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REVIEW

APPLICATION OF LIQUID CRYSTALS IN CHROMATOGRAPHY[a]

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CONTENTS

1. INTRODUCTION

This review constitutes the second part of a survey of liquid crystalline stationary phases for gas chromatography, the first part of which dealt with the properties and applications of liquid crystals as stationary phases in gas chromatography[1]. When the first part was written, it was not expected that it would have a continuation. In recent years, however, many new papers have been published on the application of liquid crystals in chromatography. Not only have studies been continued in those

[a] This work is dedicated to Professor Hans Kelker who was the first to introduce liquid crystals in chromatography.

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countries and centres in which they were started earlier, but also research now is being undertaken elsewhere. Particularly interesting is the rapid development of studies on liquid crystal stationary phases in China.

Recent studies have concentrated on new analytical applications of liquid crystal stationary phases and the use of new liquid crystal phases, not only rod-like but also disc-like. Apart from the still most common applications of liquid crystals as stationary phases in gas chromatography, they are also finding application in supercritical fluid chromatography and liquid chromatography. In the latter instance, liquid crystals are not only useful as stationary phases in chromatographic columns but may also be used for visualizing thin-layer chromatograms.

In the recent years our knowledge of the properties of liquid crystal stationary phases has increased considerably, and so has the range of their practical, often routine, applications. In Poland, for instance, there are laboratories in which liquid crystals have been used for some time for the separation and determination of the isomers of polynuclear hydrocarbons. In addition, our knowledge of other possible applications of liquid crystals in chromatography and elsewhere has also expanded. For instance, the experience from chromatography has been utilized in piezoelectric detectors[2–4]. It has been shown that a quartz resonator coated with a liquid crystal layer reacts in different ways to isomers of substances differing in molecular structure and detects them with varying sensitivity.

Research on liquid crystal has been developing very rapidly both as regards the synthesis of new substances and the knowledge of their properties and applications, as indicated in the recent years in general monographs and reviews[5–12] and in those devoted to chromatographic applications[13–18]. In one of the reviews the separation properties of cyclodextrins and liquid chrystals as stationary phases in gas chromatography are compared[19]. About 3.6% of recent publications concerned with gas chromatography are devoted to liquid crystal stationary phases[20].

In future research on liquid crystals as chromatographic materials the efforts of organic chemists synthesizing liquid crystals should be combined more closely with those of analysts, chromatographers, physical chemists and physicists studying the properties of liquid crystals. Such cooperation might contribute to a better knowledge of the relationships between the structure of the liquid crystal molecule and its separation properties, and hence to the development of new materials with the required, optimum features. The lack of such cooperation was clear at the 12th International Liquid Crystal Conference (Freiburg, 1988), where the application of liquid crystals in chromatography was not even mentioned[21]. However, several papers on the application of liquid crystals were presented at the 17th International Symposium on Chromatography (Vienna, 1988)[22].

This review encompasses work that was published or became available after the first review was written[1]. A few of these were, in fact, included in the first review at the last moment, but it was not possible at that time to give them more detailed consideration. The fundamental properties of liquid crystals are not discussed here as they were considered in the first review.

2. NEW LIQUID CRYSTALS USEFUL IN CHROMATOGRAPHY

The number of new liquid crystals synthesized is very large but most of them are

designed for use in displays, although some of them may be applied succesfully in chromatography[23]. So far, however, only a small number of the known liquid crystals have been used in this field.

In recent years a range of liquid crystal polymers have been obtained[24]. Among them, siloxane polymers are gaining particular significance in gas chromatography. Many of the latter have been synthesized intentionally for that purpose.

2.1. Polysiloxanes

Siloxane polymers are among the best known stationary phases for gas chromatography[25–27], and the production of liquid crystal siloxane polymers is a logical step in the development of liquid crystal stationary phases and of stationary phases in general. Today several kinds of liquid crystal siloxane polymers are known. Most often they are obtained by adding to the siloxane polymer backbone mesomorphic groups that impart liquid crystal properties to the polymer. Such groups are bonded to the backbone by means of flexible aliphatic chains (spacers). However, mesomorphic compounds when bonded to the backbone do not always yield a liquid crystal polymer, and it is also possible that a liquid crystal is obtained after non-mesomorphic groups are bonded to the siloxane backbone. The properties of the liquid crystal siloxane polymer are influenced by the length of the polysiloxane chain, the number of the mesomorphic substituents, the kind of mesomorphic group and the length of the spacer bonding the mesomorphic groups with the backbone[28]. Alkane or alkene chains are used as spacers[29].

When synthesizing liquid crystal polymers from polymethylsiloxanes it was found that if the initial polymers used have different molecular weights but the mesomorphic compounds are the same, the resulting liquid crystal polymers have different melting points but the same mesophase to isotropic liquid transition temperature[30]. The phase transition temperatures depend on the number of methyl groups bonded to the siloxane backbone; the greater the number of these groups, the lower is the nematic–isotropic liquid transition point. It is expected that by selecting a suitable length of the siloxane backbone, mesomorphic groups, spacers and numbers of non-mesomorphic groups, it will be possible to obtain polysiloxanes with melting points close to ambient temperature and clearing points above 300°C.

Biphenylcarboxylic esters of polysiloxanes constitute a large group of liquid crystal polymers[29,31,32] and several procedures for their synthesis for chromatographic applications have been described[28,30–32]. Not only nematic but also smectic–nematic and smectic polymers are suitable as stationary phases[29,32,33]. Polymers with the cholesteric mesophase are also known[29], although to a much smaller extent.

Polymers with mesogenic groups in the side-chains are usually non-crystallizing. The lower limit of their liquid crystal state is the glassy to mesophase transition temperature above which a segment mobility appears owing to the lability of the particular fragments of the macromolecule. In the temperature range from the glassy to mesophase transition temperature to the clearing point the polymer is in the liquid crystal state. In the temperature range of the mesophase one can distinguish the flow temperature, below which the polymer is an elastomer, in which state it is unsuitable as a stationary phase. The non-crystallizing polymers do not freeze on cooling but at a characteristic temperature (for each of them) they become glassy, preserving their mesophase structure in the course of further cooling. The liquid crystal polymers

usually reveal a wider range of the mesophase than the corresponding monomeric liquid crystals.

The polymers have a high thermal resistance and show good behaviour in the column; further improvement in their properties is possible by cross-linking in the column[28]. Azo-*tert.*-butane has been succesfully used as the cross-linking agent. The cross-linked stationary phases are more stable, but the efficiency of the columns decreases by as much as 30%.

Some formulae of liquid crystal polysiloxanes are given in Fig. 1 as examples.

Among the siloxane polymers, attention should be drawn to the phthalocyanine derivatives of siloxane polymers that have not so far been tested in gas chromatography[34]. These compounds show exceptionally high chemical and thermal stability, a wide range of the mesophase and very low viscosity in some temperature ranges, which makes it probable that they will make excellent stationary phases.

2.2. Polyacrylates

These polymers are obtained from the corresponding acrylates by radical polymerization with the use of 2,2′-azoisobutyronitrile (AIBN) as initiator[35]. The scheme of this reaction is shown in Fig. 2.

From monomeric acrylates, which provide smectic and nematic mesophases, liquid crystal polymers are obtained with only the nematic phase, but wider and occurring at higher temperatures. The mesophase range of some of these polymers exceeds 200°C.

2.3. Isothiocyanates

The opinion seems to prevail that liquid polymers are, in general, better stationary phases than are monomeric liquid crystals. In addition to the advantages already mentioned, the better arrangement of the liquid crystal polymer molecules on the surface of the capillary column walls compared with monomeric liquid crystals is emphasized. However, this is not so in every instance, and the practical importance of some monomers is still significant, *e.g.*, azo- and azoxyarylethanes[36].

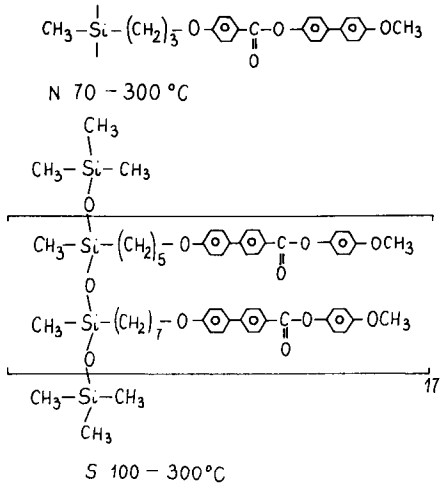

Fig. 1. Structure of liquid crystalline polysiloxanes.

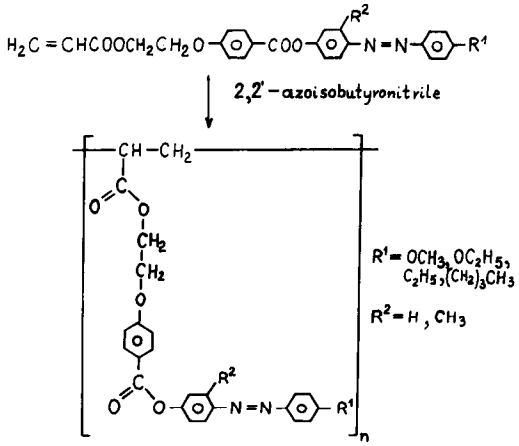

Fig. 2. Scheme of synthesis and structure of polyacrylates[35].

Great hopes can be set on the recently obtained liquid crystals belonging to the isothiocyanate group[37,38]. Some compounds belonging to this group reveal very wide ranges of the mesophase, comparable to those of the polymers. These compounds are chemically and thermally stable, have low viscosities and show good behaviour in capillary columns. Their advantage is that they are obtained more easily than the siloxane polymers. It can therefore be expected that some monomeric liquid crystal stationary phases will be competetive with polymeric liquid crystals.

Fig. 3 shows the formulae of several liquid crystal isothiocyanates.

2.4. Disc-like (discotic) liquid crystals

The study of disc-like liquid crystals as stationary phases is very interesting not only from the theoretical point of view but also increasingly for purely practical reasons. These liquid crystals have found application in chromatography only recently, although they have been known since 1977[39]. Today it is known that the rod-like structure of the molecule is not a necessary condition for a compound to be a liquid crystal; the molecule may be flat or disc-shaped[12]. Disc-like liquid crystals are known

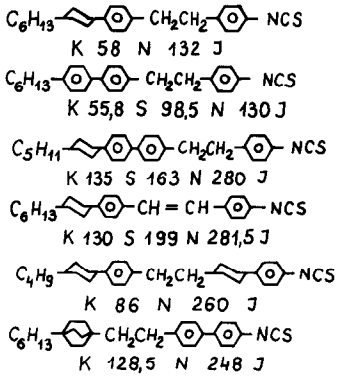

Fig. 3. Formulae and temperatures of phase transitions of liquid crystalline isothiocyanates.

which are derivatives of benzene[40,41], triphenylene[42,43], truxene[44,45], rufigallol[46,47], tetraphenylpyrylidenepyran and tetraphenylpyrylidenethiopyran[48,49], naphtalene[50], phthalocyanines[51,52] and other compounds[53,54]. Polymeric disc-like liquid crystals have been reported[55]. The disc-like liquid crystals reveal ranges of the mesophase narrower by several tens of degrees than those of the rod-like type. The discotic liquid crystals studied so far as stationary phases have very narrow ranges of the mesophase[56,57].

Fig. 4 shows a general scheme of molecules of discotic liquid crystals and the structures of the mesophase. The structure may be nematic, twisted nematic (cholesteric) or columnar[58–60]. The columnar structure, which may be considered as corresponding to the smectic structure, has several modifications.

A large number of known liquid crystals, their characteristic molecular structures and their fundamental properties have been surveyed by Demus and Zaschke[61].

3. COLUMNS WITH LIQUID CRYSTAL STATIONARY PHASES

In recent years there has been a strong trend toward the use of liquid crystals in capillary columns. This is in accordance with the general growth in importance and increasing application of capillary columns in gas chromatography[62–65]. An additional reason for the spread of capillary columns is that the efficiency of normal analytical columns filled with liquid crystals is usually lower than that of columns filled with conventional stationary phases. This lower efficiency is due to the high viscosity of the liquid crystal stationary phases. To improve the efficiency of columns filled with liquid crystals, the latter are mixed with conventional isotropic stationary

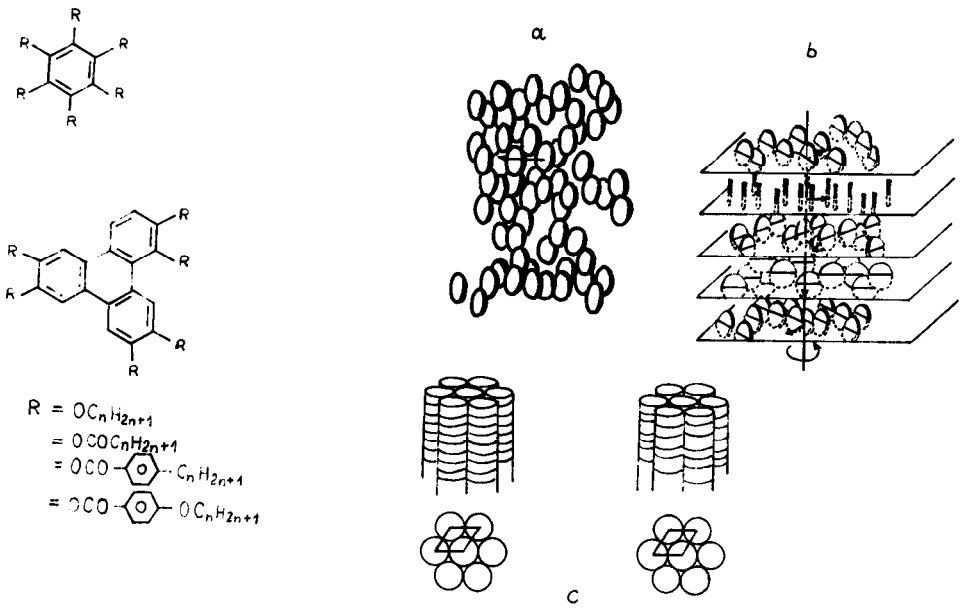

Fig. 4. Example of formulae and structures of the disc-like liquid crystal mesophase: (a) nematic; (b) cholesteric; (c) columnar.

phases, *e.g.*, SE-30, SE-52, SE-54 or OV-17[66–69]. Mixing of two liquid crystal stationary phases does not lead to a notable improvement of the column efficiency, which remains intermediate between the values obtained for the individual components of the mixture[70].

When the liquid crystal is deposited in a capillary column, the efficiency of the system becomes sufficiently high to allow, very good separations of complex mixtures to be achieved, considering the high selectivity of the liquid crystal stationary phases. The high selectivity of liquid crystals even makes it possible to use much shorter columns than those filled with conventional stationary phases. This is becoming easier because with liquid crystals of low viscosity, which are becoming increasingly available, the efficiency of the columns obtained is comparable to that of columns filled with conventional stationary phases.

However, despite this general trend towards capillary columns, in many instances liquid crystals continue to be used in conventional analytical columns.

3.1. Conventional analytical columns

In most instances Chromosorbs or Chromatons, usually silanized or acid washed, have been used as supports. Less frequently silica gels, *e.g.*, Silochrom 80[71], or glass beads[72,73] were applied. The liquid crystals (low or medium temperature) are applied to the conventional supports usually in amounts of 10–15% and seldom more than 20%, *e.g.*, 24.5%[74]. High-temperature liquid crystal stationary phases are applied to the conventional supports in an amount of 5%. This is related to the volatility of these substances; if the high-temperature phases, applied at about 300°C, were used in larger amounts bleeding of the phase from the columns might render the analysis impossible. This is particularly important when the substances to be analysed are presented in the sample in trace amounts, *e.g.*, in the analysis of polycyclic hydrocarbons. In the analysis of these hydrocarbons the liquid crystal phases may be deposited on glass beads[72,73], which were sometimes pretreated with sodium dodecylbenzenesulphonate[72]. The amount of the stationary phase (*e.g.*, BMBT) deposited on the beads was small (up to 0.025%)[73].

In most instances the liquid crystals are deposited on the supports from solutions in commonly used volatile solvents, but also in formic acid[75]. If active supports of high surface area and high adsorption potential such as Silochrom 80 are used, a special procedure for applying the liquid crystal is recommended, in which a few percent (*e.g.*, 6%) of the liquid crystal is deposited several times from solutions of increasing concentration[71]. This concentration should be adjusted depending on the properties of the adsorbent used as the support. The columns with a filling prepared in this way have a higher efficiencies than those with the filling prepared in the conventional manner. In addition, the efficiency may be higher at temperatures corresponding to the solid state than at those of the smectic or nematic. With columns with fillings prepared by the multi-step procedure the retention times of the chromatographed hydrocarbons (heptane, toluene) are shorter and the peaks are narrower and more symmetrical than with columns with fillings prepared in a one-step operation.

Some high-molecular-weight, high-temperature liquid crystals are poorly soluble and were deposited on the supports from suspensions in a solvent. Sometimes also the powdered stationary phase was mixed with the support, *e.g.*, for 48 h[76,77]. During conditioning of the column (at temperatures above the melting point) the liquid

crystal was distributed on the surface of the support, and the properties of the column did not differ from those with a filling prepared by deposition of the liquid crystal from a real solution.

By applying various liquid crystals in different systems, the optimum heights of the columns equivalent to a theoretical plate obtained were, *e.g.*, 0.6–1.8 mm[78,79]. When the columns were filled with a mixture of a liquid crystal and a conventional isotropic phase (*e.g.*, BBBT + SE-30), their efficiency depended on the quantitative composition of the mixture and on the column preparation procedure applied[66]. The height equivalent to a theoretical plate then varied, in various columns, from 0.9 to 1.6 mm.

The columns used had similar diameters (2–4 mm) and lengths (up to several metres) to those with conventional stationary phases. Sometimes, however, they were much shorter, *e.g.*, 0.7 m[72].

3.2. Capillary columns

Capillary columns make it possible to combine the high efficiency of such columns with the high selectivity of liquid crystals. The wide use in chromatographic practice of capillary columns filled with liquid crystals is envisaged, although it is realized that the general requirements regarding the reproducibility, thermal stability and wettability of the walls is greater with capillary columns than packed columns[80].

Liquid crystal stationary phases are deposited on the capillary walls from solutions by the generally used dynamic or static methods in layers 0.1–0.3 μm thick[64,65]. When applying the liquid crystals by the dynamic method, 20%[81] or 10%[82] solutions were used, and in the static method less concentrated solutions, *e.g.*, 0.25%[83].

The superdynamic method of Berezkin and Korolev[62] developed for conventional phases in which stationary phases are deposited on capillary walls, might be successful with liquid crystal phases also. This method makes it possible to obtain columns with good reproducibility and properties. The columns have 3500–5500 theoretical plates per metre and capacity factors of 2.2–2.4.

If the liquid crystal is poorly soluble but easy to produce, then its synthesis is conducted directly *in situ*. This method was applied, for instance, with liquid crystal Schiff bases (BPhBT)[84]. The solutions of the substrates, in this instance of an aldehyde and an amine, were mixed in suitable proportions and the mixture was introduced into the column, where it was heated to the required temperature at which the reaction took place. The resulting product was deposited on the column walls, whereas the unreacted substrates and solvent were removed from the column.

The efficiency of the column and its separation ability depend on the kind of liquid crystal used, its molecular structure and the related polarity, and also on the way in which the capillary walls have been pretreated and on the amount of the liquid crystal deposited on their surface[85–88]. The walls of glass columns were preliminary etched with hydrogen chloride and coated with barium carbonate. Often additional deactivation was achieved by applying a Carbowax layer, which improved the column efficiency. When liquid crystals obtained from azo or azoxy compounds were deposited on the walls of columns deactivated with Carbowax, their efficiency exceeded 3000 theoretical plates per metre and their stability was higher. The selectivities of the columns treated and untreated with Carbowax were, however, similar.[85]

Matišova *et al.*[87] studied the dependence of the properties of a liquid crystal

stationary phase on the thickness of the film on the column walls. They measured the phase transition temperatures of the liquid crystals, the column capacity factors, relative retentions and retention indices of the chromatographed substances for columns only etched with gaseous hydrogen chloride for 2 h at 330°C and for columns additionally deactivated with Carbowax 20M. The column efficiency depended considerably on the kind of reagent used for deactivating the column walls. Good columns, with 900–1000 theoretical plates per metre (as measured for anthracene at 230°C), were obtained by depositing BBBT on column walls deactivated with triphenylsilylamine[89]. When diphenyltetramethyldisilazane was used, the number of theoretical plates reduced to 500 per metre.

The column efficiency also depends on the phase in which the liquid crystal is used. For instance, for a brass column at temperatures corresponding to the mesophase and an isotropic liquid, 900–1300 theoretical plates per metre were obtained. whereas in the temperature range of the solid, the number of theoretical plates was only 100–150 per metre[81].

The column efficiency is strongly affected by the composition of the deposited mixed phase[68]. An increase in the proportion of the liquid crystal (BBBT) in the common isotropic phase (SE-52) lowered the column efficiency (in terms of the number of theoretical plates per metre) as follows: SE-52, 3381; BBBT – SE-52 (20:80), 1710; and BBBT – SE-52 (50:50), 858. When 20% of BMBT or 20% of BMxBT was added to SE-52, the column efficiency was 1600 or 1100 theoretical plates per metre, respectively.

Mainly glass capillary columns are used, although recently fused-silica columns have also been applied. Metal columns (steel, copper or brass)[81,82,90,91] are rarely used. The length of the columns varied for 4 m[81] to about 100 m.

When considering the choice of a chromatographic column, it should be realized that the separation of the components of a mixture depends much more on the kind of mesophase used and its molecular structure and range than on the kind and length of the column. Examples of the characteristics of capillary columns filled with liquid crystal stationary phases are given in Table 1.

4. FACTORS AFFECTING THE SEPARATION OF COMPONENTS OF MIXTURES ON LIQUID CRYSTAL STATIONARY PHASES

4.1. Kind of mesophase of the liquid crystal

In recent years, the mechanisms of chromatographic separations on liquid crystal stationary phases have seldom been studied, mainly because the general theoretical principles of such separations are known. However, it is not easy to describe the phenomena that occur at the molecular level and this aspect has not yet been completely elucidated. This task was undertaken and largely solved by Martire[102], who gave an exhaustive treatment of the problems connected with the mechanisms of the separation of substances of different molecular shape on liquid crystal stationary phases with various molecular structures. He proposed a theory based on the crystal lattice model in which statistical mechanics are utilized. Account was taken of molecules of the chromatographed substances of various shapes: thin and thick rods, plates, grains and semi-flexible chains. The liquid crystals considered were those whose molecular structure makes them low- or high-temperature types. These were

TABLE I

EXAMPLES OF CHARACTERISTICS OF CAPILLARY COLUMNS FILLED WITH LIQUID CRYSTAL STATIONARY PHASES

Liquid crystal stationary phase	*Temperatures of phase transitions[a] (°C)*	*Column (m × mm I.D.)*	*Method of filling, solution concentration (%)*	*Wall surface treatment*	*Test substance*	*Temperature of column testing (°C)*	*Number of theoretical plates (N)*	*Capacity factor of test substance (K)*	*Ref.*
MEPSIL IVB	C139N319I	Glass, 15 × 0.25	Static, 1.6 mg/cm^3	20% HCl, HMDS	Chrysene	230	2450/m		30
Polysiloxane	C118S300I	Fused silica 10 × 0.32	Static	N_2 at 250°C, 2h			4000/m	15	31
PMMS	C70N300I	Fused silica 19 × 0.32	Static, 0.3		Triphenylene	220	2200/m		67
CABHC	C119N232I	Glass, 24 × 0.26	Static	$BaCO_3$, Carbowax			75 000		85
PBO	C63N94I	Glass, 62 × 0.25	Dynamic, 2.5	HCl, N_2, 160°C	*trans*-2-Tetradecene	74	180 000 (160 000 eff.)	16.2	86
BBBT	C188N303I	Glass, 10 × 0.22	Static 2 mg/cm^3		Anthracene	230	900–1000/m		89
MEAB	C95.8N148I	Glass, 23 × 0.25			3-Phenyltridecane	140	60 000 (54 000 eff.)	17.4	92
MEAB	C95.8N148I	Glass, 20 × 0.25	Dynamic	HCl(g), N_2 150°C	*trans*-2-Tridecene	95	230 000 (170 000 eff.)	6.2	93

MEAB	C95.8N148I	Glass, 80 × 0.25	Dynamic, 3				215 000	5	94
MEAB	C95.8N148I	Glass, 63 × 0.25	Dynamic, 5				130 000	5	94
EBO	C97N112I	Glass	Dynamic, 5			50	106 000	2,1	95
EBO	C97N112I	Glass	Dynamic, 5			40	25 000	3,2	95
PBO	C63N94I	Glass, 100 × 0.25	Dynamic	HCl, 360°C			350 000	5	96
PBO	C63N94I	Glass, 100 × 0.25	Dynamic		1-Undecene	56	430 000 (360 000 eff.)	10.2	97
PBO	C63N94I	Glass, 100 × 0.25			*n*-Nonane	40	290 000 (170 000 eff.)	3.3	98
MEPSIL–SE-30		Glass, 60 × 0.25	Static		benz[*a*]anthracene	240	3210/m		99
MEPSIL		Glass, 60 × 0.25	Static		Chrysene	230	2450/m		99
HCFITFE	C58N132I	Glass, 15.5 × 0.3	Static	18% HCl, $BaCO_3$, Carbowax		61	700/m real		100
HBITFE	C55.8S98.5-N130I	Glass, 20 × 0.3	Static	18 % HCl, 150°C, $BaCO_3$		100.5	1700/m real		100
ITFPCBE	C135S163N-280I	Glass, 12.5 × 0.3	Static	18% HCl, $BaCO_3$, Carbowax		165	700/m real		101

[a] C = Crystal; S = smectic; N = nematic; I = isotropic liquid.

molecules with a rigid, rod-like core with semi-flexible side-groups. The effect of packing, connected with the molecular structure of the chromatographed substance, was considered to be an important element of the interactions.

The theory of separations on nematic liquid crystals is developed best, as it is on these that the best chromatographic separations are obtained. The theory of separation on smectics is much poorer, although there are more and more reports regarding the good chromatographic properties of some of them[28,29,31, 103]. In general it is assumed that smectics with a low degree of ordering of the mesophase (S_A. S_B) have better separation properties than those with a high degree of ordering. This problem requires more exhaustive studies, however.

The recent discovery of liquid crystals with re-entrant phases has created new possibilities for studying the relationship between the effectiveness of separation and other properties of liquid crystal stationary phases (*e.g.*, the column efficiency and the capacity ratio) depending on the kind of mesophase.

The re-entrant phases occur as a result of the effect of an anomalous sequence of phases shown by some liquid crystals. In such a case the nematic, cholesteric, smectic A and smectic C phases may appear twice in different temperature ranges. As a result, the same kind of phase (*e.g.*, a nematic) will appear at temperatures both higher and lower than another phase (*e.g.*, a smectic A). The phase appearing at the lower temperature is called the re-entrant phase. This phenomenon is related to the association of the liquid crystal molecules with the cyano terminal group and an alkyl or alkoxy group with at least eight carbon atoms. Most frequently the following sequence of phases with the re-entrant phase is observed: NS_AN_{re} or $NS_AN_{re}S_{Are}$ (where N = nematic; S_A = smectic A; N_{re} = nematic re-entrant; S_{Are} = smectic A re-entrant). However, instances are also known where only the low-temperature N_{re} phase occurs without the nematic phase at the higher temperature; in this event the following sequence of phases is observed: S_AN_{re}, $S_AS_CN_{re}$, $S_AS_CN_{re}S_{Cre}$ or $S_AN_{re}S_C$[104,105]. Re-entrant mesophases are observed not only in rod-like but also in disc-like liquid crystals[106].

The use of re-entrant phases in chromatography has so far been little studied[105,107–109]. Liquid crystals with a re-entrant phase can be used in a wide temperature range if they reveal a linear relationship between retention and transition temperature from one kind of phase to the other. An example of such a liquid crystal is 4-(4-nonyloxybenzoyloxy)-4′-cyanobenzene, which shows N, S_C and also N_{re} and S_{Cre} phases. This liquid crystal has a mesophase range of 67–220°C and when supercooled to 55°C preserves the S_{Cre} phase[108] (see Fig. 5). A similar, linear relationship between retention and temperature was observed[109] at the S_A–N_{re} phase transition. The observed[107] relationship log V_R = f(t) was, however, non-linear and connected with phase transition.

Some new interesting findings show that liquid crystal stationary phases can be used beyond the range of the true mesophase, *i.e.*, in the form of a solid, supercooled below the melting point, and in the form of an isotropic liquid, above the clearing point. Several examples are known of the use of liquid crystals in the solid state[71,110–112]. Karabanov *et al.*[110] described the determination of impurities in diethyl sulphide, which were separated much better on the solid than on the mesophase. The possibilities and limitations of using liquid crystal stationary phases in the solid state and in the mesophase have been described by Dmitreva and Gabitova[111].

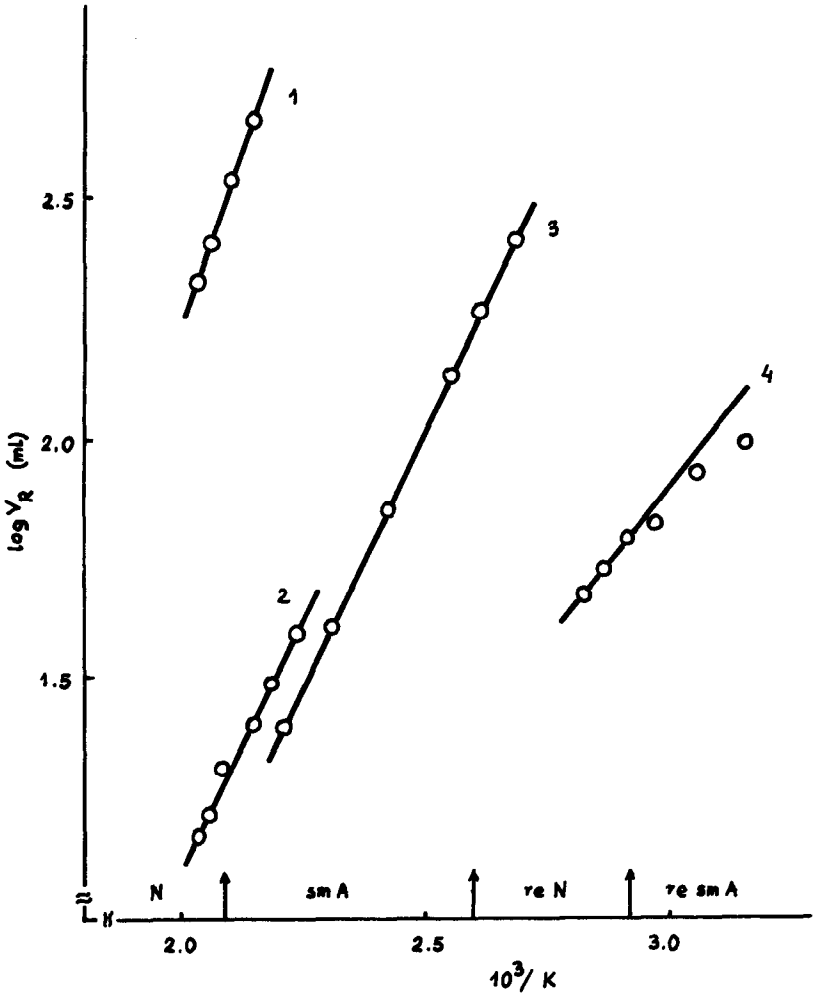

Fig. 5. Relationship between log (retention volume) and temperature for (1) phenanthrene, (2) *m*-chloroacetophenone, (3) *p*-dibromobenzene and (4) *o*-xylene[108].

In most instances the separations carried out on a solid stationary phase are much poorer than those conducted in the mesophase range and sometimes even poorer than those in which the isotropic liquid is used. For instance, the separation of isomers of trimethylbenzene on 4-butanoyloxy-4'-nitroazoxybenzene deposited in an amount of 20% on Chromaton N AW was possible with both the mesophase and the isotropic liquid but not on this stationary phase in the solid state[113]. The isotropic liquid is only seldom used, chiefly in those instances when the clearing point is not very high and the stationary phase reveals good thermal stability in the column. Industrial mixtures of methyl esters of C_8–C_{18} fatty acids were separated on methoxyethoxyazoxybenzene in short capillary columns at temperatures corresponding to the isotropic liquid[81].

In isothermal chromatography we usually utilize the initial temperature range

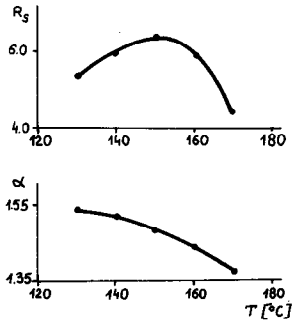

Fig. 6. Relationship between resolution (R_s) and relative separations (α) and temperature for anthracene and phenanthrene solutes on liquid crystalline polysiloxane stationary phase[30].

of the mesophase (above the melting point). The whole mesophase range or a large part of it is used when the temperature is programmed. Often also use is made of the mesophase supercooling range in which the ordering of the liquid crystal structure is greater than that in the true mesophase[76,79,87,93,96,114,115]. The supercooling may be very stable. For instance, after 12 months of mesophase supercooling at ambient temperature and 24 h at 0°C, the separations of mixtures were no worse than directly after supercooling[76].

The supercooling of the liquid crystal and the stability of the supercooled state are affected by, among other things, the molecular structure of the liquid crystal and also by the presence of lateral substituents[76,79,114,115]. The stability of the supercooled state is improved by the presence of halogen substituents.

Liquid crystal siloxane polymers are also liable to supercooling[30]. The relative retentions, α, of the chromatographed substances increase with supercooling and the resolution, R_S, achieves a certain optimum value (see Fig. 6). It is assumed that the corresponding temperature is the lowest point at which the stationary phase preserves its practical usefulness.

The possibility that a liquid crystal is supercooled depends on the thickness of its layer. The supercooled state is more stable when the liquid crystal film on the

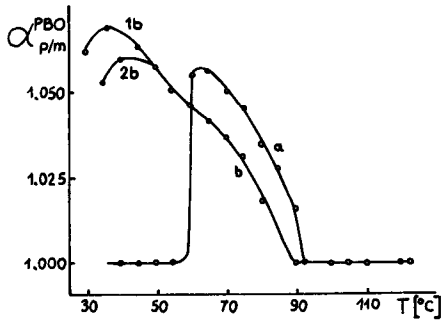

Fig. 7. Variation of the relative retention of *p*- and *m*-xylene ($\alpha_{p/m}$) on 4-*n*-pentylacetophenone (O-4-n-pentyloxybenzoyl oxime) (PBO) on heating the column (previously conditioned at ambient temperature) to the isotropic liquid temperature (a) and successive cooling to 30°C (b); 1b, cooling from the isotropic liquid (122.5°C); 2b, cooling from the mesophase (70°C).

capillary column wall is thicker[87]. If that film is thin (less than 40 nm), supercooling does not take place.

It has been found that the selectivity of a liquid crystal stationary phase in the supercooled state may depend on whether the cooling is started from the mesophase or from the isotropic liquid. It is more advantageous when the isotropic liquid is subjected to supercooling (see Fig. 7)[96].

Another method of chromatography on liquid crystals below their melting point is to use mixed phases. The separations on mixed phases are usually better than those on the pure components of the mixtures[70,79,116,117]. An important, if not the most important, reason is that mixtures, especially eutectic ones, have lower melting points and probably a greater ordering of the mesophase[118–121], which leads to a better selectivity of such mixtures at temperatures lower than those which can be used with the single phases. This has been shown, for instance, for binary and ternary liquid crystal stationary phases[70,122]. It is possible to predict the retention of the substances chromatographed on mixtures of liquid crystals if we know their retentions on the individual components. This relates both to liquid crystal–liquid crystal[123] and liquid crystal–isotropic stationary phase[124] mixtures.

In general it may be ascertained that the chromatographic separation proceeds according to different mechanisms at different temperatures corresponding to different states of the phases. Thus, by a skilful choice of the column temperature, one can obtain good separations of the components of different mixtures[74].

4.2. Molecular structure of the liquid crystal and of the chromatographed substance

A knowledge of intermolecular reactions between the liquid crystals and the chromatographed substances is important for the understanding of the phenomena taking place in the chromatographic column. The structure of the molecules and their polarity and polarizability affect, among other things, the solubility of the chromatographed substances in the liquid crystal[125]. In the course of dissolution complexes may be formed[126]. Many studies have dealt with the solubilities of non-mesogenic substances in liquid crystals[127–129] but they rarely refer directly to chromatography[122,130–134].

Quite recently an important paper was published in this field[135], concerning the thermodynamic properties of 22 solutes at infinite dilution in the smectic, nematic and isotropic mesophases of 4,4′-bis(heptyloxy)azoxybenzene. The thermodynamic properties were discussed in relation to the solute–solvent (liquid crystal) interactions as conditioned by the degree of order in the liquid crystal.

The process of dissolution dominates in the column during chromatography on a liquid crystal. However, as liquid crystals are mostly phases of medium polarity, the mechanism of the retention of the substances chromatographed on them is usually a combination being accompanied by adsorption. Nevertheless, the contribution of adsorption to the total retention is usually much smaller than that of dissolution.

Studies on the dissolution of chromatographed substances in N-(4-hexyloxybenzylidene)-4′-toluidine have led to the conclusion that the selectivity of the liquid crystal stationary phase is determined by the structure of the liquid crystal molecule influencing the creation of specific intermolecular reactions with the chromatographed substance and by the ordering of the mesophase[136]. The effect of the molecular structure of twelve liquid crystals based on azo and azoxy compounds on their

selectivity and polarity and the efficiency of conventional analytical columns in which these liquid crystals were deposited was studied by Szulc and Witkiewicz[79].

The relationship was studied between the molecular structure and the retention of the test substance for a set of nine other liquid crystals (4-alkoxycarbonyl-4'-nitroazoxybenzenes)[137]. In general, azoxybenzenes have better separation properties than azobenzenes[78,96], and among azoxybenzenes those which have different terminal substituents are more selective than those with the same substituents[94,96]. The inferior selectivity of the azo compounds probably occurs because they are planar and have a more compact structure into which the chromatographed substances penetrate only with difficulty.

The properties of the liquid crystal stationary phases depend both on the structure of the main chain of the molecule and on the terminal substituents which strongly affect the polarity of the molecules[138–140]. However, an equally important or even greater effect on the chromatographic properties of liquid crystals is exerted by the lateral substituents present in the molecule[76,79,114,115,139,140–144]. These substituents not only affect the intermolecular reactions between the liquid crystal and the chromatographed substance but also the liquid crystal – liquid crystal reactions. The lateral substituents also affect the selectivity of the liquid crystal owing to the changes they produce in the distance between its molecules[140]. This relates not only to monomers but also to polymers[145].

The direct quantitative correlation between the retention of the chromatographed substances and their molecular structure has been considered in several studies[78,146,147]. It is generally assumed that the ratio of the length to the smallest transverse dimension of the molecule, l/b (shape factor), is a decisive quantity for the retention of chromatographed substances on liquid crystal stationary phases. However, Suprynowicz *et al.*[146] chromatographed dimethylnaphthalenes on 4-ethyl-4'-(*p*-methylbenzoyloxy)azobenzene and on the basis of the results called into question the truth of this opinion. They suggested that the retention of dimethylnaphthalene isomers is directly related to the geometry of arrangement of the methyl groups in the molecule. This provoked polemics with Lamparczyk *et al.*[148,149].

Considering the shape-factor of twelve monomethylbenz[*a*]anthracenes and their separation on OV-17 stationary phase, Lamparczyk[147] predicted their retention and separation on BBBT and BABT liquid crystals.

The chromatography of olefinic hydrocarbons (pheromones) on liquid crystal derivatives of cholesteryl cinnamate has shown that the separation of geometric isomers of these compounds is affected either positively or negatively depending on the position of the multiple bond in the pheromone molecule[150].

The factors affecting the retention of polynuclear hydrocarbons and their weakly polar analogues containing sulphur in the molecule on a smectic siloxane polymer were studied[33]. In addition to the vapour pressure and volatility, the molecular structure of the chromatographed substances considerably affected the retention. The l/b ratio had a greater effect on the retention than other shape factors of the molecule, and this effect was more pronounced with a smectic than a nematic, the selectivity of the former being better. The shape of the molecule was, however, sufficiently important to affect the retention as determined from the l/b ratio.

The mutual effect of the molecular structures of the liquid crystal and the chromatographed substance was ascertained when determining the gas hold-up time

from the non-adjusted retention times of *n*-alkanes and methyl esters of fatty acids[151]. Methyl esters of mono- and bicyclic acids were chromatographed on the smectic and nematic phases of a high-temperature liquid crystal and the dependence of their retention on the molecular structure was studied. It was found that the selectivity is greatest at temperatures corresponding to the nematic range[75].

Little information is available on the mechanism of the separation ability of discotic liquid crystal stationary phases[56,57]. The existing information concerns only liquid crystal hexasubstituted triphenylene and benzene derivatives. A better knowledge and the generalization of the properties of this group of stationary phases require further studies of the already tested and other discotic liquid crystals.

The general principles underlying the interaction of the ordered structure of disc-like liquid crystals with molecules of chromatographed substances are the same as for rod-like liquid crystals. The molecular structure of discotic liquid crystals and their ordered structure are, however, the cause of the retention times of chromatographed substances whose molecules have an oblong shape on these liquid crystals being shorter than those of compounds with a compact molecular structure (cyclic–discotic). Such disc-like molecules are retained longer by the discotic liquid crystal stationary phase the flatter they are. The conclusions drawn for rod-like liquid crystals regarding good fitting, strong interaction with and easy penetration of such molecules into the ordered structure of the mesophase remain true for disc-like liquid crystals. The effect of planarity is probably even greater with discotic liquid crystal stationary phases. This has been shown for cyclohexane, cyclohexene and benzene and also for other compounds. Cyclooctatetraene (b.p. 142°C) is eluted after cyclooctane (b.p. 148.5°C), whose molecules are less flat. The importance of the planarity of the molecule is seen from the fact that the retention of cyclooctatetraene is almost the same as that of cyclooctanone (b.p. 200°C).

The separations of xylene isomers obtained on disc-like liquid crystal stationary phases are not as good as those obtained on rod-like phases. Even the order of elution is not changed with respect to conventional phases[56]. However, something is unclear here, as Goozner and Labes[152] reported that the solubilities of xylene isomers in mixtures of disc-like liquid crystals vary considerably. It is improbable that it will be possible to explain these differences only by the effect of the support.

Compounds with a linear structure of the molecule dissolve better than cyclic compounds in discotic liquid crystals. This has been shown for *n*-nonane and cyclooctane, which have similar boiling points (151 and 148°C, respectively) but very different retention times, cyclooctane being eluted second. If the chromatographed molecules do not have a disc shape (*e.g.*, *m*- and *p*-xylene isomers), the differences in their structure are immaterial and the boiling points are decisive for the order of their elution.

The ability of disc-like liquid crystal stationary phases to separate geometric isomers deserves attention. The separation of *cis*- and *trans*-decalin on a triphenylene derivative liquid crystal is illustrated in Fig. 8. The separation shown is very good and was obtained in a much shorter time than that on cyclodextrin as the stationary phase[56,153].

The relationship between the molecular structure of the chromatographed substances and their retention has been widely studied also with conventional stationary phases. Some results and conclusions from these studies may be applicable to liquid crystal stationary phases[154,155].

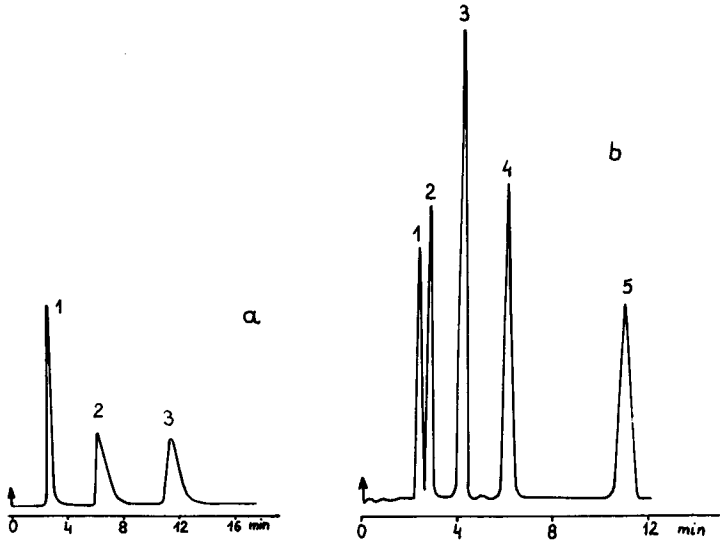

Fig. 8. Comparison of separation of a mixture of (1) *trans*-decalin, (2) *cis*-decalin, (3) tetralin, (4) naphthalene and (5) diphenyl. (a) Glass column (2 m × 4 mm I.D.) packed with 0.10 mol-% of β-cyclodextrin in formamide solution deposited on Celite; column temperature 70°C[153]; (b) glass column (2.1 m × 4 mm I.D.) packed with 10% of triphenylene hexa-4-octylbenzoate deposited on Chromosorb W AW; column temperature 198°C[56].

4.3. Effect of the support

Although great attention has been paid to the practical applications of liquid crystal stationary phases, relatively little concern has been devoted recently to fundamental studies of the interactions of the liquid crystals with the surface of the support. Such studies, however, will improve our knowledge of the properties of liquid crystal stationary phases and have not been totally abandoned, although sometimes they did not have a direct relation with gas chromatography[156].

The effect of the surface of the substrate on which the liquid crystal is deposited in the chromatographic column is rarely accounted for in analytical practice. This is also the case with other stationary phases. However, this effect may be important and should be taken into account. Usually the substrate has an adverse effect on the separations, although sometimes this effect may be positive. These problems are considered in detail by Berezkin[157].

The surface of the support or the column wall may not only contribute substantially to the retention of the chromatographed substances but may also influence the orientation of the liquid crystal molecules in various ways. Some information on this subject may be gathered from studies connected with the design of liquid crystal displays[158]. In view, however, of the special treatment of the surface of glass used in these displays, the possible correlations are very limited. A better knowledge of the effect of the condition of the surface and of the arrangement on this surface of the liquid crystal molecules should allow us to obtain systems with an optimum arrangement of the liquid crystal molecules in the column and hence with optimum properties as regards chromatographic separations.

The activity or neutrality of the surface of the support on which the liquid crystal is deposited has an effect on the mechanism of retention of polymethylbenzenes and *n*-alkylbenzenes[96]. Practice has shown that the use of an active support with a developed specific surface area (Silochrom, 80 $m^2 g^{-1}$) may give better separations of dimethylmercury and its contaminants and of diethyl sulphide and its contaminants on the nematic *p*-*n*-butyloxybenzoic acid deposited on that support compared with the same liquid crystal deposited on Chromaton N AW[71,110,159].

The distribution of the liquid crystal on the support and hence the properties of the whole system are affected not only by the chemical character (silanized or non-silanized surface) and porous structure of the support, but also by the amount of the liquid crystal deposited on its surface[160,161]. The effect of the support surface also manifests itself by the changes in the phase transition temperatures of the deposited liquid crystal. This effect is related to the conditions under which the column filling is heat treated. During heating, a redistribution of the liquid crystal on the support takes place and as a result the properties of the system are changed[96,162,163]. In some instances conditioning at high temperatures leads to a more advantageous ordering of the liquid crystals in the column. Therefore, if this treatment is not long enough or is conducted at an insufficiently high temperature, sometimes the selectivity of the column may change in the course of its use[164]. It should be borne in mind that the occurrence of this phenomenon is related to the kind of liquid crystal used and the properties of the surface on which it has been deposited.

The selectivity and also other properties of the system depend strongly on the kind of support used and on the amount of the liquid crystal deposited on it[165,166], as shown in Fig. 9. The selectivity also depends on the thickness of the liquid crystal layer on the capillary column wall and on the character of the wall surface[87,90,91,96]. The reproducibility and reliability of the retention data are the better the more inactive is the surface of the capillary wall and the greater is the thickness of the liquid crystal layer (> 140 nm)[87].

The liquid crystal molecules may occur on the support in two states[162,163,166]: as a film on the surface or in bulk form in the capillaries. The proportion of the two states influences the properties of the system and depends on the kind of the support and the kind and amount of the deposited liquid crystal.

Studies of the dependence of retention on the coverage of the support make it possible to determine the thickness of the monolayer of the liquid crystal on the support surface and hence to establish how the molecules of the liquid crystal are arranged on the surface[160,161]. It has been calculated that the molecule of the liquid crystal shown schematically in Fig. 10 occupies an area 0.806 nm^2 on the surface of a silanized support. If the length of the bonds in the molecules is taken into account, it can be calculated that the liquid crystal molecule may occupy maximally about 1.2 nm^2 in the planar position and minimally 0.21 nm^2 in the homeotropic position. The surface area of the flat, rigid part of the molecule (hatched in Fig. 10) is about 0.75 nm^2. By comparing these values it can be seen that the molecule may lie on the surface of the support planarly on its rigid, flat part with the hydrocarbon chain raised upwards. In this way a sort of modified surface of the support is formed on which successive layers of the liquid crystal are deposited. The formation of a monolayer is not, however, a sufficient condition for a further uniform distribution of the liquid crystal on the support surface. As the amount of the liquid crystal on that surface increases it accumulates in the pores with diameters greater than 1 μm[160,161].

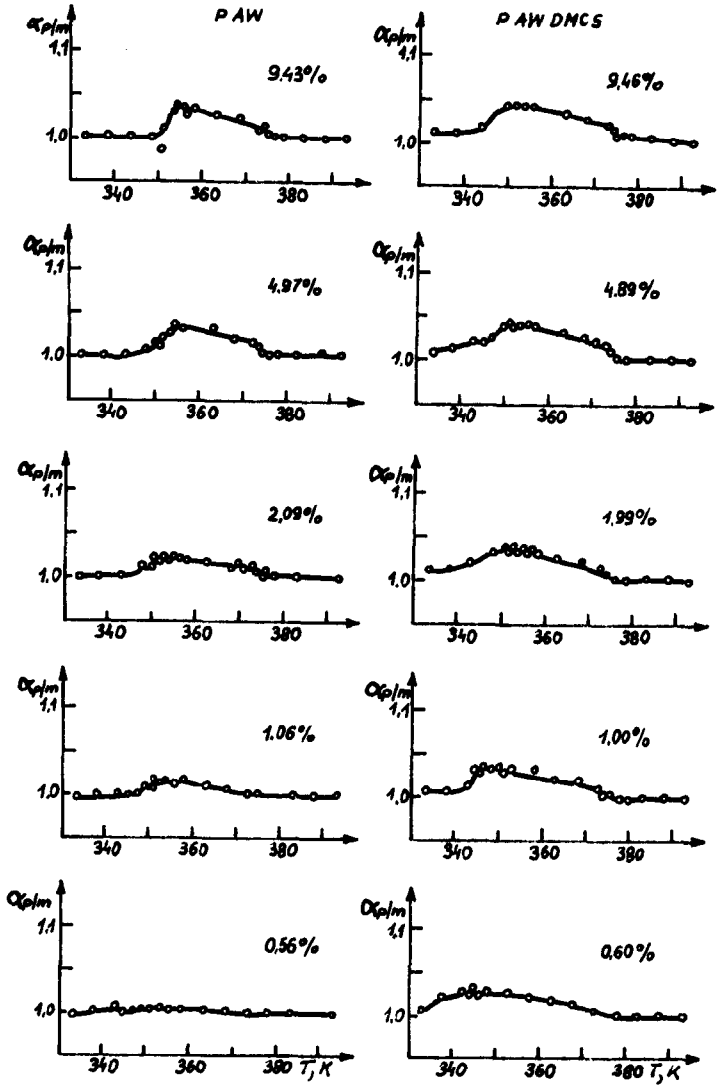

Fig. 9. Relationship between relative retention of *p*-xylene and *m*-xylene and column temperature with different amounts of liquid crystalline stationary phase deposited on Chromosorb P AW and Chromosorb P AW DMCS[165].

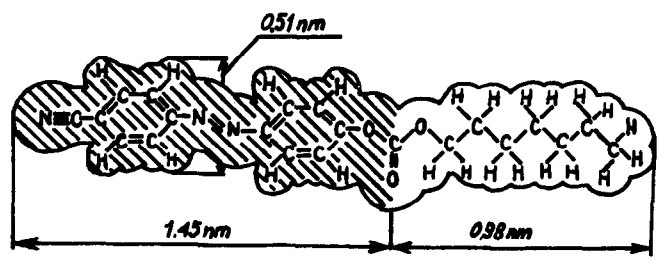

Fig. 10. Liquid crystalline 4-cyano-4'-*n*-heptyloxyformyloxyazobenzene molecule[160].

The interaction of the support surface with the liquid crystal stationary phase may give specific effects. One is a lowering of the melting point (by 7°C) of part of the liquid crystal owing to its contact with the silanized surface of various diatomite supports. The occurrence of this effect was detected for two liquid crystals prepared from the 4-cyano-4'-*n*-alkoxycarboxyazobenzenes with seven and nine carbon atoms in the alkyl chain[162,163,167]. The lowering of the melting point is due to the formation, under the influence of the support, of a layer of the phase with a crystalline structure different from that of the bulk liquid crystal beyond the support. This effect is not related to the kind of substance chromatographed but depends on the kind and amount of the liquid crystal deposited on the support and is a feature of the liquid crystal–silanized support system. It appears when the amount of the liquid crystal on the support exceeds *ca.* 3% and manifests itself by a new phase transition not observed thermo-optically. Hence this effect differs from the normal interactions of the liquid crystal with the support, which at small coverages of the support manifest themselves by a shift of the phase transition connected with the liquid crystal melting point and not by a new phase transition.

The variation of the specific retention volume accompanying the considered effect with the amount of the liquid crystal on the support is illustrated in Fig. 11. Below the melting point of the crystal (83°C), an additional phase transition appears at 76°C. The increases in retention related to the latter transition are approximately equal for all three supports. The increase in retention related to the second transition (at 83°C) depends on the amount of the stationary phase on the support. This effect is not observed for non-silanized supports. It follows that the interaction of the silanized support with the two considered liquid crystals is different from that of the non-silanized supports.

Fig. 12 and 13 show the relationships between the retention volume of *o*-xylene,

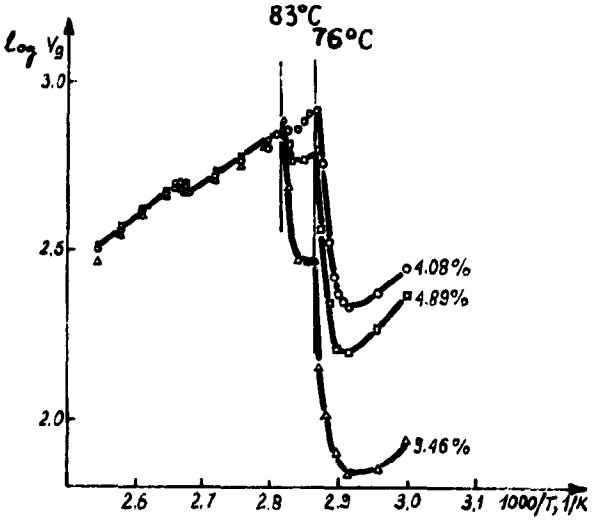

Fig. 11. Variation in retention volume of *o*-xylene with temperature on liquid crystalline 4-cyano-4'-*n*-heptyloxyformyloxyazobenzene deposited in different amounts on Chromosorb P AW DMCS. The additional phase transition at 76°C is visible[167].

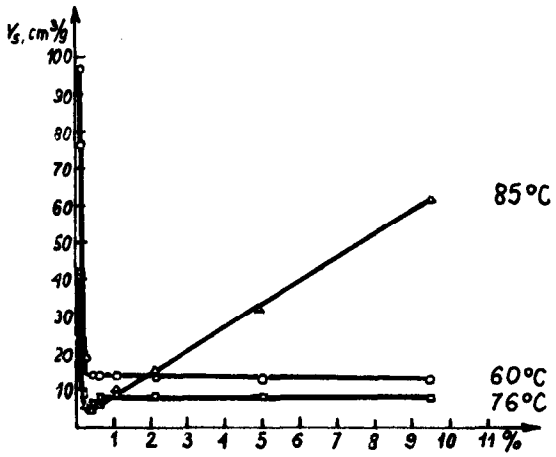

Fig. 12. Variation of the retention volume of *o*-xylene with the amount of liquid crystalline 4-cyano-4'-*n*-heptyloxyformyloxyazobenzene deposited on Chromosorb P AW[160].

calculated in terms of the support mass (V_S), and the amount of 4-cyano-4'-*n*-heptyloxycarboxyazobenzene for fillings in which Chromosorb P AW and Chromosorb P AW DMCS were used as supports. The dependence of the retention volume on the amount of the liquid crystal is given at the temperatures corresponding to the solid (60°C), mesophase (85°C) and the additional phase transition observed on silanized surfaces. The differences between the columns with silanized and non-silanized supports are visible at each of these temperatures. The results indicate that in some chromatographic systems the inactive silanized support may show a specific activity which may considerably affect the properties of such systems.

The effect of the support on the properties of liquid crystal stationary phases is also observed when these phases are supercooled. A given liquid crystal may undergo stable supercooling on one support and resist supercooling on another. On some supports liquid crystals undergo stronger supercooling than in their absence, but

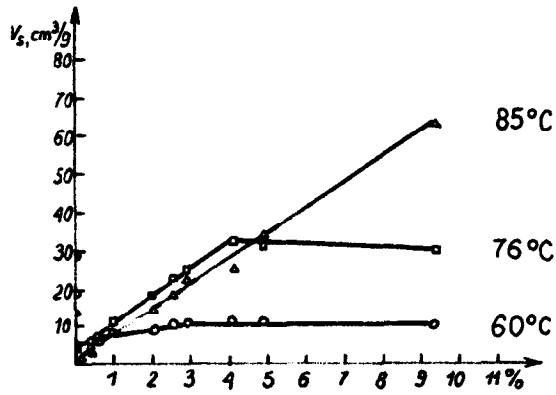

Fig. 13. Variation of the retention volume of *o*-xylene with the amount of liquid crystalline 4-cyano-4'-*n*-heptyloxyformyloxyazobenzene deposited on Chromosorb P AW DMCS[160].

opposite examples are also known. This depends on the properties of the whole liquid crystal – support system, which may manifest themselves in that below a certain amount of the liquid crystal on a silanized support supercooling does not take place at all or is very unstable, whereas above a certain coverage of the support with the same liquid crystal, characteristic of a given system, supercooling by several tens of degrees below the melting point may be stable. This characteristic coverage for 4-cyano-4′-*n*-heptyloxycarboxyazobenzene and silanized Chromosorb G, P and W was about 2.5%, 3% and 8%, respectively.[165]

The character of the surface of the support considerably affects the relative retention of the chromatographed substances[167]. On silanized and non-silanized Chromosorb P the relative retentions are greater at the same, small coverages of the supports with the liquid crystal stationary phase on the silanized support. The observed difference decreases with increasing coverage of the support (see Fig. 9).

5. PRACTICAL APPLICATIONS OF LIQUID CRYSTAL STATIONARY PHASES

Progress in the improvement of the physico-chemical properties and separation abilities of liquid crystal stationary phases and improvements in the efficiencies of columns filled with these phases has led to a continuing increase in the use of these phases in chromatographic practice.

Most examples of separations relate to various kinds of isomers, mainly positional isomers of benzene and naphthalene derivatives, *cis* and *trans* isomers of unsaturated hydrocarbons and isomers of polynuclear hydrocarbons and their derivatives. All these compounds often occur in mixtures of industrial importance. The separations of these substances on liquid crystal stationary phases are usually better and often faster than those on common isotropic stationary phases. This also relates to such highly selective isotropic stationary phases as cyclodextrins[56,153] and polysiloxanes.

Liquid crystals allow the separation of complex mixtures not only in the conventional way. For instance, Watabe *et al.*[168] developed a method for identifying and determinating components in the overlapping peaks of two or even three substances. They used a capillary column, provided with an internal and an external electrode, filled with 4,4′-di-*n*-amyloxyazoxybenzene to which they applied a constant electric field. Under the action of this field polar compounds are adsorbed in the column, the amount of the substance adsorbed increasing with the electric field. The adsorption is also affected by the dielectric constant and structure of the chromatographed substance and the kind of liquid crystal used.

5.1. Separation of isomers of benzene and naphthalene derivatives

For the separation of isomers of benzene derivatives, the use is recommended of methoxyazoxybenzene (MEAB), a nematic with the mesophase ranging from about 90 to 150°C and classified in the group of medium-temperature liquid crystal stationary phases. MEAB is the most frequently tested and practically used liquid crystal[74,81,82,90–98,169–174] and can also be applied when supercooled to 70°C[93]. Vigdergauz and co-workers are of the opinion that this liquid crystal should be included in the set of stationary phases recommended for the universal system of chemical analysis[74,175–177]. They suggested that 4,4′-ethoxypropoxyazoxybenzene should also be included in that set[132,178].

Alkylbenzenes are well separated on MEAB[90,92,93,169,170]. For instance, a mixture of C_{10}–C_{13} was succesfully separated on MEAB in a 23-m capillary in the mesophase temperature range[92], and at 100°C a good separation of 22 C_{14}–C_{17} alkylbenzenes was achieved in 22 min[170]. Xylene and ethylbenzene isomers can be separated in a 20-m column filled with MEAB in 70 s[93]. For the separation of a similar mixture, in addition to MEAB its eutectic mixture with 4,4′-azoxyphenetole was also used[172].

On MEAB a high relative retention of *p*- and *m*-xylene ($r_{p/m}$) is obtained, being 1.12–1.13 in the mesophase range. On supercooling, the value of $r_{p/m}$ at 80°C is 1.14 and at about 40°C it is 1.25[96,173]. The supercooled state at the latter temperature is, however, probably unstable.

The *p*- and *m*-xylene isomers are often used for measuring the selectivity of columns with liquid crystal stationary phases in view of the difficult separation of these isomers on common stationary phases. Some workers, however, are of the opinion that these isomers are not ideal test substances, as in the chromatographic system the interactions between the liquid crystal stationary phase and the chromatographed substance connected with their polarity are much greater than solute–solvent steric hindrance[83]. Some observations and conclusions regarding the differences in the retentions of *meta* and *para* disubstituted benzene derivatives on common stationary phases may be helpful when the mechanism of separations of these isomers on liquid crystal stationary phases is considered[179].

Apart form MEAB, other liquid crystal stationary phases also give very good separations of alkylbenzene isomers. For instance, the separations of alkylbenzenes obtained on 2-methyl-4′-*n*-butyl-4″-ethoxybenzoyloxyazobenzene in an analytical aluminium column were better than those obtained on SE-30 or OV-17[180]. A mixture of benzene, toluene, ethylbenzene, xylenes, isopropylbenzene, styrene and *n*-propylbenzene is better separated (with a different order of elution) and in a shorter time on 4-*n*-pentylacetophenone (O-4-*n*-ethyloxybenzoyl oxime)[95] than on a heavy alkylated benzene stationary phase modified with Bentone 34[181]. Various alkylbenzenes have also been successfully separated in a 1-m conventional analytical column on the liquid crystal hydroquinone *p*-heptoxybenzoate[182].

Other liquid crystals have also been used for separations of mixtures containing isomers of alkylbenzenes[183–185].

Very good separations of alkylbenzenes are obtained on recently obtained liquid crystal isothiocyanates[100]. The mesophase ranges of some of these liquid crystals compare well with those of the MEAB mesophase. Others reveal much wider ranges of the mesophase and may be used in capillary columns with temperature programming for the separation of mixtures containing components differing significantly in boiling temperature. Thus, on isothiocyanate stationary phases, mixtures containing alkylnaphthalenes in addition to alkylbenzenes may be separated[100]. For instance, dimethylnaphthalene isomers, and also pairs, which are very difficult to separate on other stationary phases, were separated.

The separation of methylnaphthalenes and of nine dimethylnaphthalenes on 4-ethyl-4′-(*p*-methylbenzoyloxy)azobenzene was studied by Suprynowicz *et al.*[146]. A liquid crystal containing a naphthalene ring in its molecule was applied to the separation of α- and β-naphthols and certain dihydroxynaphthalenes, and the effect was better than when OV-17 or OV-225 was used[186]. The results were better when the hydroxynaphthalenes were converted into acyl derivatives.

Very good separations of various positional and geometric isomers, including methylnaphthalenes, dichlorobenzenes and pheromones, were obtained when the liquid crystal stationary phase was used in the supercooled state[187].

5.2. Separation of alkene and alkane isomers

The isomers of alkenes and alkanes can be separated on conventional stationary phases especially when long capillary columns are used. The above described MEAB liquid crystal is useful for separating alkenes. This asymmetric azoxybenzene was compared with other symmetric and asymmetric azoxybenzenes and with methoxyethoxyazobenzene for the separation of C_{15}–C_{17}. MEAB showed the best separating properties[94].

On the MEAB mesophase and supercooled phase in a capillary column good separations of C_{15}–C_{18} alkanes and of mixtures of alkene isomers and cyclic hydrocarbons[91] and also C_{10}–C_{14} alkenes[93] were obtained. A change in retention occurs in which *trans*-5-decene is eluted before *trans*-4-decene. In general, it can be concluded that the *trans* isomers are retained longer than the *cis* isomers on liquid crystal stationary phases[96]. In Fig. 14 it is shown that the analysis of *n*-pentadecene isomers and *n*-pentadecane is much faster when chromatography is carried out on MEAB instead of conventional stationary phases[96,188].

A procedure for the analysis of hydrocarbon mixtures has been patented. One of the claims is the use of MEAB[189,190] and of a eutectic mixture of two other liquid crystal stationary phases[191].

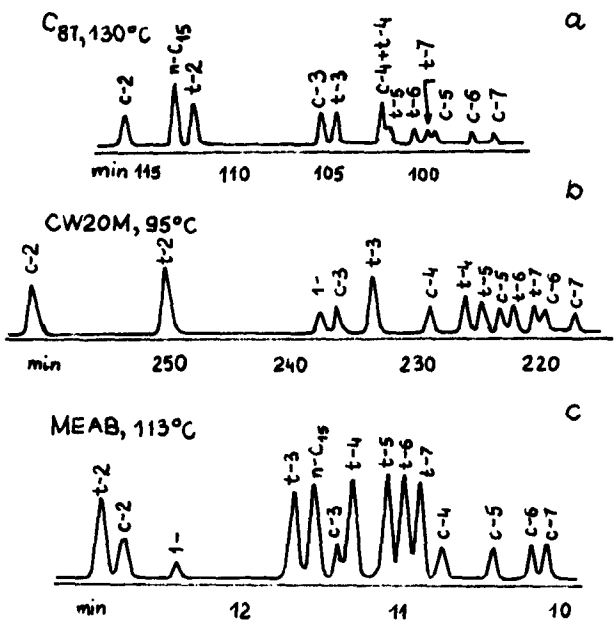

Fig. 14. Chromatograms of the separation of *n*-pentadecene isomers on columns coated with (a) Apolan-87 (200 m × 0.25 mm I.D., N = 670 000 plates), (b) Carbowax 20M (300 m × 0.25 mm I.D., N = 400 000 plates) and (c) MEAB (90 m × 0.25 mm I.D., N = 200 000 plates). c- = *cis*-isomer; t- = *trans*-isomer; n-C_{15} = *n*-pentadecane; 1- = *n*-pentadecene[173].

In general, 4-*n*-pentylacetophenone (O-4-*n*-pentyloxybenzoyl oxime; PBO) is less selective than MEAB, but the isomers of C_9–C_{11} *n*-alkenes and *n*-alkanes are separated better on it than on MEAB[97]. The diastereoisomers of C_8–C_{10} alkanes are separated better on PBO than on conventional stationary phases[98]. It has been shown that PBO has advantages as a stationary phase in the separation of alkyne isomers[192]. As in other systems, alternation of retention of the particular isomers was observed depending on their molecular structure (even or odd number of atoms in the molecule and position of the multiple bond in the chain). The separation of alkynes on a liquid crystal is easy as they are eluted in the order of corresponding to the shift of the triple bond from the centre towards the end of the chain, the selectivity increasing in the same order. Particularly good results were obtained when alkynes with ten or more carbon atoms in the molecule were separated[192].

C_{18}–C_{20} isoprenoid diastereomeric alkanes are separated worse on PBO than on isotropic phases[98]. In contrast, diastereomeric arylalkenes are separated better on a cholesteric liquid crystal than on a non-polar isotropic stationary phase[193]. By comparing the results obtained it can be concluded that if an isomer with a "more elongated" racemic molecule is eluted from a conventional stationary phase in the same or longer time than an isomer with a "less elongated" *meso* molecule, then these isomers are separated better on a liquid crystal stationary phase. This situation prevails with C_8–C_{10} hydrocarbons. In contrast, if the "more elongated" isomers is eluted earlier, then its shift beyond the "less elongated" isomer, typical of liquid crystal stationary phases, becomes diffcult and therefore the separation on liquid crystals is worse[98].

The separation of a multi-component mixture of paraffinic hydrocarbons in a common 1-m analytical column filled with the hydroquinone ester of *p*-heptoxybenzoic acid, although requiring a long time, is an illustration of the separation possibilities with liquid crystal stationary phases[182].

5.3. Separation of mixtures of benzene and aliphatic hydrocarbon derivatives containing heteroatoms

Apart from hydrocarbons, many other organic compounds of different polarity and volatility, including those with oxygen in the molecule, may be analysed on liquid crystal stationary phases.

As regards non-alkyl benzene derivatives, the separation was studied of dichlorophenol isomers on 4,4'-dimethoxyazoxybenzene (DMAB) and on isotropic stationary phases. Although the separation on DMAB was generally good, the separation of 2,6- and 2,5-dichlorophenols proved impossible. No succes was achieved in this respect when the above liquid crystal was mixed with dioctyl phthalate or silicone KF-54[194]. The separation of dichlorophenols also presented problems when 4,4'-diethoxyazoxybenzene was used as the stationary phase[195]. In contrast, chlorophenol isomers and the esters of *p*-hydroxybenzoic acid were well separated on liquid crystal polyacrylates[145].

Among other compounds chromatographed on liquid crystal stationary phases are lower aliphatic amines[196], pesticides[197], mono- and bifunctional impurities in terephthalic acid and dimethyl terephthalate[198], organic compounds in aqueous medium[199], alcohols, esters, glycols[200], pentanol isomers, esters[201], diene aliphatic alcohols and their acetates[202], gemfibrosil [2,2-dimethyl-5-(2,5-xylyloxy)valeric acid] con-

taining an isomeric contaminant[203], isomers of C_3–C_5[132], C_1–C_5 and C_3–C_8 aliphatic alcohols[111], pheromones[204] and oxygen-containing 4,4-dimethyl-1,3-dioxane synthesis products[112]. On polymeric liquid crystal siloxanes, *cis* and *trans* isomers of saturated fatty acid esters were separated[99], the *trans* isomers being retained longer than the *cis* isomers.

5.4. Separation of polynuclear hydrocarbons

Gas chromatography with liquid crystal stationary phases allows the analysis of various mixtures of polynuclear hydrocarbons. Such analyses have become fairly common, on the one hand in view of the high toxicity of these compounds, many of them being carcinogenic[205], and on the other because liquid crystal stationary phases are particularly well suited for this analysis as they allow the separation of isomers.

Recently, polymeric siloxane liquid crystal stationary phases have become increasingly used for separating polynuclear hydrocarbons[28–33,35,67,99,103,206–209]. These phases are suitable for use in capillary columns[80], primarily glass and fused-silica columns. They are usually used individually, although sometimes they are mixed with isotropic siloxane polymers, *e.g.*, SE-30 (polydimethylsiloxane)[99]. It is interesting that most of these polymers are smectics and that they reveal very good separation properties[28,29,31–33,103,206,207,209,210]. In addition to siloxane polymers, polymers without silicon atoms in the molecule are also used for this purpose[35,145]. All polymers can be used when the temperature is programmed over a wide range. Some of them may be used for a longer time at 280°C and a shorter time at 300°C.

The nematic siloxane polymers reveal mesophase ranges of 70–300°C[67] or 91–319°C[99], and their separation properties are superior to those of SE-52. This has been shown by way of example for methyldibenzothiophenes and tetranuclear aromatic compounds with sulphur in the ring, and also for other polyaromatic hydrocarbons and their methyl isomers. For the last separation columns filled with SE-52 and a liquid crystal were combined in different configurations, various separations being obtained[67].

On smectic liquid crystal siloxane polymers the following separations were, for instance, carried out: polychlorodibenzodioxine and polychlorodibenzofuran isomers[103,208], hydroxy thiophene derivatives (both primary and modified by treatment with trimethylsilylimidazole)[206] and isomers of methylchrysene and methylbenz[*a*]anthracene[32]. On the polysiloxane smectic phase, dimethyldibenzothiophene isomers[29], coal tar polynuclear hydrocarbons[28], isomers of methylphenanthrene, methylchrysene, hydroxydibenzothiophene, aminophenanthrene and of other compounds[31] are separated better than on SE-54. Naikwadi *et al.*[210] separated, in addition to polynuclear hydrocarbons and polychlorinated compounds, also halogen-disubstituted benzene derivatives. The separation of mixtures on liquid crystal siloxane polymers, as on other stationary phases, depends on the molecular structure of these polymers. It has been found that an increase in the number of mesomorphic groups in the smectic polymers leads to a higher smectic–isotropic liquid phase transition temperature and to a higher selectivity towards isomers. However, this is accompanied by a slow decrease in the efficiency of the system. We can illustrate this effect by using a mixture of methylphenanthrene and methylcarbazole isomers by way of example[28].

It has been found that an analogy exists between the dependence of retention on

the shape of the molecule of the substance being chromatographed on the liquid crystal smectic siloxane polymer in gas chromatography and on the polymeric octadecylsilane (C_{18}) in liquid chromatography[209]. The similar order of elution of isomeric polynuclear hydrocarbons in both instances indicates that the surface of the polymeric C_{18} phases is more ordered than that of the monomeric type[211]. The similarity of the selectivities on reversed phases in liquid chromatography and on liquid crystal stationary phases in gas chromatography may be extended beyond polyaromatic compounds by suggesting that the excellent separations of other isomeric compounds on liquid crystals may also be achieved in liquid chromatography on polymeric C_{18} phases.

It should be noted that the separations of polynuclear hydrocarbons on polymeric smectics are so good that they have been used to establish the quantitative composition (with the use of an internal standard) of standard solutions of these hydrocarbons[207].

On liquid crystal polyacrylates, in addition to mixtures of aromatic compounds also the isomers of chlorophenols and esters of *p*-hydroxybenzoic acid and of methoxynaphthalenes and naphthols have been separated[35,145,212]. Polyacrylates have also been used as stationary phases in supercritical fluid chromatography in glass and fused-silica capillary columns with carbon dioxide as the mobile phase. These stationary phases resist pressures of 200 MPa at 160°C. They have also been used to separate the isomers of naphthols and phenylphenols[213]. It can be expected that liquid crystal stationary phases will be increasingly used in supercritical fluid chromatography as this method develops further, its current development being very rapid and promising[214–218].

In the analysis of polynuclear hydrocarbons, monomeric liquid crystals from the group of Schiff bases, mainly BMBT, BBBT, BPhBT and BHxBT, find continued application[68,69,72,73,219–224]. In view of their features, these stationary phases have been chiefly used in conventional analytical columns[69,72,73,219–223] and only seldom in capillary (fused-silica) columns[68,84,224]. On these phases separations were effected of, *e.g.*, biphenyl polychloro derivatives[222], hydrocarbons containing 3–6 rings in the molecule and their derivatives and also isomers[72,73,219–221,223]. Some hydrocarbons were isolated from graphitized carbon black[72] and airborne particulates[221,223]. On BBBT phase mixed with SE-52 (1:1), 4-5-ring hydrocarbons containing sulphur in the molecule were separated[68]. The mixed phases BMBT – OV-17 and BBBT – SE-30 were used in combined columns or in one column to determine the content of anthracene, phenanthrene and carbazole in carbochemical products[69]. It has been shown in this connection that gas chromatography gives superior determination to polarography and UV spectrometry.

Anthracene, phenanthrene and other polynuclear hydrocarbons, including benzopyrenes, have also been separated on other high-temperature liquid crystal stationary phases with very good results[77,143,144,225–231].

The earlier considered liquid crystal polysiloxane stationary phases reveal very good separating properties and high thermal stability. Some of them, however, have the disadvantage that it is almost impossible to use them in the whole range of the mesophase and supercooled state as their viscosity is high at low temperatures[30]. This disadvantage does not apply to liquid crystal isothiocyanates, which are characterized by a wide range of the mesophase, comparable to that of the siloxane polymers, and

which have a low viscosity. Owing to these properties the liquid crystal isothiocyanates are potentially superior to liquid crystal Schiff bases as stationary phases. The easier synthesis, good solubility and good coating of capillary column walls are also features that place isothiocyanates ahead of siloxane polymers. Studies of the liquid crystal isothiocyanates as stationary phases were started only recently, so not all their properties are yet known. However, the first results regarding the separation of polynuclear hydrocarbons are very promising[101,232].

In discussing the analyses conducted on liquid crystal stationary phases, it should also be noted that various chromatographic methods have been used for testing the purity of and separating liquid crystal mixtures, *e.g.*, thin layer chromatography[233,234], high-performance column chromatography combined with mass spectrometry[235], gas chromatography[236] and supercritical fluid chromatography[237].

To assess chromatographic separations on liquid crystal stationary phases one can use the universal retention indices[174,178,238].

In Table 2 examples are given of separations on liquid crystal stationary phases and the parameters of the chromatographic processes. In Figs. 15-17 examples of chromatograms of separated compounds are shown.

6. APPLICATIONS OF LIQUID CRYSTALS IN LIQUID CHROMATOGRAPHY

6.1. Column chromatography

It has already been mentioned that liquid crystal siloxane polymers find application in supercritical fluid chromatography[213]. A recent paper dealt with the use of liquid crystals in column liquid chromatography[241]. It described the behaviour and properties of two liquid crystals, *viz.*, 4-ethoxybenzylidene-4′-*n*-butylaniline (EBBA) and cholesteryl oleate (ChO). The mesophase range of EBBA lies in the range 35.5–77.5°C and that of ChO in the range 20–33°C. These liquid crystals were deposited on Silasorb-600 (silica gel) in amounts of 20% and 40%, respectively. The phase transition points of these liquid crystals measured chromatographically were lower than those measured thermo-optically. With EBBA the clearing point was about 30°C lower. This lowering of the phase transition points was related to the adsorption of the liquid crystals on the surface of silica gel, although this has not been confirmed. Possibly the effect of hexane used as the mobile phase, which could influence the properties of the mesophase, was wrongly neglected. This seems the more likely as the properties of the mesophase are influenced even by the presence or absence of a gas[242].

With an increase in temperature the capacity factor of nitrotoluene isomers increased on both liquid crystals (EBBA and ChO). However, no distinct differences in the α values of *o*- and *m*-dinitrobenzene (DNB) at the melting point of EBBA were observed on uncoated Silasorb and Silasorb coated with the EBBA liquid crystal, although there were certain differences in the course of the plot of $\alpha_{o/m\text{-DNB}}$ *versus* temperature.

Examples have been given of the separation of nitrotoluene isomers on ChO (see Fig. 18). The separation was better the higher the column temperature. However, this separation should not be related directly to the liquid crystal properties of ChO as at least two separations (better) were obtained beyond the mesophase range. It is difficult to explain the results obtained and the mechanism of the observed phenom-

TABLE 2

EXAMPLES OF LIQUID CRYSTAL STATIONARY PHASES AND MIXTURES SEPARATED ON THEM[a]

Stationary phase	*Transition temperature to phase (°C)*			*Column (m × mm I.D.)*	*Temperature of column (°C)*	*Separated substances and separation time (min)*	*Ref.*
	Smectic	*Nematic*	*Isotropic*				
4-Methoxy-4'-ethoxyazoxybenzene (MEAB)		91	150	Glass capillary		22 C_{14}–C_{17} alkylbenzenes and *o*-dialkylbenzenes obtained from dehydrogenation of *n*-alkanes (32)	19
					140	20 C_{10}–C_{13} phenylalkanes (17.5)	
4-*n*-Pentylacetophenone (O-4-*n*-ethyloxybenzoyl oxime) (EBO)		97	112	Glass, 48 × 0.25	40	9 C_6–C_9 aromatic hydrocarbons (2.5)	
Polysiloxanes	98		226	Fused silica, 12 × 0.2	120–200	5 methylphenanthrene isomers	28
	103		288				
	92	246	286	Fused silica, 12 × 0.2	120–230	4 methylcarbazole isomers	
	120		230–260	Fused silica, 13 × 0.2	40–280	16 polycyclic aromatic hydrocarbons in a coal tar	
	60		226				
Polysiloxane (PMPS-1,2)	130	219	235	Fused silica, 18 × 0.3	80–200	7 isomeric dimethyldibenzothiophenes	29
Polysiloxane	118		300	Fused-silica capillary	70–170	5 isomeric methylphenanthrenes (19)	31
				Fused-silica capillary	70–250	6 isomeric methylchrysenes (38)	
				Fused-silica capillary	70–210	4 isomeric hydroxydibenzothiophenes (29)	
				Fused-silica capillary	70–210	5 isomeric aminophenanthrenes (30)	
				Fused-silica capillary	120–220	4 nitrogen heterocyclics and 5 *n*-alkanes (20)	
				Fused-silica capillary		5 isomeric four-ring polycyclic aromatics	
Polysiloxane	104		308	Fused silica, 10 × 0.2	40–250	16 methylbenz[*a*]anthracene and methylchrysene isomers (30)	32
	95		306				

Polysiloxanes	130 118	219	235 300	Fused silica, 10-30 × 0.2 or 0.3		Polycyclic aromatic compounds and slightly polar sulphur heterocyclics	33
Polyacrylates		75	246	Glass, 32 × 0.25	130	Diphenylmethane, biphenyl, 1,2-diphenylethane, 1-methoxynaphthalene, 2-methoxynaphthalene, fluorene, *o*-terphenyl, triphenylmethane, phenanthrene, anthracene (37)	35
		69	230	Glass, 30 × 0.25	150	*o*-, *m*- and *p*-toluic acids (15)	
		70	270	Glass, 32 × 0.25	180	1- and 2-naphthol (11)	
		85	291	Glass, 31 × 0.25	135	Methyl, ethyl, propyl, butyl, pentyl and hexyl benzoates (14)	
Mixture of two disc-like liquid crystals (1:1)				Glass, 2.1 × 4	78	Pentane, hexane, cyclohexane, methylcyclohexane, octane (4.5) Cyclohexane, cyclohexene, benzene, cyclooctene, cyclootatetraene, cyclooctadiene (15) Heptane, octane, nonane, *p*-xylene, *o*-xylene, decane (16)	57
N,N′-Bis(*p*-methoxybenzylidene)-α,α′-bi-*p*-toluidine (BMBT) + GE SE-30 N,N′-Bis(*p*-butoxybenzylidene)-α,α′-bi-*p*-toluidine (BBBT) + GE SE-30				Steel, 2 × 2	230	Technically pure anthracene, (naphthalene, 1- and 2-methylnaphthalenes, diphenyl, dimethylnaphthalene, diphenyl oxide, fluorene, triphenylmethane, phenanthrene, anthracene, carbazole, fluoranthene, pyrene (19)	66
Polysiloxane (PMMS)		70	300	Fused silica, 19 × 0.32	120–260	16 methyldibenzothiophenes and four-ring aromatic sulphur heterocyclics (31)	67
					120–260	17 compounds of the sulphur heterocyclics fraction of a coal liquid (40)	
					120–270	32 polycyclic aromatic hydrocarbons (57)	
					230	6 methylchrysene isomers (16)	
PMMS + SE-52					230	6 methylchrysene isomers (21)	
BBBT + SE-52 (1:1)				Fused silica, 19.6 × 0.32	220	12 four-ring aromatic sulphur heterocyclics (10)	68
					230	10 five-ring aromatic sulphur heterocyclics (25)	

(Continued on p. 68)

TABLE 2 (*continued*)

Stationary phase	*Transition temperature to phase (°C)*			*Column (m × mm I.D.)*	*Temperature of column (°C)*	*Separated substances and separation time (min)*	*Ref.*
	Smectic	*Nematic*	*Isotropic*				
Mixture of three liquid crystals				Glass, 3.1 × 3	104	Mono- and dialkylbenzenes (20)	70
BBBT	159	188	303	Glass, 0.7 × 3	210	Polycyclic aromatic hydrocarbon fraction of a carbon black (300)	72
BMBT		181	320	Glass, 1.9 × 2	190	17 tricyclic and tetracyclic aromatic hydrocarbons (35)	73
				Glass, 3 × 2	240	Mixture of isomers of pentacyclic aromatic hydrocarbons	
				Glass, 1.9 × 2	260	Mixture of isomers of hexacyclic aromatic hydrocarbons	
4,4′-Biphenylene dibenzoate		250	350	Steel, 1 × 2	240	Isomeric four-ring compounds (44)	77
					280	Isomeric five-ring compounds (7)	
				Steel, 3 × 2		Isomeric disubstituted benzenes and naphthalene derivatives	
MEAB		91	150	Brass, 10 × 0.35	178	10 methyl esters of fatty acids (40)	81
				Brass, 6 × 0.35	160	10 alcohols (14)	
				Brass, 8 × 0.35	180	9 cyanides of fatty acids (28)	
MEAB		97	147	Steel, 50 × 0.25	120	Isomers of chlorotoluene (8)	82
4-*n*-Pentylacetophenone (O-4-*n*-heptylbenzyl oxime)		41	69.5	Glass capillary		Isomers of C_8 cyclic and aromatic hydrocarbons	83
BBBT		188	303	Glass, 18 × 0.25	200–260	Methylchrysene isomers (44)	84
					200	3 four-ring polycyclic aromatic hydrocarbons (18)	
					280	Benzopyrenes, perylene and benzofluoranthenes (41)	
N,N′-Bis(*p*-phenylbenzylidene)-α,α′-bi-*p*-toluidine (BPhBT)		257	403	Glass, 20 × 0.25	280	10 polycyclic aromatic hydrocarbons (25)	
4-*n*-Pentylacetophenone (O-4-*n*-pentyloxybenzoyl oxime) (PBO)		63	94	Glass capillary		*cis* and *trans* isomers of *n*-tridecenes and *n* tetradecenes	86
BBBT		188	303	Glass, 10 × 0.22	280	Phenanthrene, anthracene, pyrene, benz[*a*]anthracene, chrysene (5)	89

				Glass, 10 × 0.22	280	Mixture of 4–6 polycyclic aromatic hydrocarbons (55)	
MEAB		97	147	Steel, capillary		C_5–C_{18} *n*-alkanes, isoalkanes and alkylcyclic hydrocarbons	91
MEAB		91.5	150	Glass, 23 × 0.25	90	20 C_{10}–C_{13} phenylalkanes (44)	92
					140	20 C_{10}–C_{13} phenylalkanes (15)	
MEAB		95.8	148	Glass, 20 × 0.25		Isomers C_8 alkylbenzenes and *cis*, *trans* isomers of C_{10}–C_{14} *n*-alkenes	93
MEAB		95.8	148	Glass capillary		*cis* and *trans* isomers of C_{15}–C_{17}	94
4-Propoxy-4′-ethoxyazoxybenzene		101.5	145			*n*-alkenes	
4-Propoxy-4′-propoxyazoxybenzene		115.5	123.6				
4-Butoxy-4′-ethoxyazoxybenzene		100	146				
4-Methoxy-4′-ethoxyazobenzene		132.2	134.5				
EBO		56	93	Glass capillary	40	Benzene, toluene, ethylbenzene, *m*- and *p*-xylene, isopropylbenzene, *o*-xylene, *n*-propylbenzene, styrene (2.5)	95
MEAB		95.8	148	Glass, 20 × 0.25	95	Isomers of C_8 alkylbenzenes (1.2)	96
				Glass, 86 × 0.25	80	Isomers of C_{10}–C_{13} *n*-alkenes (44)	
EBO		56	93	Glass, 48 × 0.25	40	Benzene, toluene, ethylbenzene, *m*- and *p*-xylene, isopropypbenzene, *o*-xylene (2)	
PBO		63	94	Glass, 100 × 0.25	56	25 isomeric C_9–C_{11} *n*-alkenes and the corresponding *n*-alkanes (72)	97
PBO		63	94	Glass, 100 × 0.25	40	Diastereomeric C_8–C_{10} alkanes (30)	98
Polysiloxanes (MEPSIL)				Glass, 15 × 0.25	140–200	12 fatty acid methyl esters (16)	99
MEPSIL + SE-30 (1:1)				Glass, 15 × 0.25	100–290	20 polycyclic aromatic hydrocarbons (56)	
					240	Isomeric methyl-substituted benz[*a*]-anthracenes (22)	
					275	Isomeric methyl-substituted benzo[*a*]pyrenes (32)	
Isothiocyanates		58	132	Glass, 15.5 × 0.3	50	Alkylbenzenes and dialkylbenzenes (16)	100
					127	Dialkylnaphthalenes (24)	
	55.8	98.5	130	Glass, 20 × 0.3	100.5	Alkyl- and dialkylbenzenes (10)	
					129.5	Isomers of dimethylnaphthalenes (25)	
Isothiocyanate	135	163	280	Glass, 12.5 × 0.3	246–279	13 polycyclic aromatic hydrocarbons (33)	101

(Continued on p. 70)

TABLE 2 (*continued*)

Stationary phase	*Transition temperature to phase (°C)*			*Column (m × mm I.D.)*	*Temperature of column (°C)*	*Separated substances and separation time (min)*	*Ref.*
	Smectic	*Nematic*	*Isotropic*				
SB-smectic				Glass, 25 × 0.32	180–280	2,3,7,8-Class congeners of polychlorodibenzodioxins and polychlorodibenzofurans	103, 164
4-(4-Nonyloxybenzoyloxy)-4′-cyanoazobenzene		70	250	Glass, 1.5 × 3	220	Fluorene, phenanthrene, anthracene (11)	108
					140	*m*- and *p*-dibromobenzene, *m*- and *p*-chloroacetophenone (6)	
					75	3 xylene isomers (2.5)	
					55	3 xylene isomers (3.5)	
4-*n*-Butyloxybenzoic acid		147	160	Glass, 2 × 3	100	Methane, methyl bromide, ethyl bromide, ethyl iodide, ethyl acetate, ethanol diethyl sulphide (15)	110
2-Cyano-5-phenylpyrimidine		121	128	Glass, 2.2 × 3	80	Benzene, toluene, ethylbenzene, *m*- and *p*-xylene, isopropylbenzene, *o*-xylene	111
					124	4 isomers of butanol	
					80	8 C_1–C_5 alcohols	
					80	6 C_3–C_5 alcohols	
					124	methyl ethyl ketone, methyl propyl ketone, methyl butyl ketone	
4,4′-Azoxyphenetole		138	168	Glass, 6 × 3	92	6 heterocyclic compounds of the dioxane and pyrane group (16)	112
4,4′-Ethoxypropoxyazoxybenzene		102.5	148			Isopropanol, *n*-propanol, isobutanol, *n*-butanol, isopentanol, *n*-pentanol	132
Bis(4-methylene-4′-*n*-butoxy-azobenzene		201	310	Steel, 1 × 4	243	13 polycyclic aromatic hydrocarbons (60)	144
Bis(4-methylene-4′-*n*-butoxy-3′-cyanoazobenzene)	197	212	283	Steel, 1 × 4	220	11 polycyclic aromatic hydrocarbons (50)	
					180	1- and 2-naphthylamine (8)	
Bis(4-methylene-4′-*n*-butoxy-3′-methylazobenzene		115	204	Steel, 1 × 4	120	1- and 2-ethylnaphthalene (12)	

Polyacrylates	95	250	Glass, 35 × 0.25	160	10 aromatic hydrocarbons (29)	145
	70	270	Glass, 32 × 0.25	140	3 chlorophenol isomers (8)	
				190	4 alkyl *p*-hydroxybenzoates (10)	
4-*n*-Butyloxybenzoic acid	147	160	1 × 3	150	3 isomers of trimethylbenzenes (7)	159
			2 × 3	149	Impurities in dimethylmercury (4.5)	
				149	Impurities in diethyl sulphide (57)	
4,4′-Azoxyphenetole	138	168	Copper, 50 × 0.36	138	Octane, benzene, toluene, *m*-, *p*- and *o*-xylene (30)	169
MEAB	96	148	Copper, 50 × 0.36	100	Octane, benzene, toluene, ethylbenzene, *m*-xylene, isopropylbenzene, *p*- and *o*-xylene (70)	
MEAB	91.5	150	Glass, 23 × 0.25	100	22 C_{14}–C_{17} alkylbenzenes (32)	170
MEAB	91	150	Copper, 40 × 0.35	108	Hexane, benzene, toluene, ethylbenzene, xylene isomers (28)	172
MEAB	91	150	Glass, 90 × 0.25	80	Isomeric C_{10}–C_{13} *n*-alkenes (44)	173
				113	*n*-Pentadecene isomers (13)	
2-Methyl-4′-*n*-butyl-4-(4″-ethoxybenzoyloxy) azobenzene	107	199	Aluminium, 1.84 × 4	107	13 mono-, di- and trialkylbenzenes (21) 3 vinylbenzenes (28)	180
Hydroquinone bis(*p*-heptyl-oxybenzoate)	121	195	1 × 3	150	20 aromatic hydrocarbons (24) Isomeric C_6–C_{19} *n*-alkanes (125)	182
2,6-Naphthalene bis(*p*-*n*-heptyloxycinnamate)	158	291	Glass, 1 × 3	160	1- and 2-naphthol	186
				170	1- and 2-naphthyl acetate 1- and 2-naphthyl propionate	
				225	1- and 2-naphthyl benzoate	
2-Methyl-4′-methoxy-4-(*p*-methoxycinnamoyloxy) azobenzene	149	298	Glass, 2.6 × 2.6	135 (solid)	1- and 2-methoxynaphthalene (6)	187
				135 (nematic)	1- and 2-methoxynaphthalene (30)	
2-Methyl-4′-ethoxy-4-(*p*-methoxycinnamoyloxy)azobenzene	154	300	Glass, 2.6 × 2.6	104–170	9,11-Tetradecadienyl acetate isomers (insect sex pheromones) (55)	
2-Methyl-4′-*n*-butyl-4-(*p*-methoxycinnamoyloxy)azobenzene	109	253	Glass, 2.6 × 2.6	60	*m*- and *p*-xylene (9)	
				110	*m*- and *p*-dichlorobenzene (5)	
MEAB	93	150	Glass, 80 × 0.25	113	13 *cis* and *trans*	188

(Continued on p. 72)

TABLE 2 (*continued*)

Stationary phase	*Transition temperature to phase (°C)*			*Column (m × mm I.D.)*	*Temperature of column (°C)*	*Separated substances and separation time (min)*	*Ref.*
	Smectic	*Nematic*	*Isotropic*				
						n-pentadecene isomers (13)	
MEAB		96	148	Glass, 90 × 0.25	87	47 isomers of C_9–C_{13} *n*-alkenes and C_9–C_{13} *n*-alkanes (45)	189
MEAB		93	150	Steel, 50 × 0.25	97	15 isomers of C_9–C_{11} *n*-alkenes and C_9–C_{11} *n*-alkanes (23)	190
						26 isomers of C_9–C_{11} *n*-alkenes, C_9–C_{11} isoalkenes and C_9–C_{11} *n*-alkanes	
4-*n*-Pentylacetophenone-(O-4-*n*-pentyloxybenzoyl oxime) (PBO)		63	94	Glass, 90 × 0.25	70	*n*-Decyne isomers (12)	192
						n-Undecyne isomers (17)	
				Glass, 62 × 0.25	70	*n*-Tridecyne isomers (27)	
					80	*n*-Tetradecyne isomers (35)	
Cholesterol *p*-chlorocinnamate				Glass, 26 × 0.2	175	Diastereomeric arylalkenes (17)	193
				Glass, 13.6 × 0.2	190	Diastereomeric arylalkenes (54)	
4,4′-Azoxyphenetole		136	167	Glass, 2 × 2	138	4 isomers of pentanol	201
N,N′-Bis (*p*-methoxybenzylidene)-α,α′-bi-*p*-toluidine (BMBT)		181	320	Glass, 1.8 × 2	135	Gemibrozil containing 2,4-xylyloxy isomer contamination (13)	203
					160	2,5-Xylenol containing 2,4-xylenol contamination (7)	
SB-smectic				30 × 0.25	30–280	104 polycyclic aromatic hydrocarbons (52)	207
Polysiloxane				Fused silica, 20 × 0.25	100–270	Polychlorinated biphenyls and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (39)	208
SB-smectic				Fused silica, 20 × 0.25	100–270	9 disubstituted benzene isomers (11)	210
						8 chlorinated compounds (95)	
						17 isomeric polycyclic aromatics (69)	
						25 isomeric polycyclic aromatics (46)	
						80 isomeric polycyclic aromatics (72)	

2-Methyl-4′-methoxy-4-(4″-methoxycinnamyloxy)-azobenzene		126	262	2.6 × 2.6	150 90°C for 20 min then programmed at a rate of 2°C/min	Methoxynaphthalenes (18) 6 isomeric insect sex pheromone (67)	212
Polymer		95	280	Glass, 38 × 0.25	215 225	Naphthols (13) Dihydropyrene, fluoranthene, pyrene (14)	
Polymer		88	245	Glass, 45 × 0.15	180 149	10 aromatic compounds (43) 12 aromatic compounds by supercritical fluid chromatography (SFC) (42)	
Polyacrylates		75	246	Glass, 45 × 0.15	149	Diphenylmethane, diphenyl, diphenylethane, 1-methoxynaphthalene, 2-methoxynaphthalene, fluorene, *o*-terphenyl, triphenylmethane. phenanthrene, anthracene by SFC (27)	213
		95	250	Fused silica, 10 × 0.1	100 150	*o*-Phenylphenol, 1- and 2-napthol, *m*- and *p*-phenylphenol by SFC (12) 2,6-, 2,5-, 3,5- and 3,4-xylenol (15)	
N,N′-Bis(*p*-methoxyphenylbenzylidene)-α,α′-bi-*p*-toluidine (BABT)		253	370	Glass, 1.8 × 2	235	N-Trimethylsilylimidazole ethers of monohydroxybenzo-[*a*]pyrene isomers (21) Methylbenzo[*a*]pyrene isomers (33)	219
N,N′-Bis(*p*-phenylbenzylidene)-α,α′-bi-*p*-toluidine (BPhBT)		257	403	Glass, 1.8 × 2	270	N-Trimethylsilylimidazole ethers of monohydroxybenzo-[*a*]pyrene isomers (21) Methylbenzo[*a*]pyrene isomers (34)	
BPhBT + N,N′-bis(*p*-hexyloxybenzylidene)-α,α′-bi-*p*-toluidine (1:1)				Glass, 1.5 × 2	120–255	14 polycyclic aromatic hydrocarbons (59) 10 polycyclic aromatic hydrocarbons (59)	221
N,N′-Bis(*p*-butoxybenzylidene)-α,α′-bi-*p*-toluidine (BBBT)	159	188	303	1.2 × 3	190–265	28 polycyclic aromatic hydrocarbons in benzene extract of coal fly ash (65)	
Bis(4′-ethyldiphenylidene)-1,2-di(4-aminophenyl)ethane	234	282	400	Steel, 2 × 2.2	285	10 polycyclic aromatic hydrocarbons (25)	226

(Continued on p. 74)

TABLE 2 (*continued*)

Stationary phase	*Transition temperature to phase (°C)*			*Column (m × mm I.D.)*	*Temperature of column (°C)*	*Separated substances and separation time (min)*	*Ref.*
	Smectic	*Nematic*	*Isotropic*				
						Anthracene oil (phenanthrene, anthracene, fluoranthene, pyrene, chrysene, benzo[*e*]pyrene, perylene, benzo[*a*]pyrene) (25)	
4′-(*trans*-4-Propylcyclohexyl)-4-biphenylcarboxylic acid 4′-(*trans*-4-pentylcyclohexyl)-4-biphenyl ester	200	224	462	2 × 2.2	290	*p*-5-(*cis*/*trans*-4-Pentylcyclohexyl)-2-pyrimidinylbenzonitrile (16)	228
4′-(*trans*-4-Pentylcyclohexyl)-4-biphenylcarboxylic acid *trans*-4-(*p*-cyanophenyl) cyclohexyl ester		148.6	347	2 × 2.2	150	Methyl oleate (*Z*)-isomer and methyl elaidate (*E*)-isomer (21)	
4′-{*trans*-4-[2-(4′-(*trans*-4-pentylcyclohexyl)-4-biphenylyl)ethyl]cyclohexyl} 4-biphenylcarbonitrile		194.5	440	2 × 2.2	230–300	Phenanthrene, anthracene, fluoranthene, pyrene, benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene (28)	
Polymer	195		265	Glass, 1.7 × 4	230	Benzene, naphthalene, diphenyl, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene (22)	
Bis(4′-ethyldiphenylidene)-1,2-di(4-aminophenyl)ethane	234	282	400	Glass, 1.1 × 4	238	11 polycyclic aromatic hydrocarbons (12)	231
					282	11 polycyclic aromatic hydrocarbons (10)	

Bis(4'-penthyldiphenylidene)-1,2-di(4-aminophenyl)ethane	130		387	Glass, 1.1 × 4	316	11 polycyclic aromatic hydrocarbons (7)	
Polysiloxanes	43		177			Xylene isomers	239
	75		176			Xylene isomers	
	67		156			Xylene isomers	
BPhBT		257	403			Chrysene, benzo[*a*]pyrene, benzo[*e*]pyrene, benz[*a*]anthracene	240
Polysiloxane		139	319	Glass, 15 × 0.25	180	Fluorene, phenanthrene, anthracene (3.5)	30
					235	Fluoranthene, pyrene, 1,2- and 2,3-benzofluorene, triphenylene, benz[*a*]anthracene, chrysene (9.5)	
					275	Benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*e*]pyrene, perylene, benzo[*a*]pyrene (11)	
					140–280	17 polycyclic aromatic hydrocarbons (54)	
Hexapentyloxytriphenylene (disc-like from 65°C)			118	Glass, 2.1 × 4	70	Heptane, octane, nonane, *p*- and *o*-xylene, undecane (29)	56
					78	Cyclooctene, cyclooctane, cyclooctatetraene, cyclooctadiene (17)	
Triphenylene hexa-4-octylbenzoate (disc-like from 137°C)			206	Glass, 2.1 × 4	198	*trans*- and *cis*-decalin, tetralin, naphthalene, diphenyl (12)	

[a] A few examples of very good separations of polynuclear aromatic hydrocarbons mixtures by gas chromatography on BPhBT (SP-301) liquid crystal stationary phase are given in Supelco Bulletin 773D (1983). Four excellent chromatograms of androstanol and androstanediol and -triol isomers and also estrogens and sulphur-containing polycyclic aromatic hydrocarbons are shown in refs. 270 and 271. The chromatograms were obtained by supercritical fluid chromatography.

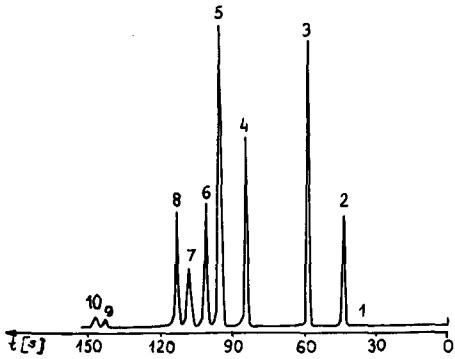

Fig. 15. Separation of alkylbenzenes on 4-*n*-pentylacetophenone (O-4-*n*-ethyloxybenzoyl oxime) in a glass capillary column (48 m × 0.25 mm I.D.) at 40°C. 1 = Methane; 2 = benzene; 3 = toluene; 4 = ethylbenzene; 5 = *m*-xylene; 6 = *p*-xylene; 7 = isopropylbenzene; 8 = *o*-xylene; 9 = *n*-propylbenzene; 10 = styrene[95].

ena. It is intriguing, for instance, why an increase in temperature improves the separation of the isomers, as this conflicts with the properties of liquid crystal stationary phases observed in gas chromatography, where the best separations are obtained at a temperature several degrees above the liquid crystal melting point.

The results obtained of applying liquid crystals in column liquid chromatography are promising although not necessarily related to their liquid crystal properties. The observations made require conformation and complementing, however. Further studies are also required. The use of siloxane polymers as column fillings seems interesting. Such polymers should be obtained by bonding the monomeric liquid crystal with the surface of silica. Suitable reagents that would allow such polymers to be obtained are under investigation[30].

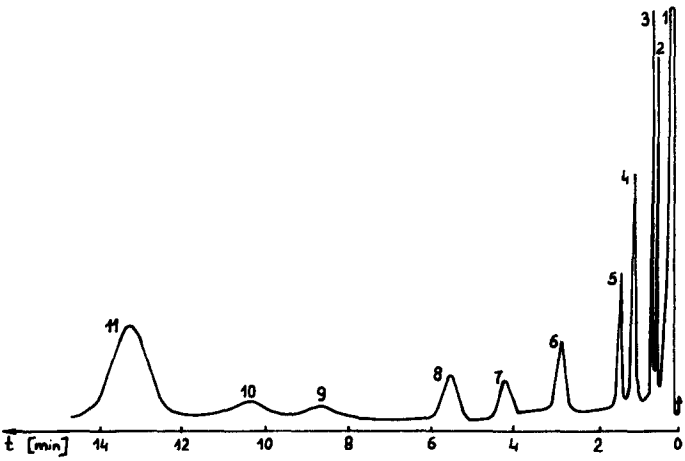

Fig. 16. Separation of hydrocarbon mixture on bis(4′-ethyldiphenylidene)-1,2-di(4-aminophenyl)ethane deposited in an amount of 5% on Chromaton N AW DMCS. Glass column (1.1 m × 4 mm I.D.); column temperature, 282°C. 1 = Benzene; 2 = phenanthrene; 3 = anthracene; 4 = fluoranthene; 5 = pyrene; 6 = triphenylene; 7 = 1,2-benzanthracene; 8 = chrysene; 9 = naphthacene; 10 = perylene; 11 = 3,4-benzopyrene[231].

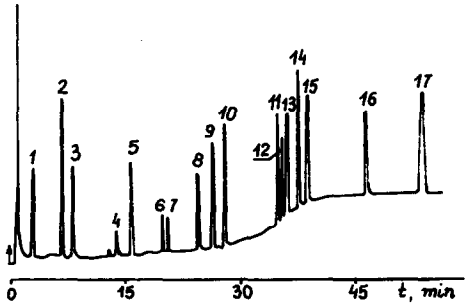

Fig. 17. Separation of polycyclic aromatic hydrocarbons on polysiloxane liquid crystalline stationary phase in a Pyrex glass column (15 m × 0.25 mm I.D.); column temperature, 140°C for 3 min, 140–280°C at 4°C/min. 1 = Fluorene; 2 = phenanthrene; 3 = anthracene; 4 = fluoranthene; 5 = pyrene; 6 = 1,2-benzofluorene; 7 = 2,3-benzofluorene; 8 = triphenylene; 9 = benz[*a*]anthracene; 10 = chrysene; 11 = benzo[*b*]fluoranthene; 12 = benzo[*k*]fluoranthene; 13 = benzo[*e*]pyrene; 14 = perylene; 15 = benzo[*a*]pyrene; 16 = 1,2,3,4-dibenzanthracene; 17 = benzo[*ghi*]perylene[30].

6.2. Thin-layer chromatography

Liquid crystals may be used in liquid crystal detectors for visualizing thin-layer chromatograms[243–246]. Until recently, liquid crystal detectors were used for detecting air pollutants[247]. Their practical value is small, however, as they are not selective. The good detectability and short response time are advantages, and the lack of selectivity presents no obstacle in using liquid crystal detectors for detecting the components on a chromatographic plate after their separation.

Liquid crystals may be used to detect various substances as their properties are changed by those substances. A small amount of the admixture disturbs the order of the liquid crystal structure, and a large amount of a foreign substance destroys that structure completely and the liquid crystal is converted to the isotropic liquid. The amount of the admixture that destroys the ordered structure of the mesophase is the

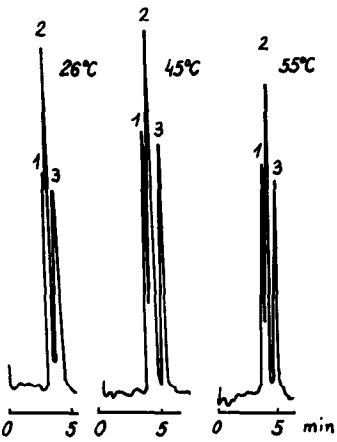

Fig. 18. Chromatograms of nitrotoluene isomers (1 = *ortho*; 2 = *meta*; 3 = *para*) at different temperatures. Column, 40% cholesteryl oleate on Silasorb-600, 5 μm (10 cm × 0.4 mm I.D.); eluent, hexane at a flow-rate of 1.5 cm^3/min[241].

smaller the closer the temperature at which this occurs is to the liquid crystal clearing point. The changes in the ordering of the liquid crystal structure affect the physicochemical properties, *e.g.*, optical, of the liquid crystal. These changes are greatest on transition from the mesophase to the isotropic liquid. The detector is therefore so designed (the liquid crystal is so selected) that the transition to the isotropic liquid is produced by the least possible amounts of the substances to be detected.

A liquid crystal detector may be produced by impregnating the pores of porous foil with a nematic liquid crystal[243,244]. The foil may have a thickness of 0.12 μm and a pore diameter of 0.12 – 0.15 μm, the surface pore density being, *e.g.*, 4.5%. The detector, when observed in polarized light, has a characteristic colour. Its intensity and homogeneity depend on the thickness of the liquid crystal layer (*i.e.*, on the foil thickness) and on the diameter and surface density of the pores.

In order to detect a substance on the chromatographic plate a detector is used with dimensions the same as those of the plate. After developing the chromatogram in any chromatographic chamber, the plate is sprinkled with water. Next the liquid crystal detector is placed on the chromatographic plate and pressed to the plate at a pressure of 0.3 – 0.4 MPa for 1–2 min. Such conditions are optimal for obtaining good mapping of the chromatogram on the detector[245]. The detector is pressed to the plate in a chromatographic pressure chamber[248] or in a special device of similar design[245]. It is recommended, however, that devices be used in which the pressure is exerted on the detector and plate by means of compressed gas. In such devices the required pressure is achieved much quicker than in devices in which water is used as the pressure medium. The best contact between the detector and chromatographic plate is ensured if an elastic foil is used as the pressure transfer medium.

The sprinkling of the adsorbate with water prevents the transfer of the liquid crystal from the foil to the adsorbent and facilitates the migration of the substances to be detected to the liquid crystal in the foil.

After the detector has been removed from the chromatographic plate it is placed between crossed polarizers, when spots of different colours than that of the detector become distinctly visible. These spots correspond to the spots on the chromatographic plate as regards shape and position.

The detectability of the chromatographed substances depends primarily on their solubility in the liquid crystal, the detectability being better the greater is the solubility. Some pesticides may be detected in a chromatographic plate in amounts of 10^{-7} – 10^{-8}g in the spot. This detectability is better than or comparable to that achieved by other methods. Liquid crystal detectors allow compounds to be visualized that cannot be detected at the required low level by a colour reaction or UV radiation. The sensitivity of the liquid crystal detector depends on the kind of liquid crystal used (the best are nematics), its clearing point, temperature and the kind of substance to be detected.

Thin-layer chromatograms should be visualized at ambient temperature. From the characteristics of liquid crystal detectors it follows that these detectors are most sensitive at temperatures close to the clearing point of the liquid crystals used. The clearing points of the liquid crystals used in the detectors should therefore lie in the range 295–305 K. In addition to single liquid crystals, their mixtures may also be used. In some instances it is advisable to heat the liquid crystal detector to increase its sensitivity. At the maximum sensitivity of the detector the spot of the detected sub-

stance has a diameter 1–2 mm greater than that detected by usual chemical methods.

The liquid crystal detector makes it possible to determine the amount of a substance in a chromatographic spot. Advantage is taken here of the linear variation of the clearing point of the liquid crystal with the amount of the isotropic substance (to be determined) dissolved in the mesophase. This relationship may serve as a means for determining the distribution of the concentration of the substance in the spot or the apparent better separation of the spots on the chromatogram. This is possible because at a low sensitivity of the detector the sites of greatest concentration of the chromatographed substance, *i.e.*, the centres of the spots, appear first. The connection and subsequent spreading of the spots proceeds as the sensitivity of the detector increases and sites of low concentration of the substance to be detected are revealed.

In the course of detection only part of the substance to be detected passes from the adsorbent (chromatographic plate) to the foil saturated with the liquid crystal. Hence it is possible to repeat the visualization of one chromatogram several times. When the chromatogram is visualized by the liquid crystal method, the chromatographic plate does not become contaminated and the substances being determined do not undergo chemical conversion. The method is therefore suitable for visualizing chromatograms in preparative thin-layer chromatography.

The chromatographic plate visualized by means of a liquid crystal detector may be used several times after regeneration with a solvent of high eluting power, preferably in a pressure or continuous flow chamber.

The use of liquid crystal detectors for visualizing chromatograms allows the wide laboratory application of plates with adsorbents of various colours and not only the white type that were used almost exclusively hitherto. One can mention here, for instance, various carbon adsorbents differing considerably in their properties from the white type and also between each other. At least some of them may be suitable for thin-layer chromatography.

The liquid crystal visualization method has several advantages over the method of visualizing chromatograms on carbon plates advanced by Prochazka and Starka[249], who used aluminium plates with a silica gel layer on which the spots were mapped and detected by a conventional method. The latter method is less sensitive and slower than the liquid crystal method.

7. FINAL REMARKS

The publications discussed in this review show that the application of liquid crystals in chromatography is an important development. Despite the opinions of some sceptics the liquid crystals now have a static position in chromatographic practice, I am convinced that developments in this field will continue rapidly. We now have at our disposal a wide range of liquid crystals which have not yet been tested as stationary phases. Some of them seem very promising in this respect. New liquid crystals are continuously becoming available and I believe that the best of them are yet to come.

So far only thermotropic liquid crystals have been used as stationary phases in chromatography, and no use has been made of lyotropic liquid crystals. The application of lyotropic liquid crystals as stationary phases seems promising as it has been

shown that gas chromatography may be used for determining the phase diagrams of these liquid crystals[250].

It has been suggested that, analogously to conventional isotropic stationary phases, colloidal stationary phases including a liquid crystal as a component could be used. These colloidal phases make it possible to control the sorptive capacity and the general selectivity of chromatographic columns over a wide range. This is due to the change in the contribution of dissolution to the adsorption ratio at the phase boundaries in the general retention of chromatographed substances. The colloidal stationary phases include mineral filling agents which adversely affect the ordering of the mesophase structure and hence the selectivity of the liquid crystal stationary phase. This decrease in selectivity varies, however, depending on the kind of liquid crystal (probably depending chiefly on its molecular structure). The efficiencies of columns with colloidal stationary phases including liquid crystals are higher than those of columns with normal liquid crystal stationary phases. As the above information was taken from the only paper so far published on this subject[251], further research is necessary to improve our knowledge of colloidal liquid crystal stationary phases and especially to allow some generalizations regarding their properties and practical usefulness.

From this review of studies published in recent years it can be seen that work dealing directly or indirectly with the use of liquid crystals in chromatographic practice for separating multi-component mixtures has dominated. Much less attention has been devoted to the testing of liquid crystals by gas chromatography. Only one paper reported studies of the thermotropic properties of polymeric liquid crystals by reversed-phase gas chromatography[252].

Investigations on the use of liquid crystals for visualizing thin-layer chromatograms have hardly been started and there is a lot to be done in this respect. It remains to be established whether the liquid crystal method of detection in thin-layer chromatography is of real practical use and what its range of applicability is. There are probably other possibilities of utilizing liquid crystals in chromatography.

Some work, mainly by Chinese authors, has not been considered in this survey[253–269].

8. SUMMARY

On the basis of the literature published since 1982, the applications of liquid crystals in chromatography are reviewed. The properties are described of new liquid crystals that may be used as stationary phases in gas chromatography; the most important in this group are liquid crystal siloxane polymers, followed by isothiocyanate liquid crystals and disc-like liquid crystals. The properties are discussed of conventional and capillary columns in which liquid crystal stationary phases are applied and the effects are considered of various factors that influence the separation of components of mixtures on these stationary phases. Among these factors, the effects of the kind of mesophase of the liquid crystal used, the structure of the liquid crystal molecule and that of the chromatographed substance and the constitution of the surface of the substrate (support, wall of the capillary column) on which the liquid crystal has been deposited are given special consideration. A large part of the review is devoted to practical applications of liquid crystal stationary phases, examples being given of separations of isomers of benzene and naphthalene derivatives, of polycyclic

hydrocrabons and of other compounds. Attention is drawn to the possibility of using liquid crystals, apart from in gas chromatography, also in liquid chromatography and especially in thin-layer chromatography for visualizing the chromatograms.

NOTE ADDED IN PROOF

Recently, various articles on liquid crystals in chromatography appeared[272–274].

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