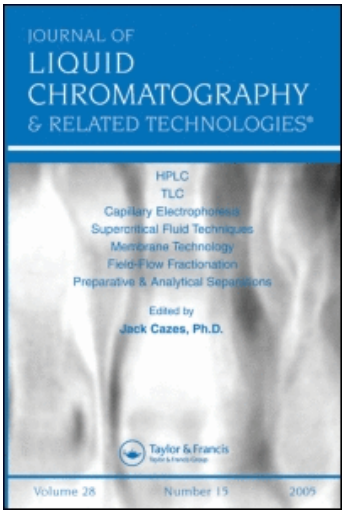

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THERMOTROPIC LIQUID CRYSTALLINE SIDE GROUP POLYMERS AS STATIONARY PHASES IN HIGH PERFORMANCE LIQUID CHROMATOGRAPHY. II. THE MECHANISM OF SEPARATION

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Abstract

Liquid crystalline side group polymers support coated on silica gels have been applied as stationary phases in high performance liquid chromatography. It has been possible to show that also in liquid chromatography, separations based on the mesophase structure can be observed in analogy to gas chromatography. From results of separations in which temperature, flow rate, sample concentration and the solvent strength of the mobile phase were varied, this work derives views on the fundamental mechanisms involved. In addition, it will be shown that different mechanisms are probably involved in the separation of steroids and dinitrobenzene isomers on these stationary phases.

Introduction

To date, reports on the application of liquid crystalline substances as stationary phases in high performance liquid chromatography (HPLC) are rare (1-5). In these reports it has been shown that also in HPLC, certain separation properties can be observed due to the special structure of liquid crystals (2,5). The results of temperature-dependent measurements correlate with the phase transition temperatures of the liquid crystalline components. However, due to the plasticizing effect of the liquids used as mobile phase, the transition temperatures observed in HPLC are lower. The results were obtained with steroid as well as dinitrobenzene isomer or nitrotoluene isomer samples in non-polar, partly modified, mobile phases on low-molecular and polymeric liquid crystals which were coated on silica gel.

This work reports on the separation of steroids and dinitrobenzene isomers on acrylate-based liquid crystalline side group polymers (LCP) coated on silica gel. In the measurements, influential parameter such as temperature, flow velocity, sample concentration and the solvent strength of the modified mobile phase were varied. The results of these measurements form the basis for the discussion of the separation mechanism.

Experimental

The liquid crystalline side group polymers used are polyacrylates (PAC_n) and were synthesized through radical polymerization by Siebke (6). The ester group consists of a alkyl spacer with a length n of two to six methylene units, phenyl hydroxy-benzoate as the mesogenic group and a methoxy terminal-group. Depending on their spacer length, the glass transition temperature of these polymers is between 300 and 350 K. The nematic-isotropic transition temperatures are within the range from 370 to 400 K. With the exception of PAC_2 (only nematic liquid crystalline phase) the polymers form a smectic and a nematic phase.

In order to apply these polymers as a stationary phase, they are adsorptively coated to irregular silica gel particles manufactured by Amicon in Witten; this silica gel has a particle diameter of 10 μm, a 50 nm nominal pore diameter and 75 m^2/g of specific surface area. In addition, a polyisobutylene acrylate (PiBA, Aldrich, Steinheim) was also used as a stationary phase on silica gel so that the separating properties of the LCPs could be compared with those of this non-liquid crystalline polymer, having the same backbone.

The preparation of the stationary phases, column preparation and the use of the HPLC apparatus has already been described (5). The HPLC measurements were carried out with steroids (testosterone, 17-methyl testosterone, (-)5-androstene-3β,17β-diol hydrate and 1,4-androstadiene-3,17-dione) in cyclohexane with 5 % by volume 2-propanol and the three dinitrobenzene isomers in n-heptan with 0,05 % by volume 1-heptanol. Sample compounds were obtained from Aldrich. The steroids were detected with a differential refractometer and the isomers with a UV filter photometer (254 nm). Columns (120mm x 4mm I.D.) were obtained from Säulentechnik Dr. Ing. Herbert Knauer GmbH, Berlin.

Results and Discussion

The sample compounds are eluted in the order of increasing dipole moment (for dinitrobenzene isomer separation) and increasing number of carbonyl groups conjugated to double bonds (for steroid separation), respectively. On methyl testosterone (steroid separation) the screening effect of the methyl group on the polar group is also obtained. Table 1 gives the order of elution using the example of column 226C20-1 (PAC_6). The capacity factor k' is used to quote retention.

TABLE 1

Order of elution on the column 226C20-1 (PAC_6) at 298 K

Steroid separation (0,495 cm^3/min flow rate)		Isomer separation (1,01 cm^3/min flow rate)	
Sample	k'	Sample	k'
Androstendiol hydrate	0,46	p-Dinitrobenzene	2,93
Methyl testosterone	0,77	m-Dinitrobenzene	4,21
Testosterone	1,05	o-Dinitrobenzene	7,85
Androstadienedione	2,13		

Temperature Dependence

The temperature-dependent measurements were evaluated using a Van't Hoffs equation (7) (equation I).

$$\ln k' = -\frac{\Delta G}{RT} + \ln \phi = -\frac{\Delta H}{R\,T} + \frac{\Delta S}{R} + \ln \phi \qquad (I)$$

Here ΔH and ΔS are the enthalpy and entropy changes, respectively, in the chromatographic processes. The phase ratio ϕ was assumed not to be affected by temperature changes.

Figure 1 shows a Van't Hoff plot of data obtained with androstadienedione and o-dinitrobenzene samples on unmodified silica gel (column 220-11), PiBA (column 227C20-1) and PAC_6 (column 226C20-1 and -4) as stationary phases. Deviations from the expected linearity occur within the whole temperature range. Whereas the deviations for columns 220-11, 227C20-1 and 226C20-4 (o-dinitrobenzene) can be attributed to a change in the adsorption-desorption equilibrium for the polar modifier on the stationary phase, the bend points (322 K and 336 K) in the curve for column 226C20-1 can be correlated with the phase

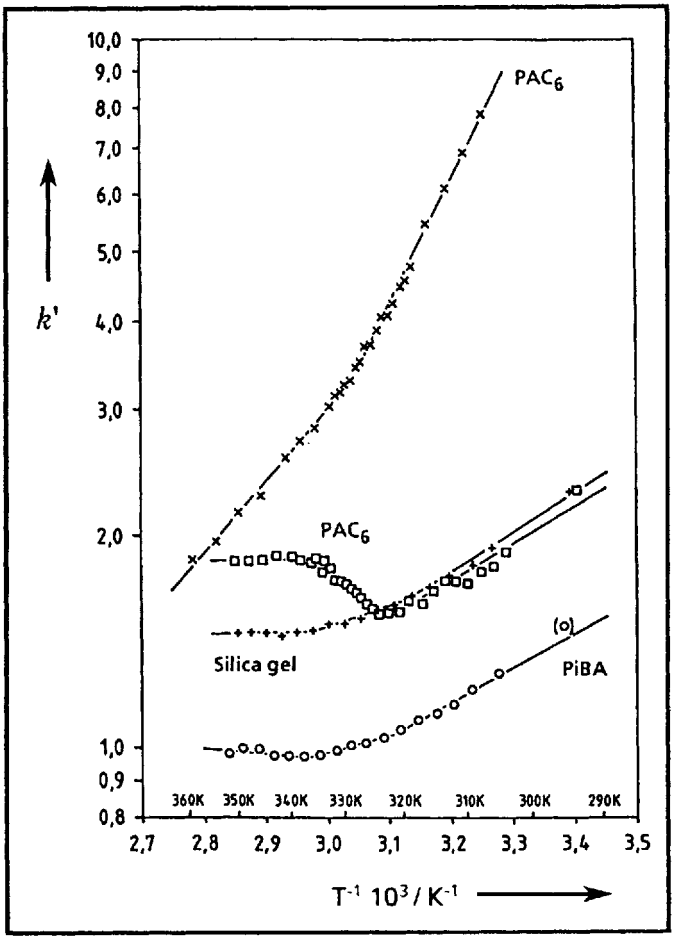

FIGURE 1: Van't Hoff plot for androstadienedione: columns 226C20-1 (PAC_6, □, 1,5 cm^3/min), 220-11 (carrier silica gel, +) and 227C20-1 (PiBA, o) at a flow rate of 0,5 cm^3/min; and for o-dinitrobenzene: column 226C20-4 (PAC_6, x) at 1,0 cm^3/min

transition temperatures (5) of the PAC_6 (smectic 360 nematic 387 isotropic). This curve pattern on PAC_6 column for steroid separation was also expected for isomer separation on column 226C20-4 but has never been observed. The curve pattern obtained for steroid separation is independent of the flow velocity of the mobile phase.

Similar deviations from linearity of the Van't Hoff plot have also been observed with RP 18 phases (9). These transitions are explained with phase changes, i.e. a type of melting in the alkyl phase, or they have been interpreted as an additional transition from an adsorption-dominated to a partition-dominated separating mechanism (10).

Flow Velocity-Dependent Band Broadening

The flow velocity influences the zone spreading experienced by a sample band within the column. The plate height H of the column is a measure for the band broadening. The velocity-dependent band broadening is described by the equations of Van Deemter (11) and other workers such as Giddings, Knox and Horváth (12) with three and more parameters. The equation that most accurately described the relationship between H and u over a velocity range from 0,02 to 1,0 cm/s is that of Van Deemter (12):

$$H = A + \frac{B}{u} + C\,u \qquad (II)$$

with A - eddy diffusion term,
B - longitudinal diffusion term,
C - mass transfer term and
u - flow velocity (linear).

Data plotted for the samples benzene as well as androstenediol hydrate and testosterone in a mixture are shown in figure 2. Benzene has with $k' \approx 0{,}15$ such a low retention that it can practically be regarded as being unretarded. The capacity factors for androstenediol hydrate and testosterone are approximately around 0,5 to 0,6 and 1, respectively.

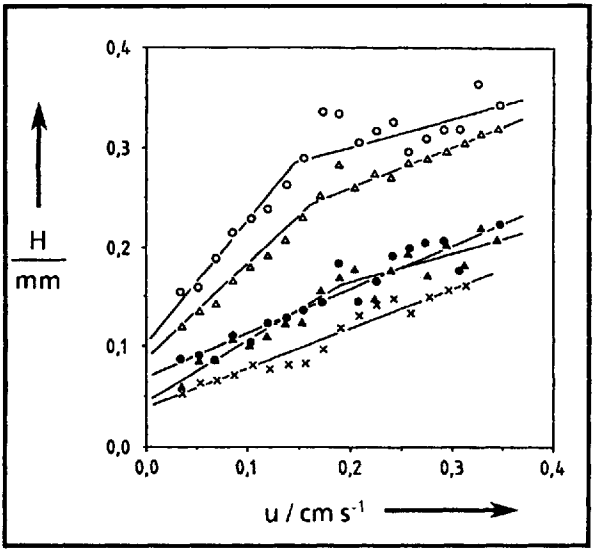

FIGURE 2: Flow velocity-dependence of the band broadening (van Deemter equation, equation II) for the columns 224C20-2 (PAC_4, benzene - x), 222C20-2 (PAC_2, testosterone - o and androstenediol hydrate - ●) and 223C20-1 (PAC_3, testosterone - Δ, androstenediol hydrate - ▲) at 298 K

In the velocity range being investigated, the Van Deemter equation (equation II) should describe the experimentally determined data of H and u. It is noticed first, that the longitudinal diffusion term B can here be neglected. While the linear Van Deemter equation sufficiently describes the curve path for benzene, two linear ranges with different slopes can mostly be observed for the steroids. The intersections are within the velocity range of $0{,}185 \pm 0{,}03$ cm/s. The average slope ratio is $2{,}6 \pm 0{,}8$:1. Simple equations with three or two parameters, respectively, are no longer sufficient for describing these curve path. Since it is very difficult to calculate the coefficients for multiparameter models

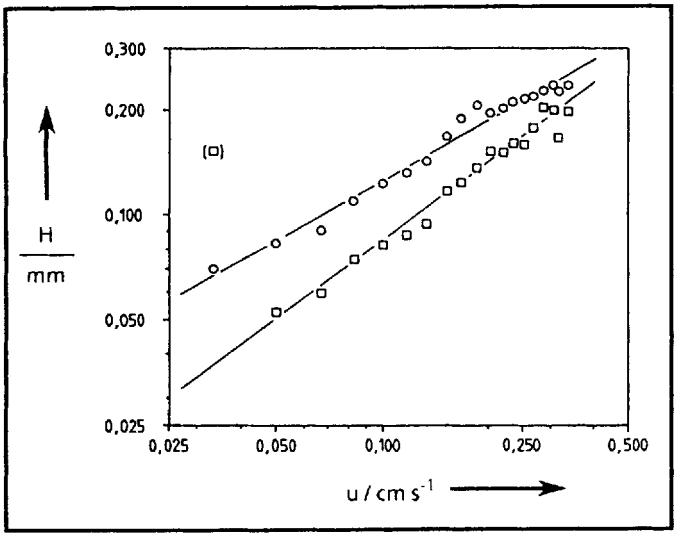

FIGURE 3: Plate height against linear flow velocity in according to equation IV for testosterone (O) and androstenediol hydrate (□) of the column 224C20-3 (PAC_4) at 298 K

(13) analytically, Snyder (13) has suggested an empirical relationship for describing the curve path.

$$H = D\, u^{0,4} \qquad \text{(III)}$$

Coefficient D corresponds to the plate height for a linear flow velocity of 1 cm/s. Data presented by other authors (14,15) have shown that the value of the exponent is not always 0,4. Often, for modern HPLC columns this value is in the range 0,3 to 0,6. Equation III is thus stated generally as

$$H = D\, u^{v} \qquad \text{(IV)}$$

Figure 3 shows the log-log plot for column 224C20-3 (PAC_4) according to equation IV. Linear curves are obtained. For androstenediol hydrate,

exponent v has the value 0,45; for testosterone it is 0,58. The mean value for both substances for all the columns measured is 0.5. The D coefficients are within the range 0,25 to 0,55; they are always higher for testosterone than for androstenediol hydrate. A dependence of the plate height on the capacity factor can be observed. The higher the retention, the higher the plate height will be. For steroid separation, this would indicate diffusion controlled mass transportation in the stagnating mobile phase (16).

These measurements were performed using steroid samples only. In view of the long retention times involved, it seemed of little sense to investigate the isomers. It would also be difficult to evaluate them.

Concentration Dependence

Besides flow velocity, sample concentration is another factor which can influence the plate height and thus the separation performance of the column. As can be seen in figure 4 for the example of androstadienedione, the plate height increases with increasing concentration. At the same time, a reduction in the retention time is observed. A qualitative comparison between the curves for adsorption chromatography with convex instead of linear isotherms (17) and partition chromatography on heavily loaded columns (18) show no difference. Although two different separation mechanisms are involved, the dependences are almost the same. The measurements under discussion show that the concentrations must be above the range of linear capacity, in which retention time and plate height are almost independent of the concentration.

A lowering of the capacity factor indicates a reduction of the number of "free" active centers in the stationary phase, since, with the growing concentration of the sample solution, the former are increasingly coated with sample molecules. Further sample molecules then apparently find less active centers for interaction.

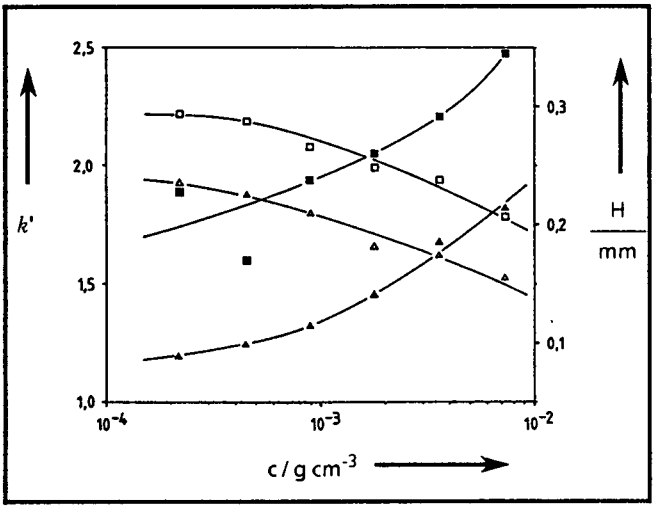

FIGURE 4: Dependence of the capacity factors and the plate height of sample concentration on the columns 222C20-3 (PAC_2, k' - □, H - ■) and 225C20-1 (PAC_5, k' - Δ, H - ▲) by a flow rate of 0,5 cm^3/min at room temperature

If a sample mixture is injected instead of a pure substance, the capacity factor for each compound increases. It seems strictly speaking not a concentration effect; instead, it is caused by intermolecular interaction between the sample molecules in the stationary and the mobile phase. The sample molecules in the stationary phase increase its capacity.

Variations of Solvent Strength

If, as in the case above, the mobile phase is a modified system, the modifier concentration has a large effect on the solvent strength of the mobile phase and thus on the separation behaviour. Table 2 shows the elution strength ε^0 of the four components on silica gel. The elution strength for 1-heptanol ist not tabulated and has been estimated in

TABLE 2

Elution strength of the four liquids used for mobile phase in accordance to Snyder (19)

Component	ε^0
Cyclohexane	0,03
2-propanol	0,63
n-heptane	0,01
1-heptanol	0,35

accordance with Snyder (19,20) from equation V using the data for the cross-sectional area requirements A_s of an adsorbed molecule on surface and the dimensionless free energy of adsorption S^0 of a sample component of the substance during adsorption out of pentane on an adsorbent with standard activity $a^{\#} = 1$.

$$\varepsilon^0 = \left(\frac{S^0}{A_s}\right)_{s=E} \tag{V}$$

Equation VI can be used to calculate the solvent strength ε_{ab} of a binary mixture (19,20).

$$\varepsilon_{ab} = \varepsilon_a^0 + \frac{lg\left(x_b\, 10^{a^{\#} n_b\left(\varepsilon_b^0 - \varepsilon_a^0\right)} + 1 - x_b\right)}{a^{\#} n_b} \tag{VI}$$

$a^{\#}$ is the adsorbent surface activity function, n_b is the effective molecular area of an adsorbed modifier molecule b and x_b is the molar fraction of the modifier. Figure 5 shows the relationship between the solvent strength and the varied volume proportion of the modifier for both mobile phases.

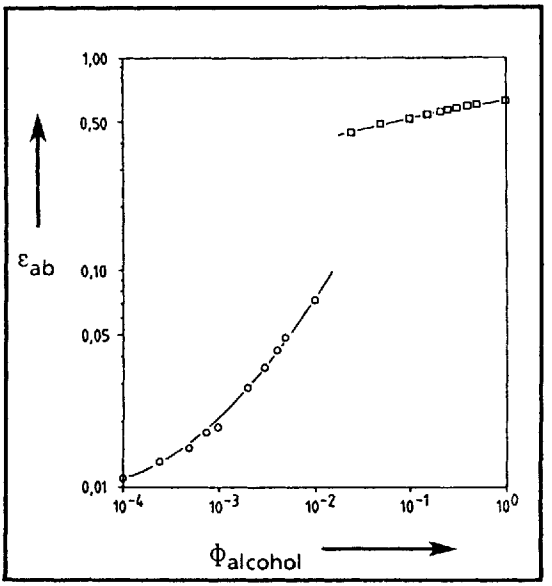

FIGURE 5: Solvent strength of the systems n-heptane/1-heptanol (o) and cyclohexane/2-propanol (□) against volume fraction of alcohol, respectively

Although in the case of heptane-heptanol mixture the volume fraction of alcohol is lower by a factor of approximately 100, the solvent strength is only lower by a factor of approximately 10.

Figure 6 shows the change in retention behaviour with increasing solvent strength of the mobile phase at room temperature. The capacity factor decreases exponentially with increasing solvent strength. If the modifier fraction is higher than a certain value, some individual sample compounds are eluted together. The polar modifier is adsorbed on the active centers of the stationary phase and shields the surface. The interface towards the eluent becomes less polar and the sample molecules are eluted more rapidly. An additional effect arises in the cyclohexane

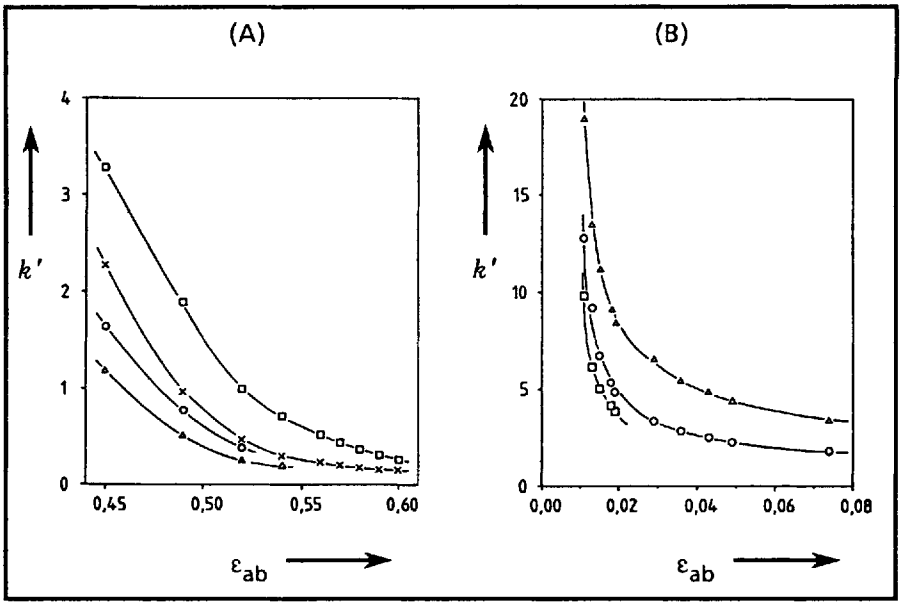

FIGURE 6: Solvent strength dependence of the capacity factors of the components in the sample mixture: (A) 226C20-1 (PAC_6, steroid separation) und (B) 225C20-7 (PAC_5, isomer separation)

2-propanol mixture: with increasing alcohol content, the steroids become more soluble in the eluent.

The following two models (21-23), which assume that the separation mechanism is based on adsorption chromatography, attempt to describe the retention behaviour for variations in the fraction of modifier:

- *The solvent interaction model:*

  When the surface of the adsorbent is fully coated with modifier (mono layer), no displacement of adsorbed modifier molecules by sample molecules takes place. Retention is mainly determined by interactions between molecules of the mobile

phase and the sample. No change in the interactions with the stationary phase arises as the proportion of modifier increases. When the surface is completely covered with modifier molecules, there is a linear relationship between the inverse of the capacity factor and the volume fraction of the modifier.

- *The competition model* (24):

The solvent molecules and the dissolved molecules compete for adsorption sites on the surface. In binary mixtures, "solvent" refers to the modifier. When the capacity factor is log-log plotted against the mole fraction of the modifier, k' is constant for very small mole fractions. This is followed by a transition range until a linear dependence with a negative gradient is reached.

Figure 7 shows data plotted according to the solvent interaction model. The conditions required for both sample mixtures are not maintained for the whole range. This model is thus not able to describe the sorption process in the column.

A presentation using the competition model is shown in figure 8; it meets the linearity requirement for isomer separation completely. In this case the model does seem to describe the retention. In the case of steroid separation, the linearity requirement is met over the whole range of eluent composition, only if individual steroids are being eluted. When first androstenediol hydrate and methyl testosterone and at a higher modifier fraction also testosterone are being eluted together, the slope of the resulting curve changes at this point. Only androstadienedione is the exception in this case. The curve slope does not change. Thus this model appears to be only partly applicable for steroid separation.

View on the Mechanism of Separation

If the order of elution of the samples is used as a basis for consideration, then both separations should take place in accordance with the

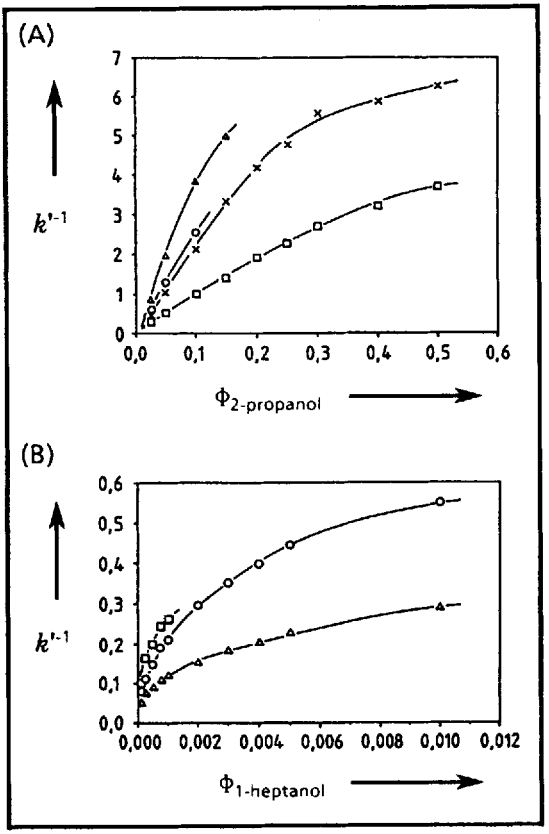

FIGURE 7: Use of the solvent interaction model: (A) steroid separation [testosterone (X), methyl testosterone (O), androstenediol hydrate (Δ) and androstadienedione (□)] on the column 226C20-1 (PAC_6), (B) isomer separation [o- (X), m- (O) and p- (Δ) dinitrobenzene] on the column 225C20-7 (PAC_5)

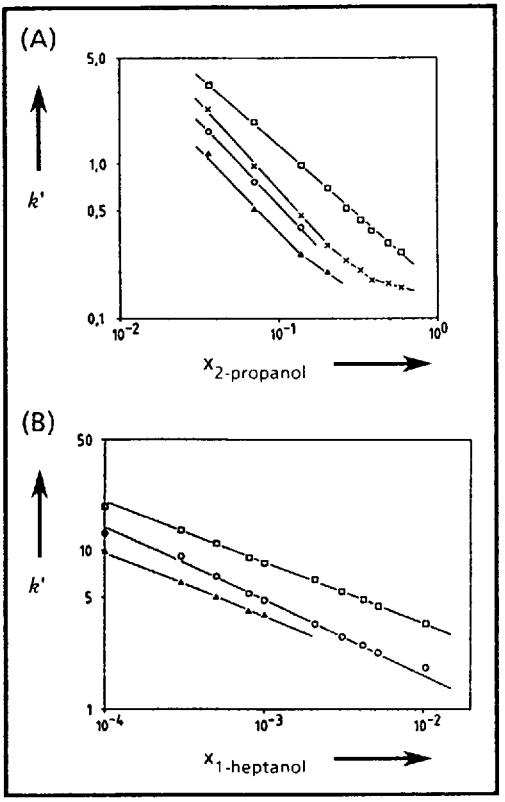

FIGURE 8: Use of the competition model: (A) steroid separation [testosterone (X), methyl testosterone (O), androstenediol hydrate (Δ) and androstadienedione (□)] on the column 226C20-1 (PAC_6), (B) isomer separation [o- (X), m- (O) and p- (Δ) dinitrobenzene] on the column 225C20-7 (PAC_5)

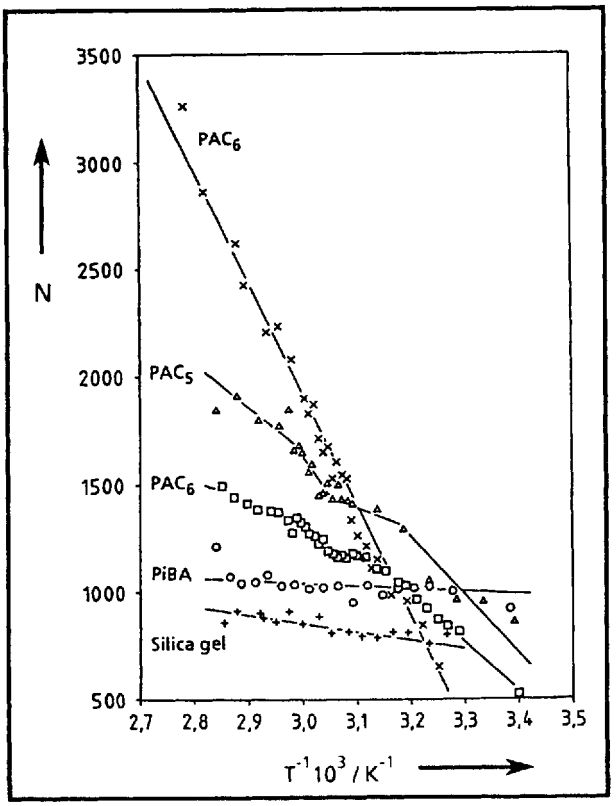

FIGURE 9: Number of plates against the inverse temperature: isomer separation (o-dinitrobenzene) - column 226C20-4 (PAC_6, x) at flow rate of 1,0 cm^3/min and steroid separation (androstadienedione) - columns 225C20-2 (PAC_5, Δ), 226C20-1 (PAC_6, □), 227C20-1 (PiBA, o) as well as 220-11 (silica gel, +) at flow rate of 0,5 cm^3/min

adsorption mechanism. However, a close look at the retention behaviour in dependence of the fraction of modifier does give room for doubt, at least with respect to the steroid separation on the physisorbed PAC_n phases. The surface should already be fully coated when the percentage of modifier is between 0,5 to 1,0 %, and the capacity factors should only drop slightly when the fraction of modifier is increased (25,26), as is the case for the isomers. Besides adsorption, there must be other interactions which play a part in steroid separation - in contrast to isomer separation. This is also confirmed by the temperature dependence of the number of plates N, as shown in figure 9 for a few examples.

The curves found for the adsorbed polymer phases differ from each other quite clearly. Whereas in the stationary phases with motionless active centers (for example with the PiBA phase or also silica gel) the number of plates remains virtually constant if temperature rises, it increases for the LCP phases. Since the mobility of the flexible side group increases with increasing temperature, the length of each individual interaction becomes succesively shorter, and the establishment of equilibrium occures faster. Therefore the number of plates increases, as is the case with isomer separation. However, in isomer separation the number of plates increases five-fold for a temperature increase from approximately 300 to 350 K, while it increases by only a factor of 2 to 2,5 for steroid separation.

It is probable that isomers are separated on the physisorbed liquid crystalline phases by the adsorption mechanism. This must involve physisorption processes with relatively strong interactions, because the calculated enthalpy changes are on the upper limit for sorption enthalpy changes of approximately -30 to -40 kJ/mol. In comparison with it the separation enthalpy changes for steroids only reach a range from -15 to +50 kJ/mol (5). No continuous increase can be observed in the relationship between N and T^{-1}, besides in steroid separation. Similar slope changes are observed in retention behaviour with tem-

perature on the LCP phase (see figure 1, column 226C20-1), which for liquid crystalline polymer phases are attributed to strutural changes of the polymer at the surface (5). Then it appears that, in this case, separation may take place by the partition mechanism - probably in combination with adsorption; because for partition the separation is mainly depended on the structure of the stationary phase, but for adsorption chromatography only the number and accessibility of the active centers are solely important.

Conclusion

In HPLC, steroid separations on liquid crystalline stationary phases probably take place via a partition mechanism. It may be that this dominating mechanism is accompanied by some adsorption effects. On the other hand, the isomer separation of dinitrobenzenes occurs almost exclusively by the adsorption mechanism. These conclusions are the results of investigations in which temperature, flow velocity, sample concentration and volume fraction of the polar modifier of the mobile phase were varied.

Acknowlodgement

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