7	55	DaX14	3.7
14	BdY3	5.2	28	BcY9	2.0	42	CeY10	2.0	56	EaX12	4.9
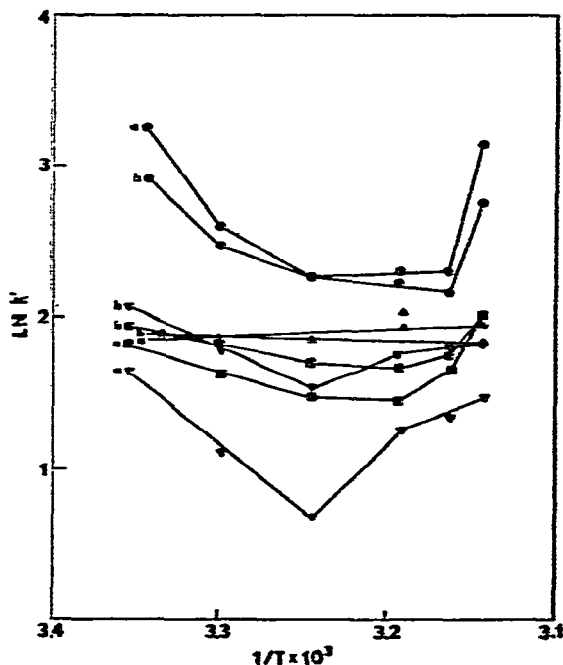
Fig. 4. Representative plots for various normal-phase surface types. Columns: ● = silica; ▲ = n-butyl; ■ = 2-carbomethoxyethyl; ▼ = 3-cyanopropyl. Test solutes: a = N,N-dimethylaniline; b = o-chloroaniline. Mobile phase: water saturated n-hexane.

results as functions of temperature range and surface type for nine test solutes are summarized in Table III. For a given temperature the magnitude of the error decreased as polarity of the surface was decreased (Table III). The non-polar n-butyl surface was affected only moderately by changes in modifier concentration resulting from temperature alterations. Additionally, each of the polar surfaces exhibited non-uniform error as a result of changes in distribution of modifier between surface and mobile phase. This is seen as a change in slope for plots shown in Fig. 4 for the silica, 2-carbomethoxyethyl and 3-cyanopropyl columns.

TABLE III

ERROR IN k' DUE TO ± 1°C TEMPERATURE FLUCTUATION FOR NORMAL-PHASE SYSTEMS

Column: A = silica; B = n-butyl; C = 2-carbomethoxyethyl; D = 3-cyanopropyl. Mobile phase: n-hexane saturated with water at temperature of measured point. a = 25°C; b = 45°C; c = value obtained in optimum range silica 35–43°C; n-butyl total range; 2-carbomethoxyethyl 35–40°C; 3-cyanopropyl 40–45°C. Compounds: 1 = biphenyl; 2 = nitrobenzene; 3 = o-chloroaniline; 4 = m-chloroaniline; 5 = p-chloroaniline; 6 = N,N-dimethylaniline; 7 = N-methylaniline; 8 = 2,6-dimethylaniline; 9 = aniline; 10 = 2,6-dimethylphenol.

No.	Run	Error (%)	No.	Run	Error (%)	No.	Run	Error (%)	No.	Run	Error (%)
1	Aa1	20.3	24	Ac4	2.3	47	Ca7	8.4	70	Cc10	3.9
2	Aa2	18.4	25	Ac5	2.3	48	Ca8	4.6	71	Da1	8.1
3	Aa3	22.1	26	Ac6	3.0	49	Ca9	6.8	72	Da2	9.4
4	Aa4	23.0	27	Ac7	1.0	50	Ca10	3.1	73	Da3	11.2
5	Aa5	24.4	28	Ac8	0.6	51	Cb1	30.0	74	Da4	11.4
6	Aa6	33.7	29	Ac9	2.7	52	Cb2	14.8	75	Da5	12.5
7	Aa7	28.5	30	Ac10	4.4	53	Cb3	27.4	76	Da6	19.0
8	Aa8	26.7	31	Ba1*	0.7	54	Cb4	30.1	77	Da7	15.8
9	Aa9	27.0	32	Ba2*	0.6	55	Cb5	32.7	78	Da8	16.9
10	Aa10	23.3	33	Ba3*	0.5	56	Cb6	36.1	79	Da9	14.2
11	Ab1	62.4	34	Ba4*	1.0	57	Cb7	33.6	80	Da10	10.3
12	Ab2	56.7	35	Ba5*	0.7	58	Cb8	30.0	81	Db1	5.2
13	Ab3	65.0	36	Ba6*	1.5	59	Cb9	28.6	82	Db2	2.4
14	Ab4	61.4	37	Ba7*	0.6	60	Cb10	13.7	83	Db3	3.2
15	Ab5	64.6	38	Ba8*	0.7	61	Cc1	2.9	84	Db4	2.8
16	Ab6	93.3	39	Ba9*	0.6	62	Cc2	1.6	85	Db5	3.7
17	Ab7	82.0	40	Ba10*	3.5	63	Cc3	1.4	86	Db6	8.8
18	Ab8	78.2	41	Ca1	1.3	64	Cc4	1.7	87	Db7	6.1
19	Ab9	71.2	42	Ca2	5.0	65	Cc5	1.9	88	Db8	5.2
20	Ab10	47.9	43	Ca3	5.1	66	Cc6	0.9	89	Db9	2.0
21	Ac1	3.6	44	Ca4	4.6	67	Cc7	1.0	90	Db10	1.1
22	Ac2	1.4	45	Ca5	5.3	68	Cc8	1.8			
23	Ac3	2.5	46	Ca6	6.8	69	Cc9	2.2			

* Linear relationship of ln k' vs. $1/T$ for total temperature range studied (25–45°C).

The normal-phase systems examined are typical of the cases where small fluctuations in column temperature result in significant errors in retention measurements. The errors made in k' for a one degree change are summarized in Table III. These are listed for the four surface types, as well as operating range. In the case of the unmodified silica surface, the influence of temperature on solute retention was found to be least significant between 35 to 43°C. In this range, when compared to

either temperatures below or above, improved precision in k' measurements were observed for a given level of fluctuation in column temperature. Although this same variability in error was true for the other polar surfaces, it was found not to be true for the n-butyl (non-polar) surface where precision was constant over the total range studied (25 to 45°C).

CONCLUSION

Generally in liquid chromatography it is accepted that temperature is not an effective parameter for modifying selectivity, since this can be accomplished more easily by changes in solvent composition. However, the effect of temperature should not be overlooked. Under certain conditions thermal instability can affect significantly solute retention. Using other conditions temperature fluctuations are of only minor importance in assuring a given level of precision in k'. The degree of significance is dependent both upon the mobile phase and the stationary phase employed.

For reversed-phase systems the precision of retention measurements are good to excellent. The largest deviations in retention as a function of temperature instability occur when a totally aqueous mobile phase is employed. Even in these systems minimal errors are observed.

Normal-phase systems, including silica, and bonded-phase polar packings are more dramatically affected by changing thermal conditions. The largest degree of error in solute retention measurements is observed when the chromatographic system is comprised of a polar stationary phase used in combination with polar mobile phase modifiers. As either/or both the polarity of the surface and modifier decrease, thermal changes affect solute retention to a decreasing degree. The smallest effect is noted for non-polar hydrocarbon type surfaces.

In addition to the above considerations the level of precision may vary as a function of temperature. Although this can occur in both the reversed-phase and normal-phase mode, non-linear behavior is generally not observed under typically employed reversed-phase conditions. However, non-linear behavior is not uncommon for normal-phase chromatography. These results thus indicate that temperature control is not only necessary but also that the position on the retention vs. temperature profile curve is extremely important in determining the level of precision in measuring solute retention in those cases where polar surfaces are employed as normal-phase packings.

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