Position-specific isotope analysis of propane by mid-IR laser absorption spectroscopy

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Abstract

Intramolecular or position-specific carbon isotope analysis of propane ($^{13}CH_3 - ^{12}CH_2 - ^{12}CH_3$ and $^{12}CH_3 - ^{13}CH_2 - ^{12}CH_3$) provides unique insights into its formation mechanism and temperature history. The unambiguous detection of such carbon isotopic distributions with currently established methods is challenging due to the complexity of the technique and the tedious sample preparation. We present a direct and non-destructive analytical technique to quantify the two singly substituted, terminal ($^{13}C_t$) and central ($^{13}C_c$), propane isotopomers, based on quantum cascade laser absorption spectroscopy. The required spectral information of the propane isotopomers was first obtained using a high-resolution Fourier-transform infrared (FTIR) spectrometer and then used to select suitable mid-infrared regions with minimal spectral interference to obtain the optimum sensitivity and selectivity. We then measured high-resolution spectra around $1384\,cm^{-1}$ of both singly substituted isotopomers by mid-IR quantum cascade laser absorption spectroscopy using a Stirling-cooled segmented circular multipass cell (SC-MPC). The spectra of the pure propane isotopomers were acquired at both $300\,K$ and $155\,K$ and served as spectral templates to quantify samples with different levels of ^{13}C at the central (c) and terminal (t) positions. A prerequisite for the precision using this reference template fitting method is a good match of amount fraction and pressure between the sample and templates. For samples at natural abundance, we achieved a precision of $0.33\,‰$ for $\delta^{13}C_t$ and $0.73\,‰$ for $\delta^{13}C_c$ values within $100\,s$ integration time. This is the first demonstration of site-specific high-precision measurements of isotopically substituted non-methane hydrocarbons using laser absorption spectroscopy. The versatility of this analytical approach may open up new opportunities for the study of isotopic distribution of other organic compounds.

Introduction

Natural hydrocarbons are composed of various isotopocules that vary in the number of isotopic substitutions (singly, doubly) or positions of isotopic substitutions (site-specific). Many light hydrocarbons contain hydrogen and carbon isotopes substituted in energetically non-equivalent positions, e.g., in the methyl $-CH_3-$ and methylene $-CH_2-$ groups of propane C_3H_8. Isotope fractionation due to chemical, physical, or biological processes occur primarily at these reactive sites because of the different

rates at which their bonds break/form among the involved isotopomers[1]. This intramolecular isotope distribution is often associated with biosynthetic pathways, kinetic processes, and environmental conditions. Thus, the $^{13}C/^{12}C$ fractionation between the central $-CH_2-$ and terminal $-CH_3-$ position of propane (C_3H_8) can, e.g., serve as a useful tool for determining the thermal maturity of thermogenic gases and their formation mechanisms. Indeed, intramolecular isotopic analysis, also referred to as position-specific isotope analysis (PSIA), has been shown to provide unique information to constrain the origin and the biosynthetic pathways of carbohydrates[2–4]. Currently, there are only two analytical approaches available to perform such analysis[5]. Nuclear magnetic resonance (NMR) is a powerful technique to observe site-specific isotope effects in organic compounds[6,7]. However, its low sensitivity requires large sample sizes (up to hundreds of mmol), which strongly limits applications in environmental sciences. Methods based on isotope ratio mass spectrometry (IRMS) have recently been developed to analyze the intramolecular ^{13}C composition of propane. These are based either on gas chromatography-IRMS[2] or on ultra high-resolution mass spectrometry[8]. Both approaches involve laboratory-based instrumentation and the samples are unavoidably destroyed either before (e.g. pyrolisis) or during analysis. Nevertheless, they were successfully applied to measure the intramolecular ^{13}C distribution of natural gas samples of thermogenic origin[9–12]. These pioneering studies revealed that thermogenic propane exhibits systematic variations in the carbon isotope structure. However, more detailed research and widespread applications are required to better understand the various processes and pathways that occur in natural gas systems. These would be strongly supported by an alternative analytical technique that is capable of high throughput and in-situ deployment.

Optical absorption techniques are known to provide precise and accurate information about the intramolecular properties of the molecule, and they are increasingly used in isotope research[13–17]. In case of more complex molecules, however, the optical absorption techniques face two main challenges: larger molecules tend to have congested and overlapping spectral features, and line-by-line assignments are often not available in spectral databases. While high-resolution IR spectra of natural propane exist[18,19], spectral information of propane's site-specific isotopomers is lacking.

In this paper, we describe an alternative approach based on direct absorption laser spectroscopy for isotopomer specific analysis of propane. The missing spectral information of site-specific ^{13}C propane isotopomers is first generated using Fourier-transform infrared (FTIR) spectroscopy between 600 and $1520\,cm^{-1}$. Then a narrow spectral range is identified for high-resolution absorption measurements using single-mode laser source. The congested and highly overlapping spectra are analyzed by a compound fitting technique that allows for high precision retrieval of the amount fractions of the different isotopomers even without the knowledge of the line-by-line spectral data. The performance of this method is investigated through measurements of isotopically spiked propane samples, while its feasibility for geochemical applications is demonstrated by retrieving the site-specific isotope ratios ($\delta^{13}C_c$ and $\delta^{13}C_t$) in natural propane diluted to $10\,\%$ in N_2 at a sample size of a few micromoles.

Samples and Methods

Samples: High purity ($99\,\%$) gases of site-specific propane isotopomers, $^{13}C_t$ and $^{13}C_c$, (Cambridge Isotope Laboratories, Inc.) were purchased in $0.5\,L$ lecture bottles. These gases were used to measure the FTIR absorption spectra as well as to prepare custom gas samples with distinct propane isotopomer compositions for laser spectroscopic measurements. In addition, we used natural propane diluted in N_2 at $10\,\%$ and $50\,\%$ in pressurized cylinders ($10\,L$, PanGas AG), as starting material, denoted subsequently as 'RefA' and 'RefB' (see Table 1).

In total, eight gas samples with different enrichments of $^{13}C_c$ and $^{13}C_t$ were prepared volumetrically based on pressure measurements

Table 1: Volumetrically prepared propane samples with various compositions of site-specific (central and terminal ^{13}C) isotopomers. The values indicate the amount fraction of the species along with their estimated uncertainty. The 'natural' samples, indicated as 'Ref', are assumed to contain all isotopomers at natural abundance. These were used as provided by the gas supplier.

Sample ID	Natural (%)	$^{13}C_t$ (%)	$^{13}C_c$ (%)	Purpose
S#1	7.93 ± 1.7	0.82 ± 0.32	0.82 ± 0.32	isotopically spiked samples for analysis
S#2	-	-	15 ± 5.6	high-resolution QCLAS spectra
S#3	-	15 ± 5.6	-	
S#4	49.00 ± 9.8	2.00 ± 0.7	-	line selection
S#5	49.00 ± 9.8	-	2 ± 0.7	
S#6	-	-	15 ± 6	reference template generation
S#7	-	30 ± 8	-	
RefA	10 ± 0.10	-	-	(incl. validation)
RefB	50 ± 10	-	-	dilution

(LEO3, Keller), with a precision of ± 1 mbar. The composition of these samples is given in Table 1.

Before sample preparation, the gas handling system was evacuated down to 0.5 mbar using a membrane pump (N920, KNF). Then, the target gas sample was expanded into a buffer volume of 5.1 mL, before being cryogenically transferred to a 10 mL stainless steel cylinder (SS-4CS-TW-10, Swagelok). Sample S#1 was prepared by adding 260 mbar of $^{13}C_t$ and $^{13}C_c$ each to 5 bar of 'RefB'. We further diluted this sample with 26 bar of N_2 (purity 4.5). The preparation of samples S#2 and S#3 involved merely a dilution step. These samples were used to generate high-resolution spectra of the pure site-specific propane isotopomers at high and low temperatures. Samples S#4 and S#5 were prepared by enriching 'RefB' at 2 bar by either $^{13}C_t$ (S#4) or $^{13}C_c$ (S#5), each at 40 mbar. The purpose of these samples was to allow the identification of absorption signatures belonging to the site-specific isotopomers.

Experimental

FTIR Spectra: Spectral information of the propane isotopomers were obtained from pure samples of $^{13}C_t$ and $^{13}C_c$ using the high-resolution FTIR spectrometer (IFS 125HR, Bruker) at PTB, Braunschweig, Germany[20]. A White-type multipass cell of 85.03 cm optical path length was filled with pure propane samples at pressures ranging between 20–35 mbar. The cell was temperature stabilized at 295.8 ± 0.2 K using a refrigerated circulator (Julabo HE25). Before the measurements, the cell and gas manifolds were purged with N_2 (purity 4.5) and conditioned with propane samples. The FTIR spectra were recorded with a resolution of 0.0035 cm^{-1}.

Figure 1 shows the acquired absorbance spectra of the pure site-specific ($^{13}C_c$ and $^{13}C_t$) propane isotopomers and natural propane in the $600 - 1520$ cm^{-1} range. There are 8 clearly distinguishable vibrational modes in this spectral range. Their assignment is taken from Shimanouchi[21]. Using these FTIR spectra of propane, we performed a detailed survey to identify the most promising range for high-resolution quantum cascade laser absorption spectroscopy (QCLAS) measurements. The range selection was made based on the following criteria: i) strong absorption features, ii) reduced overlapping/superimposed absorption features, iii) distinct absorptions from the main and minor propane isotopomers, and iv) all relevant absorption features within the tuning range of single-mode QCLs (typically a few cm^{-1}).

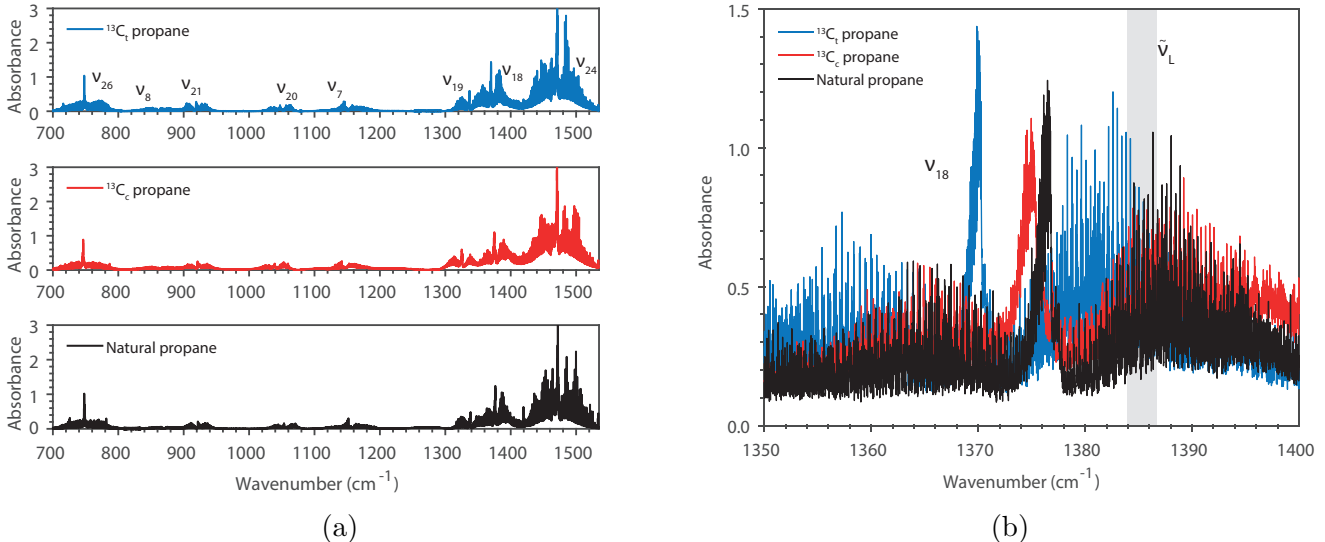Although the ν_{24} vibrational band (a degen-

Figure 1: (a) FTIR spectra of natural propane (black), pure site-specific $^{13}C_c$ (red) and $^{13}C_t$ propane isotopomers (blue) in the $600 - 1520\,cm^{-1}$ range. (b) The ν_{18} symmetric deformation band of propane, which was identified as the most suitable range for laser spectroscopic analysis. $\tilde{\nu}_L$ indicates the spectral window investigated by the QCL (grey shaded area).

erate deformation mode) has the strongest absorption features, the isotope shift effect between the vibrational bands of the various isotopomers is negligible. Thus, the absorption features of the individual isotopomers are highly overlapped, making the distinction between them very difficult. Additionally, this band is close to the bending vibrational mode (ν_2) of water located at $1595.5\,cm^{-1}$, which creates a further challenge when measuring propane samples that contain some level of humidity. The second strongest absorption band is around $1375\,cm^{-1}$. This corresponds to ν_{18}, a symmetric deformation mode, which is shown in Figure 1b. This spectral window satisfies all the above-mentioned selection criteria and thus it is highly suitable for the quantitative position-specific analysis of propane. More specifically, we targeted the R-branch of the vibrational mode and systematically searched these region. In the selection process we paid special attention to potential spectral interferences from contaminating species that may occur in natural samples. The strategy is described in more detail in the next section.

QCLAS setup: The spectrometer is an adaptation of a previous setup described in detail by Nataraj et al.[17] A schematic of the optical setup is shown in Figure 2. Briefly, it uses a distributed feedback quantum cascade laser (DFB-QCL, Alpes Lasers) as light source emitting in the spectral region around $1385\,cm^{-1}$. The laser is operated in intermittent continuous wave (iCW) mode[22], and it is scanned over $0.4\,cm^{-1}$ at a rate of $6.5\,kHz$ using a custom-made laser driver[23]. The laser beam is coupled into a $145\,mL$ volume segmented circular multipass cell (SC-MPC) with an effective optical path length of $6\,m$[24]. The SC-MPC is sealed with two $8\,mm$-thick gold-plated copper lids, and placed inside a custom-made vacuum chamber, which is evacuated down to $2 \times 10^{-5}\,mbar$ using a turbo-molecular pump (HiCube 80, Pfeiffer Vacuum). The vacuum serves as a thermal shield towards the ambient and helps to avoid condensation at low temperatures. The cell is cooled using a vibration-controlled Stirling cooler (MT11904001AA, Sunpower-Ametek) and kept at $155\,K$.

The laser beam exits the SC-MPC after 57 reflections and it is focused onto an HgCdTe photodetector (PVM-2TE-10.6, VIGO Systems).
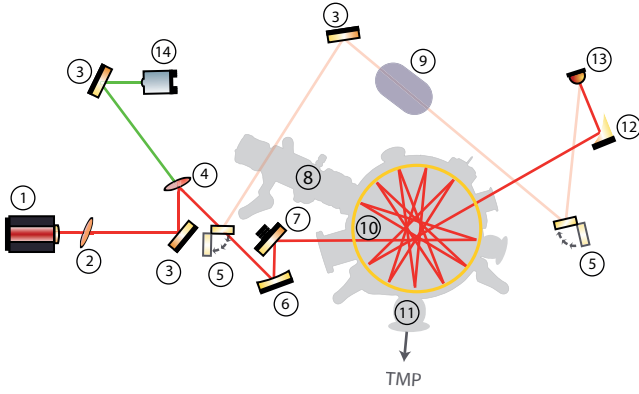
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Figure 2: Schematic of the QCLAS setup. 1. DFB-QCL with collimation optics, 2. wedged ND filter, 3. flat mirrors, 4. IR-VIS beam combiner, 5. flip mirrors, 6. concave mirror, 7. flat mirror mounted on piezo, 8. Stirling cooler, 9. etalon/reference cell, 10. SC-MPC, 11. vacuum chamber evacuated by a turbo-molecular pump (TMP), 12. off-axis parabolic mirror, 13. photodetector, 14. trace laser.

Besides the main path, we established a secondary path for measuring the transmission spectra of a 2 in long Ge-etalon and a reference gas. Switching between the two optical paths was done using an automatic mirror flipping mount (MFF102, Thorlabs).

High resolution propane spectra: High-resolution absorption spectra of propane were recorded within the entire range (about $3\,\mathrm{cm^{-1}}$) covered by the DFB-QCL. This involved consecutive scanning of the laser at different operating temperatures (from $-16\,^{\circ}\mathrm{C}$ to $18\,^{\circ}\mathrm{C}$). At each temperature step, the laser covered about $0.4\,\mathrm{cm^{-1}}$, thus, a total of seven different temperature settings were necessary to span the range from $1383.6\,\mathrm{cm^{-1}}$ to $1386.8\,\mathrm{cm^{-1}}$. Between each step, a settling time of about 5 min was necessary for the laser to fully stabilize.

The workflow to obtain these spectra is similar to that published in Brechbühler et al.[25] First, we obtained the transmission of the 'empty' cell for each spectral window by continuously purging the SC-MPC with N_2. Then the secondary optical path was used to record the transmission spectrum of the etalon with a free spectral range of $0.0245\,\mathrm{cm^{-1}}$, followed by the measurement of the absorption spectrum of ethane. The former is used to linearize the

frequency scale of the spectral scans, while the latter allows to define the absolute frequency scale using absorption line positions (with an overall uncertainty of about $0.001\,\mathrm{cm^{-1}}$) listed in the spectral database HITRAN2020[26]. For this purpose, a single pass cell of 14 cm path length was filled with 10 % of ethane (C_2H_4) diluted in N_2 at 25 mbar pressure. We used this compound because it has enough absorption features in each individual spectral window to allow a reliable frequency scale calibration. The acquisition time for the above spectra was 1 min each.

Following these steps, the propane samples were introduced into the SC-MPC at a constant flow rate and at a constant pressure of 5.00 ± 0.02 mbar, which was maintained by the pressure controller (PC-series, Alicat), placed about 40 cm upstream of the SC-MPC. The inlet flow of 1 mL/min was adjusted by a metering bellows valve (SS-4BMG, Swagelok). The sample line was made of stainless steel tubing (1/16 in). A multi-port selector valve (VICI Valco Instruments) was used to switch between the different gases.

Using the above procedure, we consecutively measured and then stitched the individual spectral windows of propane at both room (300 K) and low (155 K) temperatures. This workflow was applied to the samples 'RefA', S#2, and S#3, corresponding to natural propane and (diluted) pure site-specific ($^{13}C_t$ and $^{13}C_c$) propane isotopomers, respectively.

Figure 3 shows the combined high-resolution spectra of both the central (S#2) and terminal (S#3) ^{13}C isotopomers of propane measured at 155 K and 300 K. For comparison, the spectra acquired with the FTIR are also shown in the inset of Figure 3a. It highlights the benefit of the higher spectral resolution that the laser spectrometer has over the FTIR and reveals the spectral richness of propane that gives access to a plethora of spectral details. This allows for an unambiguous identification and quantification of the different isotopomeric species even within a narrow spectral window that can be easily covered by a single-mode DFB-QCL. Furthermore, we observed that broadband spectral features present at room temper-
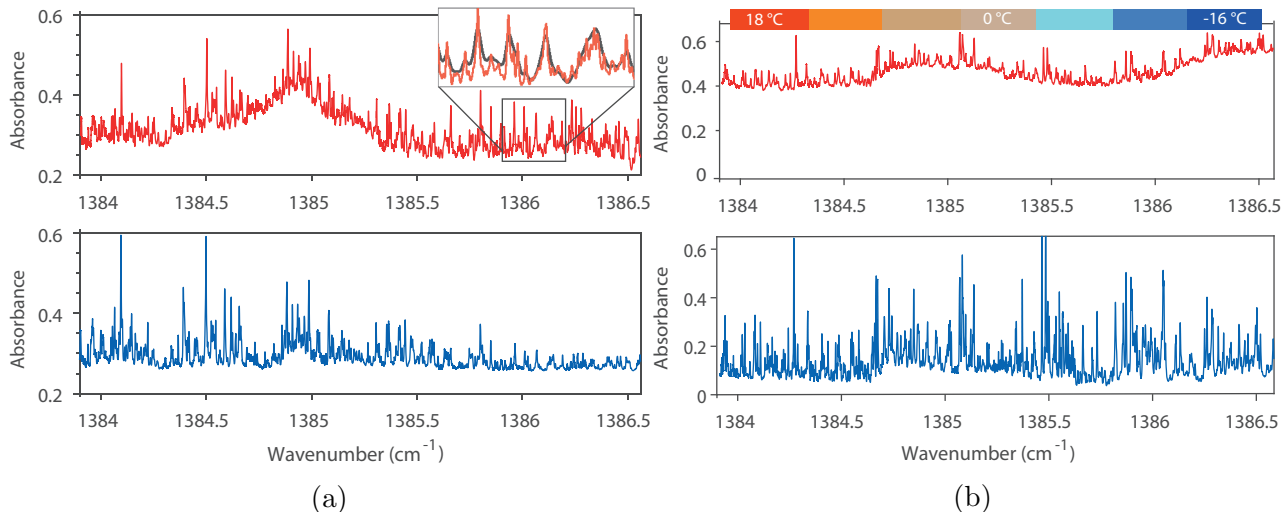
Figure 3: Absorbance spectra of central $^{13}C_c$-propane (a) and terminal $^{13}C_t$-propane (b) measured by QCLAS using the samples S#2 and S#3, respectively, at a constant pressure of 5 mbar. The spectra were acquired at 300 K (red) and 155 K (blue). The inset shows a comparison of the FTIR (black) and the QCLAS (red) spectrum. The laser scanning frequency as a function of operating temperature is shown in (b) top graph.

ature are efficiently suppressed by lowering the gas temperature. In addition, the fine structures, i.e., the sharp absorption features are significantly stronger that facilitate a better selectivity. Therefore, for the rest of this study, we consider only the measurements performed at 155 K.

As a next step, we measured the samples S#4 and S#5 that are enriched in only one of the site-specific ^{13}C isotopes (Table 1). This allowed us to identify potential regions in which the contribution of the spectral features belonging to the various isotopomers is more pronounced, even if the overall absorption is dominated by the main isotopologue. In addition, we considered potential spectral interferences from contaminating species that are expected in natural samples. Hydrocarbons, such as methane, ethane, propene, pentane, and butane are largely limiting factors for the mass spectrometric analysis due to their impact on the carbon fragment ions used for quantification. To minimize their effect, the natural samples need to undergo tedious purification based on cryogenic distillation. Based on spectral simulation of the above mentioned compounds, we found that the range between 1384.4–1385 cm^{-1}

is nearly free of interfering absorptions and allows a quantitative analysis of the propane isotopomers even in the presence of large quantities (tens of percent) of contamination. This high tolerance of the spectroscopic technique against contamination is clearly an asset for natural samples as it allows for a high throughput and simple sample handling, while minimizing potential fractionation effects.

Data Analysis: Propane exhibits congested and overlapping signatures that hamper a line-by-line assignment. This renders the classical spectral fitting inappropriate. Therefore, we developed an alternative fitting approach[25,27] using recorded spectra of pure propane isotopomeric species as a fitting template. For this, we generated spectral templates of the natural propane and the site-specific (terminal and central ^{13}C) propane isotopomers. These were obtained by the procedure described above, and using the samples S#6, S#7, and 'RefA' at pressures of 3.00 ± 0.02 mbar. The generated reference spectra were normalized such that they correspond to absorbance for a sample amount fraction of 1 ppm over an optical path length of 1 m at a pressure of 1 atm and a temperature of 296 K, as described by Brechbühler
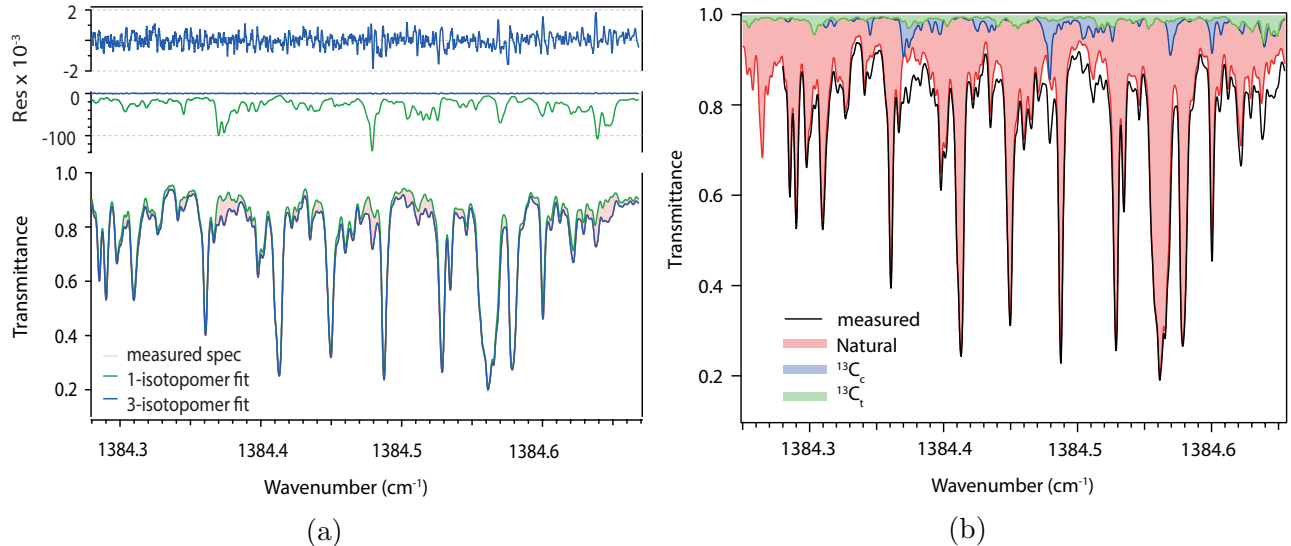
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Figure 4: (a) Measured transmittance spectrum of the isotopically spiked sample S#1 (T = 155 K and p = 3 mbar), fitted with only the natural propane (green) and fitted with all three reference templates (blue). The fit residuals corresponding to the two different cases are given in the top graph using the same color assignment. (b) Individual contributions of the 13C$_t$ (green), ^{13}C$_c$ (blue) and natural propane (red) to the measured spectrum S#1 as determined by the template fitting algorithm.

et al.[25] This normalization does not account for pressure and temperature-related effects, i.e., collisional broadening and hot-band contributions, in the absorption cross-section. It only scales the sample transmittance according to the Beer–Lambert law and the ideal gas law. The impact of this limitation on the analytical performance is discussed in the next section. The fitting of samples containing all three propane isotopomers was performed through a non-linear least-squares algorithm based on the Levenberg–Marquardt method by minimizing the sum of the squared errors between the experimental data points and the modeled spectrum. The latter is taken as the sum of the individual absorbance templates of the different isotopomers.

Results and Disscusion

An example of fitted spectra is shown in Figure 4a. To test the efficiency of the template fitting method, we considered two cases: i) using only the natural propane reference spectrum and ii) using a combination of all three isotopomers to fit the isotopically spiked sample

S#1. The effect is best seen in the fit residuals shown in the middle panel of Figure 4a. While the fitting of the measured spectrum by only one reference spectrum, i.e., natural propane, results in pronounced structures in the fit residuals, using all three reference templates simultaneously not only removes the absorption structures, but also reduces the residual amplitude by about two orders of magnitude, thereby approaching the optical fringe level of the spectrometer. This is a good indication of a well-converging fit, where the contributions of the different constituents are correctly attributed. Figure 4b illustrates the individual contributions of the various isotopologues to the measured spectrum S#1 as determined by the template fitting algorithm.

To determine the precision of the instrument on the site-specific isotopomers of propane, we measured sample S#1 (see Table 1) at 3.00 ± 0.02 mbar pressure for 30 min. Figure 5a shows the time series of measured amount fractions, χ, of the individual isotopomers of propane in sample S#1. The average values of the spectroscopically retrieved amount fractions of $\chi_{\text{Nat}} = 9.57\%$, $\chi_{^{13}C_c} = 1.23\%$, $\chi_{^{13}C_t} = 0.79\%$
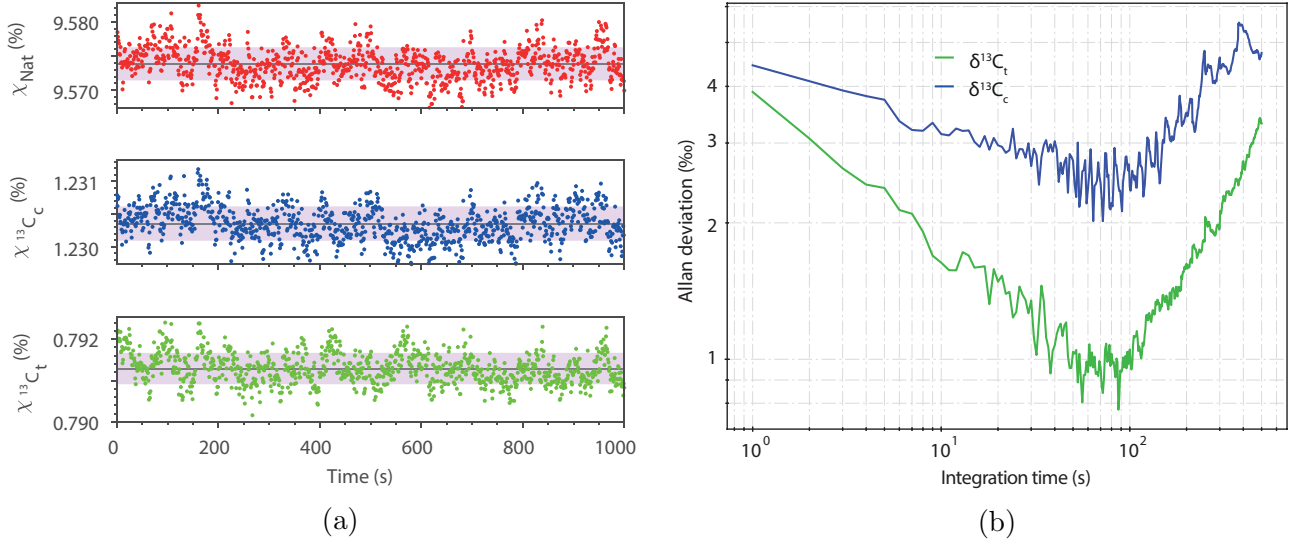
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Figure 5: (a) Time-series of the measured amount fractions of the site-specific isotopomers of sample S#1 at T = 155 K and p = 3 mbar. The shaded region shows the $\pm 1\sigma$ standard deviation (b) Allan-Werle deviation plot of $\delta^{13}C_c$ (blue) and $\delta^{13}C_t$ (green) for the isotopically spiked sample, S#1.

are within the uncertainty of the custom-made samples (see Table 1). The advantage of the laser spectroscopic technique over mass spectrometry is its capability to directly observe absolute amount fractions of the site-specific isotopomers. Thus, in principle, it is possible to determine the difference of $\delta^{13}C$ values between terminal and central C-atom positions of propane without any reference frame. Obviously, the accuracy of such measurements will be dominated by the purity of the individual propane isotopomer samples that are used for reference spectrum generation, with the main challenge being to synthesize pure ^{12}C-containing propane. One way to overcome this limitation is to adopt a pragmatic approach by defining 'RefA' as an internal reference and assuming that it is of thermogenic nature with natural abundance of $^{13}C/^{12}C = 0.011056/0.988944$ and a stochastic distribution of $^{13}C_t/^{12}C = 2^{13}C_c/^{12}C$. This allows us to assess the analytical performance of our method relative to this artificial reference frame in which the $\delta^{13}C$ of the terminal and central position carbons are by definition set to zero. Based on these considerations, we calculated the corresponding δ-values by referencing the isotopologue ratios $^{13}C_{(c,t)}/^{12}C$ to the relative isotope ratios expected from a stochastic distri-

bution in natural samples.

The Allan-Werle variance technique[28] was applied to the measured time series to determine the system's performance. Figure 5b shows the Allan deviation plots associated with the sample S#1, while Table 2 summarizes the achieved precision for the amount fractions $\chi_{^{13}C_{(c,t)}}$ and $\delta^{13}C_{(c,t)}$ within 100 s integration time for all measured samples.

Table 2: Best precision, corresponding to the Allan-Werle deviation minima, reached after 100 s integration time, of the measured amount fraction $\chi_{^{13}C_{(c,t)}}$ and $\delta^{13}C_{(c,t)}$ using various propane samples.

Sample ID	$X_{^{13}C_t}$ (ppm)	$X_{^{13}C_c}$ (ppm)	$\delta^{13}C_t$ (‰)	$\delta^{13}C_c$ (‰)
S#1	0.8	1.0	0.9	2.2
RefA	0.4	0.2	0.33	0.73

Next, we explore whether this capability can be applied with similar precision to propane samples with natural isotopic composition. Therefore, we repeated all the above measurements using sample 'RefA' (data are shown in the supplementary information).
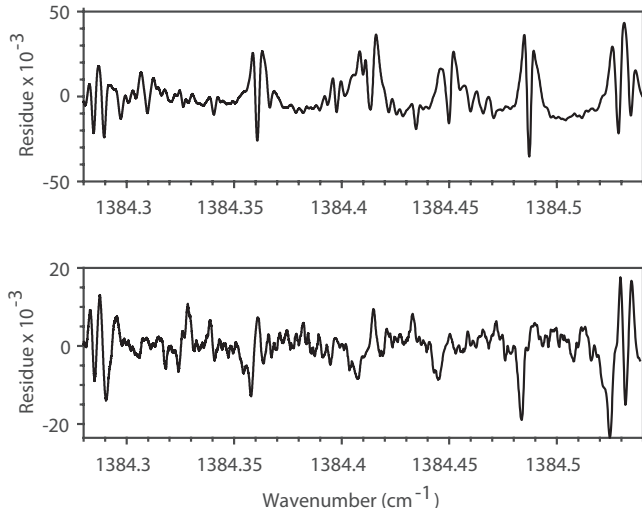
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Figure 6: The effect of pressure (top) and concentration (bottom) mismatch between measured spectrum and reference template on the fit residuals for situations with 66 % and 5-fold mismatch in pressure and concentration, respectively. The impact is mainly seen as a significant increase (a factor 20 to 50) in the peak-to-peak amplitudes compared to the optimal fit and as systematic structures around the absorption features.

In this case, the reference spectrum of natural propane corresponds to the sample spectrum, RefA, i.e. the target spectrum is fitted with itself, albeit in a procedure, which also includes the reference spectra of both site-specific isotopomers, generated using S#6 and S#7. In this best-case scenario, the fit residual reached a level of about 2×10^{-5} (1σ standard deviation). For comparison, fitting sample S#1, the fit residual is about 5 times higher, which is reflected by a loss in precision by a factor 3 for both $\delta^{13}C_t$ and $\delta^{13}C_c$. This degradation in the performance is most likely due to the mismatch in the concentration of total propane with respect to the reference template of about 26 %. Such mismatch may also lead to a slight systematic error in the sample pressure, because the pressure controller that is upstream of the SC-MPC can be dependent on the gas mixture. Accordingly, as shown in Table 2, we reach the best precision for 'RefA' with values of 0.33 ‰ (for $\delta^{13}C_c$) and 0.74 ‰ (for $\delta^{13}C_t$).

If the template does not match the sample in terms of concentration or pressure then the pressure broadening will be different between the two. The mismatch is well illustrated by fitting 50 % natural propane ('RefB') with a template that contains 10 % natural propane ('RefA'). The resulting fit residual (shown in Figure6) is about 20 times larger compared to a fit at matching concentration. Accordingly, the 1 s precision increases by more than one order of magnitude. Very similar behavior is observed when the sample 'RefA' at 5 mbar is fitted by a template (also 'RefA') at 3 mbar. The corresponding fit residual (Fig. 6) increases by a factor 20, and the 1 s precision by a factor 8. Overall, these findings underline the importance of having reference templates generated at temperatures, pressures, and concentrations that match the sample conditions well.

Note that similar effects should also apply for a mismatch involving the site-specific isotopomers. However, this impact is much more subtle, because their total contribution to the overall spectra is just a few percentage in enriched samples and less than 1 % in natural samples. This is particularly true for sample 'RefA', where the 'natural' reference template fully describes the observed spectrum (see section Data Analysis) and, therefore, the retrieved amount fractions of the site-specific isotopomers primarily reflect the random fluctuation of the system and stability of the fitting algorithm. The results obtained from the analysis of sample 'RefA' are given in the supplementary information (SI).

The achieved performance for the natural gas sample 'RefA' (see Table 2) approaches the precision level of $0.5 - 1$ ‰ reported for mass spectrometric techniques[2,10] and it is already sufficient to quantitatively detect and resolve site-preference (SP) values of natural propane samples that were observed in the range from –1.8 to –12.9 ‰[2]. Moreover, assuming a batch-mode operation, the amount of propane sample required for the site-specific carbon isotope analysis would be as small as 2 μmol, which along the fact that no tedious sample preparation is required, is highly important in the context of environmental and geochemical studies.

Finally, due to its versatility, our approach can be extended to rare isotopic species of sim-

ple molecules such as clumped CO_2 (already demonstrated by Nataraj et al. [17]), CH_4 or N_2O and other volatile organic compounds, thereby opening new perspectives in environmental sciences and fundamental research.

Conclusions

We have developed a quantum cascade laser-based absorption spectrometer relying on a low-volume Stirling-cooled SC-MPC. Despite the complex absorption signature of propane, we successfully distinguish between the individual site-specific isotopomers and quantitatively determine their contributions to the overall absorption spectrum. For samples close to the natural abundance, we report a precision of $0.33\,‰$ for $\delta^{13}C_t$, and $0.73\,‰$ for $\delta^{13}C_c$ values achieved at $100\,s$ integration time, obtained without any sample preparation. A prerequisite for achieving high precision using the reference template fitting method is a good match of amount fraction and pressure condition between the measured samples and spectral template generation. Furthermore, the method requires access to high quality and pure reference templates. In the case of propane, the pure ^{12}C-containing main isotopologue is not commercially available and challenging to synthesize. Therefore, a traceable scale of reference gases would be needed for the accuracy assessment of the technique.

Supporting Information Available

Additional results obtained by analyzing natural propane samples to validate the performance of the method.

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