Application of Multidimensional NMR Spectroscopy and Predicted NMR Databases for the Deconvolution and Analysis of Chlorinated Paraffin Mixtures

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Introduction

Chlorinated paraffins (CPs) are complex mixtures of polychlorinated n-alkanes of different chain length. Despite their many industrial applications including uses as components of lubricants, plasticizers and paint additives, both their potential effects on the environment and isomeric composition remain unknown[1]. CPs are mainly synthesized through radical chlorination of industrial alkane mixtures of different chain lengths[1]. Since this method is non-selective, as well as due to the presence of multiple different starting reagents, CP mixtures can be composed of thousands of compounds, and there is no chromatographic technique that can separate these isomers[1]. Thus, while mass spectrometry (MS) can provide valuable insights into the mass distribution of the CP mixture, the exact isomeric distribution can be very difficult to determine.

Here, we report the use of multidimensional NMR predicted spectral databases to elucidate the various isomers in a chlorinated paraffin mixture. NMR is a powerful and non-invasive tool capable of providing detailed structural information of even the most complicated systems. The chemical shift of ^{1}H and ^{13}C nuclei provides an indication of their chemical environment, which can be used to identify the chlorine arrangement in a chlorinated paraffin. Importantly, the peak capacity of multidimensional ^{1}H-^{13}C NMR spectrum is around 2,000,000, giving it the ability to overcome substantial overlap[2]. In addition, though the use of spectral databases has been well established in metabolomics, its application to the NMR of polymer mixtures has yet to be explored. By applying 2D NMR to a chlorinated paraffin mixture, we show that it is possible to profile the different isomers, assign peaks, and, with the aid of a database, perform mixture deconvolution.

Materials & Methods

Solution State NMR of CP mixtures:

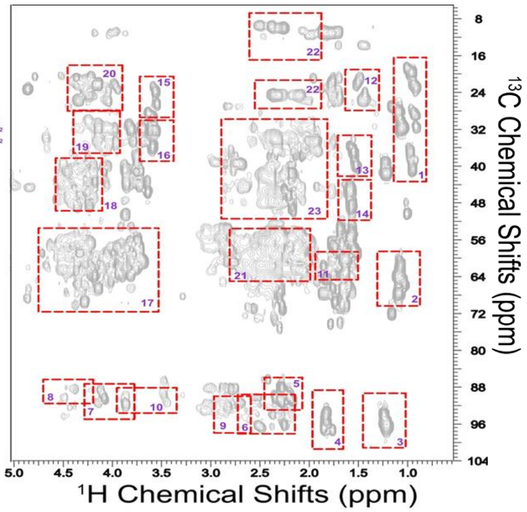
Solution-state NMR experiments were performed on a Bruker Avance III HD spectrometer equipped with a prodigy cryoprobe operating at a frequency of 500 MHz for ^{1}H (11.75 T). Commercially obtained single chain-length mixtures of chlorinated paraffins were dissolved in deuterated chloroform and loaded into a 5mm NMR tube for analysis. The temperature was fixed at 25°C. 1D ^{1}H, 1D ^{13}C, ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC spectra were gathered.

NMR spectral prediction, matching and representation:

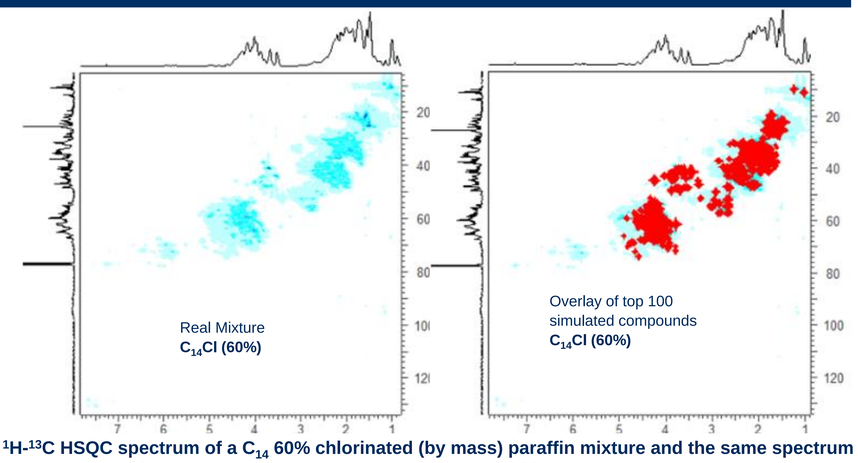
All possible saturated CPs matching the molecular formula that was identified using mass spectrometry were generated using a custom python script, resulting in over 400,000 compounds. This included C_{14} and C_{15} paraffins with varying degrees of chlorination (1-10 chlorine atoms). 1D ^{13}C NMR spectra for these compounds were generated using ACD/Labs spectrum prediction software. Using the spectra gathered for the CP mixtures, the top 1000 components were identified. Identification was performed using ACD/Labs similarity search algorithm, which compares peak location, intensity and shape. The 2D HSQC and HMBC spectra for the top 1000 compounds were then predicted. Once again comparing to the mixture, and considering the most abundant molecular formula based on mass spectrometry, the top 100 compounds were identified. To help understand the isomeric distribution, the top 1000 and top 100 compounds were displayed as a "heat map" of the probability of chlorination at that position.

2D NMR Spectra of Chlorinated Paraffins

1) CH_2Cl_2
2) $CHCl_2$-CH_2-CH_3
3) CCl_3-CH_2-CH_3
4) $-CCl_2$-$C(Cl)H$-CH_2-
5) $-CCl_2$-CH_2
6) $-CH_2$-CH_2-CCl_2-
7) $-CH_2$-$CH(Cl)$-CCl_2-
8) $-CCl_2$-$CH_2(Cl)$
9) CCl_3-CH_2-CH_3
10) CCl_3-CH_2-CCl_3
11) CH_2-$C(Cl)H$-$CH(Cl)$
12) $-CH_2$-CH_2-CH_2-
13) $-CH_2$-$CH(Cl)$-CH_2-
14) $CClH_2$-$CH(Cl)$-CH_2-
15) $-CH(Cl)$-CH_2-CH_2-
16) $-CH(Cl)$-CH_2-CH_3
17) R_2-$CH(Cl)$-$CH(Cl)$-R_2
18) $CH_2(Cl)$-CH_2-$CHCl$
19) CH_2-$C(Cl)H$-$CHCl$
20) CH_2-$C(Cl)H$-$CHCl$
$-CH_2$-$C(Cl)H$-$CHCl$-
21) $-CH_2$-$C(Cl)H$-$C(Cl)H$-
22) R_2-CH_2-CH_2-R_2
23) R_2-CH_2-$C(Cl)H$-CH_2-R_2
R_2-$C(Cl)H$-CH_2-$CH(Cl)$-R_2
R_2-$C(Cl)H$-$CH(Cl)$-CH_2-R_2
R_2-$C(Cl)H$-CH_2-CH_2-R_2
R_2-CH_2-CH_2-$CH(Cl)$-R_2

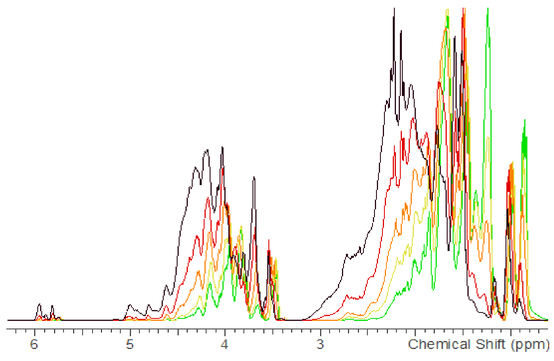


The aliphatic region of the ^{1}H-^{13}C HMBC spectrum of a 60% chlorinated C_{14} paraffin mixture, along with peak assignments. As can be seen in the spectrum, the peaks are resolved sufficiently to allow assignment of peaks from various moieties on the CPs. This allows for improved isomer determination, even in complex, multicomponent mixtures. Overall, this demonstrates the excellent peak capacity of multidimensional NMR spectra, and its effective application to CP mixtures.



^{1}H-^{13}C HSQC spectrum of a C_{14} 60% chlorinated (by mass) paraffin mixture and the same spectrum with the top 100 compounds overlaid. In general, the top 100 compounds adequately represent the mixture. The lack of representation of peaks near 6ppm(^{1}H)/70ppm(^{13}C) is due to unsaturated compounds, which were excluded from the simulated spectra due to their low concentrations (<1%).

1D Proton NMR

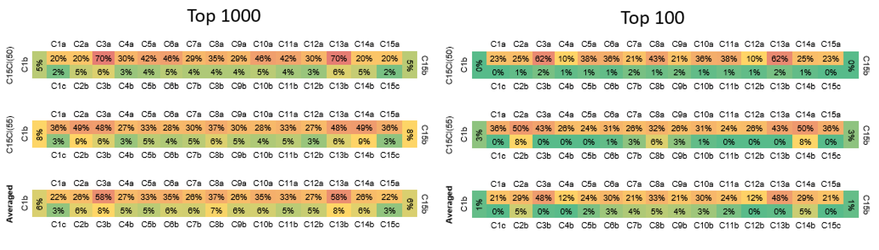


1D ^{1}H NMR for C_{14} CPs with varying chlorine contents. Chlorine content for each curve (mass %): green 40%, yellow 45%, orange 50%, red 55% and black 60%. In general, a larger degree of chlorination results in greater deshielding of the remaining protons on the carbon chain, which shifts the spectrum downfield.

Summary

1. Chlorinated paraffins are extremely complex mixtures, and cannot be separated by chromatographic techniques. However, multi-dimensional NMR combined with spectral databases allow for peak assignment, isomer identification and mixture deconvolution.
2. The top 100 constituents of CP mixtures give an adequate representation of the overall mixture, even without inclusion of unsaturated isomers, but do not reflect the full diversity of compounds.
3. Certain positions on the carbon chain, namely the 3rd from the end and middle positions, are more likely to be chlorinated than others. The 4th from the end is least likely to be chlorinated.
4. In the future, analysis could be extended to include unsaturated isomers, as well as a mixture of paraffin chain lengths, though this would be computationally difficult.

Chlorine Heat Maps



Heat maps showing chlorination probability at each carbon for a C_{15} chlorinated paraffin mixture, considering both the top 1000 and 100 compounds. The percent chlorine by mass is indicated in brackets, with the last row being the average. Based on the analysis, the third carbon from the edge of the chain is the most likely to be chlorinated, which is consistent with steric effects and the weakest C-H bond strength at this position. Further, the carbon near the symmetric centre of a chain is also likely to be chlorinated due to the folding alkane structure resulting in low steric barriers. On the other hand, the fourth carbon from the end is among the least likely to be chlorinated, also due to steric effects. The top 1000 compounds show the same trends as the top 100, but to a lesser degree, as the chlorines are more evenly distributed, and carbons with multiple chlorines are more prevalent.

References

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(2) Hertkorn, N., Ruecker, C., Meringer, M., Gugisch, R., Frommberger, M., Perdue, E. M., Witt, M. Schmitt-Kopplin, P. **2007**. *Anal. Bioanal. Chem.*, *389*, 1311–1327.

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