Uppsala University

This is an accepted version of a paper published in *Analytical Chemistry*. This paper has been peer-reviewed but does not include the final publisher proof-corrections or journal pagination.

Citation for the published paper:

Persson, A., Eilers, G., Ryderfors, L., Mukhtar, E., Possnert, G. et al. (2013)
"Evaluation of Intracavity Optogalvanic Spectroscopy for Radiocarbon Measurements"
Analytical Chemistry, Article ASAP

Access to the published version may require subscription.

Permanent link to this version:

<http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-202646>

<http://uu.diva-portal.org>

Evaluation of intracavity optogalvanic spectroscopy for radiocarbon measurements

Anders Persson[†], Gerriet Eilers[†], Linus Ryderfors[†], Emad Mukhtar[‡], Göran Possnert[†], Mehran Salehpour[†].

[†]Dept. of Physics and Astronomy, Ion physics, Box 516, 751 20, Uppsala, Sweden. [‡]Dept. of Chemistry, Ångström Laboratory, Box 523, SE-751 20, Uppsala, Sweden.

This document is the Accepted Manuscript version of a Published Work that appeared in final form in *Analytical Chemistry*, copyright © American Chemical Society after peer review and technical editing by the publisher. To access the final edited and published work see: <http://pubs.acs.org/doi/abs/10.1021/ac400905n>

ABSTRACT. Ever since the first publication of intracavity optogalvanic spectroscopy (ICOGS) in 2008, this novel technique for measuring the $^{14}\text{C}/^{12}\text{C}$ ratio in carbon dioxide has rendered considerable attention. As a result, there are currently at least five different research groups pursuing research on ICOGS. With a claimed limit of detection of 10^{-15} ($^{14}\text{C}/^{12}\text{C}$), i.e., in the same order as accelerator mass spectroscopy, achieved with a relatively inexpensive and uncomplicated table-top system, ICOGS has major scientific and commercial implications. However, during the past five years, no research group has been able to reproduce these results, or present additional proof for ICOGS's capability of unambiguous ^{14}C detection, including the authors of the original publication. Starting in 2010, our group has set up a state-of-the-art ICOGS laboratory, and has investigated the basic methodology of ICOGS in general and tried to

reproduce the reported experiments in particular. We have not been able to reproduce the reported results concerning the OG signals dependence on ^{14}C concentration and wavelength, and, ultimately, not seen any evidence of the capability of ICOGS to unambiguously detect ^{14}C at all. Instead, we have found indications that the reported results can be products of measurement uncertainties and mistakes. Furthermore, our results strongly indicate that the reported limit of detection is likely to be overestimated by two orders of magnitude, based on the results presented in the original publication. Hence, we conclude that the original reports on ICOGS cannot be confirmed, and therefore must be in error.

INTRODUCTION. Five years ago, an article describing a novel and ultrasensitive method for measuring the radiocarbon ratio ($^{14}\text{C}/^{12}\text{C}$) in carbon dioxide, was published in this journal by Murnick et al¹. The method combined four well established techniques, namely laser assisted ratio analyzer² (LARA), optogalvanic spectroscopy³, optical impedance spectroscopy⁴ and laser intracavity absorption spectroscopy⁵, into what was named intracavity optogalvanic spectroscopy (ICOGS). The method is inexpensive, user-friendly and compact, and the presented results claim that ICOGS had the potential to outperform accelerator mass spectroscopy (AMS), which currently is the predominant technique for radiocarbon measurements, not only in terms of simplicity and cost, but also in sensitivity and limit of detection (LOD).

Ever since the emergence of new applications, particularly within the bio-analytical field, radiocarbon ratio measurements have turned into a fast growing segment within AMS, diversifying its use outside the traditional field of radiocarbon dating. An example of such a new application is microdosing⁶, where a pharmaceutical drug under development is labeled with ^{14}C . The drug is then administered to a human subject in small doses, well below the therapeutic dose, to avoid issues with toxicity and side effects. Employing AMS for analysis of samples of

blood, urine, breath, tissue, biopsies etc., looking for the C^{14} labeled molecules and their metabolites, the distribution, absorption and elimination of the drug in the body can be studied. Microdosing has been shown to reduce the time-to-market of a drug by several years, and is now endorsed by the Food and Drug Administration (FDA) and the European Medical Agency (EMA). Even though applications such as microdosing have the largest commercial implications, other, more fundamental, applications of biological AMS exist, e.g., the study of cell regeneration in the human body by employing the so called bomb peak method⁷. This has accelerated the establishment of AMS laboratories fully dedicated to biomedical radiocarbon measurements. The state of the art AMS facilities have a sensitivity limit of quantification of absolute sample amounts in blood close to 120 zmol⁸ (zeptomole = 10^{-21} mole). However, AMS suffers from some major disadvantages. First and foremost, the complexity and cost of installing, operating and maintaining a particle accelerator are significant. Hence, there has long been a demand for alternative techniques for radiocarbon ratio measurements; however, no other technique has yet accomplished the required sensitivity. Secondly, there exists a lower limit to the size of sample that can be analyzed by AMS. Currently, this limit is in the order of a few micrograms⁹. In many applications, e.g., bomb-peak dating of DNA from less abundant cell types, this limit needs to be lowered, and it is doubtful if this can be accomplished with AMS. Hence, there exist both a commercial and a scientific demand for a replacement of AMS for radiocarbon analysis.

In this context, it is easy to understand why the publication of ICOGS in 2008 rendered considerable attention. In the original publication in *Analytical Chemistry*¹, and in a subsequent publication¹⁰, ICOGS is claimed to have a LOD of 10^{-15} for measuring radiocarbon ratios. It is also claimed to facilitate analysis of small samples, already comparable to what is possible with

AMS¹⁰ (<10 μg), primarily owing to the less complicated sample preparation process, where ICOGS utilize CO_2 gas samples whereas AMS require graphitization of CO_2 ¹¹. Given that this exceptional performance is accomplished with a table-top system, utilizing relatively inexpensive equipment, compared with AMS, would make ICOGS capable of outperforming AMS in almost every aspect.

ICOGS utilizes the large isotopic shifts in the vibrational transition spectrum of molecules. In small molecules, such as CO_2 , the absorption lines are narrow line-width transitions, unambiguously related to the parent molecule. For example $^{14}\text{CO}_2$, has a specific vibrational transition at 11.7677 μm (the P(20) transition between the rotational quantum level $J=19$ in first excited vibrational level of the asymmetric stretch mode, 00^0_1 , and $J=20$ in the first excited vibrational level of the symmetric stretch mode, 10^0_0), separated by hundreds of line-widths from the nearest transition in the $^{12}\text{CO}_2$ or $^{13}\text{CO}_2$ lines, greatly reducing the non-resonant absorption cross section. Moreover, the same transitions are typically utilized in CO_2 lasers. Hence, by employing optogalvanic spectroscopy with single longitudinal mode, isotope specific CO_2 lasers, it is possible to study the isotopic composition of CO_2 with great accuracy¹². This technique, often referred to as LARA, is applicable to measurements of the $^{13}\text{C}/^{12}\text{C}$ ratio in CO_2 , and was the precursor to ICOGS². In LARA, the CO_2 sample, is introduced into a plasma cell, here referred to as the sample cell, where it is partly ionized in an RF plasma. The impedance of the plasma is monitored by measuring the amplitude of the RF wave in the oscillator. The plasma is then illuminated by laser beams from a $^{13}\text{CO}_2$ and a $^{12}\text{CO}_2$ laser, respectively. The laser beams affect the impedance of the plasma via the optogalvanic effect³, and by modulating the two beams at different frequencies using choppers, their effect on the plasma can be deduced unambiguously from the Fourier transform of the measured change in the RF wave amplitude.

The major difference between LARA and ICOGS is the abundance of the detected isotopes – typically $^{13}\text{C}/^{12}\text{C}=1\%$ and $^{14}\text{C}/^{12}\text{C}=10^{-13}$. Hence, moving to ^{14}C dramatically increases the sensitivity requirement even though LARA measure the ratio $^{13}\text{C}/^{12}\text{C}$ ratio with better than per mille resolution. To accommodate this, the sample cell is relocated inside the laser cavity of the $^{14}\text{CO}_2$ laser in ICOGS¹. Somewhat surprisingly, this was shown to increase the sensitivity of the system by a factor of 10^7 . This factor is referred to as the intracavity enhancement factor. The proposed explanation of this enhancement was, in analogy with laser intracavity absorption spectroscopy¹, the increased effective interaction length of the photons inside the laser cavity. Given this analogy, ICOGS with a laser chopped at a frequency f will experience an effective interaction length, L , given by $L=c/(2f)$, where c is the speed of light. For $f=100$ Hz, $L=1500$ km. In the case of, e.g., LARA on the other hand, L is given by the extent of the plasma ($L\sim 3$ cm), meaning that the increase in effective interaction length between ICOGS and LARA is about $5\cdot 10^7$, i.e. in the same order as the observed intracavity enhancement factor. However, there exist some fundamental differences between laser intracavity absorption spectroscopy and ICOGS. For example, laser intracavity absorption spectroscopy typically employ multimode lasers, where the sensitivity enhancement is explained by mode competition in the laser medium, whereas ICOGS use single longitudinal mode lasers, where mode competition does not occur⁵. Moreover, the methods of extracting the signal are fundamentally different, where laser intracavity absorption spectroscopy studies the sample cell indirectly by measuring the change in intensity in the output of the laser due to internal absorption, whereas ICOGS studies the sample cell directly by measuring the effect of the laser on the sample electrically. If one instead regards the study of light, the situation is the opposite. Consequently, the analogy between the enhancements mechanisms in the two techniques is far from obvious.

There are currently five scientific groups pursuing research on ICOGS; Murnick et al at Rutgers NJ, US, the Lenfest Centre for Sustainable Energy at Columbia University NY, US, the company Planetary Emission Management MA, US, the Energy and Sustainability Research Institute Groningen, the Netherlands, and our group at the Department of Physics and Astronomy at Uppsala University, Sweden. Nonetheless, the number of publications on ICOGS since 2008 is very scarce. Apart from the original publication¹, a report in a conference proceeding¹⁰ containing no additional data, and the PhD thesis of one of the authors¹³ containing more elaborate descriptions of the experiments presented in Analytical Chemistry, we know of only two additional conference proceedings reporting on ICOGS measurements^{14, 15}, none of which were able to reproduce the original data. One of the latter reports is our own¹⁴, describing our experimental setup and reporting some initial results.

Starting in 2010, our group has set up a state-of-the-art ICOGS laboratory, and has, on the basis of the available publications and through private communications with the other research groups in general, and extensively with Murnick et al in particular, tried to reproduce the reported experiments. Here, we present the results of our findings.

MATERIALS AND METHODS. The experimental setup in our laboratory has been thoroughly described in a previous publication¹⁴. The schematic of the setup is described in Figure 1. At the heart of the setup is a single longitudinal mode $^{14}\text{CO}_2$ laser, which was custom made by LTG Lasertech Group Inc. (Concord, ON, Canada). The $^{14}\text{CO}_2$ laser had an extended laser cavity to make room for the sample cell and an internal chopper. The wavelength of the laser could be selected by a grating in one end, and tuned by a piezo positioned, flat, 90% reflective output coupler in the other end of the laser cavity. Generally, a laser transition was selected by tuning the grating with micrometer screws, and feedback from a CO_2 Laser Spectrum

Analyzer from Macken Instruments Inc. (Santa Rosa, CA, USA). In this study, the P(20) and P(22) transitions of $^{14}\text{C}^{16}\text{O}_2$ were employed ($\lambda_{\text{P}(20)}=11.7677\text{ }\mu\text{m}$ and $\lambda_{\text{P}(22)}=11.7916\text{ }\mu\text{m}$ ¹⁶). The wavelength was then locked at the centre of the transition, by fine-tuning the length of the laser cavity with the piezo control, with feedback from a reference cell. The reference cell was a sealed LARA sample cell containing 0.5% $^{14}\text{CO}_2$, 4.5% $^{12}\text{CO}_2$ and 95% dry air at a total pressure of 467 Pa. The piezo control could also be used for scanning the wavelength through the laser gain profile, in order to study the spectral distribution of the optogalvanic (OG) signal. The laser tube contained only 0.02 mmol of ^{14}C and was therefore not treated as a radiation hazard.

Figure 1. Schematics of the ICOGS system including optics, electronics and gas handling. Here, S/A denote spectrum analyzer, P/M power meter, M mirror, B/S beam splitter, O/C output coupler, C chopper, D/A differential amplifier, and DAQ data acquisition. Laser beams are indicated by dashed lines and data handling by solid arrows.

Two different measurements modes have been proposed for ICOGS – batch mode, where the sample cell is kept at constant pressure and volume, throughout the measurement, and flow mode, where the sample is continuously flowing through the cell¹³. In our setup, we have chosen to focus on batch mode, since this enables better separation and control of the different parameters important for ICOGS¹⁴. Moreover, experiments could be conducted in two ways where 1.) The output of the laser was tuned and locked to the center of the laser transition with feedback from the reference cell OG signal, or 2.) The laser was scanned through its gain profile, by scanning the piezo control from minimum to maximum actuation. In both kinds of experiments, the intra- and extra-cavity OG signals, and the laser power measured by a pyroelectric and a thermopile power meter, were recorded (see supporting information).

A sample could be introduced into the sample cell via a gas handling system with a base pressure of <0.5 Pa, Figure 1. The sample was introduced either in the form of pure CO₂ or a mix of CO₂ and N₂, after any water content in the gas had been removed in a -80°C cold trap. For the CO₂:N₂ mix, mixing ratios of 1:20 and 1:40 were evaluated, although most of the measurements were performed with a 1:40 mix. This decision was based on private communications with Murnick's group. The initial pressure, here referred to as the operating pressure, in the sample cell was set with a MKS Type 622 capacitance manometer with a four digit MKS PDR2000 digital readout (MKS Instruments Inc., MA, US). In this study, pressures between 15 and 100 Pa were evaluated. The maximum pressure was around 120 Pa, over which the oscillator did not manage to ignite the plasma. Moreover, three different CO₂ samples with varying ¹⁴C concentrations were used in the experiments. The concentrations were 0.29±0.0021 Modern, 3.3±0.062 Modern and 9.7±0.17 Modern, as measured in our in-house AMS facility⁸. 1 Modern corresponds to a ¹⁴C/¹²C ratio of 1.18·10⁻¹².

The measurement procedure and the post-processing of the recorded data are described in detail in the supporting information of this article. Also, the major causes of random variations in the measurements – electromagnetic interference (EMI), $1/f$ noise, and the irreproducibility of the discharge conditions – are elaborated on, and the choice of laser power and modulation frequency in the experiments are thoroughly motivated. It was found that, in repeated measurement with identical initial conditions, a $\pm 30\%$ uncertainty in the mean level of the OG signal could be expected, primarily due to the irreproducibility of the discharge conditions.

EXPERIMENTS. Six months of continuous laboratory work have been directed towards evaluating ICOGS for radiocarbon measurements. Although far from all of the collected results could be included in this study due to scope considerations, all of the collected data was consistent with the presented results, discussions and conclusion. The focus of this study was primarily to attempt to reproduce the results presented in reference 1, and, in a continuation, to investigate if ICOGS can be applied to measuring the radiocarbon content in CO_2 with adequate sensitivity. It was recognized that: 1.) Our ICOGS system may not, in every aspect, be identical to that of the original publication, and, hence, it was decided to investigate a large set of parameters, since it is unknown how these small differences might affect the signal, and 2.) The combination of parameters employed in reference 1 is not clearly defined.

Consequently, apart from the attempted reproduction of the data presented in Figures 3¹ and 4¹ in reference 1 (the superscript in the figure labels denotes a reference to the figures in reference 1, to avoid confusion with references to figures in this paper), the foundation of this study was a series of 12 experiments, at various operating pressure, gas mix, and laser transition, to cover the possible combinations in reference 1. This is shown in Table 1. In these experiments, the laser power and modulation frequency were kept constant at ~ 0.8 W and 109 Hz, respectively (see

supporting information). Each experiment consisted of 18 measurements – six each of the three ^{14}C concentrations employed in this study (0.29 Modern, 3.3 Modern and 9.7 Modern). In order to avoid systematical errors and memory effects, two measurements with 0.29 Modern CO_2 were followed by two measurements with 3.3 Modern CO_2 and so on. Between each measurement, the sample cell was pumped to the base pressure, flushed with pure N_2 , and pumped to base pressure again. Each measurement lasted for three minutes, where the plasma was allowed to stabilize for two minutes, followed by one minute of data collection. Measurements showing signs of oscillatory $\text{CO} \leftrightarrow \text{CO}_2$ reactions¹⁴ were discarded in this step. During these measurements, the laser was locked at the center of the investigated laser transition.

Table 1. Operating pressure, gas mix and laser transition of the main experiment.

Experiment	Operating pressure [Pa]	Gas mix	Laser transition
1	15	$\text{CO}_2 + \text{N}_2$ (1:40)	P(20)
2	50	$\text{CO}_2 + \text{N}_2$ (1:40)	P(20)
3	100	$\text{CO}_2 + \text{N}_2$ (1:40)	P(20)
4	15	CO_2	P(20)
5	50	CO_2	P(20)
6	100	CO_2	P(20)
7	15	$\text{CO}_2 + \text{N}_2$ (1:40)	P(22)
8	50	$\text{CO}_2 + \text{N}_2$ (1:40)	P(22)
9	100	$\text{CO}_2 + \text{N}_2$ (1:40)	P(22)
10	15	CO_2	P(22)
11	50	CO_2	P(22)
12	100	CO_2	P(22)

Moreover, most of the experiments described in Table 1 were repeated with higher laser power and lower modulation frequency. In addition, the spectral distribution of the OG signal was studied by scanning the laser through its gain profile. These experiment were conducted with different gas mixes, ^{14}C concentrations, pressure, and laser transitions, and with a maximum laser power of $\sim 2\text{W}$ and modulation frequency of 63 Hz.

RESULTS AND DISCUSSION. In reference 1, the three most compelling arguments for the applicability of ICOGS to radiocarbon measurements, is the intracavity enhancement factor together with the results presented in the Figures 3¹ and 4¹, and the main focus of this study was to reproduce these results. With regards to the intracavity enhancement factor, we typically observed an intracavity OG signal with about the same amplitude as the extra-cavity OG signal from the ^{14}C -enriched reference cell when the intra-cavity sample cell was filled with a 9.7 Modern sample. In a crude estimation, using the approximation for the OG signal presented in reference 1, where the ratio between the effective interaction lengths of the two cells was calculated based on the respective discharge conditions, an intracavity enhancement factor of roughly 10^8 was found, given the $9.4 \cdot 10^{10}$ Modern concentration of the reference sample. Although in the same order as previous reports, and close to what can be expected from the physical interpretation of the enhancement mechanism, this calculation should be regarded with great caution, given the crude approximations used in the calculation, e.g. ideal gas conditions, similar discharge conditions in the two cells, and, most importantly, that the signal stemmed purely from $^{14}\text{CO}_2$. Nonetheless, there may exist an enhancement mechanism in ICOGS, although whether or not it can facilitate radiocarbon measurements depends on the reproducibility of the results presented in the Figures 3¹ and 4¹.

In Figure 3¹, the resonance curve for the ICOGS effect, with a 9 Modern CO₂ sample, is presented. The resonance curve is shown to follow a Voigt profile, with a full-width-at-half-maximum (FWHM) of 50 MHz. However, it is unclear what the exact discharge conditions of this experiment were. Reference 1 suggest, 500 Pa pure CO₂, or possibly a mix of CO₂ and N₂, whereas a republication of the data indicate a 500 Pa mix of CO₂ and N₂ (1:20)¹³. Moreover, it is unclear if the measurements were performed in batch or flow mode, what the laser power was, and which laser transition that was studied, however, from private communications with the Murnick group we have learned that it was P(20). Many attempts to reproduce the reported resonance curve have been conducted, however, given that our system could neither handle pressures above 120 Pa, nor measurements in flow mode, we have experimented with pressures below 100 Pa in batch mode. Two representative results, with different gas mix and laser power, are presented in Figure 2. As can be seen, we were never able to reproduce the Voigt-like profile of Figure 3¹, but only observed varying distributions of background. On the other hand, the data from the enriched reference cell showed a clear Voigt profile with a FWHM of 67 MHz, as expected¹⁴.

Figure 2. Spectral distribution of the ICOGS effect (black squares) for **(a)** 5%, 9.7 Modern CO_2 in 95% N_2 at 40 Pa, and **(b)** pure, 9.7 Modern CO_2 at 100 Pa, with the laser scanned around the center of the P(20) transition (gray squares). In both cases, the OG signal was scaled by the laser power, measured by the thermopile power meter.

In Figure 4¹, the dependence of the OG signal on the ^{14}C concentration of the sample is presented. The OG signal was shown to be linearly dependent on the concentration in a range between $5 \cdot 10^{-3}$ Modern and 1.5 Modern, whereas additional measurements indicated that the signal saturated at activities above 12 Modern. In the linear interval, a sensitivity of 0.36 (0.4) V/WModern was reported. The experiment was performed with pure CO_2 at 100 Pa, with the laser locked at the P(20) transition, and the system operating in flow mode with a flow of 0.05 sccm¹³. In our reproduction, samples with ^{14}C concentrations up to just below the saturation limit were chosen, to be able to clearly distinguish the $^{14}\text{CO}_2$ OG signal over the ~30% uncertainty interval, stemming from the difficulty of reproducing the discharge conditions in repeated measurements (see the supporting information). From the reported sensitivity, the 3.3 Modern and 9.7 Modern samples were expected to yield a 2.8 and a 6.5 times higher signal, respectively,

than the 0.29 Modern sample. In order to account for the reproducibility over repeated measurements, a series of 18 measurements – six for each of the three ^{14}C concentrations – were conducted, as described above. The results are presented in Figure 3. As can be seen, no significant dependence of the OG signal on the ^{14}C concentration of the sample was observed. Moreover, the standard deviation in a single measurement was much less than the deviation between measurements with identical ^{14}C concentration, pinpointing the importance of repeated analysis of each sample. Figure 3 show that analyzing each concentration only once, could have easily rendered a significant, but false, dependence of the OG signal on the ^{14}C concentration.

Figure 3. OG signal as a function of ^{14}C concentration (left) for pure CO_2 at 100 Pa, with the laser fixed at the center of the P(20) transition. Here, the OG signal from the sample cell was scaled by the OG signal from the reference cell and distributed around the mean of the measurements with 0.29 Modern concentration. To the right are the means and the standard deviations of the 18 individual measurements.

Since it was unknown how small differences between ours and the original ICOGS system affected the OG signal, similar experiments were performed for a number of parameter combinations. The results are presented in Figure 4. Here, the post-processed data is presented together with the normal distributions for each concentration. As can be seen, no significant or systematic dependence of the OG signal on the ^{14}C concentration could be found. Moreover, most of the experiments presented in Figure 4, were repeated with higher laser power (1.2 W - 2 W) with similar result. It should be pointed out that the lack of a clear dependence between the ^{14}C concentration and the OG signal renders the calculation of the intracavity enhancement factor without merit, since the calculation requires a clear definition of how much of the OG signal stems from radiocarbon, rather than from background originating from other isotopes.

Figure 4. Post-processed OG data from experiment 1-6 (a) and experiment 7-12 (b), according to Table 1. In each panel, the time series of six measurements at three different CO_2 concentrations (0.29 Modern, 3.3 Modern and 9.7 Modern) are presented to the left, with the frequency of a fitted normal distributions for each concentration to the right.

We recognize that the most important difference between our and the original experiment probably was the difference between flow and batch mode. Although, it may be speculated that the sensitivity is lower in batch mode than in flow mode¹⁷, we would still have expected to see a significant difference between the 0.29 Modern and 9.7 Modern samples in our study, regardless of mode based on the data presented in reference 13. However, our measurements suggest the OG signal to be more or less independent of ^{14}C concentration, and that the observed differences are caused by the irreproducibility of the discharge conditions and/or chemical reactions¹⁴ and not by the ^{14}C concentration. Although the authors of the original manuscript recognize that the reproducibility of the discharge conditions is a major concern¹, they offer no quantification of its impact on their results, neither in batch, nor in flow mode. We can see no obvious reason why flow mode would yield much better sensitivity than batch mode, based on the theoretical interpretation of the ICOGS effect. However, we can see reasons why measurements in flow mode at low flow rates might render greater uncertainties than in batch mode measurements. In reference 13, the dependency of the OG signal on the flow rate was investigated, and a non-linear dependence, resembling a logarithmic increase, was found. Hence, at low flow rates (<0.1 sccm), a small deviation in flow will cause a large deviation in OG signal. Moreover, the mass flow controller used in references 1 and 13 was a MKS PFC-50/60 (MKS Instruments Inc., MA, US), with a typical accuracy of ± 0.01 sccm, i.e., 20% of the absolute flow rate in reference 1. Still, it is doubtful if this additional random error caused by the uncertainty of the flow rate, combined with the lack of information regarding the reproducibility of the signal, is enough to explain Figure 4¹, and, consequently, the reported sensitivity. A comparison between our results and those of reference 1 is presented in Figure 5. Here, all results have been scaled to the mean signal at 0.29 Modern. As can be seen, an uncertainty of more than twice what we have observed

would be required to attribute the results in Figure 4¹ to the irreproducibility of the discharge conditions, and, even if this was the case, it is highly unlikely that the four data points of Figure 4¹ would be the results of a stochastic process, given their almost perfect linearity.

Figure 5. OG signal as a function of ^{14}C concentration for the experiments of this study (white squares) plotted alongside the results from reference 1 (black squares). The dark gray region show the uncertainty interval due to the irreproducibility of the discharge conditions, and the light gray region show the typical uncertainty due to instability and drift in a single measurement.

One may claim that there could exist an additional mechanism that makes flow mode more sensitive than batch mode. However, during short periods of time – about the time it takes a molecule to pass through the plasma – it could be argued that flow and batch mode can be regarded as equivalent, and at flow rates in the 0.05 sccm range, the net velocity of the molecules is only between 0.3 cm/s and 3 cm/s, depending on the pressure. Hence, this mechanism should be visible in batch mode too, although limited to a short period of time, about 1 s - 10 s, after the

plasma is ignited. It could also be argued that diffusion should help to extend this time. However, no such mechanism has been observed in our measurements. At higher flow rates, on the other hand, previously observed dissociation of CO_2 into CO , induced by the discharge, might be mitigated by the gas refreshment from the flow¹⁴. This process will be studied more carefully in future work, since the CO_2 dissociation process might be subject to isotopic fractionation.

If the uncertainty caused by the irreproducibility of the discharge conditions limited the possibility of isolating an unambiguous ^{14}C signal in repeated batch mode measurements, it could still have been expected that clear signs of resonance would have been visible in the $^{14}\text{CO}_2$ spectrum, given the results in Figure 3¹, especially at lower pressure where the FWHM should be more narrow¹⁸. Spectroscopically, flow and batch mode are expected to be equivalent as long as the OG signal is dominated by $^{14}\text{CO}_2$, hence, a more detailed analysis of the spectral distribution of the OG signal was conducted. Figure 6 (a) show the four measured parameters in our system as a function of the piezo voltage, when the laser was scanned through the P(20) transition. As can be seen the two power meters showed more or less identical output as expected. The OG signal from the reference cell showed a clear resonance behavior, similar to Figure 3¹, however, it should be noted that the concentration of the reference sample was $9.4 \cdot 10^{10}$ Modern. The OG signal from the sample cell, on the other hand, showed a clearly asymmetric response, with dissimilar shape and centroids as compared to the other entities, which is indicative to a signal from a non-resonant background. In order to get the spectral distributions of the OG signals, they were scaled by the laser power measured by the thermopile power meter, as shown in Figure 6 (b). Now, the absorption cross section of the reference cell was clearly visible, with an FWHM of about 67 MHz. In contrast, the distribution of the sample cell data showed no signs of

resonance, regardless of ^{14}C concentration, casting more doubt on the theory that ^{14}C is responsible for any significant part of the OG-signal.

Figure 6. (a) Intracavity OG signal (black squares), reference cell OG signal (gray squares), and laser power from the two power meters (black and white circles) as a function of piezo voltage for 9.7 Modern CO_2 in N_2 (1:20) at 40 Pa. (b) Intracavity OG signal scaled by laser power for two concentrations, 0.27 Modern (black squares) and 9.7 Modern (white squares), for the same discharge conditions as in (a), along with the similarly scaled OG signal from the reference cell (gray squares).

In a previous publication, we have simulated the non-resonant background in the $^{14}\text{CO}_2$ spectrum by broadband numerical modeling of the absorption cross section of a large number of $^{12}\text{C}^{16}\text{O}_2$ and $^{13}\text{C}^{16}\text{O}_2$ transitions¹⁴. We have shown that the main contribution to the background does not, as previously thought^{1, 13}, stem from the $^{12}\text{CO}_2$ and $^{13}\text{CO}_2$ transitions closest to the $^{14}\text{CO}_2$ line, but from the transitions with the highest Boltzmann population, due to pressure broadening of the line width. In Figure 7 (a), the simulated absorption cross-section of the P(20) transition, under the conditions of Figure 6, is presented. Although the simulations suggest that it

should be possible to distinguish a $^{14}\text{CO}_2$ resonance for the three concentrations, they involved several approximations, e.g., disregarding the background from $^{12}\text{C}^{18}\text{O}_2$, as well as the (01¹1)-(11¹0) band with transitions around 11.0 μm^{19} for $^{12}\text{C}^{16}\text{O}_2$. Moreover, transitions from non-electric dipole transitions, collision excitation, and other exchange effects will also cause additional background. Consequently, the measurements should be taken as an indication of that the simulations underestimates the background, rather than vice versa.

Even though we find our results in reasonable agreement with the simulations, the question remains why we cannot reproduce the results in Figure 3¹? The peak-to-tail ratio (PTR) of Figure 3¹ is more than 16 times, whereas the PTR in one of our simulations for similar discharge conditions was 1.004. For our simulations to yield similar PTR as in Figure 3¹, the ^{14}C concentration of the sample has to be increased to the order of the concentration of the reference cell, i.e., $>9 \cdot 10^{10}$ Modern.

The only other presentation of spectral distribution measurements in ICOGS, known to us, is Figure 4.10¹⁷ in reference 13. Here, the raw data – OG signal and laser power – for a piezo scan with a pure, 0.11 Modern CO_2 sample at 147 Pa is presented. We have extracted the data from this figure manually, split the voltage axis at the minimum of the laser power, and aligned the two parts to the maximums of the laser power, yielding the spectral distribution of the measurement, as can be seen in Figure 7 (c). As can be seen – and much like in our results – this scan show no signs of resonance, but, oppositely, points to a very strong non-resonant background despite the fact that the resonance curve should have been clearly visible, taking the claimed LOD of 10^{-15} into account. Given that the simulations indicate that the ^{14}C concentration of the sample in Figure 3¹ should be similar to that of our reference cell, and that no resonance is seen, neither in our experiments, nor in Figure 4.10¹⁷, i.e., by the authors of reference 1

themselves, we speculate whether the data in Figure 3¹, mistakenly, might have been confused with data from the reference cell. If that is the case, and Figure 3¹ and Figure 4.10¹⁷ represent the spectral distribution of the OG effect in the reference cell (with a highly enriched CO₂ content) and sample cell, respectively, we can reproduce the results with fair accuracy, Figure 6 (b). Another possibility is that the data in Figure 3¹ has been confused with a scan with the ¹²CO₂ laser, in which case we too obtain a Voigt profile¹⁴.

Figure 7 (a) Simulated spectrum for P(20) with pure CO₂ with varying ¹⁴C concentration at 40 Pa. (b) Simulated spectrum for P(18) with pure, 0.11 Modern CO₂ at 147 Pa. Both (a) and (b) include resonance from ¹⁴C¹⁶O₂ and the non-resonant background from ¹²C¹⁶O₂ and ¹³C¹⁶O₂. (c) Piezo scan derived from Figure 4.10¹³ in reference 13, with pure, 0.11 Modern CO₂ at 147 Pa.

In fact, the non-resonant background in Figure 7 (c) is even more pronounced than what we have observed in our experiments, indicating the close proximity of a ¹²CO₂ or a ¹³CO₂ transition in the spectrum. This is the case for the P(18) transition of ¹⁴CO₂, which is situated only about 100 pm from the P(66) transition of ¹³CO₂. Figure 7 (b) show the simulated absorption cross-section for 0.11 Modern ¹⁴CO₂ at the P(18) transition with the discharge conditions of Figure 7 (c). As can be seen, no clear resonance is visible. Instead the ¹⁴CO₂ signal is drowned in the Lorentzian tail of the ¹³CO₂ P(66) transition, which is clearly visible in the simulated spectrum as a quadratic increase towards higher frequencies¹⁴. Moreover, the scan (Figure 7 (c)) shows a

similar distribution as the simulation (Figure 7 (b)). This raises questions regarding how the authors of reference 1 confirmed which laser transition they were studying. We have used an external spectrum analyzer for this purpose, since we have experienced that solely relying on the predefined settings of the grating micrometer screws for choosing the transition may lead to ambiguous results, depending on variations in the optical configuration of the laser cavity, caused by the introduction of the sample cell. However, although a misalignment of the laser and an, accidental, close proximity of a $^{13}\text{CO}_2$ transition in the spectrum may explain the high signal levels observed in reference 1, it could never explain the results in Figures 3¹ and 4¹.

The simulation software was also used for predicting the sensitivity of ICOGS at the different discharge conditions of Table 1. The results for pure CO_2 at three different pressures and ^{14}C concentrations are presented in Figure 8. As can be seen, the simulations suggested that a difference in signal amplitude should have been observable for 9.7 Modern CO_2 at 15 Pa compared to the other ^{14}C concentrations. However, since the results of the piezo scans imply that the simulations underestimate the non-resonant background, it is not unreasonable that Figure 4 does not show a significant ^{14}C signal, but the results can be regarded as in line with theory. Nonetheless, it is important to point out that we cannot rule out the existence of a ^{14}C signal within the 30% uncertainty region. However, the maximum sensitivity of ICOGS would then be less than 3% per Modern, i.e., the amplitude of the OG signal would be increased by less than 3% when the activity is increased by 1 Modern.

Figure 8. Simulated signal as a function of ^{14}C concentration for 15 Pa (white circles), 50 Pa (gray circles) and 100 Pa (black circles) of pure CO_2 , plotted alongside the results from reference 1 (black squares). The gray uncertainty regions are the same as in Figure 3.

Regardless of the reproducibility or validity of the results presented in reference 1, the most serious deficiency of the publication is, in our opinion, the discussion regarding the LOD of ICOGS. The International Union of Pure and Applied Chemistry (IUPAC) define LOD as the concentration, a_L , relating to the smallest measure, x_L , that can be detected with reasonable certainty for a given analytical procedure²⁰. In the case of ICOGS, a_L is given by the equation $a_L = kS\sigma_L$, where σ_L is the standard deviation of a blank OG measurement, S is the sensitivity of the procedure ($\Delta\text{concentration}/\Delta\text{OG signal}$), and k is a numerical factor chosen according to the desired confidence level. Furthermore, IUPAC recommend a 95% confidence level²⁰, i.e., $k=2$. Estimating the standard deviation in Figure 4¹ to $\sigma_L=0.02$, and treating the $4.9 \cdot 10^{-3}$ Modern sample as a blank, we estimate the LOD in reference 1 to $a_L=0.11$ Modern, or 10^{-13} . Similarly, the limit of quantitation (LOQ) is commonly defined by $a_Q=10S\sigma_L$, i.e., $k=10$, which for

reference 1 yields $a_Q=0.56$ Modern²¹. To put this number in contrast, a typical LOQ for AMS is $a_Q=0.004$ Modern¹¹. However, without any argumentation or reference to their definition of LOD, and without any support from the presented data, the authors of reference 1 states an LOD of 10^{-15} , i.e., two orders of magnitude better than what is suggested by their experiments. It could be argued that the LOD in reference 1 only is reported as an “estimation”, but, in a later publication, an LOD of “ 10^{-15} or better” is stated as a fact, even though no additional data is presented¹⁰. The fact that the authors not only state a much better LOD than what their data supports, but that this LOD happens to be the same as that of the main competing technology, namely AMS, suggests the wish is father to the thought.

It should also be pointed out that the standard deviation in Figure 4¹ do not include the uncertainty caused by the irreproducibility of the discharge conditions, which our results suggest is the major cause of instabilities in ICOGS measurements. With standard deviations from Figure 3 and sensitivities from Figure 8, we estimate the best case LOD of our system to ~ 6 Modern, although one should remember that the concept of LOD remains irrelevant until the capability of ICOGS to unambiguously quantify radiocarbon has been proven. Even if a sensitivity of a few percent per Modern should be confirmed, and the stability and reproducibility of the measurements should be greatly increased, e.g., by employing a more stable plasma source or improving the measurement procedure, we doubt that ICOGS will be able to compete with AMS, except for analysis of highly enriched samples.

CONCLUSION. We have attempted to reproduce the results of Murnick et al, claiming ICOGS to be capable of detection of radiocarbon with a $^{14}\text{C}/^{12}\text{C}$ LOD of 10^{-15} . Although sample-related signals are readily detectable, we have not been able to unambiguously identify these with ^{14}C . We have not been able to reproduce the results concerning the OG signals dependence

on ^{14}C concentration and wavelength, and, ultimately, not seen any evidence of the capability of ICOGS to unambiguously measure radiocarbon in the investigated concentration range (0.29-9.7 Modern or $^{14}\text{C}/^{12}\text{C}=10^{-13}$ - 10^{-11}). On the contrary, we have found indications that the results of reference 1 can be products of measurement uncertainties and mistakes, and we find these indications serious enough to dismiss the results. Since its publication, no research group, including the authors of the paper themselves, have been able to reproduce its result, or present any additional proof of the applicability of ICOGS to radiocarbon analysis, and we regard this as additional evidence of the validity of our conclusion.

Given the intense, but still unsuccessful attempts by several groups to reproduce the results of reference 1, along with the conference proceeding¹⁰, containing republications of its results, these results are in serious doubt. Moreover, the conclusions regarding the LOD of ICOGS seem to be motivated mostly by eagerness to become competitive to AMS; according to our findings they are overestimated by two orders of magnitude at least. Hence, we conclude that the results reported in reference 1, along with reference 10, are seriously doubtful and should be treated accordingly, and that our unsuccessful efforts to reproduce the results of the ICOGS technique should serve as a warning for potential researchers interested in its application. Unless fundamentally unfeasible, ICOGS could be approached using a more gentle and, more importantly, stable form of excitation to reduce both background and uncertainties. This could be achieved by deploying a more state-of-the-art detection method, which is currently underway at our laboratory.

ASSOCIATED CONTENT

Supporting Information. A detailed description of the measurement procedure and the post-processing of the recorded data are presented, along with a discussion of the major causes of

random error and noise in the measurements, namely EMI, $1/f$ noise, and the irreproducibility of the discharge conditions. Moreover, the choice of laser power and modulation frequency in the experiments is thoroughly motivated.

AUTHOR INFORMATION

Corresponding author: Mehran Salehpour*

*mehran.salehpour@physics.uu.se.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Funding Sources

The Swedish Research Council. (Ref. A0442201)

Notes

Any additional relevant notes should be placed here.

ACKNOWLEDGMENT

The authors wish to acknowledge the Swedish Research Council for supporting this project (Ref. A0442201). Dr. Anders Holmberg, Karolinska Institutet, Stockholm is gratefully acknowledged for providing us with the ^{14}C -labelled dextran samples.

ABBREVIATIONS

AMS, accelerator mass spectroscopy; DAQ, data acquisition; EMEA, European Medical Agency; EMI, electromagnetic interference; FDA, Food and Drug Administration; FWHM, full

width at half maximum; ICOGS, intracavity optogalvanic spectroscopy; IUPCA, International Union of Pure and Applied Chemistry; LARA, laser assisted ratio analyzer; LOD, limit of detection; LOQ, limit of quantitation; OG, optogalvanic; PTR, peak to tail ratio; RF, radio frequency; SNR, signal to noise ratio.

REFERENCES

- (1) Murnick, D. E.; Dogru, O. Ilkmen, E. *Analytical Chemistry*. **2008**, *80*, 4820-4824. DOI: 10.1021/ac800751y.
- (2) Murnick, D. E.; Dogru, O. Ilkmen, E. *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms*. **2007**, *259*, 786-789. DOI: 10.1016/j.nimb.2007.02.080.
- (3) Barbieri, B.; Beverini, N. Sasso, A. *Reviews of Modern Physics*. **1990**, *62*, 603-644. DOI: 10.1103/RevModPhys.62.603.
- (4) Stanciulescu, C.; Bobulescu, R. C.; Surmeian, A.; Popescu, D.; Popescu, I. Collins, C. B. *Applied Physics Letters*. **1980**, *37*, 888-890. DOI: 10.1063/1.91785.
- (5) Baev, V. M.; Latz, T. Toschek, P. E. *Applied Physics B*. **1999**, *69*, 171-202. DOI: 10.1007/s003400050793.
- (6) Lappin, G. Garner, R. C. *Nature Reviews Drug Discovery*. **2003**, *2*, 233-240. DOI: 10.1038/nrd1037.
- (7) Arner, P.; Bernard, S.; Salehpour, M.; Possnert, G.; Liebl, J.; Steier, P.; Buchholz, B. A.; Eriksson, M.; Arner, E.; Hauner, H.; Skurk, T.; Ryden, M.; Frayn, K. N. Spalding, K. L. *Nature*. **2011**, *478*, 110-113. DOI: 10.1038/nature10426.
- (8) Salehpour, M.; Possnert, G. r. Bryhni, H. *Analytical Chemistry*. **2008**, *80*, 3515-3521. DOI: 10.1021/ac800174j.
- (9) Salehpour, M.; Forsgard, N. Possnert, G. *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms*. **2010**, *268*, 884-886. DOI: 10.1016/j.nimb.2009.10.055.
- (10) Murnick, D.; Dogru, O. Ilkmen, E. *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms*. **2010**, *268*, 708-711. DOI: 10.1016/j.nimb.2009.10.010.
- (11) Salehpour, M.; Forsgard, N. Possnert, G. *Rapid Communications in Mass Spectrometry*. **2008**, *22*, 3928-3934. DOI: 10.1002/rcm.3808.
- (12) Murnick, D. E. Peer, B. J. *Science*. **1994**, *263*, 945-947. DOI: 10.1126/science.8310291.
- (13) Ilkmen, E. *Intracavity Optogalvanic Spectroscopy - Radiocarbon Analysis with Attomole Sensitivity*. Rutgers, Newark, NJ, 2009.
- (14) Eilers, G.; Persson, A.; Gustavsson, C.; Ryderfors, L.; Mukhtar, E.; Possnert, G. Salehpour, M. *Radiocarbon*. **2013**, *55*, [In press].
- (15) Marino, B. D. V.; Bright, M. Gronniger, G. *Proceedings of the SPIE*. **2011**, *8156*, 81560E-81560E. DOI: 10.1117/12.894532.
- (16) Bradley, L.; Soohoo, K. Freed, C. *Quantum Electronics, IEEE Journal of*. **1986**, *22*, 234-267. DOI: 10.1109/jqe.1986.1072967.

- (17) Ilkmen, E. *Intracavity Optogalvanic Spectroscopy - Radiocarbon Analysis with Attomole Sensitivity*. Doctoral thesis, Rutgers, Newark, NJ, 2009.
- (18) Demtröder, W. *Laser spectroscopy: basic concepts and instrumentation*, 3rd ed.; Springer: Berlin Heidelberg, 2003.
- (19) Hartmann, B. Kleman, B. *Canadian Journal of Physics*. **1966**, *44*, 1609-1612. DOI: 10.1139/p66-134.
- (20) McNaught, A. D. Wilkinson, A. *International Union of Pure and Applied Chemistry Compendium of Chemical Terminology*, 2nd ed.; Royal Society of Chemistry: Cambridge, UK, 1997.
- (21) Currie, L. A. *Analytical Chemistry*. **1968**, *40*, 586-593. DOI: 10.1021/ac60259a007.

For Table of Contents Only