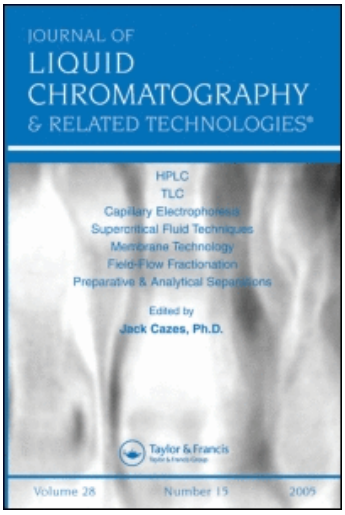
This article was downloaded by:
On: *24 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Ion Interaction: The Energetics and Mechanism of The Competitive Behavior BEtween Two Similarly Charged Molecules. 2. Temperature Effect and Energetics

Pierre R. Bedard[a]; William C. Purdy[a]
[a] Department of Chemistry, McGill University, West Montreal, Canada

To cite this Article Bedard, Pierre R. and Purdy, William C.(1985) 'Ion Interaction: The Energetics and Mechanism of The Competitive Behavior BEtween Two Similarly Charged Molecules. 2. Temperature Effect and Energetics', Journal of Liquid Chromatography & Related Technologies, 8: 13, 2445 — 2461
To link to this Article: DOI: 10.1080/01483918508076580
URL: http://dx.doi.org/10.1080/01483918508076580

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

JOURNAL OF LIQUID CHROMATOGRAPHY, 8(13), 2445-2461 (1985)

ION INTERACTION: THE ENERGETICS AND MECHANISM OF THE COMPETITIVE BEHAVIOR BETWEEN TWO SIMILARLY CHARGED MOLECULES. 2. TEMPERATURE EFFECT AND ENERGETICS

Pierre R. Bedard and William C. Purdy*

Department of Chemistry
McGill University
801 Sherbrooke Street West
Montreal, Quebec, Canada H3A 2K6

Downloaded At: 16:24 24 January 2011

ABSTRACT

The competition between two molecules of similar polarity for adsorption sites on the stationary phase is discussed in light of the effects of temperature, acetonitrile and surfactant (cyclohexylaminopropane sulfonic acid, CAPS) concentration on the retention of the thyroid hormones (3,5-diiodo-thyronine, T2; 3,3',5-triiodo-thyronine, T3 and thyroxine, T4). The data are analyzed using a second-order polynomial from which the enthalpy, entropy and heat capacity can be evaluated. The molecular motion of the analyte is reduced with an increase in surfactant concentration as determined from entropy and heat capacity calculations. This effect does not result from micelle formation but rather from molecular interaction between the analyte and a few surfactant molecules. A reduction in enthalpy from competitive and interactive behaviour is proposed. The compensation temperature is half of what is normally observed, which is related to the heat capacity effect and the data treatment.

INTRODUCTION

The energetics of the chromatographic process can be visualised as the energy change between the solvation of the solute in the mobile phase and the stationary phase adsorption

Copyright © 1985 by Marcel Dekker, Inc. 0148-3919/85/0813-2445$3.50/0

(1-3). At equilibrium, the Gibbs free energy describes the effect of temperature on equilibrium:

$$G = R\ T\ \ln K \qquad (1)$$

$$= H - T\ S \qquad (2)$$

were G, R, T, K, H and S represent the Gibbs free energy, the gas constant, the temperature in Kelvin, the equilibrium constant ($K = \Phi\ k'$, where Φ is the phase ratio), the enthalpy and entropy, respectively. The van't Hoff equation results from the rearrangement of the above equations and its usage has been demonstrated by Horvath et al. (4) within the solvophobic chromatography framework:

$$\ln k' = \frac{H}{R\ T} - S - \ln \Phi \qquad (3)$$

From thermodynamic theory (1), this equation is valid only for a small temperature range. From a practical point of view, the equation is valid when the enthalpy, entropy and the phase ratio are assumed to be reasonably constant. This assumption is valid only if the heat capacity difference between the reactants and products is negligible. Deviation from linearity results when one or more of these assumptions is not obeyed.

Non-linear van't Hoff plots have been described using the concept that a second energetic center is involved in the system. These centers have been demonstrated to involve a set of "extra reverse phase adsorption equilibria" which significantly contributed to the overall energetics. These include a)the free silanols at the surface of the stationary phase (5,6), b) a change in the conformation of the solute (7) and c) a change in the ionization of the solute or the buffer (8).

This paper concludes a series (9) of two that has studied the competion behaviour between two similarly charged molecules.

Downloaded At: 16:24 24 January 2011

Experimentally this consists of a study of the effect of a surfactant (CAPS) on the retention of some thyroid hormones at various temperatures. The focus (of this particular contribution) is on the energetics of the competition between the surfactant and the test solute, taking into account the earlier findings (9). A second-order polynomial is proposed for the analysis of the retention temperature data from which the enthalpy, entropy and heat capacity can be determined. The particular problem of evaluating the phase ratio for the entropy determination is discussed. Finally, some inferences about the events at the surface of the stationary phase are proposed.

Downloaded At: 16:24 24 January 2011

EXPERIMENTAL

The experimental set-up has been described previously (9). No changes have been made unless specified. The mobile phase was pre-heated, which otherwise might have led to splitting or deformation of the peaks resulting from the non-homogenous temperature at the inlet of the column (10). The flow rate was monitored, since the viscosity change might affect the compressibility of the mobile phase.

ESULTS AND DISCUSSION

The analysis of the temperature-retention data resulted in a non-linear van't Hoff plot. From a multiple enthalpic center point of view (as proposed by Horvath et al. (5-8)), two processes might readily be identified for the retention of the thyroid hormones. First, work is required to cross an electrostatic barrier, since both the analyte and the surface are negatively charged. Second, the molecule undergoes hydrophobic interactions with the stationary phase. The summation of both processes results in a nonlinear temperature dependency as

expressed by the summation of van't Hoff equations. However, the exact value of the contributing enthalpies can only be evaluated from extrapolations (at the inflection point and at the intercept) of the plot and the usual assumptions described above.

A Different Perspective is Proposed

From thermodynamic theory (1), the enthalpy of the process can be evaluated more properly from the the equilibrium constant and temperature data:

$$\frac{d(\ln K)}{d(T)} = \frac{H}{T^2} \qquad (4)$$

The rate of change of the enthalpy with respect to temperature arises from the heat capacity, which describes changes in degrees of freedom (i.e. phase transition). Obviously the latter is not "practical". Another method has been proposed by Osborne et al. (11) and modified by Seelig and Oppenheimer (12):

$$G = -RT\ln K = a_1 + a_2 T + a_3 T^2 \qquad (5)$$

where a_1, a_2 and a_3 are the coefficients that are evaluated using multiple linear regression. If "a_3" is not significant, the result is a rearranged van't Hoff equation. From this polynomial the following thermodynamic quantities are evaluated:

$$H = \frac{d(G/T)}{d(1/T)} = a_1 + a_3 T^2 \qquad (6)$$

$$S = \frac{d(G)}{d(T)} = -a_2 - 2 a_3 T \qquad (7)$$

$$C_p = \frac{d(H)}{d(T)} = -2 a_3 T \qquad (8)$$

This third-order polynomial is different from the Taylor's series expansion, proposed by Hammers and Verschoor (13), which resulted in the regression of five parameters and higher equations. Their criteria for selection are not sensitive in checking wheter all of the regressed parameters are significantly

Downloaded At: 16:24 24 January 2011

different from zero or significantly contribute to the overall fit (14).

The temperature dependency of the capacity ratio, k', for T0, T2, T3 and T4 has been determined for mobile phases containing 12.5% acetonitrile with no, 0.005M and 0.01M CAPS and 15% acetonitrile with no CAPS (all pH 11.5 and 0.02M PO_4). The data were analyzed using a multiple linear regression program. The problem, encountered before (15), of non-homogenous variance was not present as demonstrated by the distribution of the residuals (see figs 1 and 2).

Downloaded At: 16:24 24 January 2011

As discussed above, it is necessary to evaluate whether or not a given parameter is significant. At the onset, it was obvious that the intercept parameter was significant for all cases. The data were analyzed to test the significance of "a_2" and "a_3" by assuming a value of zero. For the case of mobile phases containing 12.5% acetonitrile without and with 0.005M CAPS, statistical and residual analysis of figures 1 and 2 show the adequacy of a second-order polynomial (note that a very similar pattern is found for both T3 and T2). For the other conditions it can be demonstrated that either the "a_2" or the "a_3" term is not significant, in other words the equation is overdefined when the regression is performed with three parameters. However, from a statistical point of view, both equations are valid (i. e. $a_2 = 0$ or $a_3 = 0$). From a thermodynamic point of view, both equations describe the situation in similar terms: for one case the entropy and entnalpy are independent of temperature and for the other a variation of less than 10% from 20 to 70° Celcius.

The phase ratio term (as $\ln\Phi$) is part of the "a_2" parameter in equation 5. Two assumptions are made: the phase

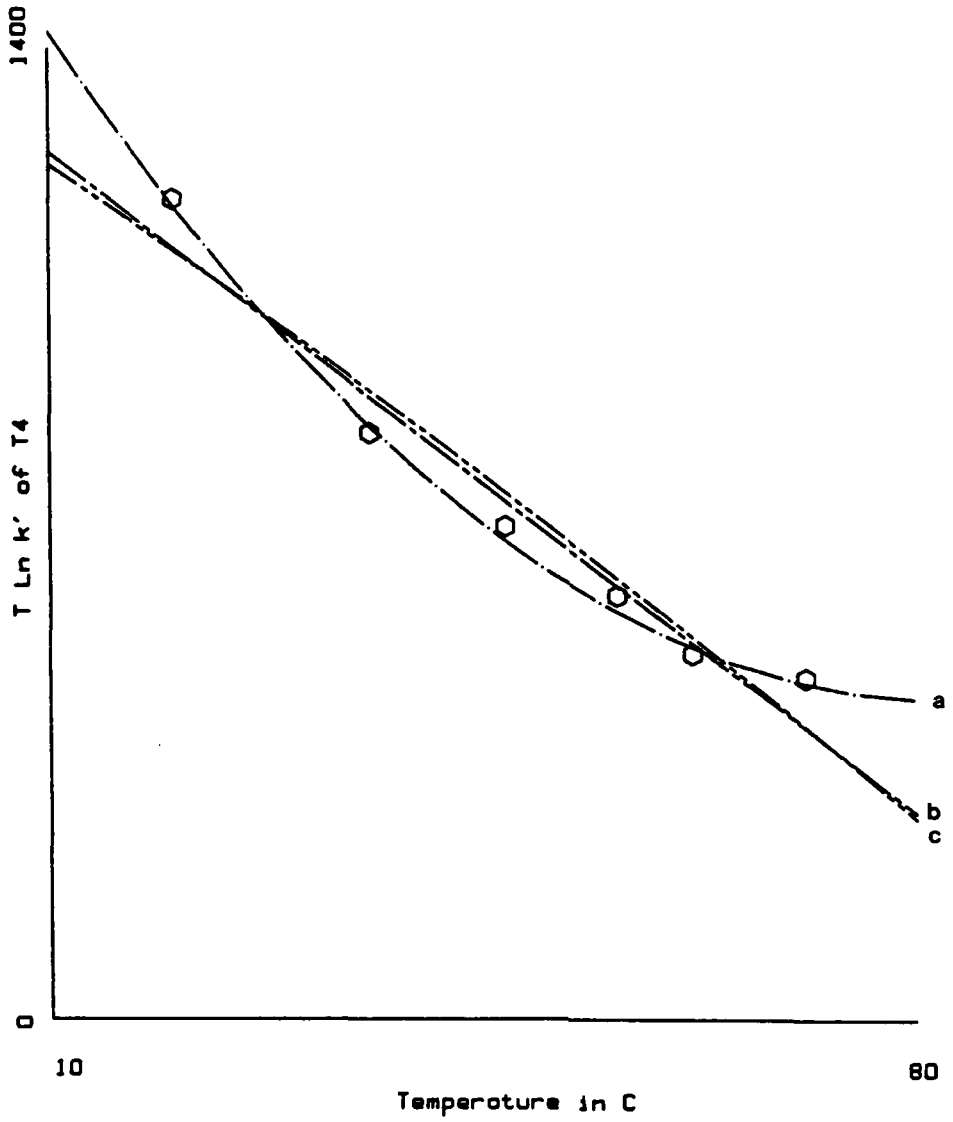


FIGURE 1. Quality of fit for different temperature dependencies vs. Ln k'. The experimental conditions are stated in the experimental section with 0.0 M CAPS and 12.5% (V/V) acetonitrile. The temperature dependency are: a) $a_1 + a_2 T + a_3 T^2$; b) $a_1 + a_2 T$; c) $a_1 + a_3 T^2$, respectively; see text.

Downloaded At: 16:24 24 January 2011

Downloaded At: 16:24 24 January 2011

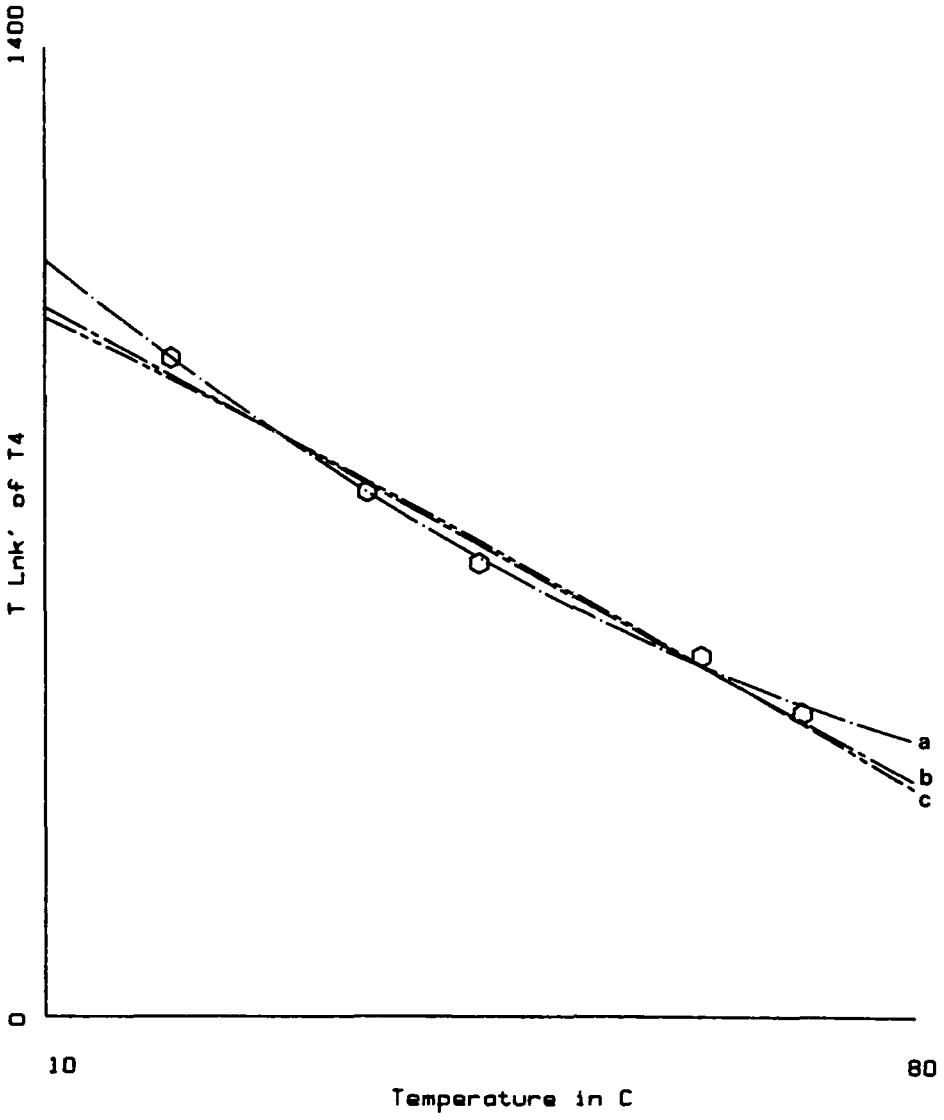


FIGURE 2. Quality of fit for different temperature dependenies on Ln k'. The experimental conditions are stated in the experimental section with 0.005 M CAPS and 12.5% (V/V) acetonitrile. The temperature dependency are: a) $a_1 + a_2 T + a_3 T^2$; b) $a_1 + a_2 T$; c) $a_1 + a_3 T^2$, respectively; see text.

ratio does not significantly change with temperature and the natural log of the phase ratio is between -3 and 3. Then the phase ratio contribution is not significant with respect to the values of "a_2". Except for 12.5% CH_3CN with 0.01 M CAPS and 15% CH_3CN, two approachs can be taken.

If "a_2" is zero, in which case the phase ratio is obviously absent, then the entropy dependency on temperature results in a variation of less than 10% over the temperature range of 20 to 70° C. However if"a_3" is zero, the calculated entropies could be

Downloaded At: 16:24 24 January 2011

TABLE 1

Results from the Regression Analysis. The chromatographic conditions are described in the experimental section. The +/- values associated with a_1 is the "standard error of the estimate" and with "a_2" and "a_3" is the "standard error" . Note "R" is the gas constant.

T	[CAPS]/ CH_3CN	a_1/R	a_2/R	a_3/R	r^2	F
4	no/12.5	23805+/-17	-131+/-17	0.185+/-0.026	.997	587
4	.005/12.5	10587+/-12	-52.5+/-12	0.0667+/-.019	.998	602
4	.01/12.5	3487+/-13	-9.06+/-.31	-	.996	878
4	no/15	3528+/-8.6	-9.26+/-.21	-	.998	2008
3	no/12.5	23743+/-26	-134+/-26	0.1903+/-.040	.993	216
3	.005/12.5	11484+/-13	-60 +/-14	0.079 +/-.021	.998	485
3	.01/12.5	3129+/-13	-8.88+/-.31	-	.995	835
3	no/14	3120+/-15	-8.88+/-.36	-	.994	616
2	no/12.5	22589+/-30	-131+/-30	0.1910+/-.047	.985	97.3
2	.005/12.5	12484+/-20	-71+/-21	0.1007+/-.033	.990	97.0
2	.01/12.5	1663+/-13	-4.9+/-.30	-	.985	269
2	no/15	2148+/-16	-6.55+/-.39	-	.990	284

offset by as much as 40% (using the above assumptions). Both can be demonstrated to be thermodynamically valid. The results from both cases are the same within experimental error. This latter fact might indicate that the second of the above assumptions should contribute to the result by less than +/- 0.5. For the remaining discussions the phase ratio will be assumed to be insignificant for this specific set of conditions. The significant equation parameters are reported in table 1 with the relevant statistics and a summary plot in figure 3 is presented.

Extra-Thermodynamic Considerations

The classical approach to extra-thermodynamic analysis, which includes entropy-enthalpy compensation plots, has been described by Leffler and Grunwald (16). Noteworthy is the caveat proposed by Krug, Hunter and Grieger (17,18) which will be addressed later. Enthalpy-entropy compensation is typically expressed as:

$$H = A\,(S) + B \qquad (9)$$

were A and B are the slope and the intercept, respectively. In other similar analyses, the nil value for heat capacity is generally assumed without being formally recognised. The heat capacity effect is explicitly accounted for in eqs 6,7 and 8. Combining eqs (6)and (7) with eq(9) would result in the description the compensation temperature as:

$$a_1 + a_3\,T^2 = A\,(-a_2 - 2\,a_3\,T) + B \qquad (10)$$

where the slope parameter is a function of the compensation temperature, T'. This yeild the following identities:

$$A = T'/2 \qquad \text{and} \qquad B = a_1 + a_2\,A \qquad (11)$$

Then it would be expected that the observed compensation temperature is half the value normally found in solvophobic chromatography. This finding and our results (the slopes are

Downloaded At: 16:24 24 January 2011

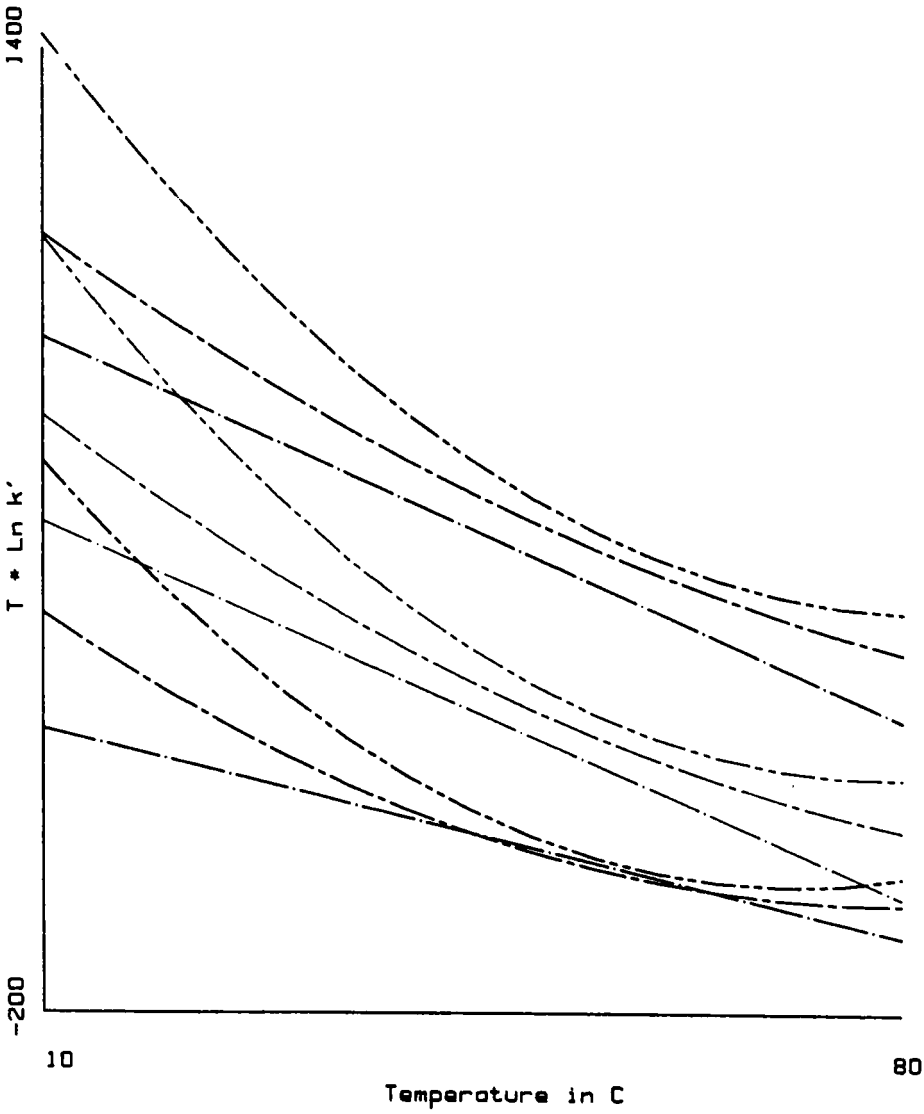


FIGURE 3. Summary of the final result for the Ln k' dependency on temperature . The experimental conditions are stated in the experimental section with T4: a) 0M, b) 0.005M, c) 0.01M; T3: d) 0M, e) 0.005M, f) 0.01M; T2: g) 0M, h) 0.005M, i) 0.01M. See text.

Downloaded At: 16:24 24 January 2011

317.7 +/- 1) compare very well with the reported approximate value of 625 K (19). Otherwise the normally observed compensation temperature would be averaged out and lead to other conclusions about the phenomenon observed.

The compensation temperature is believed to be the temperature where an increase (or decrease) in ST is exactly compensated for by a decrease (or increase) in H. These thermodynamic variations arise from modification of the mobile-phase or of molecular substituents. In practice this is never observed due to other events of lesser importance that perturb the system and that cannot be predicted by simple theory. These secondary events are observed at the compensation temperature since the main effect cancels (from compensation) itself out. Figure 3 describes this situation; at about 50° C, the free energy, for a given thyroid hormone, is the same (within 12%) for every CAPS concentration. Finally it is noteworthy that for a given concentration of surfactant, the entropy and enthalpy dependence on temperature are parallel for all thyroid hormones.

Downloaded At: 16:24 24 January 2011

The statistical compensation problem, discussed by Krug, Hunter and Grieger (17,18) had been originally explored by Leffler and Grunwald (16). Using different methods, they came to similar conclusions. In the absence of extra-thermodynamic effects the correlation coefficient between S and H was 0.998 for the temperature set used (20,50 and 70° C) (17). This is an evaluation of the expected "randomness" in the absence of extra-thermodynamic effects; a lower value indicates that an important factor is not properly accounted for, while a larger value does not necessarily indicate a successful relationship. The correlation coefficients for the entropy-enthalpy plots of the individual hormones are larger than those computed for random

events. When the regression is performed using all hormones and CAPS concentrations, the correlation (0.98) is smaller than expected for random variation alone. This probably results from the number of iodine atoms and their position on the thyronine backbone. A regression analysis of the intercept of the individual hormone entropy-enthalpy plot with respect to the number of iodine atoms ($r = 0.997$) confirms the significance of the iodine contribution. However this is only secondary to the surfactant for the overall control over k'. The free energy-enthalpy plots, as recommended by Krug et al. (17, 18), also produced correlation coefficients better than 0.999 in the temperature- [CAPS] plane. These findings suggest that the same fundamental mechanism leads to the adsorption of the thyroid hormones and that this mechanism is germane with the concept of "solvophobic chromatography".

Contrary to the recommendation of Krug et al. (17, 18), it was necessary to use the entropy-enthalpy plot for the determination of the compensation temperature. The experimental temperature and the compensation temperature were similar which would have resulted in unacceptably large variances for the compensation temperature. Also considering the significant contribution of heat capacity, analysed in a similar manner as eq 10, the enthalpy-free energy regression would have resulted in a slope equal to one.

Molecular Interpretation

A possible origin for the heat capacity effect is a change in the level of ionization of the ionophore present. Modeling was performed with an IBM personal computer taking into account various concentrations of all species, their respective pKa dependency on temperature and using enthalpy, entropy and, when

Downloaded At: 16:24 24 January 2011

available, heat capacity from various sources (20-23). The concentration of free (non-ionized) CAPS did not change by more than 10% over the chromatographically useful temperature range. Similarly, the pH did not shift by more than 0.3 units over the same range. Therefore the level of ionization of the phenol group on the thyroid hormones would not change. An example of the graphic output is shown in figure (4), note the negligible effect of the heat capacity. In this context the heat capacity observed in the chromatographic experiments is not the result of a change in ionization of the molecule present, but would originate rather from a large change in molecular motion, larger than would be accounted for by molecular vibrations. Upon adsorption, a molecule would lose some degrees of freedom due to numerous anchorage points. An increase in surrounding adsorbed surfactant would not affect the overall molecular motion of the analyte. Hence the change in molecular motion, as monitored by the heat capacity (in fact the chromatographic experiment only monitors the net difference in thermodynamic quantities between that of the solutes in the mobile phase and the stationary phase), can only originate from phenomena in the mobile phase.

As the CAPS concentration increases the molecular motion of the thyroid hormones decreases. The effect is the only mechanism that can be postulated to explain a reduction in molecular motion. Micelle formation is unlikely, considering the surfactant and acetonitrile concentrations used (24). The final analysis should explain that the thermodynamic values for both the 12.5% CH_3CN with 0.01M CAPS and 15% CH_3CN without CAPS are very similar. Both CAPS and acetonitrile could complex with the hormone in a manner similar to that proposed by Gnanasambandan and Freiser (25) for alcohols and a dye. A concentration

Downloaded At: 16:24 24 January 2011

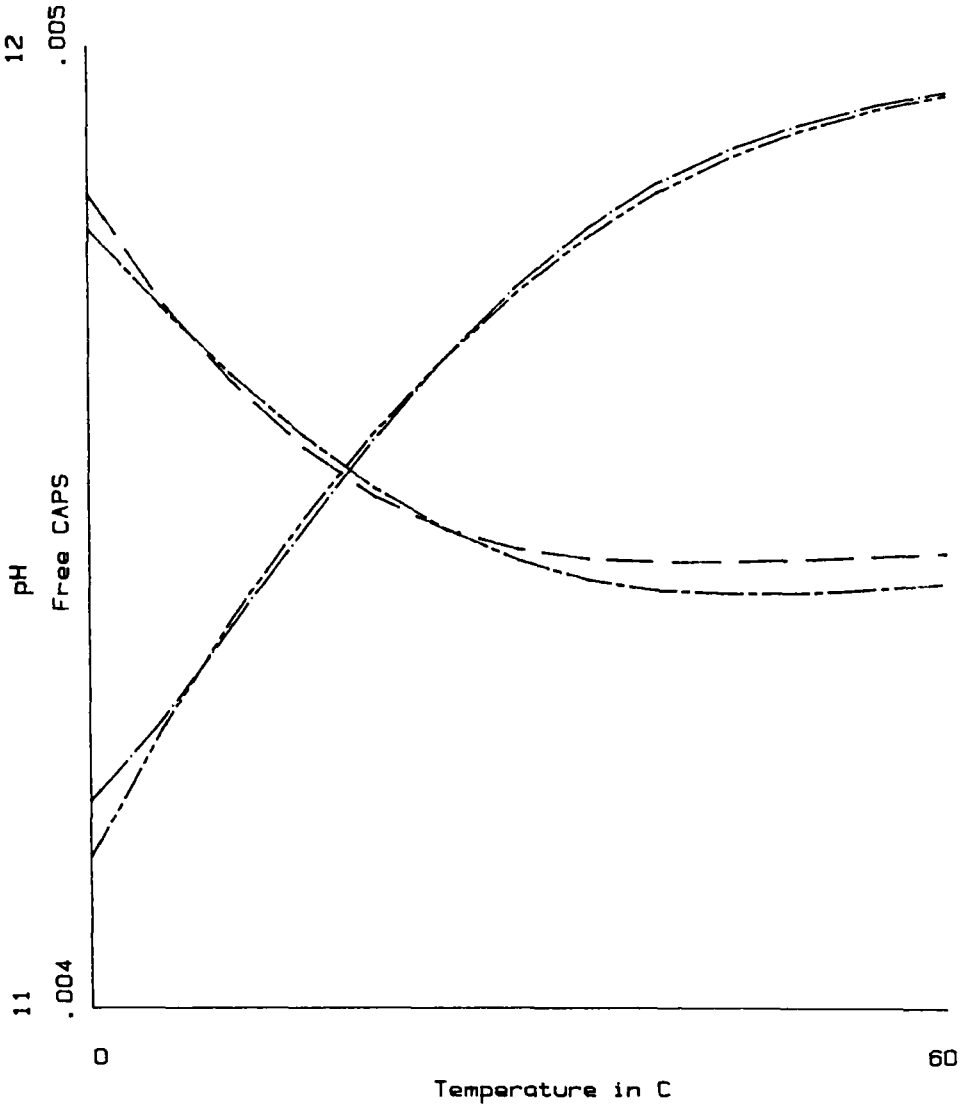


FIGURE 4. Computer simulation on the effect of temperature on pH and CAPS ionization. The initial conditions are 0.02 M phosphate, pH 11.5, 25° C and 0.005 M CAPS (total). The thermodynamic data are those of ref 21 and 22 for phosphate and CAPS, respectively. Results a) Concentration of unionised CAPS, b) pH; using the same data but assuming that the heat capacity is negligible or zero, c) Concentration of unionised CAPS, d) pH

Downloaded At: 16:24 24 January 2011

increase in either, would produce a larger and stronger cage leading to lower molecular motion. Note that using a multiple enthalpic center point of view (as proposed by Horvath et al. (5-8)), this cage production might readily be identified as the second enthalpic center.

Competition for the Stationary-Phase Sites

The reduction in enthalpy has been partially described as due to a reduction in molecular motion. The non-temperature-dependent portion of enthalpy is reduced with an increase of both CAPS and CH_3CN. Adsorption of a hormone results in desorption of the surfactant molecule (9). Energically, the net effect results from the difference in enthalpy of adsorption of both the analyte and CAPS. The more surfactant molecules that are desorbed the smaller is the net difference.

$$H_{ta} - n\,H_{ca} = H_{oc} \qquad (12)$$

In this equation; H_{ta}, H_{ca} and H_{oc} are the enthalpy of adsorption of an hormone at [CAPS]=0, the enthalpy of a CAPS molecule and the enthalpy observed, respectively, and n is the number of desorbed surfactant molecules. Then if H_{ca} = 8Kj/mole (14), up to 20 CAPS molecules should be desorbed for each hormone molecules adsorbed. This seems unlikely on the basis of molecular size alone. But it can be explained considering the interactions between the analyte and the surfactant, which could result in significant changes in the "effective" heat of adsorption of both molecules, as suspected in the previous paper (9). The acetonitrile reduces the energy of solvation in both the stationary and mobile phase as expressed by a reduction of the interfacial tension (2,3).

Downloaded At: 16:24 24 January 2011

CONCLUSION

A different perspective for the computation of entropy, enthalpy and heat capacity for non-linear van't Hoff plots is proposed, which results in an experimental and theoretical treatment that yields a temperature compensation half of what is normally expected. This is rationalized as a heat capacity effect arising from the formation of a complex between a thyroid hormone and a combination of surfactant and acetonitrile molecules in the mobile phase.

The reduction in the non-heat-capacity-dependent term of the enthalpy results from both desorption of the surfactant and ion-interaction.

Downloaded At: 16:24 24 January 2011

ACKNOWLEDGEMENTS

The authors are indebted to the Natural Science and Engineering Research Council of Canada for financial support.

REFERENCES

1- G. M. Barrow; "Physical Chemistry" 3rd ed, McGraw-Hill Book Co., New York, 1973, pp. 134-139 and 206-215.

2- Cs. Horvath and W. R. Melander; J. Chrom. Sci., 15 (1977) 393.

3- Cs. Horvath, W. R. Melander and I. J. Molnar; Anal. Chem., 49 (1977) 142.

4- Cs. Horvath, W. R. Melander and I. J. Molnar; J. Chrom., 125 (1976) 129.

5- W. Melander, J. Stoveken and Cs. Horvath; J. Chrom., 199 (1980) 35.

6-A. Nahum and Cs. Horvath; J. Chrom., 203 (1981) 53.

7-W. R. Melander , A. Nahum and Cs. Horvath; J. Chrom., 185 (1979) 129.

8-W. R. Melander, J. Stoveken and Cs. Horvath; J. Chrom., 185 (1979) 111.

9- P. R. Bedard and W. C. Purdy; J. Liq. Chrom., part I of this series.

10- B. J. Wilder; Anal. Chem., 51 (1979) 774.

11-J. C. Osborne, Jr., G. Palumbo, H. B. Brewer, Jr., and H. Edelhoch; Biochemistry, 15 (1976) 317.

12- S. Seelig and J. H. Oppenheimer; J. Biol. Chem., 257 (1982) 1378

13- W. E. Hammers and P. B. A. Verschoor; J. Chrom., 282 (1983) 59.

14- N. Draper and H. Smith; "Applied Regression Analysis", 2nd Ed., John Wiley and Sons, New York, 1981, chapter 6

15- P. R. Bedard and W. C. Purdy; in preparation

16- J. E. Leffler and E. Grunwald; "Rates and Equilibria of Organic Reactions", John Wiley and Sons, Inc., New York, 1963, chapter 9.

17- R. R. Krug, W. G. Hunter and R. A. Grieger; J. Phys. Chem., 80 (1976) 2335.

18- R. R. Krug, W. G. Hunter and R. A. Grieger; J. Phys. Chem., 80 (1976) 2341.

19- W. Melander, D. E. Campbell and Cs. Horvath; J. Chrom., 158 (1978) 215.

20- J. W. Larson, K. G. Zeeb and G. Hepler; Can. J. Chem., 60 (1982) 2141.

21- A. Vacca, A. Sabatini and L. Bologni; J. Chem. Soc., Dalton Trans., 6 (1981) 1246.

22- C. D. McGlothin and J. Jordan; Anal. Lett., 9 (1976) 245.

23- CRC Handbook of Chemistry and Physics; CRC Press, Inc., Boca Raton, Fl, USA, 1978, pg D-204

24- J. H. Knox and R. A. Hartwick; J. Chrom., 204 (1981) 3.

25-T. Gnanasambandan and H. Freiser; Anal. Chem., 54 (1982) 1282.

Downloaded At: 16:24 24 January 2011