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Intracavity Optogalvanic Spectroscopy. An Analytical Technique for ^{14}C Analysis with Subattomole Sensitivity

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We show a new ultrasensitive laser-based analytical technique, intracavity optogalvanic spectroscopy, allowing extremely high sensitivity for detection of ^{14}C -labeled carbon dioxide. Capable of replacing large accelerator mass spectrometers, the technique quantifies attomoles of ^{14}C in submicrogram samples. Based on the specificity of narrow laser resonances coupled with the sensitivity provided by standing waves in an optical cavity and detection via impedance variations, limits of detection near 10^{-15} $^{14}\text{C}/^{12}\text{C}$ ratios are obtained. Using a 15-W $^{14}\text{CO}_2$ laser, a linear calibration with samples from 10^{-15} to $>1.5 \times 10^{-12}$ in $^{14}\text{C}/^{12}\text{C}$ ratios, as determined by accelerator mass spectrometry, is demonstrated. Possible applications include microdosing studies in drug development, individualized subtherapeutic tests of drug metabolism, carbon dating and real time monitoring of atmospheric radiocarbon. The method can also be applied to detection of other trace entities.

Carbon 14 (radiocarbon) is an ideal organic tracer having an extremely low natural abundance in living systems, near 1 ppt, and a long half-life, 5730 years, ideal for clinical and laboratory tracer experiments. Until recently, almost all quantitation of ^{14}C content has been by scintillation detection of the low-energy β particle emitted in its decay. At present there is great interest in the drug development community for pharmacokinetic information on new drug entities using nontherapeutic microdoses of labeled drugs, which require much higher analysis sensitivity than is possible with scintillation counting.¹ Accelerator mass spectrometry (AMS), first developed to extend carbon dating to smaller and older samples, has become the standard method for ^{14}C atom counting in such sensitive bioanalytical tracer studies.^{2,3} Limitations to wide use of AMS for bioanalytical studies include size, cost, and complexity of the analysis system as well as the fact that samples must contain of the order of 0.5 mg of total carbon. The laser-assisted ratio analyzer (LARA) technique⁴ is based on the existence of large isotope shifts in molecular spectra, the use of fixed frequency isotopic lasers, and sensitive detection via the laser optogalvanic effect (OGE). Research and development during

the past decade has demonstrated that LARA's sensitivity and versatility make new classes of isotope ratio measurement systems possible.^{5,6}

The basic concept of OGE detection has long been used in atomic and molecular spectroscopy.⁷ It is based on the electrical response of a gas discharge to an optical perturbation. If a laser of intensity I , and frequency ν is incident on a cylindrical (length L and radius R) weak electrical discharge, the electrical response, S , of the discharge can be expressed by an integral over the laser–discharge interaction volume:

$$S = \int_0^L dz \int_0^R r dr \int_0^{2\pi} d\theta [n(r, \theta) I(r, \theta, z, \nu) \sigma(\nu) K] \quad (1)$$

The density of interacting species is n and $\sigma(\nu)$ refers to the laser–species interaction cross section. S can be any discharge parameter related to conductivity and K is a corresponding optogalvanic proportionality constant that depends on the details of the electrical discharge. To a good approximation, on resonance, for cases where light absorption is small and K is independent of n , the average electrical response simplifies to a product:

$$S = n L I A \sigma K \quad (2)$$

Here, n represents the average molecular density of interacting particles, L , the length of the interaction region, I the average laser intensity and A the average area of the laser beam. Physically, what happens is that the light changes the equilibrium distribution of species, including excited species in the discharge. This affects collision rates, including those between molecules and electrons, leading to a measurable conductivity change, S , of the ionized gas. Optogalvanic spectroscopy for isotopic analysis makes use of the fact that laser resonances in CO_2 are isotope dependent.⁸

In practice, a periodically switched cw laser in resonance with a specific molecular transition changes the population of excited molecular states, which, in turn, changes the electron density,

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temperature, or both in the discharge, giving rise to an impedance change varying with the laser intensity. There exist several significant advantages of OGE measurements compared to ion beam and other spectrometric stable isotope measurement technologies: (1) The measured parameter is electrical and periodic so that filtering and averaging techniques can be used to achieve extremely low noise and background. (2) No optical measurement is required, thus eliminating the need for collection and dispersion optics and light transducers. (3) The measured signal is proportional to the product of analyte density, path length, and laser power. In contrast with laser absorption techniques or ion counting, the laser power provides a gain, or sensitivity enhancement per molecule, so that signals for dilute isotopes can be amplified relative to the majority species. (4) The measurement system can be varied, within limits, in order to enhance signal to background ratios by controlling gas mixtures (ranging from 100 to 1% or less CO₂, balance N₂, CO₂ free air, or other inert buffer) pressure (typically 1–5 mbar) and electrical discharge power (typically 2–5 W).

Previous success using LARA for breath testing^{9–15} and environmental monitoring¹⁶ has led to the development of the technique for the detection of ¹⁴C reported here. When the laser operates at a particular transition in ¹⁴CO₂, any resonant change in the impedance of the discharge system is due to the presence of ¹⁴CO₂ in the discharge. The laser transition chosen for ¹⁴CO₂ must be well separated in wavelength from any transition in ¹²CO₂ or ¹³CO₂ (1% natural abundance) and from any transition in a CO₂ molecule with ¹⁸O (0.02% natural abundance) or ¹⁷O (0.004% natural abundance). The discreteness of energy levels and the large isotope shifts in vibrational–rotational transitions allow a line-specific ¹⁴CO₂ laser to be built. The strongest lasing transitions observed in ¹⁴CO₂ are at 11.8 and 11.3 μm, significantly longer in wavelength than lasing transitions of the other “stable” isotope CO₂ lasers. Any ¹⁴CO₂ molecule in the lower or upper laser level is automatically in resonance (absorption or stimulated emission) with the narrow band laser, providing the sharp specificity required for isotope ratio analysis. The nearest ¹³CO₂ and ¹²CO₂ lines are separated by more than 500 line widths, leading to nonresonant cross sections reduced by ~10 orders of magnitude. The nonresonant interaction is further reduced for ¹³CO₂ and ¹²CO₂ by rotational state Boltzmann population ratios for the two isotopes. In any case, this small background effect is pressure dependent and measurable and does not seriously limit the ultimate ¹⁴CO₂ detection limit.

Extension of the LARA technique to ¹⁴CO₂ was previously established with highly enriched (nanocurie) ¹⁴C samples.¹⁷ In those studies, an isotope ratio (¹⁴C/¹²C) sensitivity of order 10^{–9} was demonstrated with a very simple external cavity configuration. A ¹⁴CO₂ laser at 11.8 μm with a maximum output power of 2 W was used. The measurement yielded signal-to-noise ratios of several hundred with short averaging times and a sensitivity limit of ~1 pmol of ¹⁴CO₂. Measurements with nonenriched carbon dioxide confirmed that no memory effect was present and that the system sensitivity was far too low for detection at modern (1.180 × 10^{–12} ¹⁴C/C) levels of ¹⁴CO₂.

Enhancement of sensitivity in laser absorption experiments often involves increased absorption length in optical cavities—most notably cavity ringdown spectroscopy¹⁸ and laser intracavity absorption spectroscopy (ICAS).¹⁹ The new standing wave technique reported here is analogous to, but with key differences from, ICAS. The most significant difference from ICAS is that detection is via the OGE rather than optical. The analyte in this case is the same as the lasing species, and an OGE exists for stimulated emission or absorption.

EXPERIMENTAL SECTION

The layout of the measurement system is shown in Figure 1. The sample cell is placed inside a laser cavity (LaserTech Group Inc. model LTG250, 15-W output with sealed ¹⁴CO₂ fill) thus subjecting it to the full saturated laser power. Also inside the laser cavity is a computer-controlled shutter used for modulating the ¹⁴CO₂ laser beam. The laser output beam can be used to monitor power and wavelength as well as for parallel external cell measurements if required. A small electronically modulated ¹²CO₂ laser (Synrad model 48-1, 5-W output) beam passes through the ¹⁴CO₂ laser output coupler and the sample cell for single-pass ¹²CO₂ measurement. This ¹²CO₂ signal is used for normalization. The quartz sample cell, 10 cm in length and 2.5-cm o.d. with ZnSe Brewster angle windows, is pumped with an oil-free vacuum pump to low pressure. In these studies, a radio frequency (rf) glow discharge is utilized because of its stability and low inherent noise.²⁰ With sample pressure held constant—typically between 1 and 5 mbar, a low-power (2–5 W) rf discharge is ignited and maintained via external copper electrodes. The oscillator power supply circuit also monitors the average rf voltage amplitude across the discharge for the OGE signal. The ¹²CO₂ and ¹⁴CO₂ laser beams simultaneously passing through the sample cell are chopped at different frequencies, and the resulting voltage variation across the cell is measured. The Fourier transform values of the voltage signal at the two different frequencies yield the ¹²CO₂ and ¹⁴CO₂ OGE signals.

The sample consists of CO₂ with a buffer (95–99%) of nitrogen or CO₂-free air, or 100% CO₂. Reference samples consist of tanks of research grade CO₂ (Air Products) with ¹⁴CO₂ less than 10^{–12} (1 modern) level as determined by AMS measurements at Lawrence Livermore National Laboratory (LLNL). Aliquots of CO₂

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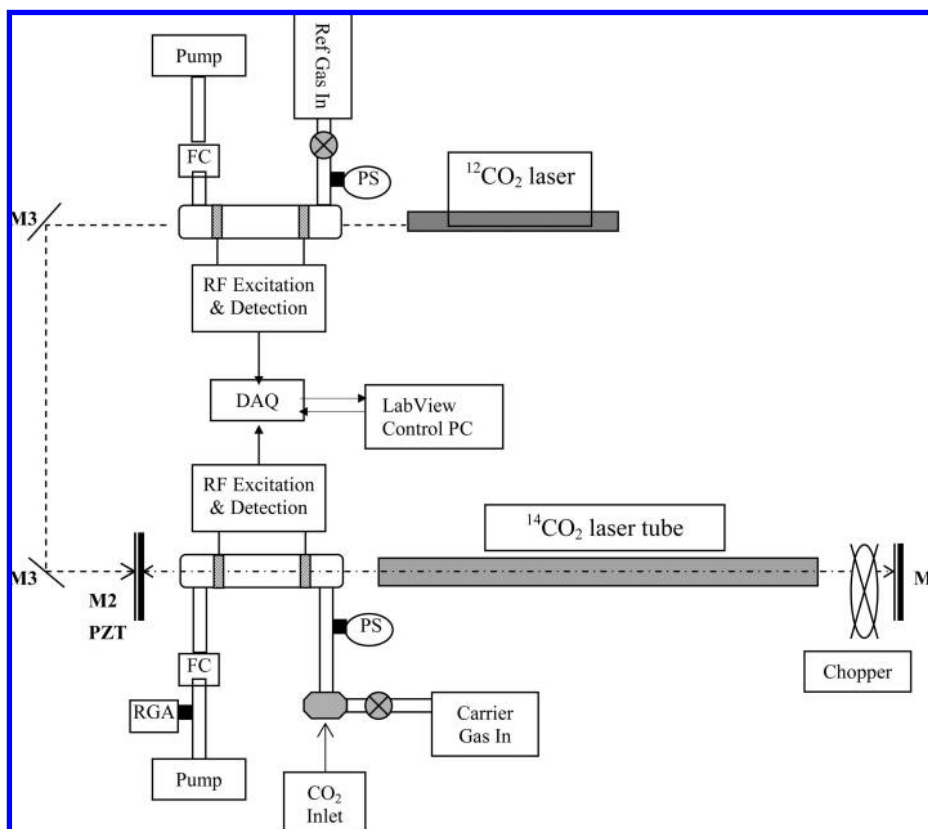


Figure 1. Experimental configuration. The OGE cell inside the cavity has Brewster windows to reduce losses. The C12 laser incident on the OGE cell provides a “C12 signal” that is used for normalization of the C14 signal. The shutter inside the laser cavity is for modulating the $^{14}\text{CO}_2$ laser. M1, high reflective mirror and grating; M2, 85% reflective output coupler; M3, gold plate mirror; PS, pressure sensor; FC, flow controller; RGA, residual gas analyzer; DAQ, data acquisition board.

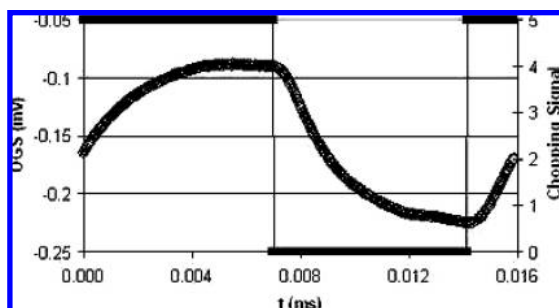


Figure 2. OGE signal in response to a laser modulated at 63 Hz. The sample is 5% CO_2 in N_2 at 10^{-11} ^{14}C enrichment. The waveform is a 5-s coherent averaged waveform.

with varying $^{14}\text{CO}_2$ concentrations were also supplied by LLNL. Measurements could be run in a sealed cell (batch mode) or continuous-flow mode with flow rates of 0.1–2 sccm.

Time-averaged OGE signals were obtained using a National Instruments Data Acquisition System. The virtual instrument designed controlled the shutter, and monitored pressure, $^{12}\text{CO}_2$ concentration, and laser wavelength data. An OGE output from the $^{14}\text{CO}_2$ laser tube was used to stabilize laser wavelength and for laser power normalization.

RESULTS AND DISCUSSION

Figure 2 shows the OGE response to an enriched sample (9 M) of 22 μmol of CO_2 containing 223 amol of $^{14}\text{CO}_2$. The figure is a 5-s coherent averaged waveform with the $^{14}\text{CO}_2$ laser chopped

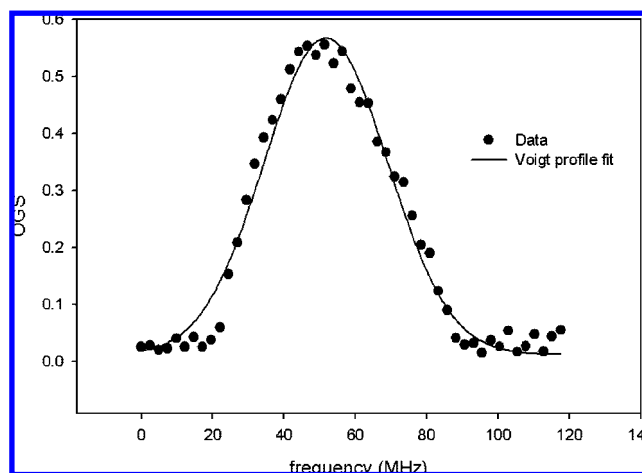


Figure 3. Resonance curve for intracavity optogalvanic effect. The solid line is a best-fit Voigt profile. The width, 48 MHz, is expected for $^{14}\text{CO}_2$ in the 5 mbar discharge at 310 K.

at 63 Hz. Figure 3 shows a resonance curve obtained by tuning the laser through its gain profile. The optogalvanic effect line shape shows the Voigt profile expected for the discharge conditions. The nonresonant background (and the peak intensity) varies with different laser lines, also as expected. Under all conditions, the observed signal is indicative of a greatly enhanced interaction length due to the intracavity standing wave. The maximum enhancement observed over the single-pass system is of order

10 million, which was initially surprising, as in ICAS studies with a single-mode laser gain enhancements only of order 100 are expected.

We believe that the observed mega-enhancement is due to the nature of the optogalvanic effect in the optical cavity. In eqs 1 and 2 above, I is the circulating internal laser power, ~ 50 W in our case, and L the interaction length is now greatly enhanced due to the standing wave. A simple estimate would have L enhanced only by the number of reflections of a single photon, where the major loss is due to the output coupler. However, for the cw laser system, loss in the OGE cell is balanced by the gain of the lasing medium keeping the internal circulating power constant. Now, the effective interaction length L_{eff} is given by $L_{\text{eff}} = c\Delta t$, where Δt is given by the time that the laser is on. For a modulation frequency of 63 Hz, Δt is 15.8 ms yielding an effective interaction length of almost 5000 km. This value is close to our observed maximum enhancement of $\sim 10^7$ for the intracavity configuration compared to single pass. In a multimode ICAS experiment, Δt is given by the spectral saturation time and can be as long as several seconds.²¹ In the ICAS experiment, what is observed is a change in laser output. The effect on laser output of the few thousand $^{14}\text{CO}_2$ molecules in the OGE analysis cell is negligible compared to the 10^{19} active $^{14}\text{CO}_2$ molecules in the laser cavity. However, all photon interactions in the intracavity cell contribute to the OGE.

The sensitivity is so great that the natural abundance of ^{14}C in ambient air can easily be detected. An estimate of the present limit of detection is of the order $10^{-15} \text{ }^{14}\text{C}/^{12}\text{C}$, similar to AMS. It is known that nitrogen is an ideal buffer gas for the OGE in CO_2 for much the same reason that nitrogen is used in the CO_2 laser. However, unlike the situation in a laser, where helium is also present to depopulate lower levels, helium is found to reduce the OGE.²² Further, we have found that air is a better buffer gas than pure nitrogen, as it inhibits CO_2 dissociation. The optogalvanic proportionality factor for $^{14}\text{CO}_2$ in eqs 1 and 2 is a complicated function of partial pressure for all gas constituents as well as discharge conditions,²³ even changing sign as the system goes from absorption to gain conditions. Also, eqs 1 and 2 assume thermal equilibrium, a situation only easily achieved in tens of milliseconds for low $^{14}\text{CO}_2$ concentration. These effects make calibration of the system over large changes in $^{14}\text{CO}_2$ concentration difficult. For radiocarbon dating of small samples and drug development studies near modern enrichment, a linear calibration region over more than 1 order of magnitude has been found using pure CO_2 as the carrier gas, at approximately ~ 1 mbar pressure with a Δt near 25 ms. Figure 4 shows the calibration from 4.9×10^{-3} modern to 1.5 modern samples with the OGE signal plotted against AMS results. A 12 modern sample showed saturation effects under the same measurement conditions. The slope of the calibration curve, 0.4, is in agreement with calculations due to the nonresonant signal from ^{13}C and ^{12}C discussed above. A detailed model of the intracavity OGE must include laser saturation effects as well as equilibration time constant effects on the OGE parameter K .

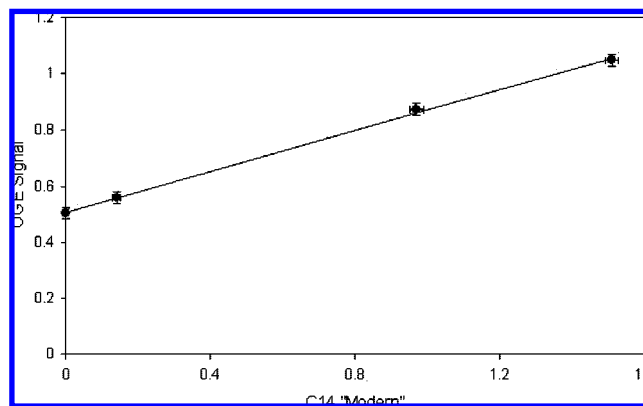


Figure 4. Calibration curve. OGE signals obtained with the ICOGS system plotted against the AMS results. The vertical error bars are determined by voltage averaging statistics.

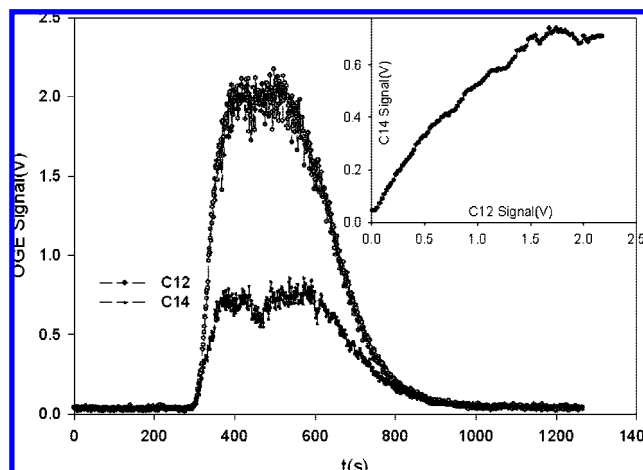


Figure 5. Variation of OGE for ^{12}C (top) and ^{14}C (bottom) as 1 mL is injected into the CO_2 free buffer gas flowing at 10 SCCM. As shown in the inset, the signals scale at low concentrations, but the ^{14}C signal saturates at a much lower concentration than the ^{12}C .

The system can run with continuous flow as shown in figure 5 for 1 mL of CO_2 injected into a gas stream of CO_2 free air as carrier. The buildup and loss of ^{12}C and ^{14}C signal is obvious. The saturation effect present when the concentration reaches $\sim 1\%$ is also obvious. Modeling of this effect using ICAS theory^{18,20} and OGE theory²⁴ is in progress.

CONCLUSION

It is believed that the sensitivity shown in these and certain AMS experiments will allow more rapid drug development by permitting early safe metabolic testing with microdoses of test compounds.²⁵ Further, such microdosing studies can be used to customize doses for individual metabolic differences. Though both ^{14}C LARA and AMS aim to quantify ^{14}C in biological samples, there are several significant differences in the technologies. For both techniques, a carbon-containing compound is first oxidized to CO_2 . The analyte in the LARA technique remains in the form of CO_2 , while AMS machines, at present, require an additional step to reduce the CO_2 sample to elemental carbon. There are significant efforts, however, to enable Bio-AMS machines to run

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effectively with a gas ion source utilizing CO₂.²⁶ Reasons for these efforts include improving efficiency via fewer processing steps, and higher throughput, as well as enabling smaller sample size. Ultimately it is desired to couple an input separation device such as a gas or liquid chromatograph with fractions oxidized to CO₂ and continuously flow through the system.

The ¹²C or ¹³C normalization is straightforward with our system for samples of any enrichment, using ¹²CO₂ and ¹³CO₂ lasers as is now done with existing breath and environmental monitoring instruments. Furthermore, the small sample required and the possibility of continuous flow or batch processing should permit development of combined instrumentation with gas chromatographs or liquid chromatographs coupled to an oxidizer as a front end.^{27,28} The measurement sensitivity is so great that carbon dating using microgram samples can easily be envisioned.

Limits of precision and sensitivity are yet to be determined but should equal or exceed those of AMS. Use of an external

calibration cell and double ratio measurements as is done to determine ¹³C variations at the part per 10 000⁶ level should also be possible with ¹⁴C. The engineering challenge will be in the handling of such small samples and eliminating the ubiquitous background ¹⁴CO₂. We further note that the technique may be extended to other trace atom or compound analysis with tunable or fixed frequency lasers.

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