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APPLICATION OF SUPERCRITICAL FLUID CHROMATOGRAPHY TO THE ANALYSIS OF LIQUID-CRYSTAL MIXTURES

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SUMMARY

Supercritical fluid chromatography (SFC) has been applied to the analysis of liquid-crystal mixtures. Most of the components have a low volatility and some classes are not very stable at high temperatures. SFC on modified high-performance liquid chromatography equipment, with packed columns and carbon dioxide as the mobile phase, was found to be a convenient, practical alternative to both gas chromatography and liquid chromatography.

The components eluted from the SFC column could readily be classified by multichannel UV detection. Carbon dioxide is an excellent mobile phase because of its transparency in the UV. However, a class of components with identical UV spectra may represent several chemical (sub-)classes. SFC retention data were used to identify the different components within a given class. The application of this strategy for the identification of components in liquid-crystal mixtures is demonstrated in this paper.

The main problems encountered in this study are the accurate control of the flow-rate under SFC conditions (especially when pressure programming is used) and the poor peak shape obtained for components that contain ether groups.

INTRODUCTION

Liquid-crystal displays are increasingly important in the electronics industry as a means of translating electronic information into a visual picture. Some examples of their use, in order of increasing size and complexity, include wrist watches, calculators, personal computers, and television screens. The most common cell used in liquid-crystal displays today is the twisted nematic cell, which is illustrated in Fig. 1[1]. In this cell, the liquid crystal is oriented at the surface of the (chemically modified) electrodes above and below the liquid-crystal compartment. The two plates are twisted at an angle of 90°, causing the liquid-crystal molecules in the cell to be arranged in the form of a twisted helix (Fig. 1a). A beam of light that is polarized by the polarizer on the top of the cell may "follow" this twist and pass through the second polarizer at the bottom, which is at a 90° angle to the one at the top. When a voltage is applied to the cell, a homeotropic phase is formed and the light that is polarized by the polarizer at the top of the cell will be blocked by the one at the bottom (Fig.

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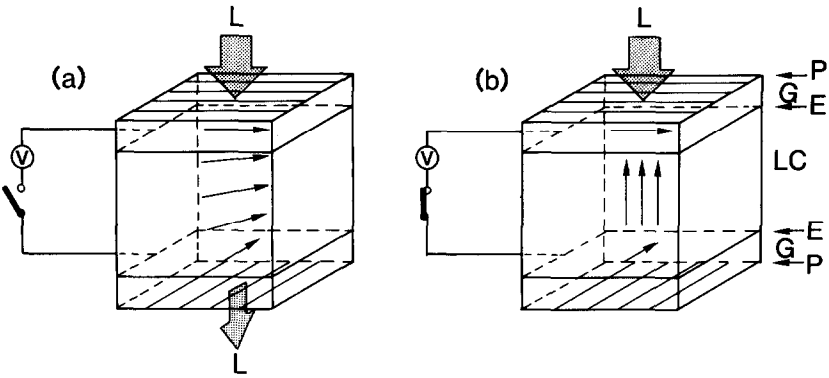


Fig. 1. Schematic illustration of a twisted nematic cell for liquid-crystal displays. P = polarizer; G = glass window; E = (surface-modified) electrode; LC = liquid crystal; – – – = director; V = voltage supply; L = light. For explanation see text.

1b). If a mirror is mounted underneath the cell, it will appear clear from the top if no voltage is applied (Fig. 1a) and dark when the cell is addressed (Fig. 1b).

A number of properties of the liquid-crystal mixture in the cell are relevant. These include the melting point, enthalpy of melting, transition point from nematic to isotropic phase, optical and dielectric anisotropy, viscosity coefficients, and elastic constants[2]. In order to find the best compromise for all these properties, mixtures of different liquid-crystal components need to be applied. Typically, such mixtures may contain between 5 and 15 components.

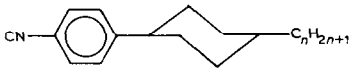
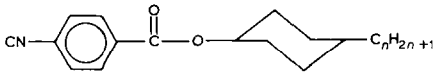
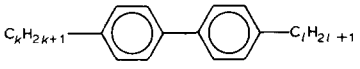
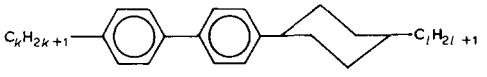
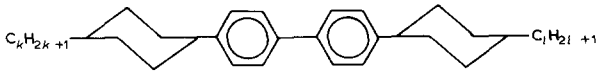
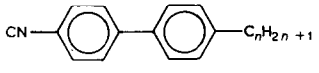
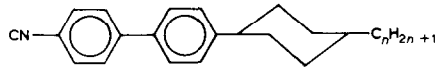
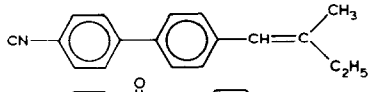
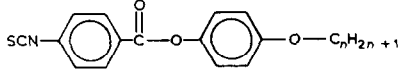
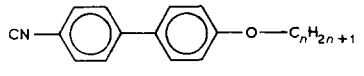
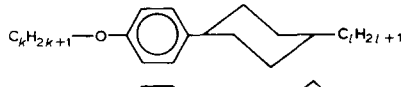
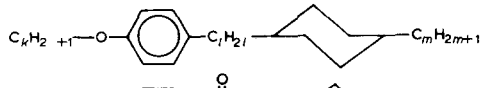
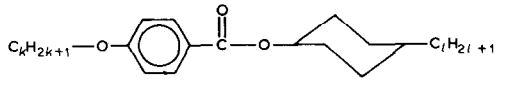
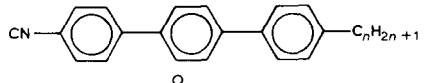
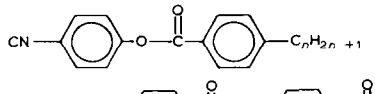
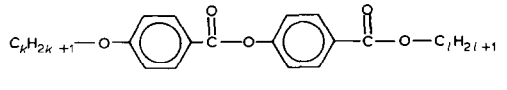
Between 15000 and 20000 organic substances are known to possess liquid-crystalline properties, *i.e.* give rise to an ordered intermediate phase in between the crystalline (solid) and the liquid state. However, only a small fraction of these compounds shows sufficient stability in combination with the required physical properties, so that they may be applied in displays. Moreover, the liquid-crystal components commonly used are part of a small number of chemical classes. Within one such class, the different components often differ in the length of an aliphatic hydrocarbon chain. This is illustrated in Table I, which lists the structures of some of the most common classes of components in liquid crystal mixtures.

Table I also lists the maxima in the UV spectra for the different classes of components. Typically, the UV spectra are very similar for compounds within one of the classes, *i.e.* the length of the alkyl chain hardly affects the UV spectrum. Therefore, we can use UV spectral information to classify liquid-crystal components. Fig. 2 shows some typical UV spectra of components from the classes A–F. However, in some cases two different chemical classes may give rise to similar UV spectra (see Table I), so that the component that is classified on the basis of its spectrum could be a member of one of several sub-classes (*e.g.* A_1 or A_2, C_1 or C_2 or C_3 or C_4, E_1 or E_2 or E_3).

Another obstacle to the use of UV spectroscopy for the identification of liquid crystals is the fact that the features of the individual components can hardly be recognized in the UV spectrum of a complex mixture (typically 5–15 components, see above). More information concerning the components of the mixture may be obtained by using other spectroscopic techniques which yield spectra with a higher

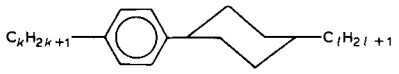
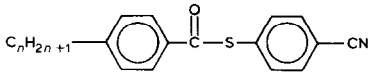
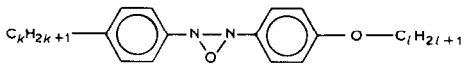
TABLE I

STRUCTURE, NAME AND UV DATA FOR THE COMMON CLASSES OF CHEMICAL COMPONENTS IN CONTEMPORARY LIQUID-CRYSTAL MIXTURES

Class	*Structure*	*Name*	*Max UV (nm)*
A_1		Cyanophenylcyclohexanes	237
A_2		Cyclohexyl cyanobenzoates	237
B_1		Alkylbiphenyls ($n = k+l$)	265
B_2		Biphenylcyclohexanes ($n = k+l$)	265
B_3		Cyclohexyl-biphenylcyclohexanes ($n = k+l$)	265
C_1		Cyanobiphenyls	280–285
		Cyclohexylcyanobiphenyls	280–285
C_3		Alkylenecyanobiphenyls ($n = 5$)	280–285
C_4		Alkoxyphenyl benzoates isothiocyanates	280
D		Aloxycyanobiphenyls	296
E_1		Alkoxyphenylcyclohexanes ($n = k+l$)	230; 287
E_2		Alkoxyphenylalkylcyclohexanes ($n = k+l+m$)	230; 287
E_3		Cyclohexyl alkoxybenzoates ($n = k+l$)	230–233; 287
F		Cyanoterphenyls	302
G		Cyanophenyl benzoates	253
H_1		Alkoxybenzoyl benzoates ($n = k+l$)	270

(*Continued on p. 124*)

TABLE I (*continued*)

Class	*Structure*	*Name*	*Max UV (nm)*
H_2		Phenylcyclohexanes ($n = k+l$)	272
I		Cyanophenyl thiobenzoates	260; 280
J		Diarylazoxy compounds ($n = k+l$)	350–360

information content (*e.g.* IR, NMR). Indeed, we routinely apply such techniques as a rapid early scan for new or unknown mixtures. This will quickly reveal whether the unknown mixture is identical to a known one. Moreover, valuable information about the presence or absence of certain classes of components may readily be obtained. For example, a peak around 1730 cm^{-1} in the IR spectrum is strongly indicative of the presence of esters in the mixture. The presence in the mixture of components that contain alkoxy groups is apparent from the occurrence of a signal in the usually void area around 4 ppm in the ^1H NMR spectrum. These and many similar (but often more hidden) bits of information are often invaluable if a classification of the components on the basis of their individual UV spectra is considered. Therefore, in real life, the situation may actually be more favourable than in the discussion below, where we will assume that we have no additional spectroscopic information to rely on.

In order to identify (and quantify) the individual components in the mixture, the application of a coupled device will be required that combines a chromatographic technique to separate the individual components with a spectroscopic (or spectrometric) technique for their identification. The most prolific of these techniques is the gas chromatography–mass spectrometry (GC–MS) combination. Indeed, GC–MS can be applied successfully for the analysis of many liquid-crystal mixtures[3]. Unfortunately, not all possible components of liquid-crystal mixtures are sufficiently volatile and sufficiently stable at elevated temperatures to allow separation by GC.

As an alternative separation method, liquid chromatography (LC) may be applied for mixtures that are not compatible with GC. However, the combination with a mass spectrometer now becomes much more complicated and it is not possible to obtain the same information content in combination with the same high degree of reproducibility as in GC–MS analysis[4]. Instead of using a mass spectrometer for identification purposes, a multichannel UV detector may be used. We have seen above that the information from a UV spectrum may often be used to group the components into chemical classes, but not to identify them within a given class. For the latter purpose, however, the chromatographic separation method provides an excellent tool, and we may use the retention times under standardized conditions to identify the components within a certain class.

In this paper, we have studied the possibility of using supercritical fluid chro-

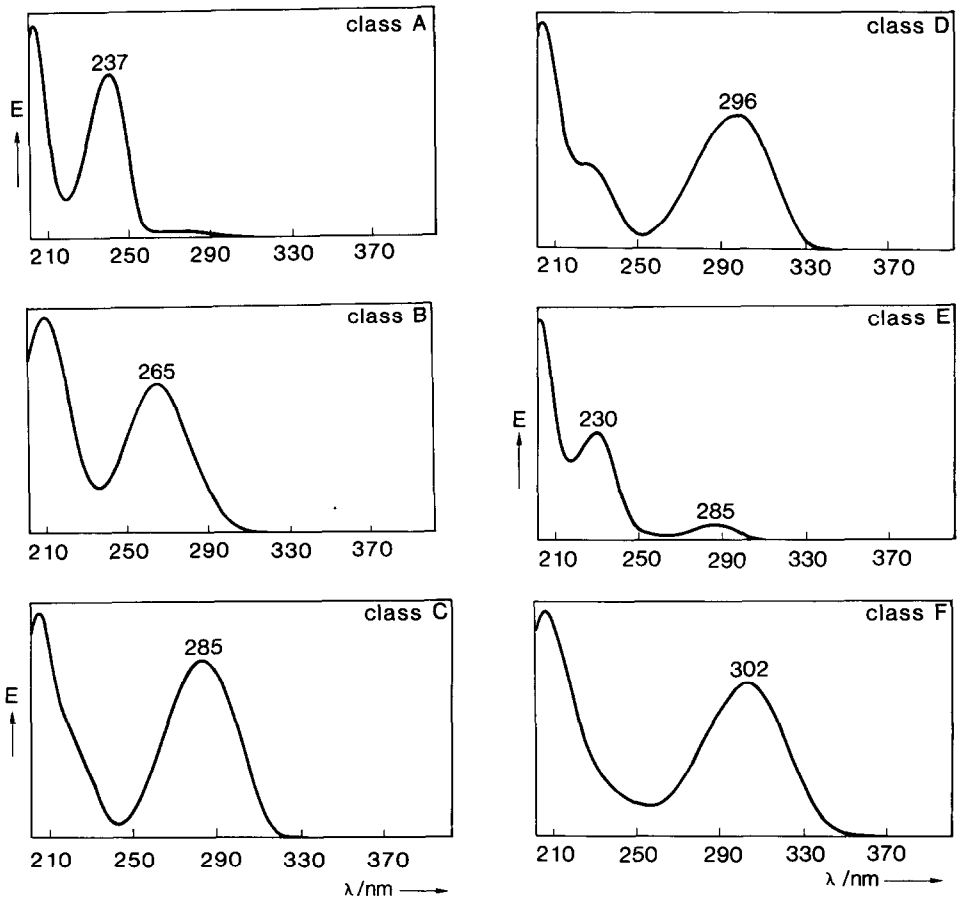


Fig. 2. Typical UV spectra for components in the Classes A–F of liquid-crystal components (for identification of classes see Table I).

matography (SFC) in combination with multichannel UV detection for the analysis of liquid-crystal mixtures. We have investigated the potential and limitations of an analysis scheme, based on: (1) classification of the different components in the mixture on the basis of their UV spectra, and (2) identification of the components on the basis of their retention times in SFC under standardized conditions. SFC potentially offers a number of advantages in comparison with LC:

(1) Separations by SFC are potentially faster than by LC.

(2) Programmed analysis in SFC can be realized very rapidly by programming the density (or pressure) of the mobile phase.

(3) SFC may be used in combination with UV spectrometric detection[5], but may also be combined with MS[6,7] or (FT)IR spectroscopy[8] much more easily than is the case for LC.

(4) In combination with any of these detection and identification methods the selection of suitable mobile phases (carbon dioxide, xenon) may lead to a much lower spectral background in SFC than in LC.

EXPERIMENTAL

The basic SFC instrument was a modified liquid chromatograph and has been described elsewhere[9]. For the present study, a multichannel (diode-array) UV-absorbance detector was installed (PU 4021 from Philips Analytical, Cambridge, U.K.). This detector made use of the same high-pressure flow-cell previously used in a conventional (PU 4020) variable-wavelength UV detector.

For recording three-dimensional data matrices ("Chromascans") a PU 4850 data station was attached to the detector. This allowed storage of complete UV spectra (190–390 nm) every 0.5 s throughout the chromatogram, and subsequent rapid access to the UV spectrum at any desired time or to the chromatogram at any desired wavelength.

A single HPLC column (CP-spher C_{18}, 8 μm particle size, from Chrompack, Middelburg, The Netherlands) was used throughout the study.

As before[9], we used pure carbon dioxide as the mobile phase throughout this study. Besides the considerable advantages of carbon dioxide as a non-flammable, non-toxic, and cheap solvent, it is also fully transparent in the UV range used in this study. This allows the use of the UV spectrometer as a virtually universal detector at 190 nm, as well as the use of spectral information in the range from 190 to 220 nm, which is usually of little value in LC because of the high background absorption of the solvent.

During the present study, the needle valve of a standard reducing valve for carbon dioxide was used to control the flow-rate. The reducing valve is not used to reduce the pressure. So far, in our experience, this is the only way to ensure reliable operation of the SFC instrument over a long period of time. The reason for this is that supercritical fluids are not compatible with most polymeric materials commonly used for O-rings, valve seats, etc. We feel that the accuracy of the flow control is one of the major limiting factors of the present instrumentation.

In the present paper, we define the SFC conditions by the column inlet pressure and the average linear velocity. In a previous publication[9] we have shown that the pressure drop over the column is an important parameter in SFC. However, it is difficult to measure the pressure drop over the column itself. We have recently noted that the pressure drop over the detector is not negligible. The average linear velocity can easily be obtained from the column length and the hold-up time ($\bar{u} = L/t_0$).

Mixtures of liquid crystals have been obtained from various sources (commercial mixtures, synthetic mixtures, display contents). Standards of pure liquid crystals used in this study were obtained from BDH (Poole, U.K.), Hoffman-La Roche (Basel, Switzerland) and from E. Merck (Darmstadt, F.R.G.). The mixtures described in this publication were synthetic mixtures, made up from the individual pure components. Before injection into the SFC column, all mixtures were diluted with a suitable solvent (tetrahydrofuran or benzene).

RESULTS

SFC of liquid-crystal mixtures

The most elegant way to obtain a chromatogram for a liquid-crystal mixture with SFC is to use a pressure (or density) program. Fig. 3 shows a chromatogram

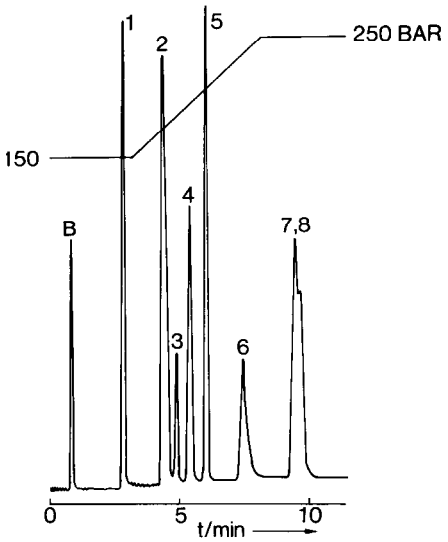


Fig. 3. Chromatogram of liquid-crystal mixture, obtained under pressure-programmed conditions. Temperature, 50°C; detection at 210 nm; average linear velocity, 0.21 cm/s; column inlet pressure as indicated in the figure.

obtained for a liquid-crystal mixture under programmed SFC conditions. A fast separation is achieved and reasonably shaped peaks are obtained throughout the chromatogram. Unfortunately, it is very difficult to obtain reproducible retention data under programmed conditions, because of variations in the flow-rate. The present instrument allows us to maintain a stable flow-rate under isobaric conditions, enabling us to measure isobaric capacity factors in a reproducible way. A stable and reproducible flow-rate under pressure-programmed conditions would require the application of more sophisticated instrumentation, such as mass-flow controllers. However, there is, a high degree of incompatibility of mass-flow controllers and high-density supercritical fluids. In general terms, it is difficult to confine the excellent solvent properties of supercritical fluids for solutes of high molecular weight solely to the column. This problem needs to be overcome in order to improve the reproducibility of SFC experiments.

Fig. 4 shows typical chromatograms of a liquid-crystal mixture, obtained under isobaric conditions. Chromatogram a was obtained at a column inlet pressure of 150 bar. It is seen that all 8 components of the mixture are eluted from the column in about 25 min. The shape of the peaks is good in the first part of the chromatogram, but very poor for the last 3 peaks. The last two components in the mixture co-elute as one peak.

Fig. 4b shows a chromatogram of the same mixture, obtained with an inlet pressure of 205 bar. All peaks appear to be eluted more rapidly from the column, and the total analysis time is about 15 min. The peak shape for the later-eluted peaks is improved but still poor. An interesting aspect of this chromatogram is that, although the retention times (capacity factors) have been much reduced, the separation does not seem to have suffered in comparison with chromatogram a. Instead, solutes 7 and 8 now appear as two partly resolved peaks in the chromatogram.

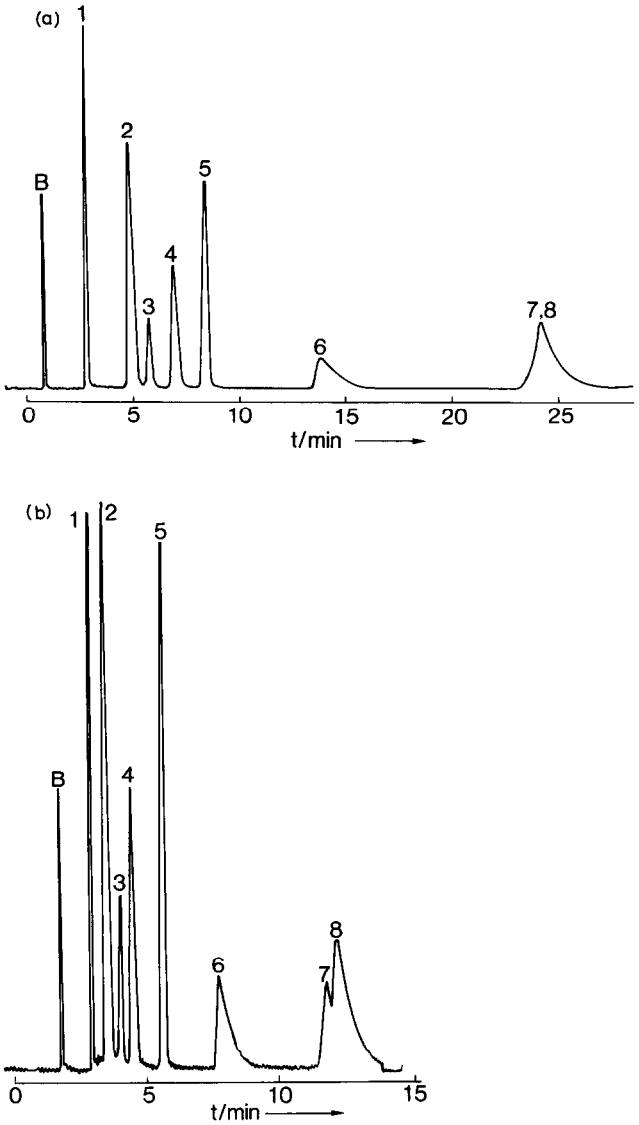


Fig. 4. Chromatograms of a liquid-crystal mixture, obtained under isobaric conditions. Temperature 50°C; detection at 210 nm. (a) Inlet pressure, 150 bar; average linear velocity, 0.21 cm/s; (b) inlet pressure, 205 bar; average linear velocity, 0.20 cm/s.

This aspect of SFC is illustrated in Fig. 5, where the retention (ln k) of some liquid crystal components is plotted against the column inlet pressure. It appears from this figure that the lines for the different solutes are roughly parallel, indicating that the selectivity (α values) between pairs of adjacent peaks in the chromatogram are roughly independent of the pressure. Hence, the resolution between adjacent peaks is not greatly reduced upon increasing the column inlet pressure, as long as the capacity factors are well above 1. However, it is expected that a further increase in the pressure will lead to a much reduced selectivity, especially within a homologous series[10].

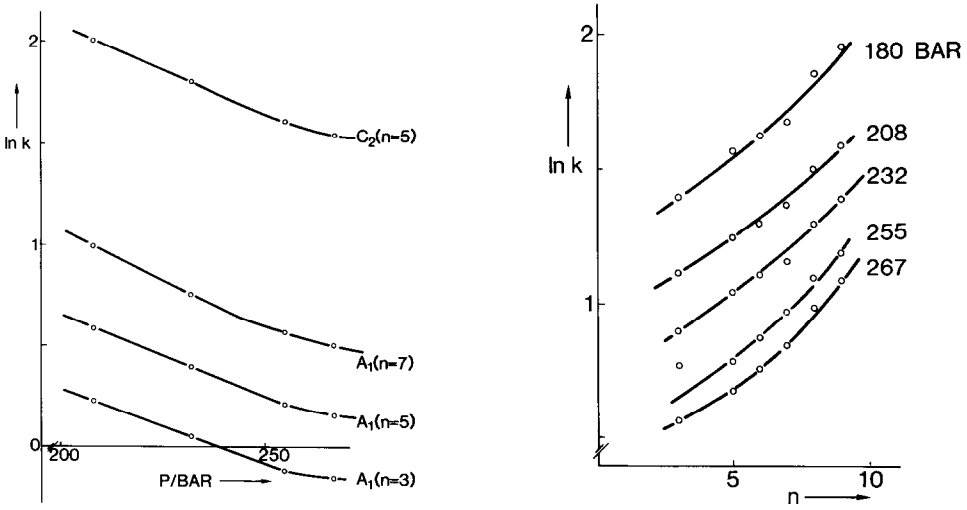


Fig. 5. Variation of the logarithm of the capacity factor with the column inlet pressure for some liquid-crystal components. Temperature, 50°C. For solute identification see Table I.

Fig. 6. Logarithm of the capacity factor, plotted against the number of carbon atoms in the alkyl chain for alkoxycyanobiphenyls (Class D in Table I) at five different values of the column inlet pressure. Temperature, 50°C; average linear velocity, 0.22 cm/s.

Standardized SFC experiments

The components within one of the classes in Table I generally show different retention times under SFC conditions. Notably, this will be the case if only the length of the alkyl chain (number of carbon atoms, n) is varied within a homologous series. For example, Fig. 6 shows plots of ln k *vs.* n for Class D (alkoxycyanobiphenyls) at five different values of the column inlet pressure. Thus, once a component has been classified and once its retention time (capacity factor) is known, it can be identified completely. For this purpose, the accuracy with which capacity factors are measured needs to be better than the selectivity. (difference in ln k values) between successive homologues. From Fig. 6 it appears that this selectivity does not vary greatly with pressure over the range studied (column inlet pressures from 180 to 267 bar). Only at higher column inlet pressures is the selectivity bound to decrease considerably[9,10].

If a class of components comprises several sub-classes (*e.g.* Class C in Table I), a ln k *vs.* n plot for this class will contain several "lines" (actually, each sub-class forms a series of points located on a line). This will generally require a much higher accuracy in the measurement of capacity factors. In some cases, it may be beneficial to compare the retention data with recorded plots at two different sets of conditions (see, *e.g.*, the behaviour of the last two solutes in Fig. 4). Of course, in the extreme case in which two different components show exactly the same retention behaviour and exactly the same UV spectra, the present strategy cannot be used to identify them. In that case, additional information will have to be obtained from other analytical techniques.

The capacity factors of a number of liquid crystal components have been accurately determined at two different values for the column inlet pressure. These data are collected in Fig. 7 for column inlet pressures of 150 and 205 bar, respectively. In

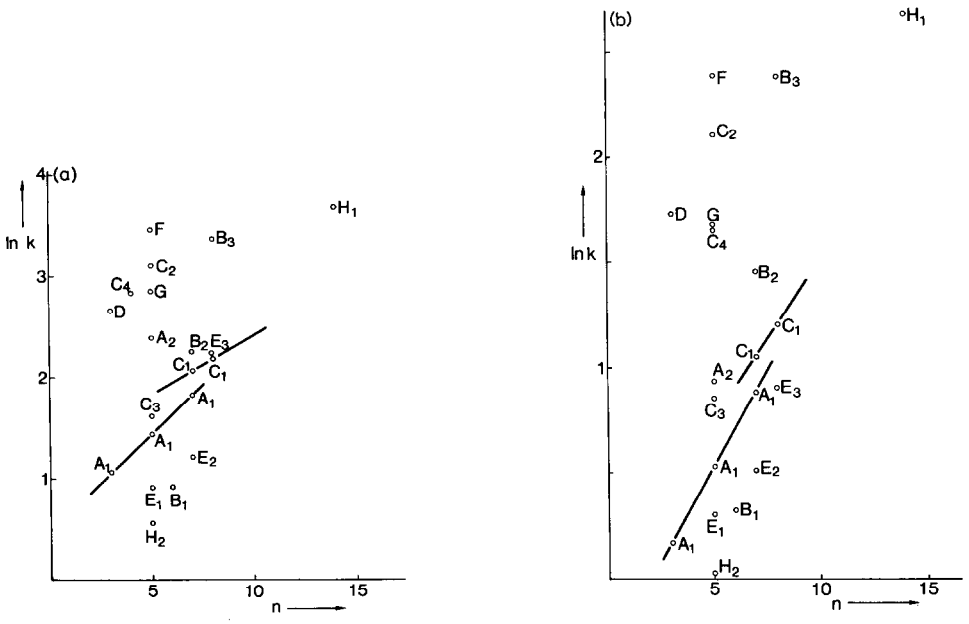


Fig. 7. Logarithm of the capacity factor, plotted against the number of carbon atoms in the alkyl chain (see Table I) for a variety of liquid-crystal components. Temperature, 50°C; average linear velocity 0.21 cm/s; column inlet pressure: (a) 150 bar; (b) 218 bar.

this figure, the logarithm of the capacity factor is plotted on the vertical axis. On the horizontal axis is the number of carbon atoms in the alkyl chain. This number corresponds to the index, n, as it appears in Table I for the different classes of compounds.

In order to obtain reproducible retention measurements, the column inlet pressure and the average linear velocity were carefully controlled. This implies that the column pressure drop (which may influence the capacity factor, see ref. 9) is implicitly kept constant. After establishing the correct inlet pressure, the flow-rate was adjusted with the needle valve, so that the hold-up time equalled the required value. Typically, for well-behaved solutes that showed sharp, symmetrical peaks, the reproducibility of the capacity factor measurements was within about 1%, corresponding to a variation of about 0.01 in ln k. The reproducibility of the retention measurements decreased slightly when the pressure was increased and much more dramatically when certain polar groups were present in the solute molecules. Notably, components that feature ester or ether (alkoxy) groups (*e.g.* Classes A_2, C_4, D, and E_3 in Table I) showed poorly-shaped peaks and a much larger variation in the retention data, as measured from the top of the peak. The later-eluted solutes in Fig. 4 possess alkoxy groups, and the consequences for the peak shape are evident from this figure.

Chromascans

Fig. 8 shows a "chromascan", which is a (pseudo-isomeric) three-dimensional plot of UV absorption *vs.* retention time and wavelength. The recorded data include two complete UV spectra per second with a resolution of 1.5 nm (128 diodes over

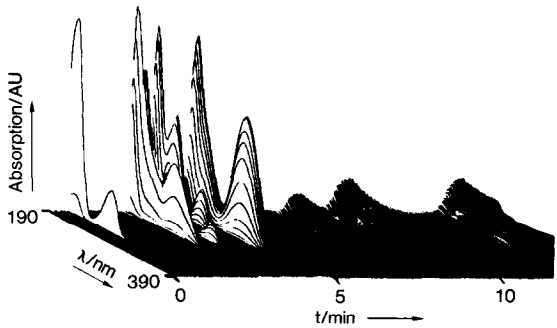


Fig. 8. Chromascan of a liquid-crystal mixture. In this pseudo-isomeric, three-dimensional plot the vertical axis shows the UV absorption. On the horizontal axis is the retention time. The third axis represents the wavelength. Column inlet pressure, 150 bar; average linear velocity, 0.20 cm/s; temperature, 50°C. For solute identification see Table II.

the 200 nm range). Hence, for the chromatogram of Fig. 8, with an analysis time of 15 min, the number of recorded data points is $15 \times 60 \times 2 \times 128 = 230400$. Not all this information can readily be visualized in a two-dimensional representation, such as Fig. 8.

Fig. 9 shows a chromatogram (horizontal section), taken from the "chromascan" of Fig. 8. At a low wavelength, such as the 210 nm selected for Fig. 9, all liquid-crystal components show a considerable absorption in the UV, and hence the detection is universal for this purpose. More clearly than in Fig. 8, the shape of the different peaks can be examined in Fig. 9. Again, the later-eluted solutes (which contain alkoxy groups) show poorly-shaped peaks.

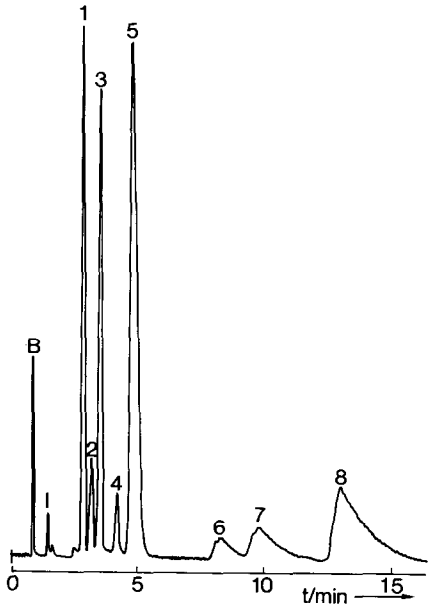


Fig. 9. Chromatogram from the "chromascan" of Fig. 8. Wavelength, 210 nm. Components are classified on the basis of UV spectra (see Fig. 10).

Fig. 10 shows the UV spectra obtained from the "chromascan" at the tops of the eight peaks in Fig. 9. The capacity factors corresponding to each of the spectra are indicated in the figure. Using these spectra and the data on the UV maxima in Table I, the individual solutes can be classified. This yields the UV classes (letters) that are shown in Table II (third column), but not the accompanying indices (subclasses).

The conditions used to record Fig. 8 were identical to those used for Fig. 7a. Therefore, the latter figure can be used to identify the different components within a class on the basis of their retention data. This procedure is illustrated in Table II. Using the classification from the third column and the retention data from Fig. 9 (fourth column in the table), the peaks can be matched against the data in Fig. 7a

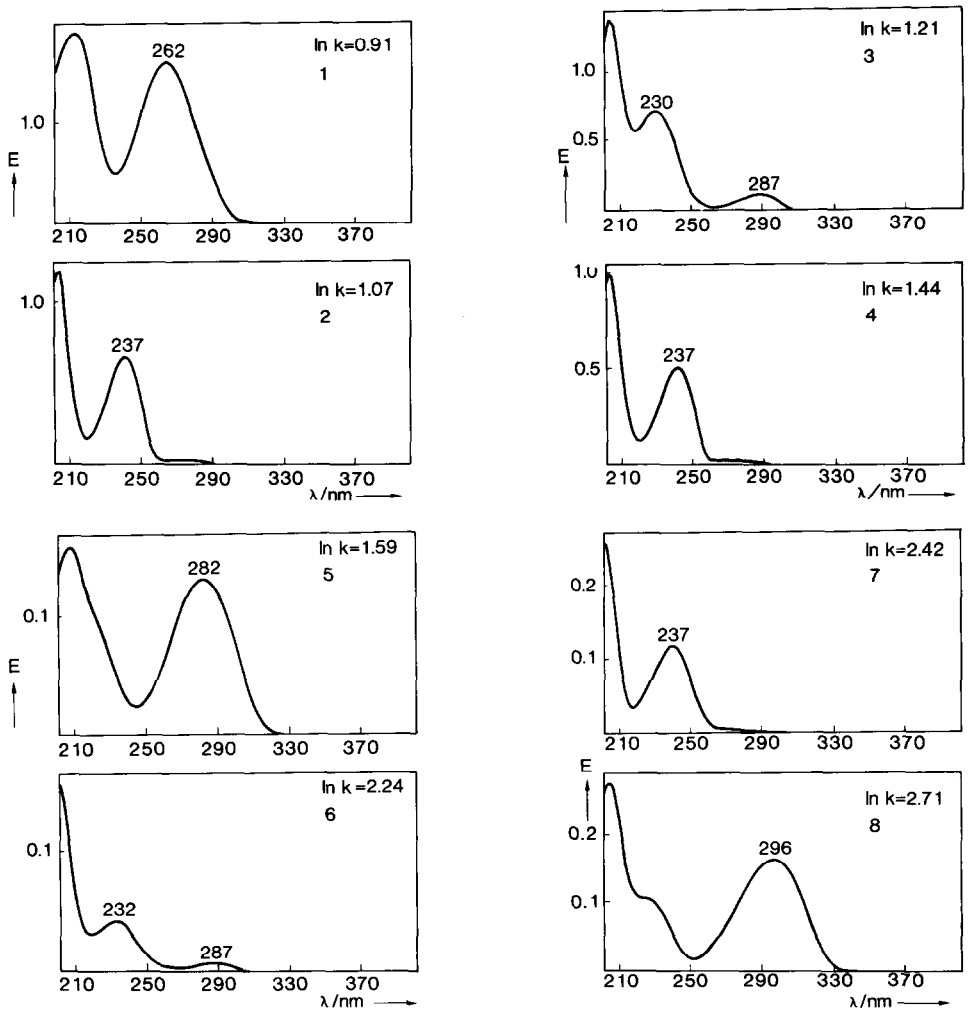


Fig. 10. UV spectra from the "chromascan" of Fig. 8, taken at the top of the 8 peaks in Fig. 9. Capacity factors as indicated in the figure. The components are classified according to the UV data given in Table I, which shows the description of classes.

TABLE II

CLASSIFICATION AND IDENTIFICATION OF THE COMPONENTS IN THE LIQUID-CRYSTAL MIXTURE SHOWN IN FIG. 8 ("CHROMASCAN"), FIG. 9 (CHROMATOGRAM) AND FIG. 10 (UV SPECTRA)

n = Number of carbon atoms in alkyl chain.

Peak no. (Fig. 9)	*Max UV (Fig. 10)*	*Class (Table I)*	*ln k (exp) (Fig. 9)*	*ln k (ref.) (Fig. 7a)*	*n*
1	262	B_1	0.91	0.92	6
2	237	A_1	1.07	1.06	3
3	230; 287	E_2	1.21	1.21	7
4	237	A_1	1.44	1.44	5
5	282	C_3	1.59	1.61	5
6	232; 287	E_3	2.24	2.24	8
7	237	A_2	2.42	2.39	5
8	296	D	2.71	2.66	3

to locate the closest solute within the same class. The retention data obtained from Fig. 7a are listed in the fifth column of Table II, and a close agreement between the two columns with ln k values can be observed. From Fig. 7a (horizontal axis) we may then derive the number of carbon atoms present in the molecule. This number is shown as the last entry in Table II. If a class comprises several sub-classes, Fig. 7a also provides the indices listed in the third column of the table.

DISCUSSION

Figs. 8–10 and Table II illustrate the procedure described in this paper for the identification of the components in a liquid-crystal mixture. It is clear from the example given that the identification of the components can be rapidly achieved as long as the individual components of the mixture are available for recording their retention times under standardized SFC conditions and their UV specta. The procedure is found to yield generally correct results, but the reliability of the identification may still be much improved if the flow-rate can be controlled better than presently possible. Ideally, (pressure-) programmed elution (see Fig. 3) should be feasible with accurately controlled and reproducible flow-rates. It is obvious that if more individual components are characterized (*i.e.* the number of points in the plots of Fig. 7a and b increases), the chances of erroneous interpretation will increase, unless the capacity factors can be determined with higher precision.

There is one major obstacle to the analysis of liquid-crystal mixtures by the method proposed here. This has already been touched upon in Figs. 4 and 9. The shape of the last few peaks in these chromatograms was seen to be poor. We generally observe poor peak shapes for all solutes in which ether or ester groups are present. This is not simply a matter of solute polarity, because cyano groups do not cause a deterioration of the peak shape. We could not improve the peak shape by reducing the sample size or by using different sample solvents. A possible explanation involves the hydrogen bonding capacity of ether groups, possibly at the surface of the stationary phase. It has been observed by several authors (*cf., e.g.* refs. 7 and 11) that the addition of minor amounts of polar modifiers to the carbon dioxide mobile phase

greatly reduces the retention and improves the peak shape for many (polar) solutes in packed-column SFC. This situation is very similar to that commonly observed in liquid–solid chromatography. Therefore, strong interactions between active adsorption sites (possibly silanol groups) and solutes containing ether groups may be responsible for the poor peak shapes observed in Figs. 4 and 9. If this explanation is correct, the problem may also be solved by using a different type of stationary phase. Indeed, the use of modifiers in the mobile phase is not usually required in capillary SFC, where polymeric stationary phases with a much larger effective layer thickness are commonly employed.

It appeared in the early stages of the project that replacing an old column by a nominally identical new one from the same manufacturer may cause a substantial alteration of the retention times obtained under standardized conditions in SFC. In our experience, the lifetime of packed columns for SFC is very long (around one year of operation). Hence, we do not have sufficient information to judge whether or not the reproducibility of column materials forms a major obstacle for the application of SFC.

CONCLUSIONS

(1) Components in liquid-crystal mixtures can be identified on the basis of their UV spectra and SFC retention times under standardized conditions. The combination of packed-column SFC with a multichannel UV detector may be used for this purpose.

(2) The reliability of the method is limited by the accuracy of the experimental capacity factors. This may be improved by improving the flow control.

(3) Solutes with ether (or ester) groups give rise to poor peak shapes under the conditions used in this work. Peak shapes may be improved either by adding polar modifiers to the mobile phase or by using different types of stationary phases.

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