Published in final edited form as:

Anal Chem. 2016 September 06; 88(17): 8714–8719. doi:10.1021/acs.analchem.6b02054.

Quantifying Carbon-14 for Biology Using Cavity Ring-Down Spectroscopy

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Abstract

A cavity ring-down spectroscopy (CRDS) instrument was developed using mature, robust hardware for the measurement of carbon-14 in biological studies. The system was characterized using carbon-14 elevated glucose samples and returned a linear response up to 387 times contemporary carbon-14 concentrations. Carbon-14 free and contemporary carbon-14 samples with varying carbon-13 concentrations were used to assess the method detection limit of approximately one-third contemporary carbon-14 levels. Sources of inaccuracies are presented and discussed, and the capability to measure carbon-14 in biological samples is demonstrated by comparing pharmacokinetics from carbon-14 dosed guinea pigs analyzed by both CRDS and accelerator mass spectrometry. The CRDS approach presented affords easy access to powerful carbon-14 tracer techniques that can characterize complex biochemical systems.

TOC image

Introduction

The scarcity of carbon-14 makes it an ideal biochemical tracer in carbon rich organic systems. Historically, radioisotopes have been used in large amounts and quantified with liquid scintillation counting^{1,2}. More recently, the exceptional sensitivity of accelerator mass spectrometry (AMS) has been used to trace small doses of nutrients, toxins, and therapeutics

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Contributions A.D.M purified the samples, made the CRDS carbon-14 measurements, and wrote the manuscript. T.J.O made the AMS carbon-14 measurements. A.D.M, T.J.O., and G.B. analyzed the data. All authors contributed to editing the final manuscript.

Competing Interests The authors declare that they have no competing financial interests.

in humans and animals^{3–10}. This technique opened new regimes of experimentation such as human phase-0 drug trials using subtherapeutic doses¹¹. However, AMS's complexity and cost have limited the proliferation of the measurement method and its derivative techniques¹².

For tracer studies in biochemical systems, carbon-14 concentrations must be accurately measured at and above the natural abundance ($\sim 1.2 \cdot 10^{-12} \text{ }^{14}\text{C}/\text{C}$). The sensitivity requirements of the measurement are dependent on dose, pharmacokinetics, and the goals of the biology experiment. For a study with moderately labeled resultant samples (approximately ten times the natural abundance for median labeled samples), correctly identifying samples labeled with carbon-14 requires a minimum detection limit of one-third the natural abundance, and accurately measuring elevated sample outliers requires a large dynamic range (at least 500 times the natural abundance). For these bio-requirements, AMS's ultimate detection limits are excessive and its complexity is unwarranted.

For these reasons, there have been several efforts to develop alternative means of carbon-14 detection^{13–17}. Cavity ring-down spectroscopy (CRDS) has emerged as a laser-based method capable of carbon-14 measurements. The most sensitive of these systems utilized laser locking to the ring-down cavity and a saturated-absorption cavity ring-down (SCAR) spectroscopic technique. This system had a minimum detection limit 60 times below what is necessary for basic biological studies¹⁸. This spectroscopic method is ideally suited to measure trace isotopes such as carbon-14, but laser-locking and saturation of the $^{14}\text{C}^{16}\text{O}_2$ target spectroscopic transition require complex laser systems that inhibit utilization by scientist and technicians without the necessary measurement expertise.

This article will present the results from a relatively simple cavity ring-down spectrometer capable of measuring carbon-14 concentrations relevant to samples originating from biological studies. This system has the sensitivity necessary to resolve carbon-14 concentration below the natural abundance and is capable of measuring and compensating for the interfering species typical of a processed biological sample.

Instrumentation

A carbon-14 spectrometer was developed that balances complexity and sensitivity for carbon-14 measurements in biological systems. It uses robust, mature hardware suitable for turnkey operation. Figure 1 shows a schematic of the experimental setup. The major components include a quantum cascade laser (QCL), high-finesse optical cavity, mid-IR detector, and a cascade refrigeration system. All of these items were sourced from commercial vendors. The data acquisition and other CRDS specific hardware are from Picarro Inc., a commercial vendor of CRDS systems. For this application, many modifications were made to the measurement and control software, but these changes do not inhibit broad utilization¹⁹. Further details can be found in the Supporting Information.

Experimental Section

Solid and liquid samples must be homogenized, dried, and then combusted to carbon dioxide for both AMS and CRDS. For traditional AMS, the carbon dioxide is then reduced to graphite prior to pressing into individual sample holders²⁰. This suite of sample preparation steps is the rate limiting process for AMS sample measurement. The CRDS system directly measures the carbon-14 content of the combusted carbon dioxide, and a biological sample can be prepared for measurement in minutes. For this study, duplicate samples were processed identically through the combustion step. AMS samples were then graphitized while CRDS carbon dioxide was purified with cryo-focusing. Carbon dioxide was passed through a dry-ice isopropanol trap to remove water and then frozen onto a liquid-nitrogen cold-finger. At this point, the remaining non-condensables, such as carbon monoxide, were evacuated. Some interfering species, such as carbon dioxide isotopologues and nitrous oxide, cannot be readily removed from the sample, and these interferers must be measured and compensated for spectroscopically.

The spectroscopic region surrounding the asymmetric-stretch band of $^{14}\text{C}^{16}\text{O}_2$ is dominated by interference from carbon dioxide isotopologues and other interfering species. Figure 2 depicts the 4 regions near the $^{14}\text{C}^{16}\text{O}_2$ transition used to make the carbon-14 measurement. The spectra is of purified carbon dioxide combusted from a glucose sample with 50 times contemporary carbon-14 levels. Panels are alphabetized by measurement order. The spectroscopic region in Panel A provides a metric of the sample's $^{13}\text{C}^{16}\text{O}_2$ concentration. A group of the least-temperature-dependent, $^{13}\text{C}^{16}\text{O}_2$ -isolated transitions were selected for measurement. The small isotopic shift and relative abundance of $^{13}\text{C}^{16}\text{O}_2$ causes this region to be dominated by higher-order and hot-band ro-vibronic transitions. The $^{13}\text{C}^{16}\text{O}_2$ concentration should be measured first or simultaneously as these transitions interfere with many of the subsequent measurements. The capability to perform CRDS measurements at low-temperatures is the most significant improvement to the experimental setup with all measurements presented here conducted near 250K. This reduces both total interference and temperature sensitivity of nearby ro-vibronic transitions. Panel B measures the $^{14}\text{N}_2^{16}\text{O}$ P(17) transition of the ν_3 asymmetric-stretching band. Panel C was used for two purposes. This region measures the $^{12}\text{C}^{16}\text{O}_2$ concentration of the sample, but the large $^{16}\text{O}^{13}\text{C}^{18}\text{O}$ and $^{12}\text{C}^{16}\text{O}_2$ peaks are also used as a wavelength reference during the measurement of the $^{14}\text{C}^{16}\text{O}_2$ transition. Panel D shows the spectrum around the $^{14}\text{C}^{16}\text{O}_2$ P(20) transition. The glucose sample with the highest $^{14}\text{N}_2^{16}\text{O}$ concentration was selected to highlight the interference in Panel D.

The cavity was maintained at fixed lengths and ring-downs were acquired at intervals of the cavity's free spectral range (FSR). Panel A uses 4 cavity lengths, while Panels B, C, and D use 2 cavity lengths. The use of two cavity lengths was selected to measure the $^{14}\text{C}^{16}\text{O}_2$ peak and the strong interference from the $^{13}\text{C}^{16}\text{O}_2$ grouping near 2209.116 cm^{-1} . Cavity lengths for the schemes of all Panels are stabilized using the peak wings in Panels A, B, and C. These regions have large changes in loss and can be used as wavenumber discriminators. Further details of this method can be found in McCartt et al¹⁹.

Each of the panels in Figure 2 represent a single measurement scheme lasting approximately 20 seconds. Each measurement scheme is comprised of 3000 ring-downs with priority given to peaks of spectroscopic interest. Measurement schemes are repeated 6 times for Panels A and B, 8 times for Panel C, and at least 25 times for Panel D. This represent a minimum total measurement time of approximately 15 minutes.

Additional details regarding sample preparation and spectrometer operation can be found in the Supporting Information.

Results and Discussion

Using the spectroscopy outlined above, we were able to accurately measure carbon-14 concentrations below contemporary levels. Figure 3 presents the comparison of CRDS and AMS measurements, and concentrations are calculated and presented in units of $F^{14}C$,

which is defined as a $^{14}C/C$ isotope ratio of $1.176 \cdot 10^{-12}$ (approximately the natural isotopic abundance)²¹. The measurements were calibrated with petrogenic (i.e., ^{14}C -free) and a 50 $F^{14}C$ glucose samples, and the linear response of the spectrometer was validated against AMS measurements up to 387 $F^{14}C$. Linearity is maintained despite variations in interferers such as $^{14}N_2^{16}O$. Approximately 700 $F^{14}C$ (measured by liquid scintillation counting) and petrogenic samples were used to measure sample-to-sample carryover (i.e., memory). The observed carryover was near the detection limit giving a ratio of better than 1 in 5000.

There are several methods to assess the sensitivity of the carbon-14 spectrometer which differ in their ability to incorporate error sources. For samples with less than 10 $F^{14}C$, the spectrometer has a precision of .225 $F^{14}C$ ($1 - \sigma$) for a 20-second measurement scheme. Two-sample confidence interval analysis gives an average limit of detection (LOD) of .14 $F^{14}C$ (one sided, $3 - \sigma$), but this does not address drift or other measurement inaccuracies. Figure 4 plots the sliding Allan deviation of petrogenic and corn-derived samples²²⁻²⁴. The minimum of the Allan deviation is proportional to the measurement error ($1 - \sigma$) for carbon-14. The averaged Allan deviation gives a LOD of .207 $F^{14}C$. While the Allan deviation captures drift during the measurement, it does not account for sample-to-sample drift or inaccuracies resulting from interfering species. Analysis of 12 “blank” petrogenic samples results in a standard deviation of .083 $F^{14}C$. This corresponds to a LOD of .273 $F^{14}C$, but again does not account for inaccuracies arising from varying concentrations of interfering species for different sample types. Finally, the residuals from Figure 3 have a standard deviation of .112 for the petrogenic, corn-derived, and glucose samples near the detection limit with concentrations less than 2 $F^{14}C$. This gives a method detection limit of .367 $F^{14}C$. Analysis of the residuals captures both drift and variations in the interfering species matrix and gives the best estimation of the complete error budget.

When attempting to measure trace isotopes or gases in the densely-populated, fingerprint region of the mid-IR, there will inevitably be interference from other species. Correctly identifying, measuring, and compensating for these interferers is critical to the accuracy of the spectrometer. As is evident in Figure 2 Panel D, loss at the $^{14}C^{16}O_2$ P(20) transition

location is dominated by interference from other species. A database of instrument conditions and spectrometer output for all measurements was collected and analyzed for correlation. This was used to refine the spectroscopic model and mitigate any experimental conditions that negatively affected the carbon-14 sensitivity. Fluctuations in loss interference due to changes in temperature and concentration of $^{14}\text{N}_2^{16}\text{O}$ and $^{13}\text{C}^{16}\text{O}_2$ make them species of particular concern. Figure 5 plots the $^{14}\text{N}_2^{16}\text{O}$ and $^{13}\text{C}^{16}\text{O}_2$ concentrations for all samples included in this study as measured by CRDS.

Interference from elevated levels of $^{14}\text{N}_2^{16}\text{O}$ decreases the precision of the $^{14}\text{C}^{16}\text{O}_2$ measurement, but a spectroscopic model can compensate for this interference and accurately determine $^{14}\text{C}^{16}\text{O}_2$ concentrations. During sample preparation, combustion, purification, and transport to the cavity, nitrous oxide from the environment can enter the sample preparation stream. While nitrous oxide can be produced during combustion, this source is relatively small for the experimental setup. Differences in sample preparation procedure and day-to-day variability resulted in $^{14}\text{N}_2^{16}\text{O}$ measurement concentrations ranging from less than 1 ppb to 57 ppb as is seen in Figure 5.

While the proportion of nitrous oxide originating from the atmosphere is unknown, it is likely that some amount of contemporary carbon dioxide is leaking into the system from the environment and distorting the carbon-14 measurement. In the worst-case scenario, attributing the entire 57 ppb $^{14}\text{N}_2^{16}\text{O}$ concentration to the atmosphere, the sample would be diluted with approximately 70 ppm of atmospheric carbon dioxide²⁵. Even for this extreme scenario, the resultant F^{14}C error is 2 orders of magnitude below our detection limit.

The spectrometer also measures and compensates for differences in carbon-13 concentrations. Variations in $\delta^{13}\text{C}$ values seen in Figure 5 are primarily the result of preferential photosynthetic fixation of $^{12}\text{CO}_2$ by terrestrial plants²⁶. The averaged $\delta^{13}\text{C}$ values presented in Figure 5 are representative of the different sample types. However, the temperature sensitivity of the overlapping $^{13}\text{C}^{16}\text{O}_2$ measurement transitions causes the statistical variability of the observed values. While the concentration uncertainty of the $^{13}\text{C}^{16}\text{O}_2$ measurement is somewhat high, the spectrometer directly measures and accurately compensates for $^{13}\text{C}^{16}\text{O}_2$ loss interference at the $^{14}\text{C}^{16}\text{O}_2$ transition location.

The conditions in the cavity ring-down experiment can result in saturation of the $^{14}\text{C}^{16}\text{O}_2$ P(20) transition. This saturation must be compensated for when assessing the carbon-14 concentration. Figure 6 shows the effective strength of the $^{14}\text{C}^{16}\text{O}_2$ P(20) transition over a range of pressures. The data presented is from a glucose sample containing approximately 387 F^{14}C . Because saturation is dependent on pressure, cavity laser power, and to some degree, temperature, sample to sample variation can cause inaccuracies. For this reason, saturation and any other inaccuracies that may arise are compensated for by two carbon-14 isotopic standards. We calibrated daily with a petrogenic sample devoid of carbon-14 and a 50 F^{14}C sample.

Samples from a pharmacokinetic case study using guinea pigs as the model organism were measured with CRDS and compared to measurements made with AMS on duplicate

samples. Guinea pigs were dosed intravenously with 100 mg/kg of an oxime acetyltransferase reactivator with tissue and plasma samples taken in a geometric time series. Figure 7 plots the carbon-14 labeled oxime concentrations measured by both methods in guinea pig liver and plasma samples. The presented oxime results correspond to carbon-14 concentrations ranging from 1 to $64 \text{ F}^{14}\text{C}$. The measurements of carbon-14 in the guinea pig samples by CRDS accurately reproduced the AMS results ($R^2 = .99739$). Pharmacokinetic parameters have been calculated for both AMS and CRDS concentration curves and presented for comparison.

After a careful study of the spectroscopic regions surrounding the $^{14}\text{C}^{16}\text{O}_2 \text{ P}(20)$ transition and characterization of the saturation, a model was developed to accurately interpret the measured spectra. Calibration with glucose samples returned a linear response to carbon-14 concentrations. The sub-contemporary carbon-14 sensitivity of the instrument can perform pharmacokinetic studies, and the guinea pig CRDS case study accurately reproduced the AMS results. This was achieved despite interference from nitrous oxide, carbon dioxide isotopologues, and other sources of inaccuracies. All of this was accomplished with mature, robust hardware, and zero day-to-day adjustments to the optical system.

Sample size is dictated by cavity volume and measurement pressure. For the current instrument, a minimum of 3 mg of carbon is needed for a discrete sample measurement. This could be reduced by a factor of 4 with a new experimental setup that utilizes lower pressures and reduces cavity dead space. Moreover, with increased CRDS sensitivity, samples could be diluted with a carrier gas enabling further reduction of sample size.

Conclusions

Simple CRDS instruments could enable the proliferation of carbon-14 tracer techniques to aid the biomedical community. In the past, low-level carbon-14 biological studies were ultimately processed by AMS facilities. While AMS systems can detect extremely low concentrations of carbon-14, they are expensive, large, and complex, and the production of graphite is time consuming. Our results suggest a tabletop-sized CRDS system can now conduct most biological studies previously performed with AMS. Indeed, the availability of simple and inexpensive carbon-14 labeling technologies in labs and hospitals could be transformative. High-throughput sequencing has produced a plethora of information for the biological sciences. Proteomic hypotheses derived from this genomic information must eventually be tested, and computational models of complex biological systems will need to be constrained by physical experiments. Carbon-14 labeling is a proven method that elegantly and quantitatively traces target species through biochemical systems, and it is highly versatile as almost all organic compounds can be labeled with carbon-14. Furthermore, micro-tracer techniques in humans have the potential to greatly reduce drug development cost, aid in the risk assessment of toxins in diets and environments, and expedite the realization of innovative health care practices such as personalized medicine. Carbon-14 detection with CRDS can provide access to this simple yet powerful technique for tracking organic compounds through biochemical systems.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors wish to acknowledge Heather Enright for the guinea pig animal work, Ed Kuhn for biological sample preparation, Mike Malfatti for pharmacokinetic calculations, and Kurt Haack for carbon dioxide sample preparation. We would also like to thank Picarro for their contribution to this work. This work was performed at LLNL under the auspices of the U.S. Department of Energy under contract DE-AC52-07NA27344, under grant R21GM111242 and at the Research Resource for Biomedical AMS, Biomedical Technology Research Resources (BTRR) under grant 8P41GM103483, which are both supported by the National Institutes of Health (NIH), National Institute of General Medical Sciences (NIGMS).

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Figure 1.
 ^{14}C CRDS Instrument Schematic. The schematic shows the optical system layout and the freezer, which houses the high-finesse optical cavity. For more details please see the Supporting Information.

Figure 2.

$^{14}\text{CO}_2$ measurement spectra. Panels A, B, C, and D represent the 4 regions near the $^{14}\text{C}^{16}\text{O}_2$ P(20) transition used during the carbon-14 measurement. Panel A shows a set of relatively temperature independent $^{13}\text{C}^{16}\text{O}_2$ transitions used for one metric of the carbon-13 concentration in the sample. Panel B shows a spectrum containing a $^{14}\text{N}_2^{16}\text{O}$ transition. Panel C measures the $^{12}\text{C}^{16}\text{O}_2$, $^{16}\text{O}^{13}\text{C}^{18}\text{O}$, and $^{16}\text{O}^{13}\text{C}^{17}\text{O}$ concentrations. Spectra from this region are also used as a wavelength reference during the measurement of Panel D. Panel D shows the $^{14}\text{C}^{16}\text{O}_2$ P(20) transition. Spectra are of carbon dioxide combusted from a glucose sample with 50 times the contemporary carbon-14 abundance. The glucose sample with the largest $^{14}\text{N}_2^{16}\text{O}$ concentration measured is presented to emphasize the locations of interference. Measurements were conducted at 20 torr and 254 K. Colors represent the loss contribution of a particular species. Black data points represent the average ring-down measurement value at a given wavelength for a single ~20 second scheme. Error bars representing the 2- σ confidence interval are present for all data points shown, but only visible in Panel D.

Figure 3. CRDS versus AMS. Comparison of $F^{14}C$ for petrogenic, corn-derived, and glucose (contemporary end elevated) samples measured by CRDS and AMS. Error bars represent the $2 - \sigma$ confidence interval calculated from 4 measurement campaigns, but in most cases are smaller than the data points.

Figure 4. $F^{14}C$ Allan deviation. Grey lines represent the sliding Allan deviation for an individual sample measurement. While the blue line is the average of all the individual sliding Allan deviations. The averaged data reaches a minimum at 794 seconds and .063 $F^{14}C$ (effective). Data was calculated from petrogenic and corn-derived samples.

Figure 5. $^{14}\text{C}^{16}\text{O}_2$ interfering species. Interfering concentrations are presented as measured by CRDS. The x-axis labels represent the source of the carbon dioxide. Liver and plasma samples are from a guinea pig animal study. The left axis and blue points represent the $^{13}\text{C}^{16}\text{O}_2$ concentration. Values are presented in delta notation with respect to Pee Dee Belemnite. Points represent the average and 2- σ confidence interval for all measurements of a particular sample type. The right axis and red points are $^{14}\text{N}_2^{16}\text{O}$ concentrations presented in parts per billion (ppb). These points represent single sample measurements. Error bars are plotted for the $^{14}\text{N}_2^{16}\text{O}$ data but are smaller than the data point for most measurements.

Figure 6. $^{14}\text{C}^{16}\text{O}_2$ P(20) transition saturation. Fractional strength of the $^{14}\text{C}^{16}\text{O}_2$ P(20) transition is plotted versus measurement pressure. The effective transition strength decreases with the collisional relaxation rate of the higher order state. Error bars are present but in most cases are smaller than the data points. Measurement temperature was 251K.

Figure 7.

Pharmacokinetic Curves. Carbon-14 concentrations for samples from a guinea pig, animal study were measured by AMS (blue squares) and CRDS (red circles). Data is presented in nanograms of oxime xenobiotic per milligram of sample. Pharmacokinetic parameters $T_{1/2}$ dist. (distribution half-life), $T_{1/2}$ elim. (elimination half-life), C_{int} (initial concentration), AUC_{0-t} (area under the curve during the experiment), AUC_{inf} (single-dose area under the curve), V_d (Volume of distribution), and Cl (Clearance) were calculated for all of the curves and are presented for comparison. Error bars represent $1 - \sigma$ measurement error for a single sample. CRDS error values originate from measured shot-to-shot noise for a single carbon dioxide sample, and AMS error values are attributed to the larger of counting statistics or variation on measurements for a single graphite target.