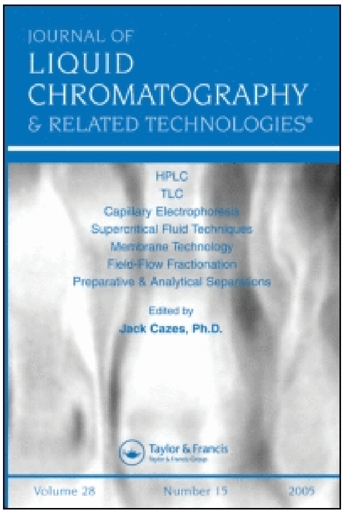

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THERMOTROPIC LIQUID CRYSTALLINE SIDE GROUP POLYMERS AS STATIONARY PHASES IN HIGH PERFORMANCE LIQUID CHROMATOGRAPHY. I. THE TEMPERATURE DEPENDENCE

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Abstract

Till now, thermotropic liquid crystalline side group polymers have been rarely used in gas chromatography and have never been applied as stationary phases in high performance liquid chromatography. This work presents the results of temperature-dependent measurements on surface modified silica gels serving as stationary phases. These silica gels have been coated with polyacrylates with mesogenic side groups of phenyl benzoate type and various spacer lengths. Steroids and dinitrobenzene isomers were used as sample substances for testing the chromatographic properties of these stationary phases. It will be shown that chromatographic characteristics correlate with the phase transition temperature of the liquid crystalline polymers.

Introduction

The first attempts to utilize low-molecular, liquid crystalline substances as stationary phases in gas-liquid distribution chromatography (GLC) were carried out by Kelker (1,2) in 1963. Since then, numerous, works and applications have been published (3-6), partially with previously infeasible separation results. Originally, mainly nematic liquid crystals were used as stationary phases, while Kraus et al. (7) were the first to conduct an intensive study of the separation properties of various smectic modifications. In order to widen and raise the temperature range of the anisotropic phases, the low-molecular liquid crystalline substances have been replaced with liquid crystalline side group polymers (8).

Besides being used for the purpose of studying and separating mixtures, gas chromatography (GC) can also be utilized for determining specific data of substances used as stationary phases. For non-liquid crystalline polymers, it is possible to determine their respective structure, i.e. the glass transition temperature, the degree of crystallinity as well as the melting temperature from the temperature-dependent retention behaviour (9-11). When liquid crystals are applied, the phase transition temperatures can be determined and it is possible to obtain informations on the structural similarities between the individual mesophases, as shown by Kraus et al. (12), who used 4-n-heptyloxybenzylidene amino-4'-cyano-biphenyl (k 346 s_E 368 s_B 384 n_{RE} 413 s_A 485 n 546 i) as an example.

In GC, the separation properties of the stationary phases depends heavily on their state of aggregation and their structure. Although in high performance liquid chromatography (HPLC) the separation should depend on the interactions between the molecules of the sample substances and both the stationary as well as the mobile phases, experiments have already been carried out in analogy to GC by using low-molecular liquid crystals as stationary phases on silica gel. The results of the experiments by Taylor and Sherman (13) who used steroids on cholesteric phases could not be related to the liquid crystalline structures of the stationary phase. However, experiments performed by Aratskova et al. (14) using dinitrobenzene isomers on a nematic (EBBA) and a cholesteric phase brought results, which can be compared to those obtained using GC. Due to the adsorption effects of the silica gel and the influence of the mobile phase, the temperature intervals of the anisotropic phases obtained with the aid of HPLC may be shifted either to higher or to lower temperatures, respectively, as compared with those of the pure liquid crystals.

This work reports the results of temperature-dependent measurements carried out on thermotropic liquid crystalline side group polymers (LCPs) on silica gel. Except for the spacer length of two methylene units, LCPs form both a smectic as well as a nematic phase. The results of the temperature-dependent series of chromatographic measurements are discussed in comparison with the phase transition temperatures of the polymers.

Experimental

Chemicals

The LCPs used were polyacrylates with mesogenic side groups of the phenyl benzoate type with a methoxy terminal-group and an alkyl spacer length *n* of two to six methylene units (PAC_n, figure 1). These polymers were synthesized by Siebke (15). Table 1 gives the phase transition temperatures (T_u) and the molecular weights, weight average (M_w), as well as the homogeneity (E) of the polymers. These results have been obtained from gel permeation chromatography using calibration curves of the same liquid crystalline polyacrylates with spacer length two (PAC_2) and six (PAC_6).

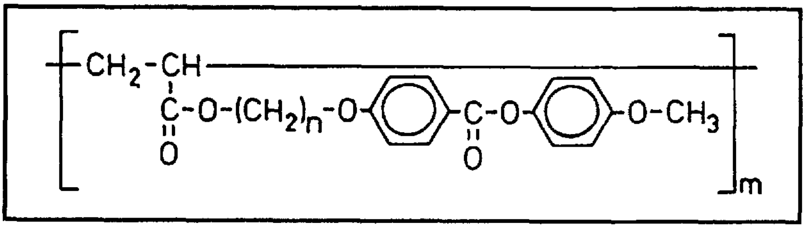


FIGURE 1: Repeated structural unit of the liquid crystalline side group polymers PAC$_n$ with spacer lengths n = 2, 3, 4, 5, 6

TABLE 1

Characterization of the polymers used as stationary phases

Polymer	Phase transition temperature$^{+)}$ T_u / K	PAC$_2$-calibration		PAC$_6$-calibration	
		M_w/g mol^{-1}	E_{GPC}	M_w/g mol^{-1}	E_{GPC}
PAC$_2$	g 337 n 391 i	279100	1,76	3408000	7,58
PAC$_3$	g 345 s 360 n 373 i	204500	1,86	2373000	10,37
PAC$_4$	g 330 s 350 n 397 i	126400	1,83	194900	2,14
PAC$_5$	g 305 s 379 n 388 i	129500	1,75	186100	1,94
PAC$_6$	g 299 s 360 n 387 i	73700	2,02	92900	2,02
PiBA$^{*)}$	g 250 i	116000	3,70		

*) fabricator data

+) g - glassy state, s - smectic, n - nematic und i - isotropic

The additional non-liquid crystalline polyisobutylene acrylate (PiBA) is an auxilliary standard made by Aldrich, Steinheim. It was also used for creating stationary phases so that the retention properties of the stationary phases obtained with LCPs could be compared to those of a non-liquid crystalline one, having the same backbone.

The solid support used is a silica gel with irregulary formed particles, manufactured by Amicon, Witten, with a nominal pore diameter of 50 nm, 75 m^2/g of specific surface area and a mean grain diameter of 10 µm.

The fluids (purity grade "for analysis", Merck, Darmstadt) used for the mobile phases were distilled over sodium or kept over molecular sieves for drying purposes. The sample substances used were obtained from Aldrich, Steinheim.

Column Preparation

The silica gels were coated with the polymers using a process devised by Jungheim and Engelhardt (16). In this process, the silica gel was suspended in benzene (PiBA: toluene) solution of polymer in an ultrasonic bath. Then the solvent was slowly evaporated until the gel became free-flowing. It was then dried in vacuo.

The 4 mm ID x 120 mm vertex columns manufactured by Säulentechnik Dr. Ing. Herbert Knauer GmbH, Berlin, were packed at room temperature using the slurry method (17) in which 2-propanol is used as the suspension medium and methanol is applied as the pressure fluid.

Instrumentation

The modular HPLC apparatus consists of equipment produced by Wissenschaftliche Gerätebau Dr. Ing. Herbert Knauer GmbH, Berlin (pump type 52.00 or 364.00, injection system - 20 µl, a column oven for 300 mm columns and a 2-channel compensation linerecorder) and by Waters in Eschborn (differential refraktometer RI model 401 and UV

TABLE 2

Characterization of the chromatographic systems

	Steroid separation	Isomer separation
Stationary phases	1) Silica gel 2) Coated (liquid crystalline) polymers	Coated liquid crsytalline polymers
Mobil phase	Cyclohexane	n-Heptane
Modifier	5 Vol.-% 2-propanol	0,05 Vol.-% 1-heptanol
Sample mixture	1) Testosterone 2) 17-Methyl testosterone 3) (-)5-Androstene-3β,17β-diol hydrat 4) 1,4-Androstadiene-3,17-dione	1) 1,4(p)-Dinitrobenzene 2) 1,3(m)-Dinitrobenzene 3) 1,2(o)-Dinitrobenzene
Flow rate	c. 0,5 cm^3/min	c. 1,0 cm^3/min
Temperature range	293 K to 353 K	293 K to 363 K
Detection	RI	UV

detector model 440) as well as a flowmeter manufactured by Phase Separation Ltd., Queensferry-Clwyd (UK), a digital thermometer of the type 7300 (Pt 100) made by Kobold in Kelkheim and a manometer produced by VDO in Hannover.

Chromatographic System

The choice of fluids used for the mobile phases and the sample substances was in accordance with the work carried out by Taylor and

TABLE 3

Datas of the modified silica gels (physisorption)

Polymers	γ_C / %	γ_P / %	d_F / nm	Identification number$^{+)}$
PAC_2	9,97	15,0	1,9	222C20
PAC_3	9,87	14,6	1,9	223C20
PAC_4	10,08	14,8	1,9	224C20
PAC_5	11,81	17,3	2,3	225C20
PAC_6	11,05	15,9	2,1	226C20
PiBA	10,63	16,2	2,2	227C20

+) Identification number for the column

Sherman (13) (steroid separation) as well as Aratskova et al. (14) (isomer separation). Table 2 presents the two systems investigated. The mobile phases selected must not be solvents for the polymers applied as the stationary phases. The alcohols had to be added to the mobile phases due to the fact that the steroids are insoluble in alkanes, so that the retention time during isomer separation would have been too long ($k' > 10$). The solvent composition for both sample mixtures differed little from that for modified mobile phases. The retention time of the resulting peaks of the two systems was taken as dead times.

Results and Discussion

Before the modified silica gels were used as stationary phases, they were characterized by elementary analysis. The mass proportion γ_P of the polymers was calculated from the carbon contents of the coated silica gels $\gamma_{C.KG\text{-}P}$ and the carbon contents of the pure polymers $\gamma_{C.P}$

$$\gamma_p = \frac{\gamma_{C,KG-P}}{\gamma_{C,P}}\ 100\% \qquad (\mathrm{I})$$

Using this value, the thickness d_F in nm of the polymer film

$$d_F = \frac{\gamma_P\ 10^3}{\left(100\% - \gamma_P\right)\rho_P\ O_{SP}} \qquad (\mathrm{II})$$

can be estimated. Here ρ_P is the density of the polymer component in g/cm^3 and O_{SP} is the spezific surface area of the silica gel in m^2/g. Table 3 lists the values and idenfication numbers of the stationary phases.

The calculated polymer proportion were illustratively checked for the stationary phase 225C20 (PAC$_5$) using differential thermogravimetry under atmospheric pressure up to 1250 K. The resulting polymer proportion of 17,5 % by weight agrees well with the calculated value of 17,3 % by weight.

The effect of adsorption forces (range approximately 2 nm) on the polar silica gel surface influenced creation and structure of the liquid crystal film (18,19). The approximately 2 nm thick polymer film was chosen under the expectation that the adsorption forces would counteract the disturbing influence of the mobile phase having on the creation of the liquid crystalline phase. Caloric measurements carried out on LCP liquid mixtures using differential thermoanalysis have shown, that the liquid crystalline phases can no longer be detected, when there is added just 10 % by weight of a liquid.

The first series of HPLC measurements for the steroid separation carried out on the coated LCP silica gel stationary phases showed that the samples are eluted in the order of increasing number of carbonyl groups conjugated to double bonds. In methyl testosterone the screening effect of the methyl group on the polar group is obtained. The capacity factors decreases linearly with increasing spacer length n (figure 2). It was found that the capacity factors on the LCP phases are lower than on

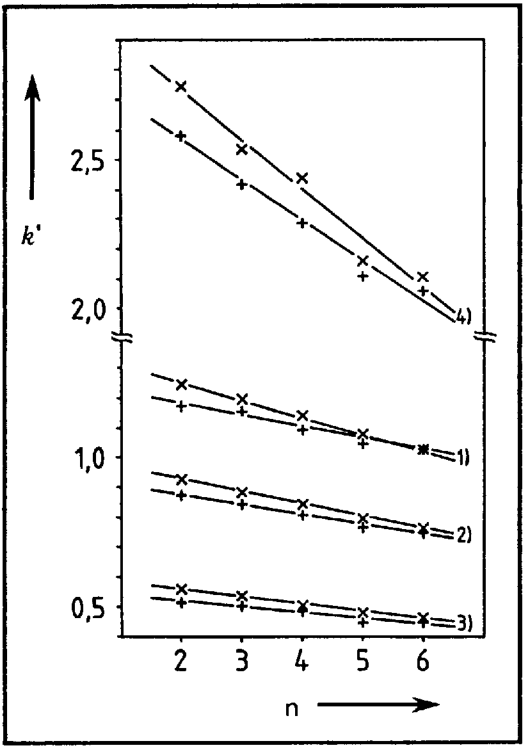


FIGURE 2: Results of the first series measurements (steroid separation, sample attachment see table 2) using the coated LCP phases, first measurement (X) and second measurement, one week later (+)

pure silica gel and higher than the values on the PiBA phase. This seems to indicate that the polarity of the stationary phases decreases from the silica gel via the PAC_2 and the PAC_6 down to the PiBA. The dinitrobenzenes are eluted in the order of increasing dipole moment.

The temperature-dependent measurements revealed a narrowing of the elution zone as well as a reduction in the retention time of the

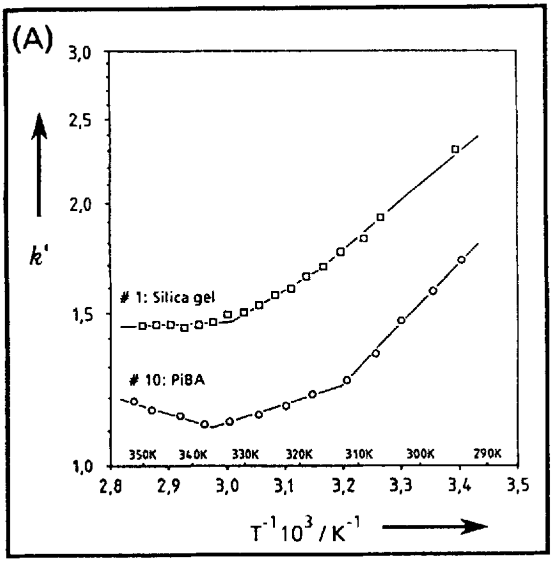


FIGURE 3: Temperature dependence of the capacity factor of androstadienedione on following columns (nomenclature see table 4):

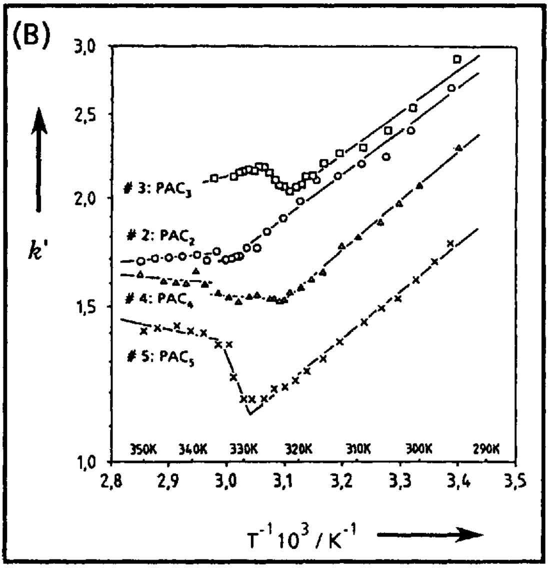
(A) - 220-11/② (□) and 227C20-2 (o),

(B) - 222C20-2 (o), 223C20-3 (□), 224C20-2 (Δ) and 225C20-4 (X)

(C) - 225C20-6/① (□), 225C20-6/② (o), 226C20-3 (Δ) and 226C20-1 (X, 1,5cm^3/min flow rate)

substances with increasing temperature. Only the data for the substances with the longest retention times of the two mixtures (1,4-androstadiene-3,17-dione und 1,2-dinitrobenzene) are used for the evaluation, since the effects described in the following are the most discernible with these substances.

The experimentally determined capacity factor *k*' is linked to the change in free enthalpy of the process via the distribution coefficient *K* (20-22)



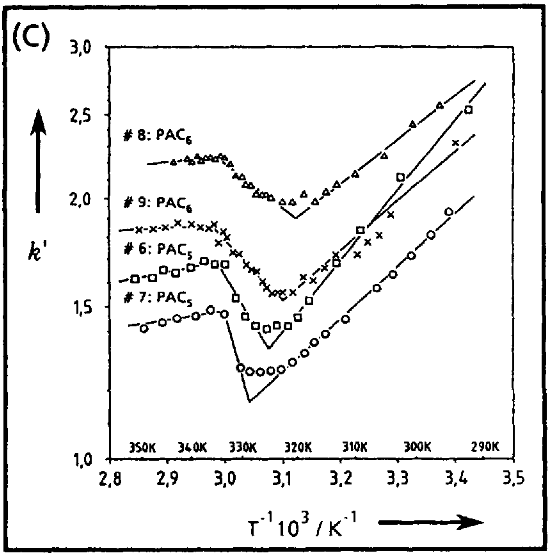


FIGURE 3 (continued)

$$\Delta G^0 = -RT \ln K = -RT \ln \frac{k'}{\phi} \qquad \text{(III)}$$

and

$$\ln k' = -\frac{\Delta H^0}{RT} + \frac{\Delta S^0}{R} + \ln \phi \qquad \text{(IV)}$$

Here, ϕ is the volume ratio of the stationary phase to the mobile phase in the column. ΔH^0 and ΔS^0 are the changes in enthalpy and entropy related to the retention process. A plot of the logarithm of the capacity factor against the inverse of the temperature is referred as a Van't Hoff plot. If the mechanism of the process is invariant over the temperature range investigated and the enthalpy change is constant, this plot yields a straight line.

The results obtained in the steroid separation are shown in figure 3. Various linear sections can be observed in the temperature range investigated. Because of this finding, the stationary phases have been divided into two groups.

The first group incorporates the pure silica gel (220) and the PiBA (227C20) coated on silica gel (figure 3A). The curves can be divided into two or three linear sections which show a decrease in slope with increasing temperature. Above 330 K to 335 K the slope can even be negative.

The second group comprises the silica gels coated with liquid crystalline polymers. The temperature dependence of ln k' of androstadienedione is entirely different on these phases as can be seen from figure 3B and 3C. The slopes for these phases either invert twice (extreme values), or the capacity factors change abruptly (discontinuous curves). These effects are obviously larger than the error range and independent of the flow velocity of the mobile phase. Besides the abrupt changes, only column 224C20-2 (figure 3B) shows a bend in the curve at 323 K, similar to the one which can be seen in the first group (figure 3A).

TABLE 4

Enthalpy change for mass transfer from the stationary phase to the mobile phase and the transition temperatures

Column number	Material	Column code+)	Enthalpy change ΔH_i in kJ/mol and temperature T_i in K				
			ΔH_1	T_1	ΔH_2	T_2	ΔH_3
1	220	11/②	-11,4	315	-7,2	333	-0,4
2	222C20	2	-9,6	332	-4,6	334	-1,1
3	223C20	3	-9,4	322	13,9	327	-3,3
4	224C20	2	-10,9	323	0,9	336	0,9
5	225C20	4	-10,3	329	29,8	334	3,8
6		6/①	-15,8	326	22,6	335	-2,6
7		6/②	-11,6	329	44,8	334	-1,8
8	226C20	3	-10,0	321	8,7	336	-1,2
9		1&)	-10,9	322	13,8	336	0,6
10	227C20	2	-13,1	313	-4,0	336	3,9
11*)	225C20	7	-31,2				
12*)	226C20	4	-35,9	322	-27,0	332 347	-20,9 -16,4

+) Number written in a circle gives the number of temperature-dependent measurement of this column,

&) 1,5 cm^3/min flow rate and *) isomer separation

Table 4 shows the transition points (bend points) for the individual linear sections, and the enthalpies calculated with equation IV for the mass transfer from the stationary phase to the mobile phase. The observed deviation from the initially linear dependence for the columns in the first group (220 and 227C20) can be attributed to a temperature dependent change in the adsorption-desorption behaviour in the system having an added modifier. Scott and Lawrence (23,24) showed, that with an increase in temperature, the adsorbed polar modifier is desorbed from the active centers of the stationary phases, so that its concentration in the mobile phase rises. Engelhardt (25) states that the modifier concentration in an n-heptane/2-propanol system rises from 0,1 % by volume at 303 K to approximately 0,5 % by volume at 353 K in the mobile phase at the end of the column. Thereby a competition occures between the activated surface and the polar mobile phase. Our measurements seem to indicate that the activation of the stationary phase is higher than the increase in the elutional force of the mobile phase, so that at high temperatures the capacity factors increase again and the enthalpy changes ΔH become positive. The intermediate ranges are transition ranges, where the active centers are probably only partially covered by adsorbed modifier molecules. The differences between the observed temperatures of the same stationary phase, noted for repetitive measurements or for different columns respectively, can be caused by differences in adsorption strengths or quantities of polymer in the columns.

The completely different behaviour of the coated PAC_n phases is also documented by the enthalpy changes, particulary in the case of spacer length 3, 5 and 6 (see table 4). These are the columns showing extreme values in their curves, while the other two show one discontinuity each. Since with one exception (column 224C20-2) the PAC_n columns do not show the behaviour described for the first group, the effects are attributed to a change in stationary phase, i.e. in the polymer phase itself.

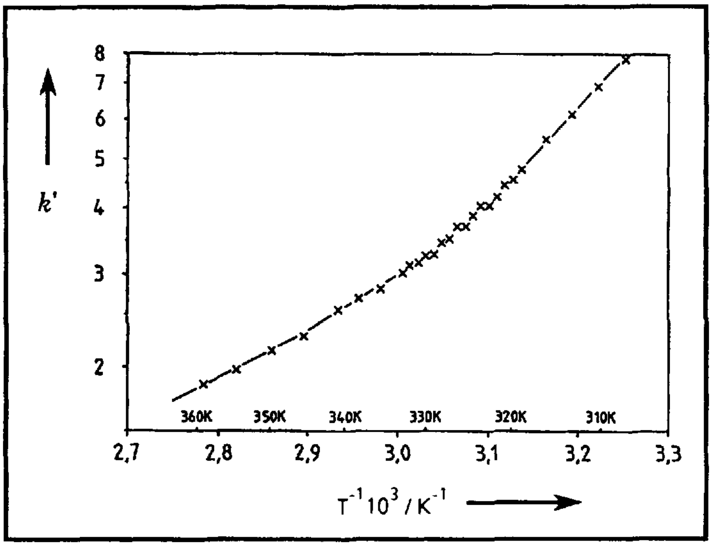


FIGURE 4: Temperature-depending measurement on the column 226C20-4 with 1,2-dinitrobenzene (isomer separation)

From the experiments on isomer separation, figure 4 shows a typical curve for 1,2-dinitrobenzene with column 226C20-4. The path of the curve resembles the curve pattern in the first group (220 or 227C20 resp.) for steroid separation. Temperature effects observed in steroid separation do not occur. Repeating experiments with different PAC_n columns produces the same result. The isomers used are molecules with many free π-electrons as well as permanent dipole moments for ortho and meta isomers. Obviously, no change in the stationary phase can be observed with these substances. The calculated enthalpy changes and temperatures of the curve bends are given for comparison to steroid separations in the last two rows of table 4 (column number 11 and 12). They differ considerably from the enthalpy changes observed in steroid separation.

TABLE 5

Temperatures of the extrem values or discontinuities and the percent decrease relative to the phase transition temperatures of the LCPs

LCP-phase	$T_{s \rightarrow n}$ in K	T_1 in K	$\Delta T_1/T_u$ in %	$T_{n \rightarrow i}$ in K	T_2 in K	$\Delta T_2/T_u$ in %
222C20				391	333	14,8
223C20	360	321	10,8	373	327	12,4
224C20	350	+)		397	337	15,1
225C20	379	328	13,5	388	334	14,0
226C20	360	322	10,5	387	336	13,3

+) Not observed

As table 5 shows, the temperature of the extreme values or discontinuities, respectively, correlate with the phase transition temperatures of the pure LCPs (in table 1). The values of $\Delta T/T_u$ give the percent decrease of the observed temperatures relative to the phase transition temperature T_u.

On the average, the temperatures are 13,1 ± 1,7% lower than the phase transition temperatures so that a relationship can be assumed.

The results of the temperature dependent chromatographic measurements for steroid separation on coated LCP phases are shown in figure 5 together with the phase transition temperatures of pure liquid crystalline polymers (values from table 5). The odd-even effect already noted by Siebke (15) can also be observed in the "conversion" temperatures obtained in chromatography. This means that the liquid crystal-

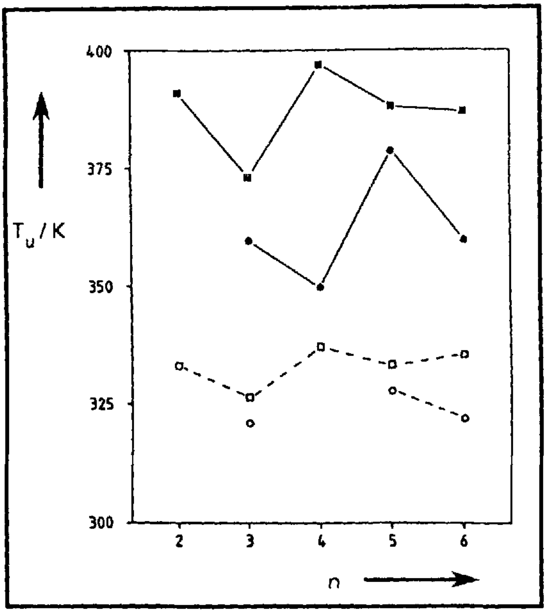


FIGURE 5: Phase transition temperatures against the spacer length n for the liquid crystalline polymers [● $T_{s \rightarrow n}$ and ■ $T_{n \rightarrow i}$] as well as the stationary phases [○ $T_{u,1}$ and □ $T_{u,2}$, steroid separation]

line polymers serving as stationary phases in HPLC must also be changing their structure at certain temperatures, a procedure which goes hand in hand with a change in the capacity factor. Due to the plasticizing effect of the mobile phase, the chromatographic "conversion" temperatures are lower than the phase transition temperatures of pure polymers. It is doubtful whether smectic phases are formed. It is much more probable that only a nematic orientation of the longitudinal axes of the mesogenes occurs. This would also explain the above described deviation in the observed behaviour of the column with PAC_4 as stationary phase. The reduced phase transition temperature (smectic-nematic) should be around 304 K for n=4. However, it is probable that a nematic-structure related orientation already exists just above room temperature so that this phase transition can not be found.

The temperature behaviour described above could not be observed on these liquid crystalline phases in isomer separations (dinitrobenzene isomers).

Conclusion

Also in HPLC certain separating properties of thermotropic liquid crystalline stationary phases adsorbed on silica gel can be observed. They can be attributed to the structure of the mesophases. The characteristic temperatures are related to the phase transition temperatures of the liquid crystalline side group polymers used. They are lowered due to the plasticizing effect of the liquid used as mobile phase.

Acknowlodgement

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Reference

1) Kelker, H., Ber. Bunsenges. physik. Chem. 67, 698, 1963

2) Kelker, H., Z. Anal. Chem. 198, 254, 1963

3) Kelker, H. and von Schivizhoffen, E., Adv. Chromatogr. 6, 247, 1968

4) Kelker, H., Adv. Liq. Cryst. 3, 237, 1978

5) Janini, G. M., Adv. Chromatogr. 17, 231, 1979

6) Kelker, H., Proc. Sino-W. Ger. Symp. Chromatogr. 1981 (publ. 1983), p. 58

7) Seifert, K. and Kraus, G., J. Chromatogr. 192, 97, 1980

8) Naikwadi, K. P., Jadhav, A. L., Rokushika, S., Hatano, H. and Ohshima, M., Makromol. Chem. 187, 1407, 1986

9) Lavoie, M. and Guillet, J. E., Macromolecules 2, 443, 1969

10) Guillet, J. E. and Stein, A. N., Macromolecules 3, 102, 1970

11) Guillet, J. E., J. Macromol. Sci.-Chem. A4, 1669, 1970

12) Kraus, G., Hong Van, T.and Weißflog, W., Z. Chem. 22, 448, 1982

13) Taylor, P. J. and Sherman, P. L., J. Liq. Chromatogr. 2, 1271, 1979

14) Aratskova, A. A., Vetrova, Z. P. and Yashin,Ya. I., J. Chromatogr. 365, 27, 1986

15) Siebke, W., Dissertation, Technische Universität Berlin, Berlin 1986, D83

16) Jungheim, M. and Engelhardt, H., Fresenius Z. Anal. Chem. 320, 694, 1985 and
Jungheim, M., Diplomarbeit, Universität des Saarlandes, Saarbrücken 1984

17) Unger, K. K., J. Chromatogr. Libr. 16, 176, 1979

18) Rayss, J., Witkiewicz, Z., Waksmundzki, A. and Dabrowski, R., J. Chromatogr. 188, 107, 1980

19) Rayss, J. and Waksmundzki, A., J. Chromatogr. 292, 207, 1984

20) Jinno, K. and Hirata, Y., J. High Resolut. Chromatogr. Chromatogr. Commun. 4, 466, 1981

21) Jinno, K. and Hirata, Y., J. High Resolut. Chromatogr. Chromatogr. Commun. 5, 85, 1982

22) Henderson, D. E. and O'Connor, D. J., Adv. Chromatogr. 23, 65, 1984

23) Scott, R. P. W. and Lawrence, J. G., J. Chromatogr. Sci. 7, 65, 1969

24) Scott, R. P. W. and Lawrence, J. G., J. Chromatogr. Sci. 8, 619, 1970

25) Engelhardt, H., J. Chromatogr. Sci. 15, 380, 1977