

Unusual effect of temperature on the retention of enantiomers on a chiral column

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ABSTRACT

The Van 't Hoff plots of data obtained by chromatographing the enantiomers of a conformationally rigid spirolactam on a commercial high-performance liquid chromatographic column packed with a stationary phase derived from (*R*)-*N*-(3,5-dinitrobenzoyl)phenylglycine are non-linear. The extent and sense of the curvature of these plots depends not only on the temperature but also on the concentration of 2-propanol in the hexane mobile phase. With low concentrations of 2-propanol, retention is seen to decrease, then increase, then decrease again as the temperature is raised. This unusual behavior is ascribed to the temperature-dependent interaction of 2-propanol with the stationary phase and/or the analyte.

INTRODUCTION

Plots of the natural logarithms of chromatographic retention factors against the reciprocal of absolute temperature are termed Van 't Hoff plots and are usually linear. In the brief explorations by ourselves and others [1-3] into the effect of temperature upon chiral recognition during liquid chromatography, non-linear Van 't Hoff plots had not been reported until rather recently when Papadopoulou-Mourkidou [4] described such non-linear behavior for the enantiomers of two pesticides.

Many of the causes of non-linear Van 't Hoff behavior are addressed in papers by Horváth and co-workers [5-7]. Basically, any reversible process which alters the enthalpy or entropy of adsorption can, in principle, give rise to non-linear Van 't Hoff plots. These processes may involve the analyte, the stationary phase, or the mobile phase. Dissociative processes such as ionization, changes in conformation, or changes in the extent to which the mobile phase interacts with either the analyte or stationary phase are examples of such reversible processes. Additionally, the presence of multiple types of retention mechanisms or multiple types of binding sites may also lead to non-linear Van 't Hoff plots. In view of the importance of conformation and solvation to chiral recognition, the frequency with which multiple retention processes can be expected, and the occurrence of multiple types of binding sites on many chiral stationary phases (CSPs), the marvel is not that an instance of non-linear Van 't Hoff behavior has been found, but that such behavior had not been reported previously. Because Papadopoulou-Mourkidou [4] used a hexane mobile phase containing but 0.2% 2-propanol as a polar modifier, we immediately suspected that this might be a

contributing factor to her observation. We also suspected that the narrow temperature range over which prior studies were conducted might also have led to the observed linear Van 't Hoff plots.

In this paper, we report an instance of extreme non-linear Van 't Hoff behavior and rationalize its occurrence.

EXPERIMENTAL

The chromatographic system used consists of a Rainin Rabbit HPX pump, a Rheodyne Model 7125 injector, a Regis covalent 250×4.6 mm I.D. (*R*)-*N*-(3,5-dinitrobenzoyl)phenylglycine column, a Milton Roy UV monitor D operating at 254 nm and a Shimadzu CR1A integrating recorder. Column temperature was controlled by immersing the column in either a stirred constant-temperature bath containing ethylene glycol or a large dewar flask filled with ice and either water or methanol. In the heated bath, temperature was controlled to $\pm 0.2^\circ\text{C}$; in the ice-methanol baths, temperatures were held to $\pm 0.5^\circ\text{C}$ of the recorded value. Void volumes were determined at each temperature from the detector response to a trace amount of 1,3,5-tri-*tert*-butylbenzene spiked into the analyte and were fitted to a linear plot. Values obtained from the plot were used in computing capacity factor k' and in k' values, the computation being performed by the "Enantiotherm" program written by C. J. Welch of this laboratory for the Apple Macintosh computer. The preparation and characterization of this analyte are reported elsewhere [8].

RESULTS AND DISCUSSION

The conformationally rigid spiro lactam **1** [8] was chromatographed at different temperatures on a commercial covalent (*R*)-*N*-(3,5-dinitrobenzoyl)phenylglycine CSP using mobile phases of hexane containing either 20, 10 or 5% (v/v) 2-propanol. Void volumes were determined using 1,3,5-tri-*tert*-butylbenzene as a marker. Retention times were recorded to 0.01 min by a recording integrator. Plots of the natural

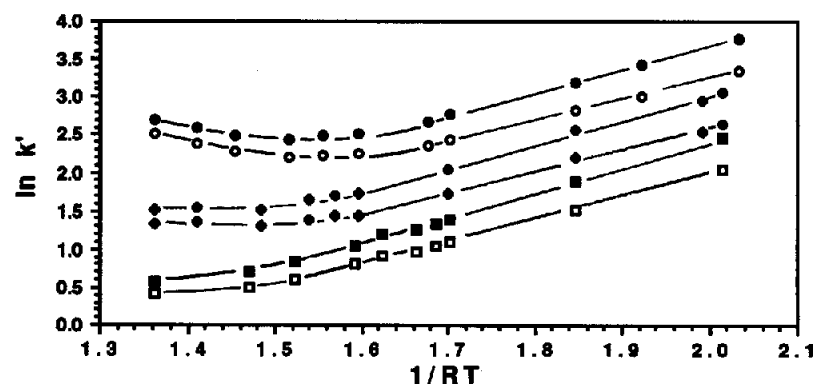


Fig. 1. Non-linear Van 't Hoff plots for the enantiomers of **1** on the chiral phase using respectively: \circ and \bullet = 5% 2-propanol in hexane; \diamond and \blacklozenge = 10% 2-propanol in hexane; \square and \blacksquare = 20% 2-propanol in hexane as an eluent.

logarithms of the capacity factors *versus* $1/RT$ (R is the gas constant; T is absolute temperature) are shown in Fig. 1.

As one normally expects, increasing the 2-propanol concentration in the mobile phase hastens the elution of the analyte enantiomers. Starting at subambient temperatures, one finds that an increase in column temperature causes a reduction in retention, linear Van 't Hoff behavior being observed *up to a point*. The temperature at which the deviation from linear Van 't Hoff behavior begins is found to decrease as the 2-propanol concentration is reduced. Note that, with the 5% 2-propanol mobile phase, retention begins to increase as the temperature is raised above *ca.* 55°C. It seems likely that similar retention increases might have been encountered, had sufficiently high temperatures been used, with the 10 and 20% 2-propanol mobile phases. However, there is so little retention with these mobile phases at the higher temperatures that the experiments were not conducted. Instead, the 2-propanol concentration was reduced to 2.5% so as to increase retention and to make possible the use of an extended temperature range. Fig. 2 shows the Van 't Hoff plots resulting from these experiments.

From Fig. 2, one may note, progressing from low to high temperature, an initial linear Van 't Hoff segment, deviation from linearity followed by an increase in retention until a maximum is reached at *ca.* 110°C, followed by a subsequent decrease in retention as the temperature is increased further. The last portion of the high-temperature segment is again (approximately) linear. How might one rationalize such a plot?

Thermodynamic data such as reported herein include contributions from many sources, contributions which are not readily segregated. Even so, plausible rationalization of the observations can be offered and finds considerable precedent in some achiral systems. At low temperatures, residual silanol groups and strands of bonded phase adsorb the 2-propanol. Since analyte adsorption requires loss of associated 2-propanol by the stationary phase and by the analyte, the determined enthalpies and entropies of adsorption contain the enthalpies and entropies of the 2-propanol loss and are accordingly reduced in magnitude. Note that below 25°C, the slopes of the $\ln k'$ *versus* $1/RT$ plots at each enantiomer are similar for each of the four 2-propanol

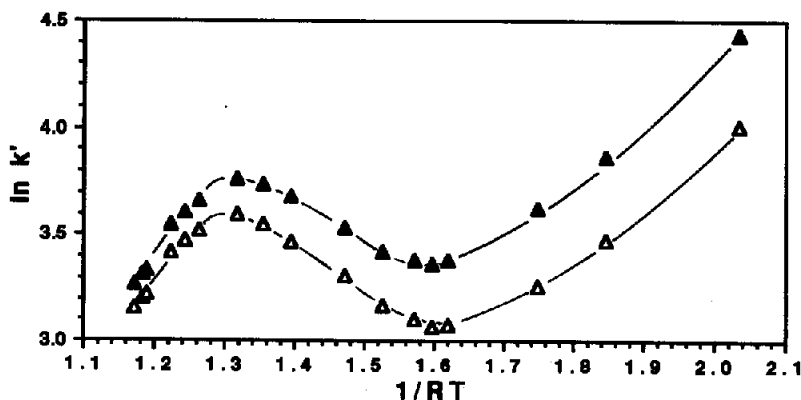


Fig. 2. Non-linear Van 't Hoff plot for the enantiomers of 1 on the CSP using 2.5% 2-propanol in hexane as an eluent.

concentrations. These slopes are the ΔH values of adsorption and are, proceeding from 20 to 2.5% 2-propanol, -3.07 ± 0.032 , -2.83 ± 0.28 , -2.72 ± 0.10 and -2.64 ± 0.18 kcal/mol for the least-retained enantiomer and -3.46 ± 0.036 , -3.19 ± 0.30 , -3.01 ± 0.08 and -2.90 ± 0.16 kcal/mol for the most-retained enantiomer. The lack of a strong dependence of ΔH on 2-propanol concentration suggests that the stationary phase and analytes are "saturated" with 2-propanol below 25°C even when but 2.5% 2-propanol is present. Analyte adsorption thus "displaces" essentially the same quantity of 2-propanol at each of the concentrations used. Note that the enthalpy of adsorption becomes slightly more exothermic as the 2-propanol concentration increases. This can be rationalized by the reasonable assumption that the return of adsorbed 2-propanol to the bulk mobile phase becomes more exothermic as the 2-propanol concentration increases. Thus, of the three processes, analyte adsorption, 2-propanol desorption, and 2-propanol "resolution", only the ΔH of the latter is dependent upon the 2-propanol concentration. Note that the loss in entropy upon analyte adsorption also increases as the propanol concentration increases. In the same order as before, the ΔS values are -8.23 ± 0.12 , -6.11 ± 1.06 , -4.36 ± 0.37 and -2.71 ± 0.66 entropy units (e.u., $\text{cal mol}^{-1} \text{ degree}^{-1}$) for the least-retained enantiomer and -8.95 ± 0.07 , -6.69 ± 0.56 , -4.70 ± 0.15 and -2.88 ± 0.29 e.u. for the most-retained enantiomer. This trend is consistent with the view that analyte adsorption returns essentially the same quantity of 2-propanol to the bulk mobile phase at each of the 2-propanol concentrations used. Since the ΔS value of the return of 2-propanol to the bulk mobile phase is determined in part by the change in the mobile phase's 2-propanol concentration, these ΔS values might be expected to be more positive at the lower 2-propanol concentrations. Here, return of a given quantity of 2-propanol procedures the greatest change in concentration. A greater positive ΔS of 2-propanol return more effectively reduces the magnitude of the large negative ΔS of analyte adsorption, thus explaining the overall trend of the observed ΔS values with a decrease in 2-propanol concentration. The correlation coefficients for the data leading to these four linear plots are 0.998 or greater.

As the temperature increases, thermal desorption of 2-propanol leads to the formation of sites at which analyte adsorption is more exergonic owing to the reduced need to displace 2-propanol. This can increase retention of the analyte provided the magnitude of the effect is sufficiently great. Such effects were noted some years ago by Maggs [9], Scott and Lawrence [10] and by Gilpin and Sisco [11] on achiral systems. At a given temperature, the number of "desolvated" sites increases as the 2-propanol concentration is reduced. Hence, as the temperature is increased, the point at which an increase in retention is first noted is found to decrease as the 2-propanol concentration is reduced. However, analyte retention can only increase until the thermal desorption of 2-propanol nears completion. Beyond this temperature, the decrease in retention expected with an increase in temperature is again observed. The enthalpies of analyte adsorption, -5.12 ± 0.90 and -5.45 ± 0.87 kcal/mol, obtained from the linear high-temperature segment of the Van 't Hoff plots exceed those obtained from the linear low-temperature segments, since there is less of the offsetting contribution from displacement of 2-propanol from the stationary phase (or the analytes) upon analyte adsorption.

Severely non-linear Van 't Hoff plots have been noted by Gilpin and Sisco [11] using polar stationary phases (silica, 2-carbomethoxyethyl, cyanopropyl) and hexane

saturated with water as a mobile phase. These concave plots were explained on the basis of "changes in modifier concentration in the mobile phase and on the surface as a function of temperature". This is in line with the views of Maggs [9] and Scott and Lawrence [10]. The explanation of the present data is simply an elaboration on this theme. Since most CSPs are rather polar, non-linear Van 't Hoff plots will doubtless be the rule when non-polar mobile phases containing polar modifiers are used and extended temperature ranges are investigated.

We hasten to add that other factors may also be involved in producing non-linear Van 't Hoff plots. Self-association of the bonded-phase strands, were it to occur, could be disrupted by increasing either the temperature or the 2-propanol concentration. Amides of N-(3,5-dinitrobenzoyl)amino acids do indeed associate in solution. This interaction is thought to favor a "head to tail" orientation [12] whereas the adjacent strands are more or less confined to a head-to-head orientation. Self-association is still conceivable, however. In any event, differential scanning calorimetric experiments with the dry adsorbent show no transition (up to 200°C) which would suggest thermal disruption of such self-association. Even so, such self-association of the strands of bonded phase cannot be totally discounted as a contributing factor to the observed effect. Under identical conditions, several other analytes do not afford the complex curves noted herein for the enantiomers of 1. Hence, the unusual behavior cannot be attributed solely to the CSP-mobile phase combination. Evidently, there is some domain into which the various equilibrium constants must fall if the Van 't Hoff plots are to resemble those described herein. Depending upon the values of the ΔH and ΔS values of the various processes, thermally promoted "desolvation" can cause Van 't Hoff plots to be concave or convex.

Three interesting points remain to be made. First, plots of the natural logarithm of the separation factor for the enantiomers against the reciprocal of absolute temperature (Fig. 3) are essentially linear for all four 2-propanol concentrations even though the 2.5% 2-propanol mobile phase produces the very non-linear Van 't Hoff plot of the retention parameter, k' . This indicates that whatever the unusual occurrence is, it affects both enantiomers equally and that chiral recognition is little influenced by the 2-propanol concentration. This seemingly rules out either a mechanism change (such

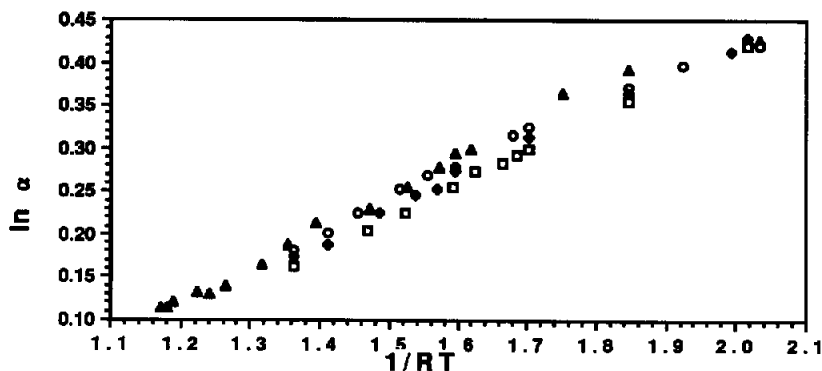


Fig. 3. Plots of $\ln \alpha$ versus $1/RT$ for the enantiomers of 1 on the CSP using, respectively: \blacktriangle = 2.5; \circ = 5; \blacklozenge = 10 and \square = 20% 2-propanol in hexane as an eluent.

as a change in the proportion of achiral vs. chiral retention processes) or the population of higher energy conformers which afford a different enantioselectivity. This behavior is to be contrasted with the non-linear natural logarithm of the separation factor, $\ln \alpha$, versus $1/T$ behavior noted recently during the gas chromatographic separation of the enantiomers of several amino acid derivatives on a CSP [13]. Second, the demonstrated difference in the thermodynamic parameters determined in the 2-propanol-desorbed region and the 2-propanol-saturated region should be given heed by those who formulate computer models for chiral recognition systems. Computer models have as yet taken no explicit cognizance of the role played by the solvent. For that reason, thermodynamic parameters calculated from these models should perhaps be compared with experimental values obtained from high-temperature "desolvated" experiments rather than those determined at ambient temperature in the presence of strongly interactive mobile phases. Finally, the ability of the chiral column to separate enantiomers is unimpaired after its use at more than 150°C for several hours, thus indicating that neither racemization nor loss of bonded phase has occurred to any significant extent. Such durability is not expected of all CSPs (e.g., protein or cellulose CSPs). Although the higher temperatures are well above the boiling point of the mobile phase at atmospheric pressure, the pressure within the system is great enough to prevent boiling in either the column or the flow cell, considerable cooling of the eluent having occurred before the latter was reached.

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