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Determination of $^{13}\text{C}/^{12}\text{C}$ Carbon Isotope Ratio

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Isotopomers $^{12}\text{CO}_2$ and $^{13}\text{CO}_2$ absorbed into polystyrene films provide narrow, sharp, and well-resolved IR absorption bands for the ν_3 antisymmetric stretching mode. This is exploited to set up an inexpensive FT-IR-based method for the measurement of the carbon isotope ratio. Accuracy of 2.5‰ $\delta^{13}\text{C}$ units is readily achieved already at a low resolution of 2 cm^{-1} .

The $^{13}\text{C}/^{12}\text{C}$ carbon isotope ratio is a chemical parameter with many important applications in several scientific areas. For example, it is a useful tool in environmental science for monitoring climatic changes¹ and human activity impact,² in archaeology for the palaeodietary reconstruction,³ in geochemistry for the surveillance of volcanic activity,⁴ and in medicine for the noninvasive test of *Helicobacter pylori* infection,⁵ yet it is an active research field of analytical chemistry.⁶ In general, the isotopic ratio is carried out analyzing carbon dioxide obtained by converting the carbon contained within a sample. Isotope ratio mass spectrometry (IRMS) has been the first technique to be used for highly accurate $^{13}\text{C}/^{12}\text{C}$ determinations.⁷ Recently, several methods for isotopic analysis based on a number of different techniques have been

reported.⁸ These include a laser-assisted ratio analyzer (LARA) technique based on the laser optogalvanic effect (OGE),⁹ nondispersive infrared spectroscopy (NDIRS),¹⁰ and mid-IR tunable laser spectroscopy.¹¹ Conventional IR absorption spectroscopy-based methods exploiting the isotope shift in spectral lines have been also previously reported.^{6b,c} Carbon isotope ratio is quoted in relative differential units $\delta^{13}\text{C}$ with respect to a standard.¹² IRMS delivers very high accuracy of 0.1‰. At present, equivalent accuracy is provided by both LARA⁹ and NDIRS¹⁰ techniques. However, in many important scientific applications such a high degree of accuracy is not needed and less sophisticated and expensive approaches could be used. In this respect, IR absorption spectroscopy represents a useful alternative.^{6b-d} According to this methodology, the $^{13}\text{C}/^{12}\text{C}$ value is determined from the ν_3 antisymmetric stretching mode absorption intensity ratio of the two most abundant species, $^{12}\text{CO}_2$ and $^{13}\text{CO}_2$. Unfortunately, the isotope effect is not large enough to resolve the broad gas-phase spectral bands and a number of difficult expedients, namely, increased resolution to detect the area of selected rotational lines,^{6b} high gas pressure systems,^{6c} and use of multipass cells,^{6d} have to be taken into account in order to get useful results. Here we illustrate an original approach that is interesting per se and makes conventional IR absorption spectroscopy easily applicable.

MATERIALS AND METHODS

All the chemicals were purchased from Aldrich. International standards, purchased from the International Atomic Energy Agency (IAEA), are as follows: IAEA-CH-3, cellulose, $\delta^{13}\text{C} = -24.5 \pm 0.2$; IAEA-C8, oxalic acid, $\delta^{13}\text{C} = -18.3 \pm 0.2$; IAEA-CO-1, marble, $\delta^{13}\text{C} = +2.5 \pm 0.1$; USGS41, L-glutamic acid, $\delta^{13}\text{C} = +37.8 \pm 0.2$.

Quartz tube (15 cm long with internal diameter of 0.4 cm) was filled with mixture of 60.0 mg of organic international standard and 300.0 mg of CuO. The tube was connected to a glass flask

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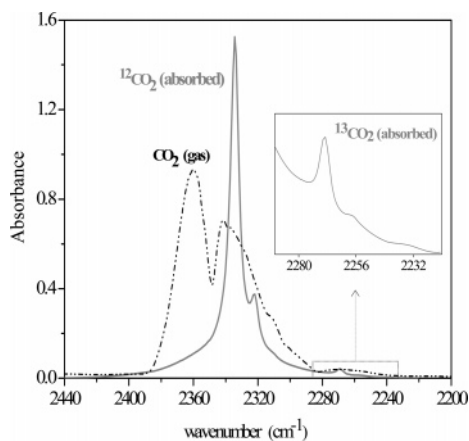


Figure 1. FT-IR spectra of carbon dioxide for the ν_3 antisymmetric stretching mode at 2 cm^{-1} of resolution. Dotted line is for the gas phase; solid line is for carbon dioxide absorbed within a polystyrene film, $150\text{ }\mu\text{m}$ of thickness, exposed for 30 min to carbon dioxide at room temperature. Both spectra are reported for the sake of comparison. The inset expands the absorption band relative to species $^{13}\text{CO}_2$.

(15 mL) containing the atactic polystyrene (aPS) film. The apparatus was evacuated and the tube heated with a Bunsen lamp to produce the carbon dioxide. A 70-mg sample of international marble was introduced into a 10-mL glass flask with aPS film linked under the cap. The flask was evacuated, and 2.5 mL of orthophosphoric acid was syringed onto marble. In both experiments, the polymeric films were left for 30 min at room temperature into CO_2 gas. The aPS film was kept from the flask and introduced in the FT-IR spectrometer.

Amorphous aPS films, with a thickness of $100\text{--}160\text{ }\mu\text{m}$, were obtained by casting from 1.5% aPS chloroform solutions at room temperature.

Transmission infrared spectra in the midrange ($4000\text{--}400\text{ cm}^{-1}$) were acquired at a resolution of 2.0 cm^{-1} and a scanning number of 32 with a Bruker Vector 22 FT-IR spectrometer, equipped with a deuterated triglycine sulfate detector. All the spectra were recorded under a N_2 atmosphere.

RESULTS

The new IR approach presented for the $\delta^{13}\text{C}$ determination is characterized by quite appealing features. The method is based on taking IR absorption spectra for the ν_3 antisymmetric stretching mode region ($2200\text{--}2400\text{ cm}^{-1}$) of carbon dioxide absorbed into a polymer containing basic functional groups. In particular, we have used polystyrene because of its good transparency within the selected spectral range, but several other common polymers can also be used as well. The present study has been carried out adopting a Fourier transform infrared (FT-IR) spectrometer; however, this is not mandatory.

Owing to the interaction with the polymer, most probably of Lewis acid–base nature,¹³ the trapped carbon dioxide shows a modified IR spectrum with respect to the gas phase; see Figure 1.

As can be observed, only the broad P and R bands¹⁴ are clearly discernible in the gas-phase rotovibrational spectrum (dotted line). Conversely, the IR spectrum of carbon dioxide absorbed within the polymer (solid line) displays narrow, sharp, and well-resolved bands centered at 2334 and 2269 cm^{-1} , assigned to $^{12}\text{CO}_2$ and $^{13}\text{CO}_2$ isotopomers, respectively. The shoulder at 2322 cm^{-1} can be assigned to the $(\nu_3 + \nu_2) - \nu_2$ hot band as reported elsewhere.¹⁵ The interaction with the polymer provides a frequency red-shift of the ν_3 absorption band of $\sim 15\text{ cm}^{-1}$, i.e., the difference between 2349.3 cm^{-1} for the gas phase¹⁶ and 2334 cm^{-1} for the $^{12}\text{CO}_2$ trapped within polystyrene, and it does not appreciably change the isotope effect ($2349.3 - 2284.5 = 64.8\text{ cm}^{-1}$ for the gas phase¹⁶), which remains nearly the same ($2334 - 2269 = 65\text{ cm}^{-1}$ as herewith reported).

The clear resolution of the ν_3 absorption bands for the two isotopomers can now be readily exploited to obtain a measure of the absorption intensities ratio. The latter is obviously connected to the concentration ratio of the two species through Beer's law¹⁴ and, then, to $\delta^{13}\text{C}$. This can be done at least in a couple of ways, hereafter indicated as cutting (C) and resolution (R) methods.

The C method, also adopted in previous works,^{6c} is to avoid any kind of extrapolation toward the baseline to work out each single peak area and to estimate the absorption intensity ratio as the quantity A^{13}/A^{12} , where A^{13} is the absorbance area between 2200 and 2275 cm^{-1} and A^{12} the full area between 2200 and 2440 cm^{-1} minus A^{13} , being 2275 cm^{-1} , the wavenumber corresponding to the absorbance minimum between the two peaks. Of course, with such a procedure, A^{13}/A^{12} will be only a crude approximation of the true absorption intensity ratio.

A better way is to resolve the two absorption bands as in the R method. In mathematical terms, the sample spectrum $S = S(\nu)$ is a function of frequency that gives the IR absorbance of the sample. Restricting the S domain to be the ν_3 spectral range of carbon dioxide, for example, $2200\text{--}2440\text{ cm}^{-1}$, S can be taken to a good approximation equal to the sum of the absorption bands of the two isotopomers. In other words, if we call $S^{12} = S^{12}(\nu)$ and $S^{13} = S^{13}(\nu)$, the two functions giving the absorbance of $^{12}\text{CO}_2$ and $^{13}\text{CO}_2$ separately, we have that $S = S^{12} + S^{13}$. The aim of the R method is to work out the two functions S^{12} and S^{13} using only experimental data. To do that, a reference $^{13}\text{CO}_2$ IR absorption spectrum, $R^{13} = R^{13}(\nu)$, for the ν_3 spectral range is used together with the position $S^{13} = dR^{13}$, where d is a damping factor to be determined according to some conditions (see later). It should be remarked that every experimental $^{13}\text{CO}_2$ reference spectrum, for example, as obtained from ^{13}C -enriched BaCO_3 ,¹⁷ contains a small band from the $^{12}\text{CO}_2$ isotopomer. However, this contribution turns out to be much less important as the damping factor d is usually quite small. The above equations can be arranged to give

$$S^{12} = S - dR^{13} \quad (1)$$

(14) See, for example: Barrow, G. M. *Introduction to molecular spectroscopy*; McGraw-Hill: New York, 1962; p 145.

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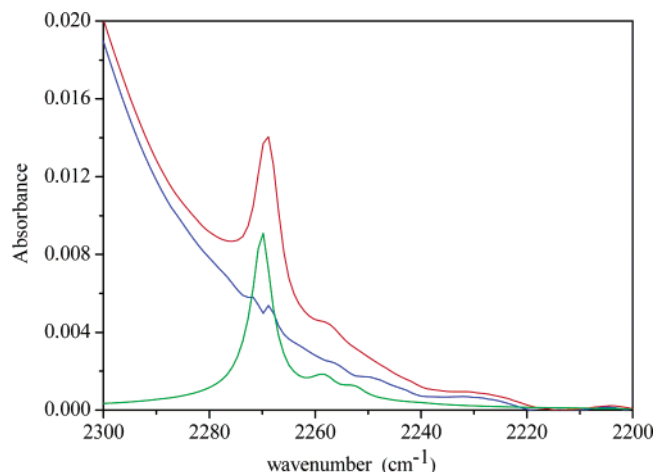


Figure 2. Expanded region of infrared spectrum showing the resolution of the $^{12}\text{CO}_2$ (blue) and $^{13}\text{CO}_2$ (green) absorption bands from the sample spectrum (red) as provided by the algorithm described in the text.

Now, a numerical algorithm can be devised to obtain d , setting its initial value to zero and adding a small increment within a loop until the absorbance minimum in S^{12} , computed according to eq 1, between the resonance frequencies of the two isotopomers disappear. More precisely, the monotone decreasing of the S^{12} curve between the $^{12}\text{CO}_2 - (\nu_3 + \nu_2) - \nu_2$ and $^{13}\text{CO}_2 - \nu_3$ resonance frequencies provides the condition for determining d . A computer program¹⁸ implementing a “damped rebound” algorithm for the determination of the damping factor d has been written. This program permits the complete resolution of the two absorption curves S^{12} and S^{13} as shown in Figure 2.

The absorption intensity ratio can be expressed again as A^{13}/A^{12} , now with A^{13} and A^{12} the integrated area from 2200 to 2440 cm^{-1} of S^{13} and S^{12} absorption curves, respectively.

A second important feature concerns the straightforward preparation of the sample and the operational procedure required to record the IR spectra. Simply, a thin polystyrene film is exposed to the carbon dioxide obtained from the sample within a pre-evacuated flask. Then, the film is placed within the FT-IR spectrometer in a clean nitrogen fluxed atmosphere, and a set of transmission spectra is taken until nearly complete gas desorption. To minimize the polystyrene signals, the last spectrum is subtracted from all the previous ones. Following this procedure, spectra like those displayed in Figure 1 (solid line) can be obtained. At variance with IRMS and traditional IR techniques, the presence of impurities, in particular water vapor, is not a major problem because of the polymer selectivity toward carbon dioxide.

To establish the accuracy of the proposed method, we have performed a large number of tests on each of four adopted international reference materials.¹⁹ Measured averaged absorption intensity ratios and relative standard deviations (RSDs) are displayed in Table 1 for both C and R methods described above.

As can be observed, in both cases absorption intensity ratios correlate quite nicely with the standard $\delta^{13}\text{C}$ value. However, the RSDs confirm the superior quality of the R method, being not

Table 1. Averaged Absorption Intensity Ratios A^{13}/A^{12} and RSDs for the International Reference Materials Adopted in the Present Work

reference materials	$\delta^{13}\text{C}$ (‰) ^a	C method ^b		R method ^b	
		A^{13}/A^{12}	RSD (%)	A^{13}/A^{12}	RSD (%)
IAEA-CH-3	-24.5 ± 0.2	0.0229	1.3	0.0131	0.6
cellulose					
IAEA-C8	-18.3 ± 0.2	0.0231	0.9	0.0134	0.7
oxalic acid					
IAEA-CO-1	$+2.5 \pm 0.1$	0.0255	1.3	0.0150	0.7
marble					
USGS41	$+37.8 \pm 0.2$	0.0280	1.4	0.0176	0.6
L-glutamic acid					

^a Certified $\delta^{13}\text{C}$ values from IAEA. ^b See text for details on C and R methods of band area integration.

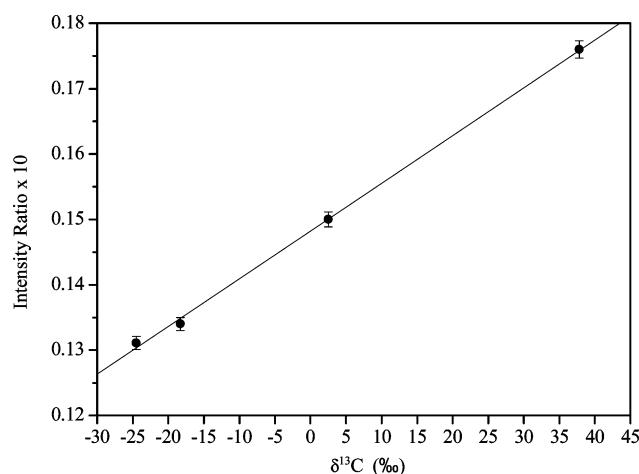


Figure 3. Linear calibration plot between averaged IR absorption intensity ratio A^{13}/A^{12} , obtained by means of the resolution (R) method (see text), and $\delta^{13}\text{C}$ values relative to the international standards used in the present paper. The error bar for the intensity ratio corresponds to the relative standard deviation of 0.7%.

larger than 0.7% in the worst of cases, to be compared with 1.4% affecting the C method. This has encouraged us to carry out the calibration line adopting the R method.

Figure 3 shows the calibration line obtained plotting the averaged absorption intensity ratios, obtained via the R method, against the reference $\delta^{13}\text{C}$ values of Table 1. The error bar is fixed to the worst case of RSD = 0.7%. As can be observed, the linear correlation is rather good (correlation coefficient $r = 0.999\,23$), leading to a standard deviation of 2.5‰ in terms of $\delta^{13}\text{C}$ units, as estimated according to straightforward error propagation. Adopting the data relative to the C method (RSD = 1.4%), the $\delta^{13}\text{C}$ standard deviation turns out to be 4‰ units.

It is noteworthy that the achieved accuracy is already sufficient to carry out a number of applications, for example, detection of *H. pylori* infection by means of the breath test. According to the literature, the $\delta^{13}\text{C}$ cutoff for positive diagnosis of *H. pylori* infection is quoted to be +5‰ at 30 min and its variation, for an infected subject, is usually reported to be in the range 20–25 (per mil) δ units after 20 min.²⁰ Since the achieved accuracy is lower than such values, our claim seems to be correct at least to establish the infection presence.

(18) The computer program is available at <http://zanasi.chem.unisa.it/download>, along with a number of examples relative to the international standards used to build the calibration plot.

(19) Stable Carbon Isotopes Standards available from IAEA.

CONCLUSIONS

We have proven how a very precious piece of information, i.e., the $^{13}\text{C}/^{12}\text{C}$ carbon isotope ratio, can be obtained by means of inexpensive FT-IR spectroscopy of carbon dioxide trapped within a polymer according to an easy and fast protocol. The calibration has been carried out using international standard materials covering a range of $\delta^{13}\text{C}$ values commonly adopted in many

scientific applications and spanning the natural occurring isotopic abundance.

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(20) These values can be found in many papers; see, for example, refs 5b and 6d and: Logan, R. *Eur. J. Gastroenterol. Hepatol.* **1993**, *5* (Suppl. 2), S46–S49.