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LIQUID CHROMATOGRAPHIC STUDY OF THE TWO PHYSICAL STATES OF A DENSELY BONDED ALKYL-SILICA AND THE CORRESPONDING RETENTION PROCESSES

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SUMMARY

The densely grafted ($\geq 4 \mu\text{mol}/\text{m}^2$) alkyl-bonded silicas, previously tested by gas chromatography, have been studied in reversed-phase liquid chromatography with water-methanol mobile phases at all concentrations and with various solutes. The same phase transition appears at temperatures depending on the composition of the mobile phase. It is interpreted as the change from an adsorption-dominating retention to a solution-dominating process. Conclusions concerning the mechanism of retention in reversed-phase chromatography are discussed and new experiments with more standard stationary phases are suggested.

INTRODUCTION

Densely covered ($\geq 4 \mu\text{mol}/\text{m}^2$) C_{18} - or C_{22} -alkyl-silicas are new materials recently prepared by means of Kováts¹ bonding techniques. By gas chromatography of an auxiliary volatile solute, these materials bring out a melting-like phase transition of the bonded layer in a temperature range that is more limited the longer is the alkyl chain^{2,3}. In addition, this transition is shifted towards higher temperatures if the grafted layer is immersed in the capillary condensate of a very polar and associated liquid, glycol or water-glycerine mixture³. Lastly, *n*-alkanes of similar chain length were proved to be inserted in the layer of bonded radicals to give a mixed two-dimensional layer, formed of the physisorbed and chemisorbed species^{2,3}. Riedo *et al.*⁴ have demonstrated the existence of such "mixed crystals" by contact angle measurements in grafted capillaries. The molecular area of an alkyl chain² is 0.21 nm^2 , exactly as for the condensed phases of long-chain fatty compounds at the surface of polar substrates. In addition, they undergo a transformation about 20°C above the transition temperature of the chemisorbed layer alone. There is interest in these phenomena for the interpretation of the retention mechanism in reversed-phase liquid chromatography³.

The experiments described in this paper support the above hypothesis. Densely covered silicas appear to be a borderline case that sheds some light on the physical

phenomena involved in retention in reversed-phase liquid chromatography, namely solubility and adsorption. Indeed, the influence of temperature on retention in liquid chromatography has been the subject of many studies recently. Several workers, *e.g.*, Nahum and Horváth⁵, have demonstrated non-linear variations of the logarithm of the capacity factor as a function of the reciprocal of the absolute temperature. These experiments do not suggest phase transitions of the bonded layer as we have shown by gas chromatography.

Most of the materials used in those experiments were either poorly characterized commercial products or bonded silicas obtained from bi- or trifunctional reagents and any comparison with our results is questionable. In addition, the complicated bonding recipes are applied to narrow-pore silicas where the curvature of the surface precludes the formation of a well defined layer.

Recently, Gilpin and Squires⁶ published $\log k'$ versus $1/T$ curves characterized by reproducible discontinuities at certain temperatures, but obviously this phenomenon has no connection with our gas chromatographic transitions: the short-chain alkyl-bonded layer undergoes a sort of relaxation effect following the change of the mobile phase. Nevertheless, these experiments and other observations related to retention volumes⁷ or column dead volume⁸ measured with different mobile phases indicate the influence of the physical state of the bonded layer.

This paper describes a reversed-phase liquid chromatographic study of the two different physical states evidenced by gas chromatographic experiments on densely bonded alkyl-silicas. The work was carried out on a C₂₂ bonded silica with larger pores and much larger particles than is usual in analytical chromatography, in order to obtain better defined layers and to clarify the interpretation. On the other hand, the use of a refractometer as a detector allowed us to work with very different solutes, polar and apolar, linear and cyclic.

EXPERIMENTAL

Choice of the alkyl chain

The preparation of the monofunctional bonding reagent, docosyldimethyl-(dimethylamino)silane and the bonding process have been described earlier^{2,3}. The choice of the C₂₂-alkyl chain* makes it possible to observe the phase transitions in a more favourable temperature range between the melting point of water and the boiling point of methanol.

Substrate

Although these transitions may be observed with densely bonded silicas with pores of 15 nm diameter (sometimes even narrower), we have preferred to work with a more macroporous substrate. Increasing the radius of curvature of the surfaces makes the diffusion of the reagent easier and consequently leads to denser and more reproducible layers. This silica is Spherosil XOB 075 made by Rhône-Poulenc (Paris, France), with a specific surface area of 94 m²/g according to laboratory measure-

* With the usual C₁₈ silicas exactly the same phenomena can be observed at around room temperature², which enhances the practical consequences of the results reported in this paper.

ments by a dynamic BET method⁹ and a mean pore diameter of the silica of about 30 nm.

The particle size (40–50 μm) is unfavourable for high efficiencies, but it was selected to test the material conveniently by gas chromatography^{2,3} at different stages of its use. In practice, the width of the peaks that corresponds to 200–250 plates for a column of 1/4 in. diameter and 20 cm length does not impede retention measurements.

The bonding density is 3.98 $\mu\text{mol}/\text{m}^2$ according to carbon analyses effected by the Service d'Analyse du CNRS (Solaise, France). This is lower than the density obtained for very wide pore silicas with the same alkyl chain length³. This may be a consequence of an effect of the surface curvature on the structure of the bonded layer. However, bonding of Spherosils with narrower pores has led to densities of more than 4 $\mu\text{mol}/\text{m}^2$. Consequently, the material used in this study was not chosen for its exceptional bonding density. Our test of a good graft is a gas chromatographic curve similar to Fig. 1 in ref. 3 (see also ref. 2, p. 103). Nevertheless, it is worth noting that for C_{18} - or C_{22} -alkyl chains the bonding density of all of our Spherosils is higher than those of almost all the materials reported in previously published papers¹⁰.

Liquid chromatography

Liquid chromatographic experiments were effected with a Chromatem pump (Touzart et Matignon, Vitry, France) equipped with a pulse damper. The flow-rate was 0.8 ml/min. The use of a refractometer (R 403, Waters Assoc., Paris, France) was of paramount importance because it made it possible to test a wide variety of solutes, which is not possible with the more usual UV spectrophotometer adopted by most authors. The column was thermostated with a standard 0.1°C laboratory liquid bath.

The dead volume was evaluated by water injection in the case of methanol and methanol-rich mobile phases. With aqueous mobile phases we used heavy water¹¹. Precise determination of the column dead volume is a not completely resolved problem in reversed-phase liquid chromatography, but our purpose was not to compare dead volumes with different mobile phases. In addition, the retention volumes reported in this paper are large and therefore the uncertainties about the dead volume do not affect the observed phenomena.

The methanol used for the mobile phase was of RSE grade, obtained from Carlo Erba (Milan, Italy). Water was doubly deionized and distilled in the presence of permanganate.

RESULTS AND DISCUSSION

Pure methanol mobile phase

Two very different types of $\log k'$ versus $1/T$ curves are observed. With short-chain linear solutes (*n*-alkanes and fatty alcohols with less than 12 carbon atoms) and even more with non-linear solutes (cyclododecane, 9-bromoanthracene) (Fig. 1), analogy with the gas chromatographic $\log V_s$ versus $1/T$ curve observed with helium as carrier gas is obvious (Fig. 2). All of these diagrams are characterized by a transition between two linear variations and an inflexion can always be seen. In the most favourable instances, a real increase appears between the two linear decreases observed when temperatures are increased. Transition is terminated between 46 and

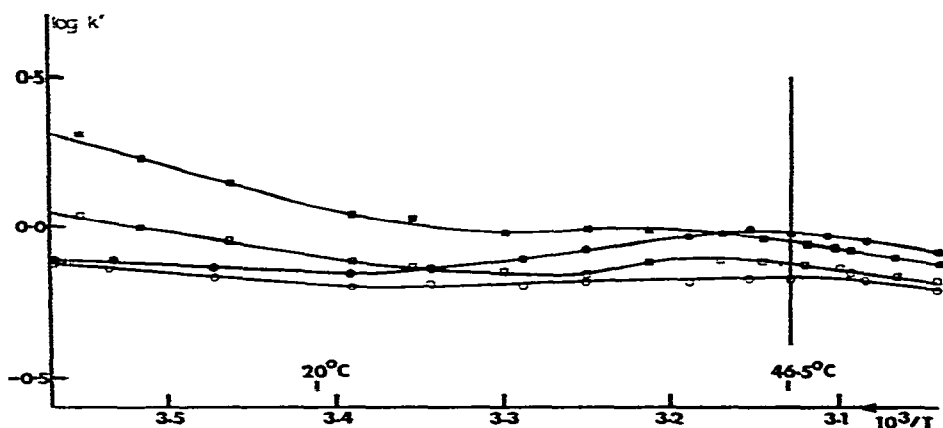


Fig. 1. Variation of $\log k'$ with $1/T$ for a pure methanol mobile phase and different short-chain and non-linear solutes: \square , *n*-undecane; \blacksquare , *n*-dodecane; \bullet , cyclododecane; \circ , 9-bromoanthracene.

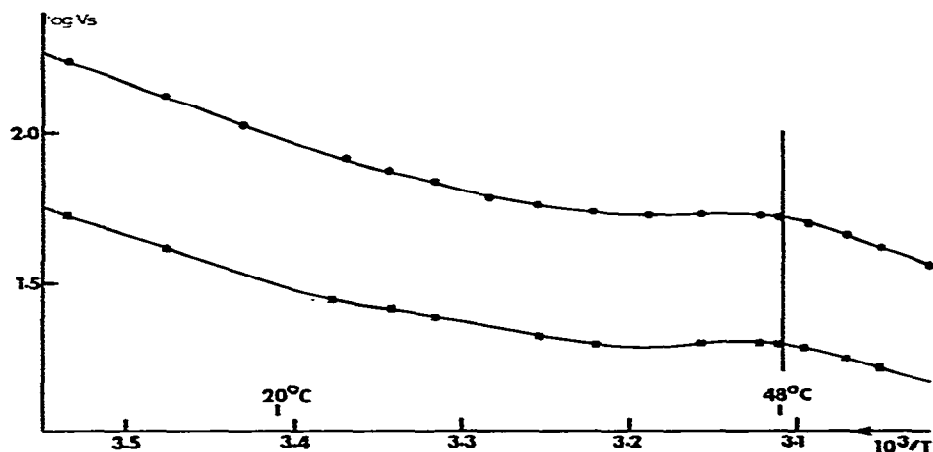


Fig. 2. Variation of the logarithm of the absolute retention volume per gram of silica, V_s , with $1/T$ for helium as carrier gas and \blacksquare *n*-hexane and \bullet *n*-heptane as solutes. The packing is the same as in the liquid chromatography experiments. Column: 15 cm \times 4 mm I.D.

47°C, that is, in the same temperature range as with helium. Obviously we observe the same phenomenon using both kinds of chromatography.

As far as gas chromatographic experiments are concerned, by analogy with well studied phenomena with physisorbed films¹²⁻¹⁴, we have concluded that the transition can be interpreted as a sort of two-dimensional melting, giving the solute access to new retention sites in the inner part of the layer of alkyl radicals².

The lack of any increase in transition temperature in liquid chromatography seems to indicate that methanol has less influence on the aggregation of the alkyl chains than the glycols tested in gas chromatography. On the contrary, a slight decrease of about 2°C shows that methanol behaves partly as an organic liquid phase³.

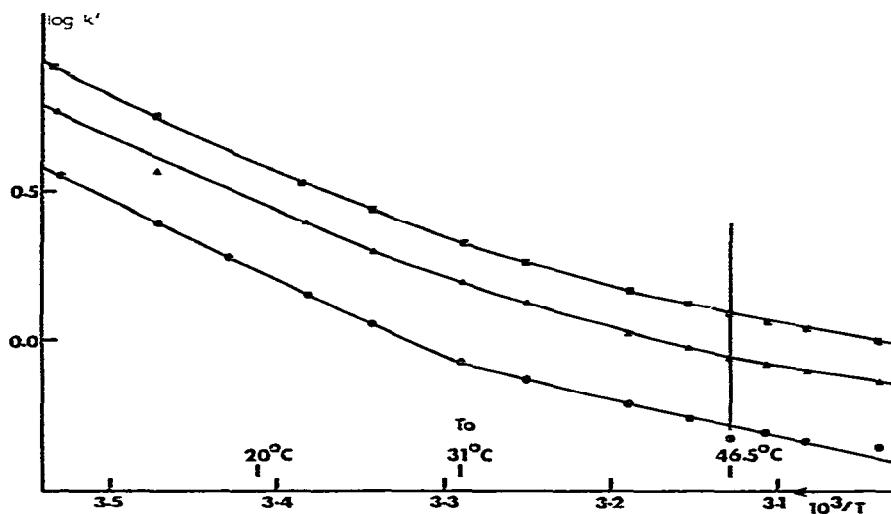


Fig. 3. Variation of $\log k'$ with $1/T$ for a pure methanol mobile phase and different long-chain linear solutes: ■, *n*-tetradecane; ▲, 1-chlorotetradecane; ●, 1-hexadecanol.

Long-chain solutes exhibit completely different behaviour in liquid chromatography (Fig. 3). The main feature of these curves is the very strong retention of these compounds at low temperatures and the very steep linear decrease in $\log k'$ up to a temperature T_0 lower than 46°C . Subsequently, the final linear decrease appears without any transition. The order of magnitude of the corresponding slope is the same as for short-chain and non-linear compounds. No very clear phenomenon can be observed at 46°C . Diagrams for C_{12} – C_{14} linear compounds present an intermediate feature with a non-linear decreasing part between T_0 and 46°C .

The anomalous behaviour of long-chain solutes is easy to interpret. These "solutes" in liquid chromatography are simply the "stationary phases" which have proved to be so easily inserted in the alkyl layer by gas chromatography of a volatile solute. However, as these experiments have shown, the thermal stability of the mixed phase (radicals and molecules) is limited if the concentration of the long-chain compound is low². For this reason, the strong retention of the solute, a consequence of its inserting tendency, terminates at relatively low temperature under the effect of thermal agitation. Between this temperature and 46°C there is a compensation between the decrease in the insertion effect and the increase in the retention connected with the phase transition of the normal bonded layer.

Water-methanol mobile phases containing less than 50% water*

Replacing methanol by methanol-rich mixtures as the mobile phase does not radically modify the phenomena. However, the capacity factor increases rapidly as a consequence of the increasing hydrophobic interactions. Testing long-chain solutes becomes a very difficult task.

Nevertheless, replacing the preceding solutes with shorter homologous compounds results in curves with an intermediate increasing part, very similar to Fig. 1, as

* Volume/volume before mixing.

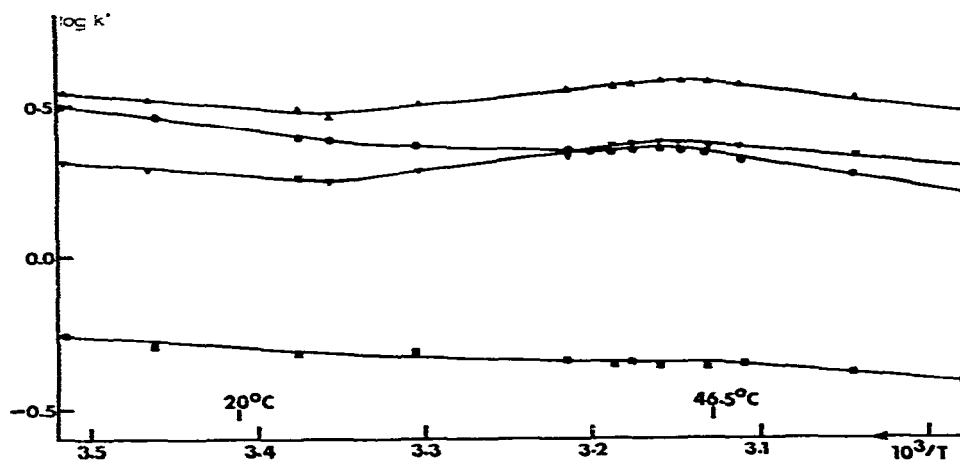


Fig. 4. Variation of $\log k'$ with $1/T$ for a methanol-water mobile phase (70:30, v/v) and different linear solutes: ●, 1-decanol; ■, 1-hexanol; ▲, *n*-hexane; ▼, *n*-pentane.

Fig. 4 shows for a particular composition of the mobile phase. The end temperature of the transition remains at 46°C.

Water-methanol mobile phases containing more than 50% water (excluding pure water)

At low temperatures, the linear decrease and the transition for short-chain compounds are replaced by a roughly horizontal line (Fig. 5). A linear decrease appears at higher temperatures, but from about 55% water its initial temperature is suddenly shifted from 46 to 53–54°C and does not change further with higher concentrations of water.

This shift has to be compared with the increase in the end temperature of transition observed by gas chromatography of an auxiliary volatile solute when the

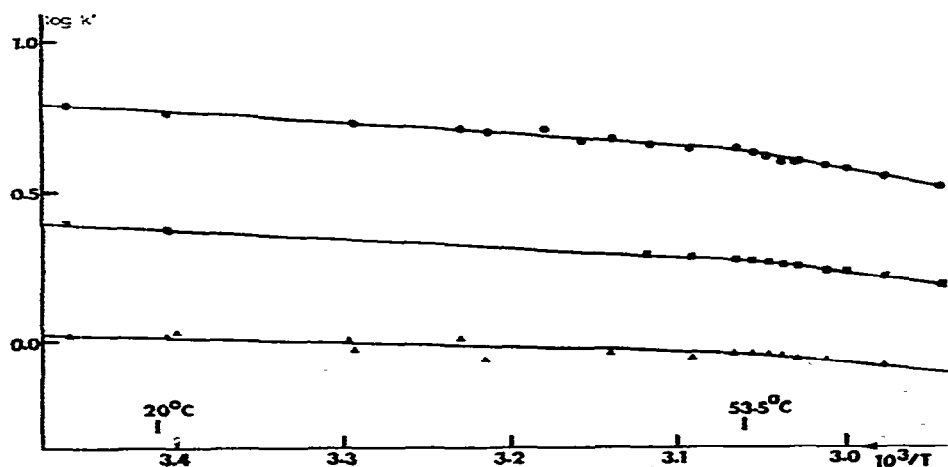


Fig. 5. Variation of $\log k'$ with $1/T$ for a methanol-water mobile phase (35:65, v/v) and different linear solutes: ●, 1-hexanol; ■, 1-pentanol; ▲, 1-butanol.

bonded silica is covered with a glycol or a glycerine–water mixture³. In fact, 54°C is lower than the temperature obtained in gas chromatographic experiments with the more associated liquid phases, namely 59°C. However, a rigorous comparison is difficult because we do not have good measurements with water or methanol as the stationary phase on bonded substrates by gas chromatography. In fact, retentions increase as the water concentration increases. Therefore, we are obliged to replace fatty alcohol solutes with homologous compounds with shorter chains and to give up the *n*-alkanes because of their insufficient solubility.

Pure water mobile phase

Qualitatively, replacing the preceding methanol–water mixtures with pure water as the mobile phase does not result in a dramatic change in the diagram. In fact, the capacity factors are still higher (Fig. 6a). However, the transition temperature (or rather, a break temperature) remains at 53–54°C. However, if the precise values of the retentions are investigated, it becomes essential to specify the order of the experiments with the different mobile phases.

The above described experiments are carried out with the same column and progressively higher concentrations of water in the mobile phase. Provision is made for equilibration of the packing material with any mobile phase. The interval between the experiments amounts to several hundred times the dead time. Accordingly, returning to pure methanol as the mobile phase leads to recovery of approximately the same capacity factors.

In contrast, if the column is first swept with methanol and dried thoroughly*, the use of pure water as the mobile phase leads to much lower retentions, as Fig. 6a shows. We can conclude that it is very difficult to eliminate the last traces of methanol included in the bonded layer. Our results agree well with several published observations^{15,16} on the strong and non-linear adsorption of the organic constituents and modifiers used in mobile phases. These adsorbates have been considered to play a part in the retention process by reversed-phase liquid chromatography.

In addition, it is worth noting that an increase in log capacity ratio (k') at intermediate temperatures may be observed with pure water as the mobile phase, as with methanol-rich mixtures. This increase is not very marked for linear fatty alcohols, but is much more perceptible for non-linear compounds (Fig. 6b). The slopes of the linear parts of the diagram vary from one solute to another, but with all solutes the transition is terminated at 53–54°C. However, our purpose is not to interpret these variations.

DISCUSSION

It is generally accepted that the mobile phase–mobile phase and mobile phase–solute interactions and their relative values for different solutes are the basis of separations by reversed-phase liquid chromatography. The theory of these separations has been established by Horváth *et al.*¹⁷ on the basis of the hydrophobic interaction concept. In addition, most workers consider that the separations may be complicated

* Advantage was taken of the opportunity to control the good condition of the column by a gas chromatographic test.

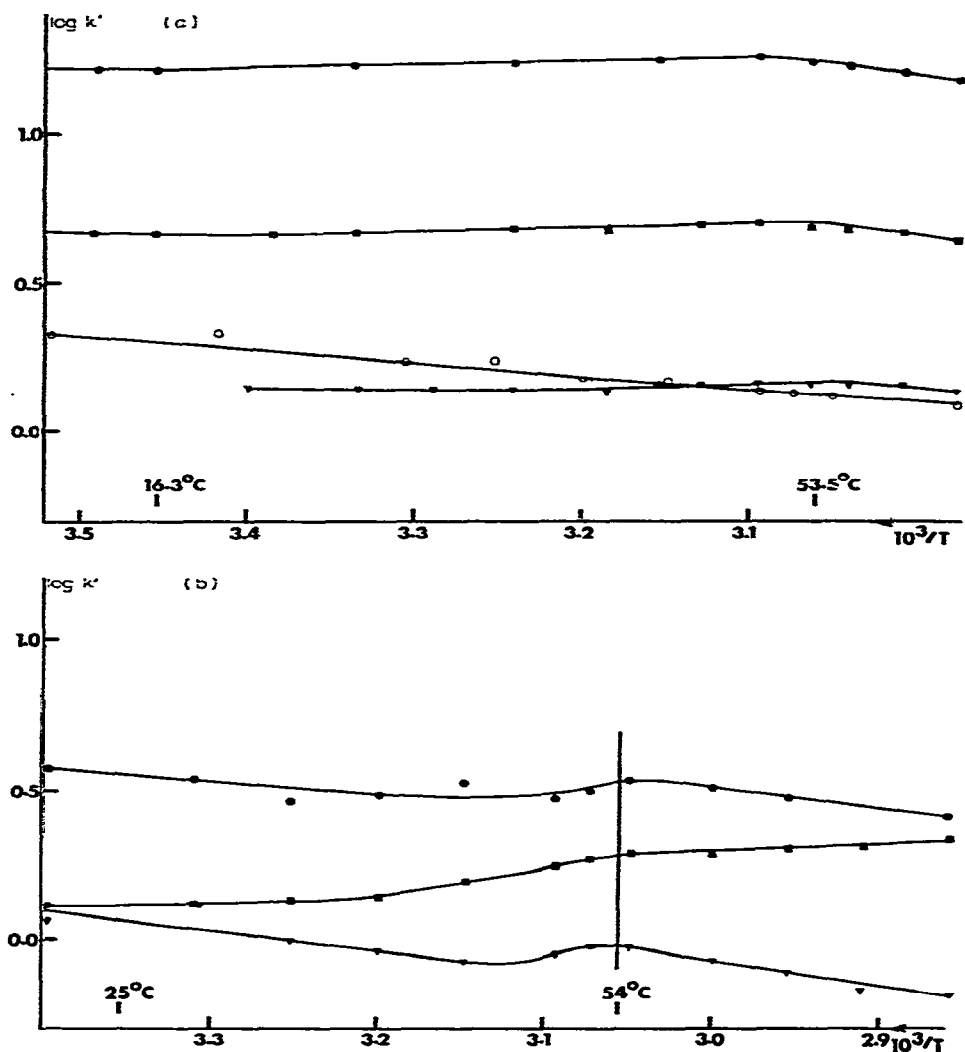


Fig. 6 (a). Variation of $\log k'$ with $1/T$ for a pure water mobile phase after a series of methanol-water experiments and linear solutes: ●, 1-pentanol; ■, 1-butanol; ▼, 1-propanol. After drying of the column, solute (○) 1-pentanol. (b) Variation of $\log k'$ with $1/T$ for a pure water mobile phase (used after drying of the column) and different non-linear solutes: ●, methyl isobutyl ketone; ■, diethyl ether; ▼, cyclohexanone.

by the parasitic influence of the adsorbing centres of the silica that escaped the silanization (in spite of the highly deactivating properties of the water molecules). The influence of the physisorbed organic material has also been demonstrated recently^{15,16}.

However, neglecting the influence of the stationary phase is somewhat paradoxical. Anyone who would call into question this influence could usefully examine Fig. 7, in which we have assembled the curves of $\log k'$ versus $1/T$ obtained for various solutes and pure methanol as the mobile phase. The order of elution is completely

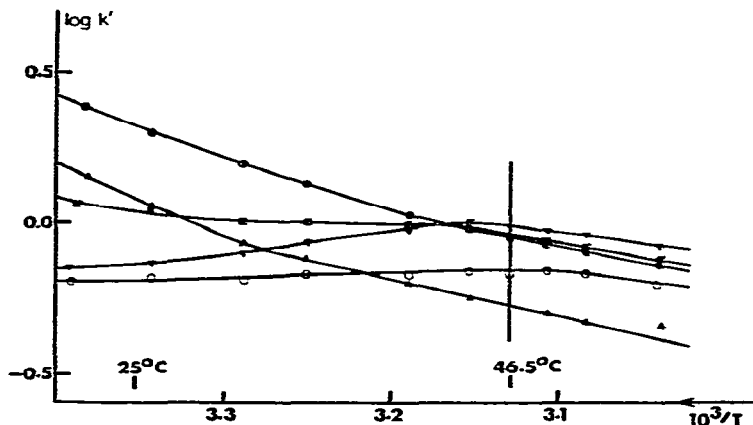


Fig. 7. Variation of $\log k'$ with $1/T$ for a pure methanol mobile phase and various solutes: ●, 1-chlorotetradecane; ■, *n*-dodecane; ▼, cyclododecane; ▲, 1-hexadecanol; ○, 9-bromoanthracene.

modified within 20°C without further change in the selectivities at higher temperatures. Neither the hydrophobic interaction theory nor any non-specific adsorption process is able to explain such an abrupt change. In addition, the difference in the retention properties of the linear and non-linear solutes is obvious from our experiments.

If the separations depend on solvophobic interactions, the magnitude of the retentions depends strongly on the amount of stationary phase that is able to interact with the solute molecules.

This situation is shown very clearly in the simplified theory recently published by Jandera *et al.*¹⁸, who proposed an empirical expression of ΔG , the free energy of transfer of a mole of solute from the mobile to the stationary phase. Thereafter, the calculation proceeds as follows:

$$\log k' = \log \varphi - \frac{\Delta G}{2.3 RT} \quad (1)$$

(see also ref. 17), or

$$k' = K\varphi \quad (2)$$

where K is a "partition coefficient" and φ a "phase ratio". However, Jandera *et al.*¹⁸ did not decide on the nature of the retention phase, surface or volume and, accordingly, they adopted a partly arbitrary definition of φ . Nevertheless, controversy in connection with the nature of the retention phenomenon, adsorption or solubility, is only partially academic.

It is academic because of the fact that the "sorption" process by the bonded layer can be neither a pure adsorption process nor a real solubility phenomenon. It cannot be a pure adsorption process because sorption by an organic material cannot be a pure surface process as it is on carbon or silica. Very strong insertion of long-chain alkanes in our grafted silica at low temperatures is one more demonstration of

this fact. "Sorption" by a monolayer also cannot be a solution process. Gas chromatographic specific retention volumes by liquid-expanded layers of amphiphilic compounds at the surface of silica have been shown to be much lower than the corresponding three-dimensional ones¹².

However, the above controversy does exist because it is possible to modify the nature of the phenomenon by changing the value of parameters such as the temperature or the composition of the mobile phase. In this instance φ is a function of T . Phenomena observed with our densely bonded C₂₂-alkyl silica exemplify this situation.

The above-mentioned "transition" is the transition from an adsorption-dominant retention at the surface of a "solid" monolayer to a solution-dominant retention in a liquid expanded-like layer.

However, the influence of the mobile phase composition on these phenomena is clear, as shown by the changes in the transition temperature observed in both gas and liquid chromatography when the bonded silica is immersed in an associated liquid. Obviously solvophobic interactions have an effect on the solute molecules and also on the bonded radicals. The first effect is responsible for the increase in retention with increasing water concentrations. The second effect is responsible for the decrease in retention in pure water when all the methanol has been removed from the graft.

The usual bonded silica does not give rise to phase transition. As we have shown in a previous paper (ref. 2, p. 103) that a less densely bonded layer remains in the "liquid" state at any temperature, our conclusion would be logically in favour of a solution-dominant retention in the usual reversed-phase liquid chromatography with alkyl-bonded silicas.

However, agreement between our measurements and the conclusions of many workers quoted in this paper lead us to think that the physical state of the chains and, consequently, the "phase ratio", may also vary under the conditions of current analytical practice, by virtue of the "solvation" or "crystallization" of the layer.

As Karch *et al.*⁸ wrote in 1976, "It is extremely dangerous to discuss the mechanism of chromatographic separations with reversed-phases without defining with great accuracy the quality of the stationary phase used". Unfortunately, this quality is more difficult to guarantee as the density of the layer decreases, especially if well defined large-pore silicas are preferred. Perhaps progress in this field will involve the careful synthesis of average density equally distributed grafts.

REFERENCES

- 1 E. sz. Kováts, *Ger. Pat.*, DE 29 30 516 A1 (1979).
- 2 D. Morel and J. Serpinet, *J. Chromatogr.*, 200 (1980) 95.
- 3 D. Morel and J. Serpinet, *J. Chromatogr.*, 214 (1981) 202.
- 4 F. Riedo, M. Czencz, O. Liardon and E. sz. Kováts, *Helv. Chim. Acta*, 61 (1978) 1912.
- 5 A. Nahum and C. Horváth, *J. Chromatogr.*, 203 (1981) 53.
- 6 R. K. Gilpin and J. A. Squires, *J. Chromatogr. Sci.*, 19 (1981) 195.
- 7 R. P. W. Scott and C. F. Simpson, *J. Chromatogr.*, 197 (1980) 11.
- 8 K. Karch, I. Sebastian and I. Halász, *J. Chromatogr.*, 122 (1976) 3.
- 9 B. Pommier, F. Juillet and S. J. Teichner, *Bull. Soc. Chim. Fr.*, (1972) 1268.
- 10 K. K. Unger, *Porous Silica*, Elsevier, Amsterdam, 1979, Table on p. 104 and following pages.
- 11 H. Engelhardt, *High Performance Liquid Chromatography*, Springer, Berlin, 1979, pp. 48 and 127.
- 12 J. Serpinet, *J. Chromatogr. Sci.*, 12 (1974) 832.
- 13 G. Untz and J. Serpinet, *J. Chim. Phys.*, 73 (1976) 912.
- 14 G. Untz and J. Serpinet, *Bull. Soc. Chim. Fr.*, (1976) 1742.
- 15 R. M. McCormick and B. L. Karger, *Anal. Chem.*, 52 (1980) 2249.
- 16 E. H. Slaats, W. Markovski, J. Fekete and H. Poppe, *J. Chromatogr.*, 207 (1981) 299.
- 17 Cs. Horváth, W. Melander and I. Molnár, *J. Chromatogr.*, 125 (1976) 129.
- 18 P. Janderá, H. Colin and G. Guiochon, *Anal. Chem.*, 54 (1982) 435.