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Liquid Chromatography–Solid Phase Extraction–NMR (LC–SPE–NMR) Analysis of Liquid Crystalline Mixtures

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Abstract : We have performed Liquid Chromatography–Solid Phase Extraction–NMR (LC–SPE–NMR) analysis for liquid crystalline mixture and elucidated the structures of selected components by NMR spectra. Combining the results of one-dimensional ¹H experiments as well as homonuclear and heteronuclear two-dimensional experiments, we could analyze the molecular structure of the liquid crystal singles whose structure had not been interpretable by mass spectrometry alone.

Keywords : Liquid crystal, NMR, LC-SPE-NMR, tetrahydropyran

INTRODUCTION

Liquid crystal display (LCD) devices utilize the anisotropic electro-optical property of the liquid crystalline materials to display the visual information on the screen. In modern LCD devices, a series of digital data are applied to the array of thin film transistors (TFT) fabricated onto a sheet of glass, which controls the electric field across the liquid crystalline materials inside the LCD cells. This electric field changes the orientations of the liquid crystal molecules, which consequently control the polarization of the light that passes through each pixel. This polarization change induced by the

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motion of liquid crystal molecules eventually displays the desired information onto the screen with the help of the pair of polarizing films attached onto both sides of the LCD panel.

Liquid crystals used in modern commercial LCD's are not from single compounds; rather, they are often mixtures of a dozen of liquid crystalline components, commonly referred as "singles". This use of liquid crystalline mixtures in LCD devices is mandated to expand the operational temperature range of the LCD devices. Commercially viable LCD devices should operate in at least 50 K of temperature ranges, whereas pure single liquid crystals often have the nematic phase range of less than 20 K.^{1,2}

Chemical analysis of liquid crystals in LCD devices therefore requires separation step before the structural elucidation of a particular component in the liquid crystalline mixture. Separation and subsequent structural analysis by standard gas chromatography–mass spectrometry (GC–MS) technique is the first-line analytical method for most cases of liquid crystal analysis in LCD. However, there are certain cases that standard GC–MS alone is not enough, particularly if the component of interest cannot be analyzed with gas chromatography or has structure too complex to be analyzed with mass spectrometry. Liquid chromatography–mass spectrometry (LC–MS) can solve part of the problems associated with GC–MS, but for routine application to liquid crystals LC–MS still has many obstacles, including the issues with the ionization of liquid crystal analytes. Electrospray ionization (ESI) method is usually not applicable to most liquid crystals due to the polarity issues. Recently, atmospheric pressure chemical ionization (APCI) method showed some success for the

analysis of liquid crystals in LCD devices.^{3,4,5} Recently, atmospheric pressure photoionization (APPI) method has been reported to successfully ionize lower polarity liquid crystals.⁴ However, there are cases when the structure of the analyte component is not known altogether, in which the component may be either an impurity or a deliberately incorporated constituent.

Liquid chromatography–nuclear magnetic resonance (LC–NMR) spectroscopy can serve as a more universal alternative when structural analysis for components with limited information is required.⁶ However, a few issues need to be solved before hyphenation with liquid chromatography. First, unlike mass spectrometry, the analytes of interest usually need to be collected from many HPLC runs for NMR analysis to overcome the intrinsically low sensitivity of NMR. HPLC eluents further dilutes the analytes, not only lowering the analyte signals but also adds the overwhelming solvent signals in NMR spectra, if the standard non-deuterated solvents are used as eluent. Some researchers have tried to employ deuterated eluents for HPLC run and then preconcentrate the eluents through solid phase extraction (SPE) and on-line column trapping.^{7,8} However, use of expensive deuterated solvents needs to be minimized in corporate environment, particularly if dozens or hundreds of HPLC runs are needed to obtain sufficient amount of sample for NMR analysis. An alternative approach uses non-deuterated solvent as HPLC eluent, and then removes the non-deuterated solvent later, and finally transfers the sample with deuterated solvent for NMR analysis. This approach significantly saves the consumption of deuterated solvents, but requires more

sophisticated equipment for collection, preconcentration/drying and transfer to the NMR spectrometer.

In this article, we presented the analysis of a single component in liquid crystalline mixtures by online liquid chromatography–solid phase extraction–NMR (LC–SPE–NMR), with non-deuterated solvent as HPLC eluent and later removed before NMR analysis.

EXPERIMENTAL

Two liquid crystal mixtures, hereafter referred as MLC-A and MLC-B, were obtained from the same supplier and used without further purification. Preliminary GC–MS analysis showed that these two liquid crystals had very similar composition, with all but one common component with the similar composition as determined by GC peak area ratio. The only differing components in two liquid crystal mixtures, hereafter referred as MS-A and MS-B, showed similar mass fragmentation patterns in GC–MS and APCI-MS results with $m/z = 14$ difference on some peaks including molecular ion peaks, suggesting the similar molecular structures between the two singles with one methylene (CH₂) unit difference.

For most of the work presented here, we used an integrated LC-SPE-NMR/MS system provided by Bruker Corporation. The system consists of (1) Agilent 1200 HPLC (Agilent, USA), (2) Spark Prospekt 2 SPE (Spark, the Netherlands), (3) Bruker Avance III 500 (Bruker Biospin, Germany), and (4) Bruker Esquire 4000 ESI/APCI MS (Bruker Daltonics, Germany), along with associated

controllers and software. For NMR analysis, we used 3 mm SEI microflow probe (Bruker Biospin, Germany), which had an active volume of 30 μL and received the sample by either manual injection or automated transfer through standard HPLC liquid handling system.

The LC-SPE-NMR experiment was performed in two steps. First, a series of HPLC-SPE run was repeated 16 times to collect sufficient amount of analyte of interest into a SPE cartridge. The liquid crystal was diluted into acetone by 50-fold, and 5 μL of this diluted sample was injected for each HPLC run. The injected sample was eluted through 15 cm Agilent Eclipse XDB-C18 column (Agilent, USA), with pure HPLC-grade acetonitrile used as eluent at 0.7 mL/min. Standard UV detector signal at 254 nm was used to identify the peak of interest. After the HPLC run became stable and the retention times of the peaks were stabilized, the SPE system was programmed to trap the analyte of interest onto the same SPE cartridge (a Spark reverse phase (RP) SPE cartridge for standard 96-well system) using both the retention time and UV detector signal for peak identification. This preconcentrated analyte was dried overnight to remove the non-deuterated HPLC eluent solvent. Next, SPE-NMR step was performed by transferring the trapped and dried analyte from the SPE cartridge into the microcoil NMR probe inside the NMR magnet with benzene- d_6 (Sigma-Aldrich, USA) as deuterated NMR solvent, after rinsing the microflow NMR probe with the same deuterated solvent and drying it. All of the steps mentioned above were automatically performed with HyStar (Bruker Biospin and Bruker Daltonics, Germany) integrated controlling software. A standard one-dimensional ^1H NMR spectrum as well as ^1H - ^1H double-quantum-filtered correlation spectroscopy

(DQF-COSY) and ^1H - ^{13}C heteronuclear single-quantum correlation (HSQC) spectra were obtained to elucidate the structure of the analyte of interest. All of these spectra presented in this paper were processed with iNMR.⁹

RESULTS AND DISCUSSION

The one-dimensional ^1H NMR experiments were performed with the two analytes MS-A and MS-B without any solvent suppression method. The spectrum of the single MS-A is shown in Fig. 1, showing peaks of benzene (7.30 ppm, with the pair of its ^{13}C satellite peaks), the solvent of choice in NMR experiment, as well as peaks from acetone (solvent before HPLC injection), acetonitrile (HPLC eluent), and water. Most of the analyte peaks were resolvable and of comparable quality to standard tube-type liquid NMR results, although the multiplet structure was not always easily interpretable with LC-NMR results.

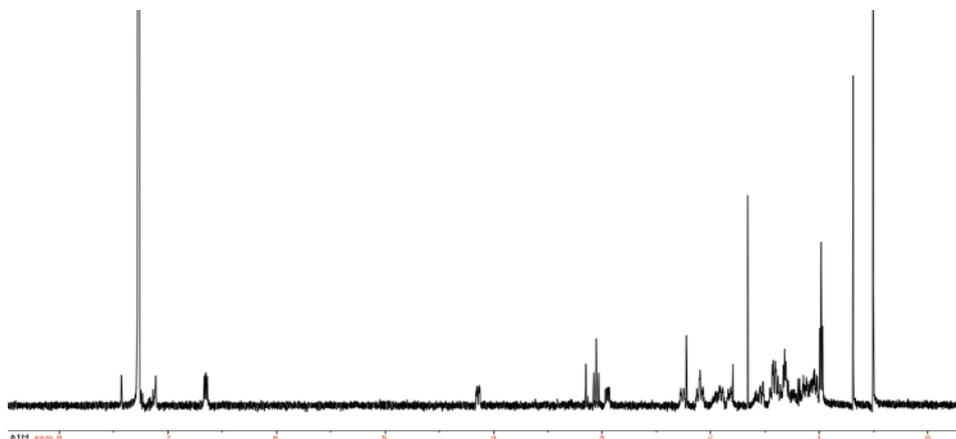


Figure. 1. The one-dimensional ^1H NMR spectrum of the single MS-A.

The most notable feature of the spectra is the surprisingly few peaks in the aromatic proton region. Except for the benzene peak at 7.30 ppm and its satellites, the two analytes MS-A and MS-B both showed only one multiplet at 6.6–6.7 ppm. Considering that most organic liquid crystal singles used in LCD's and other applications have multiple aromatic rings connected in series to enhance their anisotropic molecular properties, this spectra feature was quite exceptional as NMR spectrum of liquid crystalline materials. Some liquid crystal singles employ cyclohexane rings in place of benzene rings, but neither MS-A nor MS-B showed characteristics of cyclohexane-only singles in GC-MS analysis.

Two-dimensional ^1H - ^1H DQF-COSY (of MS-A, shown in Fig. 2) and ^1H - ^{13}C HSQC (of MS-A, shown in Fig. 3) experiments were also performed for additional structural information. These two-dimensional experiments failed to reveal any additional hidden peaks at the aromatic region, suggesting that the ^1H peaks at 6.6–6.7 ppm are perhaps the only spectral lines in the aromatic proton region for these particular analytes. The aliphatic peaks at < 2.0 ppm still did not provide much structural information yet without the knowledge about the backbone structure of the liquid crystal singles.

A decisive clue about the backbone structure came from the spectral feature of oxygenated carbon-proton peaks in the ^1H - ^{13}C HSQC spectrum. Although it is common to employ ether-like structure in liquid crystal backbones, the GC-MS fragmentation pattern of the two singles significantly deviated from those of the widely used alkoxybenzene-type singles, which usually

fragmented in markedly straightforward way. Further, in chain-like alkoxybenzene structures the chemical shifts of the protons attached to the oxygenated carbon should be around 4.0–4.5 ppm, whereas 1D and 2D proton NMR spectra of these two singles have peaks at both 3.0–3.2 ppm and 4.2 ppm, coupled with each other. Furthermore, ^1H - ^{13}C HSQC spectrum of MS-B shows three cross peaks, with two peaks (3.1–3.2 ppm and 4.2 ppm) associated with one oxygenated carbon and one peak (3.0–3.1 ppm) associated with the other oxygenated carbon.

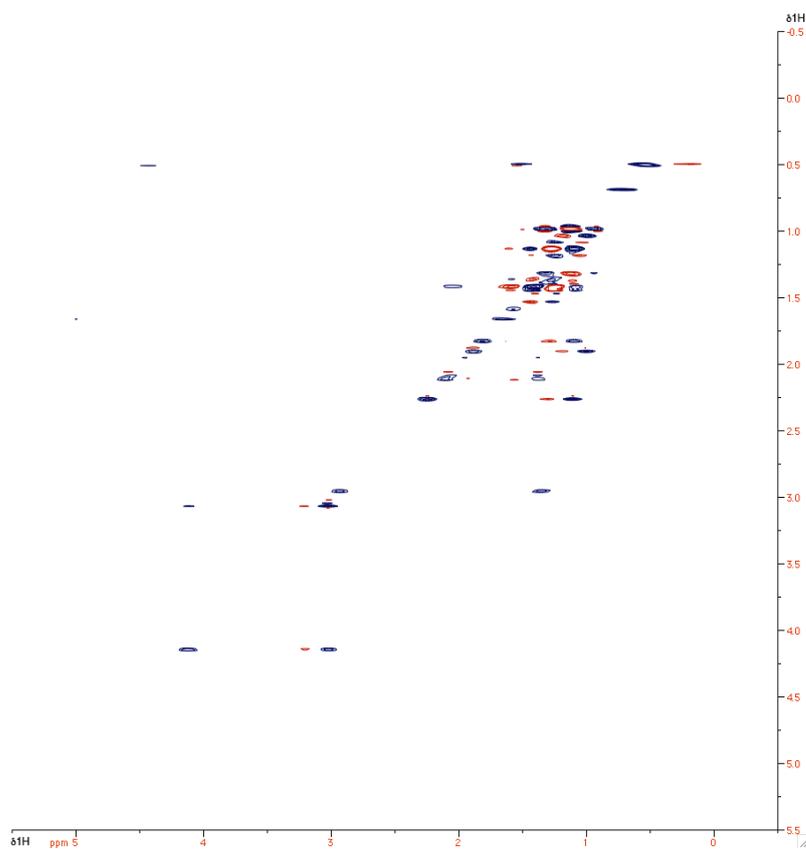


Figure. 2. ^1H - ^1H DQF-COSY spectrum of the single MS-A.

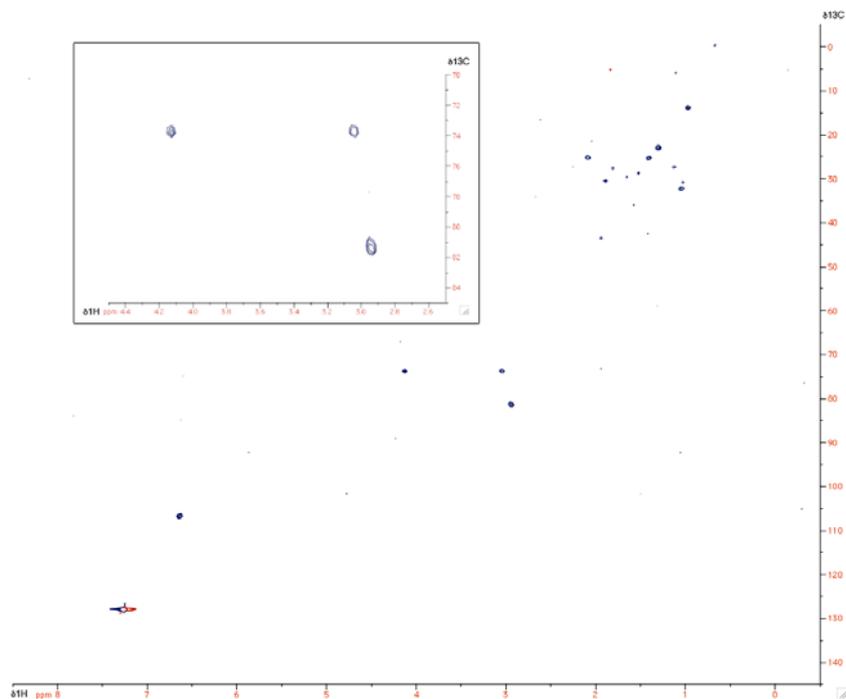


Figure. 3. ^1H - ^{13}C HSQC spectrum of the single MS-A. The inset shows the spectral region of 2.5 ppm < $\delta^1\text{H}$ < 4.5 ppm and 70 ppm < $\delta^{13}\text{C}$ < 85 ppm with expansion.

These spectral features strongly suggested the tetrahydropyran-like structures for the possible molecular structures of the two singles, MS-A and MS-B. In fact, there are already several examples in natural compounds—particularly among carbohydrates—with axial protons next to oxygenated carbon atoms in pyranose rings, and these compounds shows similar NMR spectral features. A notable example is that of D-maltose, whose axial protons shows chemical shifts of 3.2–3.9 ppm regardless of whether their neighbouring carbon atoms are oxygenated inside or outside the rings.¹⁰ Axial protons in D-glucose and D-galactose are also known to behave similarly.¹⁰ An axial proton

attached to an exocyclic oxygenated carbon in a steroid ring also showed chemical shift at < 4 ppm.¹¹ Considering the absence of equatorial hydroxyl groups of our liquid crystal singles, the values of the chemical shifts of axial protons below 3.2 ppm seem reasonable.

Use of less rigid structures such as tetrahydropyran rings for liquid crystal backbones has been suggested in liquid crystal conferences¹² but so far the tetrahydropyran structures have not been routinely employed in commercial applications in LCD devices. Although the liquid crystalline materials with tetrahydropyran structures seem to potentially lower the operating electric field due to their polar nature, chemical stability and solubility/miscibility issues may have hindered its widespread use. However, much of the exact reasons about the adoption/rejection of particular materials have often been kept as trade secret among liquid crystal suppliers and are not widely available in public.

Although we may have succeeded in suggesting the most probable backbone structures of the previously unknown singles, a full interpretation of the single structure by LC-NMR alone is still a daunting task. One of the major obstacles is the lack on more powerful carbon-backbone assigning tools such as 2D INADEQUATE experiments due to the limited amount of samples. The use of isotope-labeled samples can mitigate the problems in biological systems such as proteins and metabolites, but in industry samples such as liquid crystals in LCD devices, this option is seldom viable.

CONCLUSION

We showed that LC-SPE-NMR can be used to separate, collect, concentrate, and analyze a particular component in liquid crystalline mixtures. Despite the limited amount of the samples, standard 1D and 2D experiments with microflow NMR equipment could provide enough information to elucidate the backbone structure of the liquid crystal molecules previously not analyzable by mass spectrometry alone.

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