ECS Transactions, 41 (18) 1-7 (2012)
10.1149/1.3684414 ©The Electrochemical Society

# Detection of Acetone on Human Breath using Cyclic Voltammetry

Perry Motsegood and Johna Leddy*

Department of Chemistry, University of Iowa, Iowa City, IA 52242,
johna-leddy@uiowa.edu

A cyclic voltammetric measurement protocol for acetone concentration collected in the vapor phase and measured in solution is demonstrated for acetone concentrations across the human physiological range, 1 $\mu$M to 10 mM at platinum electrodes in 0.5 $M$ $H_2SO_4$. Effects arise through adsorption of acetone from the gas phase onto a platinum surface and hydrogen in acidic solution within the voltammetric butterfly region. The protocol is demonstrated to yield breath acetone concentration on a human subject within the physiological range and consistent with ketone urine test strip.

## Introduction

Acetone is a metabolite of fat catabolism and serves as an indicator for various physiological states related to ketosis, such as diabetes, low carbohydrate diet, and weight loss (1). Acetone can provide an alternative to glucose monitoring. Under these physiological stresses, fat consumption increases in cells, as do ketone levels in the blood. The main ketone bodies used for ketotic markers are 3-hydroxy-butyrate (3-HB), acetoacetic acid (acac), and acetone (2). Of these, acetone is the only ketone body that appreciably partitions from the blood into the breath.

Human breath is a gas complex mixture comprised of numerous organic small molecules. A report by Phillips lists the relative abundance for the top three most abundant volatile organic compounds (VOCs) to be isoprene (48.60%), 1,2-pentadiene (15.00%), and acetone (14.59%) (3). Although multiple thiols fill the top ten, the reported relative abundances are lower than acetone by a factor of seven or greater.

Qualitative methods are available for blood, urine, and breath (1). In clinical settings, the preferred blood measurement is not for glucose but for one of three ketone bodies, specifically 3-HB, whereas over the counter urine test strips, Ketostix® (Bayer) as an example, monitor acac. Breath analysis typically involves a gas chromatograph and mass spectrometer, making a rapid, yet portable, device infeasible. Electrochemistry offers the possibility of an acetone breath sensor that is portable and would not require extensive and costly equipment. Cyclic voltammetry is used to examine the competitive adsorption in 0.5 $M$ $H_2SO_4$ between acetone and free protons. Adsorption and electrochemical reduction of acetone on platinum has been studied mechanistically in 0.5 $M$ $H_2SO_4$ using cyclic voltammetry (4), (5), (6). The adsorption of acetone in a 0.5 $M$ $H_2SO_4$ solution alters the line shape of the butterfly region by blocking adsorption sites normally available to hydrogen. During this process, adsorbed acetone is reduced or desorbs from the surface. These previously blocked adsorption sites are now occupied by hydrogen and the signature hydrogen reduction wave is regenerated. This effect on lineshape is utilized to correlate concentration of acetone on breath.

Here, the direct electrochemical reduction of adsorbed breath acetone on platinum in 0.5 $M$ $H_2SO_4$ is reported, a calibration plot is generated, and a human breath sample is tested.

## Experimental

Details of the experiments and voltammetric protocols follow.

### Reagents

Deionized water from a Milli-Q plus water filtration system (Millipore) was used . Acetone calibration solutions were prepared from HPLC grade stock and serial dilution. Concentrated $H_2SO_4$ was diluted to 0.5 $M$. All reagents were purchased from Fisher Scientific and used as received. Artificial 'lungs' were prepared by mixing 500 mL acetone:water solutions in a 1 L glass bottle with a Teflon/silicone piercable septa (Industrial Glassware, Millville, NJ). Gas samples are generated by partition of acetone from solution into the headspace above the sealed vessel. The partition is done from equal volumes of acetone solution and headspace. The acetone solution contained the appropriate acetone:water concentration. Partition at room temperature was allowed for two hours prior to use of the vapor sample to simulate humidified breath samples. Because the partition of acetone into the headspace is directly proportional to solution concentration, all concentrations of vapor phase samples used in the calibration process are reported as the acetone solution concentration in water.

### Electrode Preparation

The three electrode electrochemical cell consisted of a platinum disk electrode (2 mm dia, CH Instruments), a platinum mesh counter electrode (1 $cm^2$), and a Ag/AgCl reference electrode. A computer controlled CH Instruments 760B bipotentiostat was used to make all measurements. The working electrode was mechanically polished using 1 $\mu m$, 0.3 $\mu m$, and 0.05 $\mu m$ alumina polish for 30 seconds each on a micropolishing cloth (Buehler, p/n 40-7218), rinsed with water, and then electrochemically cleaned in 0.5 $M$ $H_2SO_4$ by scanning between -0.2 $V$ and +1.5 $V$ at 500 $mV/s$ for 25 cycles to generate the signature hydrogen reduction 'butterfly' wave on platinum.

### Gas Sample Procedure

Once the electrode is electrochemically cleaned, a blank (acid only) scan is performed to generate a baseline. The electrode is removed from the acid solution, rinsed with DI water, and dried with ultrahigh purity dry nitrogen. An acetone vapor sample is manually delivered directly to the platinum surface using a valved polypropylene syringe at a height of 3-5 $mm$ above the electrode surface with a flow rate of ~0.5 $mL/s$. The electrode is reinserted to the 0.5 $M$ $H_2SO_4$ solution and rescanned using the same parameters as the blank scan. The potential limits for the cleaning and sampling are summarized in Table I.

End breath samples were generated by exhaling for a total of 40 seconds. The first 30 seconds of breath were discarded and the last ten seconds were delivered directly to

Table I: **Voltammetric parameters for electrochemical cleaning and gas sample scans.**

| | Cleaning Scan | Sample Scan |
|---|---|---|
| $E_{initial}$ $(V)$ | +0.2 | +0.2 |
| $E_{low/final}$ $(V)$ | -0.2 | -0.2 |
| $E_{high}$ $(V)$ | +1.5 | +1.5 |
| Scan rate $(V/s)$ | 0.5 | 0.1 |
| Segments | 51 | 3 |

the electrode surface. Breath was focused onto the electrode surface using a common plastic straw with a 6 $mm$ inner diameter and 20 $cm$ length. Once the breath sample was delivered, the electrode was reinsterted to acid solution for analysis. Approval for the use of human subjects was received from the University of Iowa Internal Review Board. The subject had been fasting for 14 hours prior to sample collection.

## Results and Discussion

Data and analysis for calibrants and breath sample follow.

### Headspace Analysis

In Figure 1, the cyclic voltammetric response in 0.5 $M$ $H_2SO_4$ is shown for analysis of headspace gas of a low concentration (1 $\mu M$) solution immediately after the sample is introduced. The scan starts at 0.4 V and sweeps negative and then positive to 1.5 V. the final sweep segment is then negative back to -0.2 V. On the third segment, the well resolved butterfly region for hydrogen adsorption is apparent. There is a final oxidative sweep. In Figure 2, and enlargement of the reductive portion of the butterfly region is shown.

On the initial reductive sweep, the waves for hydrogen are disrupted by the acetone adsorbed from the vapor sample. Thus, on the first sweep, acetone adsorption is apparent. After the full oxidative and the return reductive sweep, the acetone has been fully electrolyzed to yield a butterfly consistent with only hydrogen adsorbed. The complete electrolysis of acetone is consistent with the bottom portion of the butterfly being coincident on the second and fourth segments.

In Figure 3, the cyclic voltammetric response in 0.5 $M$ $H_2SO_4$ is shown for analysis of headspace gas of a high concentration acetone (10 $mM$) is shown immediately after the sample is introduced. The signal for the 10 mM solution differs from the 1 $\mu$M solution. In both cases for the initial segment, the first wave of the butterfly is suppressed and the second wave is altered relative to the pristine hydrogen adsorption wave.

In Figure 4, the voltammetric response for the adsorbed human breath sample in 0.5 $M$ $H_2SO_4$ is shown. Voltammetric characters are consistent with the responses for the acetone samples above. On the first segment, the first wave is suppressed and the second wave is altered. On the third segment, the voltammetric response is that characteristic of only hydrogen adsorbed on platinum, as for the prior samples.

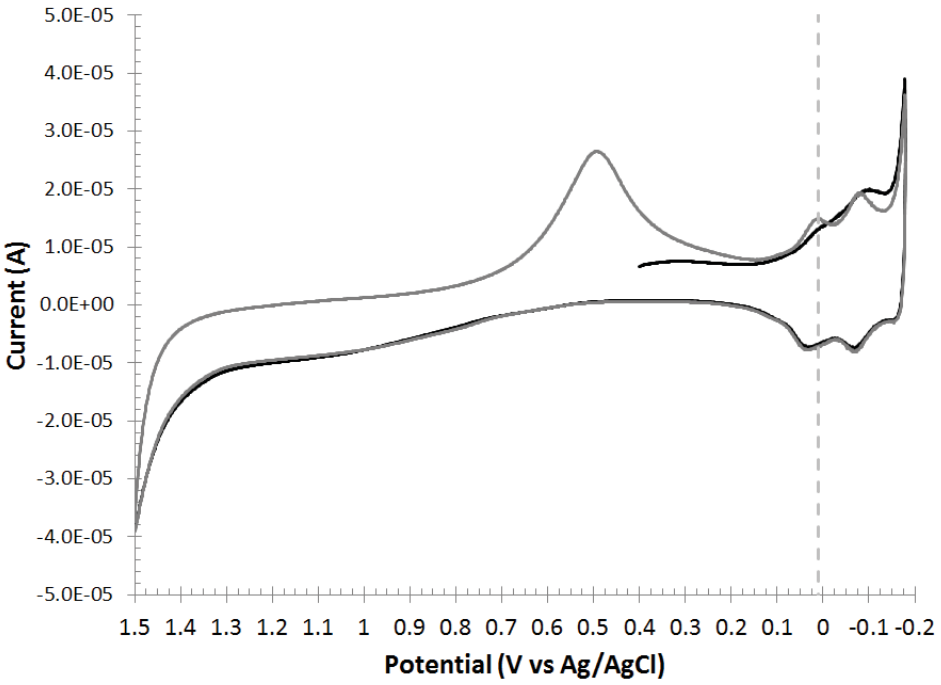


Figure 1: Overlay of headspace analysis scans in 0.5 $M$ $H_2SO_4$ for 1 $\mu$M acetone:water solution over the full sample scan range. Scanning twice through the hydrogen reduction region provides acetone headspace data (black) followed by hydrogen reduction (gray). The vertical dotted line at +0.05 V depicts where the sample data is extracted.

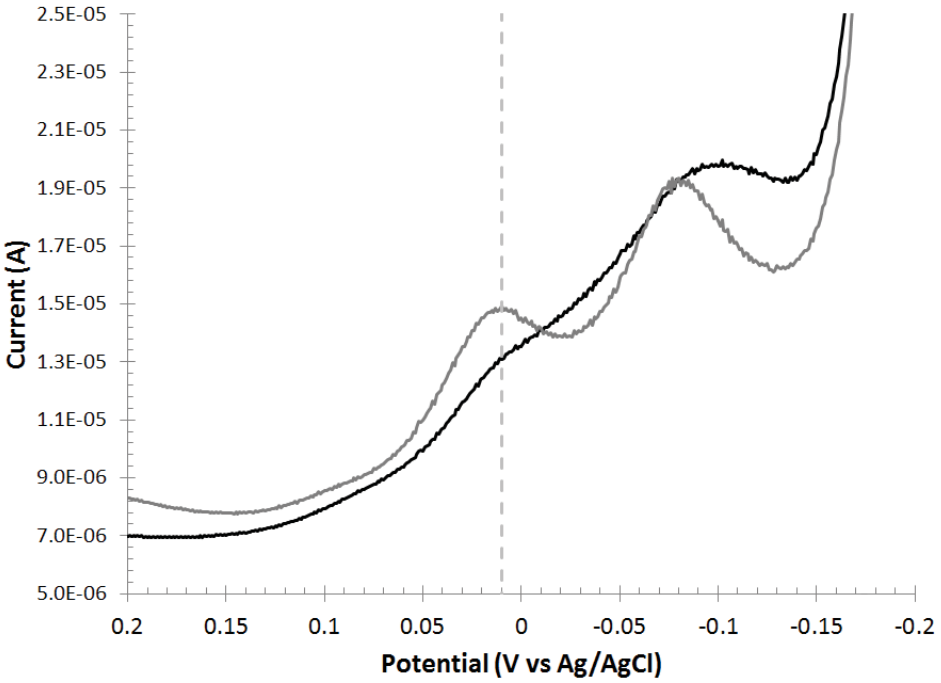


Figure 2: Overlay of subsequent headspace scans in 0.5 $M$ $H_2SO_4$ through the hydrogen reduction region, 1 $\mu$M acetone in water. First pass (black) through this region reduces adsorbed acetone, while the second pass (gray) regenerates the hydrogen reduction wave.

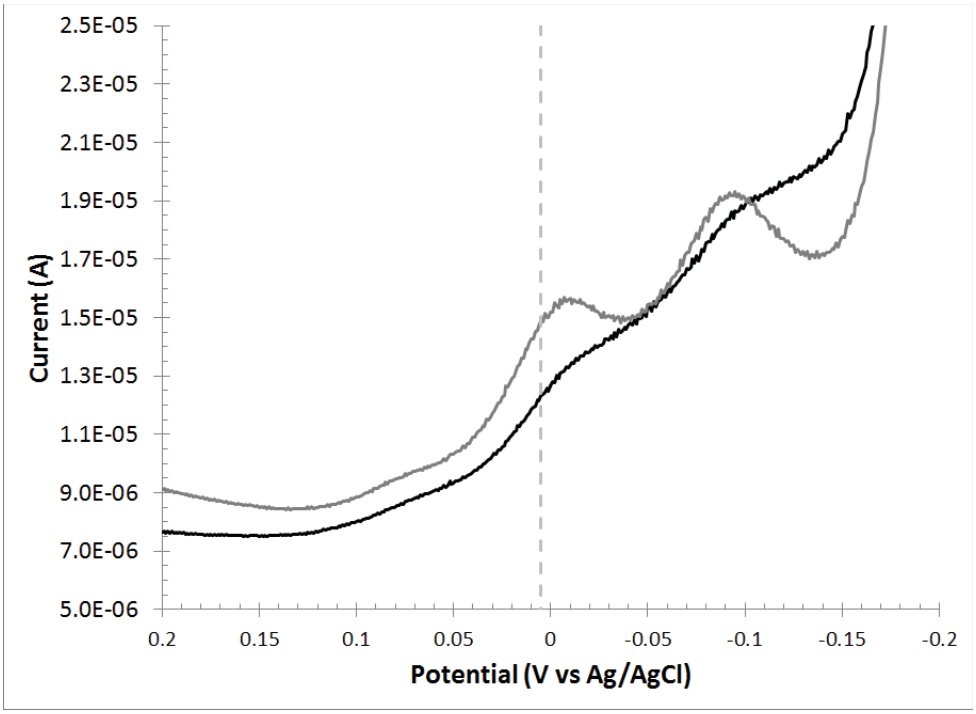


Figure 3: Overlay of subsequent headspace scans in 0.5 $M$ $H_2SO_4$ through the hydrogen reduction region, 10 $mM$ acetone in water. First pass (black) through this region reduces adsorbed acetone, while the second pass (gray) regenerates the hydrogen reduction wave.

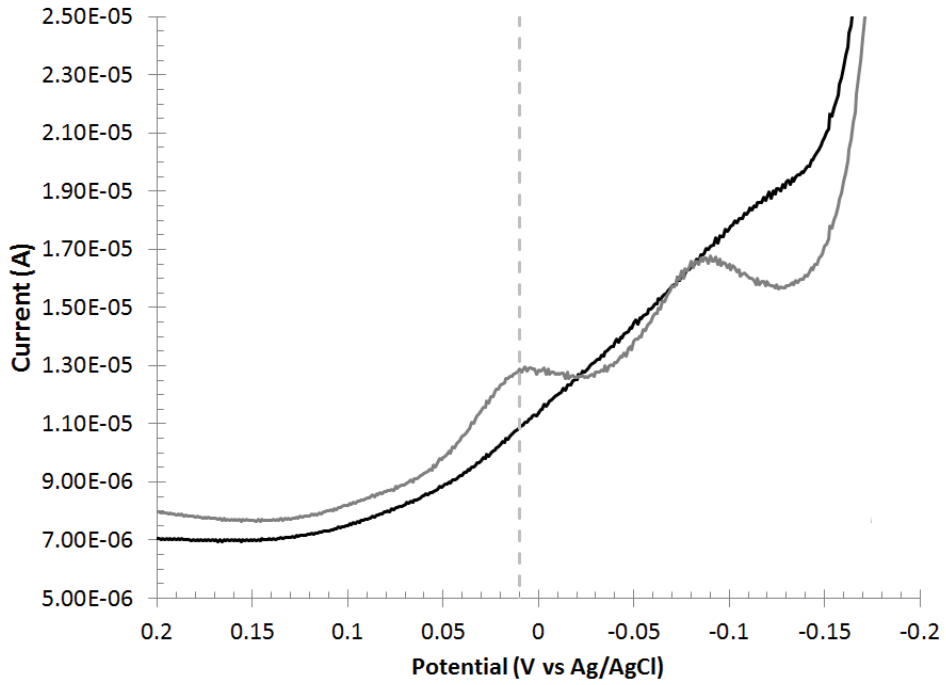


Figure 4: Overlay of subsequent human breath scans in 0.5 $M$ $H_2SO_4$ through the hydrogen reduction region. First pass (black) through this region reduces adsorbed acetone, while the second pass (gray) regenerates the hydrogen reduction wave.

Calibration Curve

The calibration data, shown in Table II, are generated for solution phase acetone concentrations from 1 $\mu$M to 10 mM in decade increments. The analyzed current, $\Delta i\,(\mu A)$ is derived from the plots as the difference in the current on the first and third segments at +0.005 V. Current difference values are reported as absolute values.

Table II: **Extracted current difference values for calibration and breath samples.**

| Sample | [acetone (M)] | log[acetone (M)] | $\Delta i$ ($\mu$A) |
|---|---|---|---|
| water vapor | 0 | – | 1.40 |
| 1 $\mu M$ | 1.00E-6 | -6 | 1.40 |
| 10 $\mu M$ | 1.00E-5 | -5 | 1.50 |
| 100 $\mu M$ | 1.00E-4 | -4 | 1.60 |
| 1 $mM$ | 1.00E-3 | -3 | 1.90 |
| 10 $mM$ | 1.00E-2 | -2 | 2.40 |
| **breath** | $4.6 \times 10^{-4}$ | -3.33 | **1.80** |

The calibration data are linearized as $\Delta i$ versus $1/\log_{10}[acetone]$ as shown in Figure 5 by the solid markers.

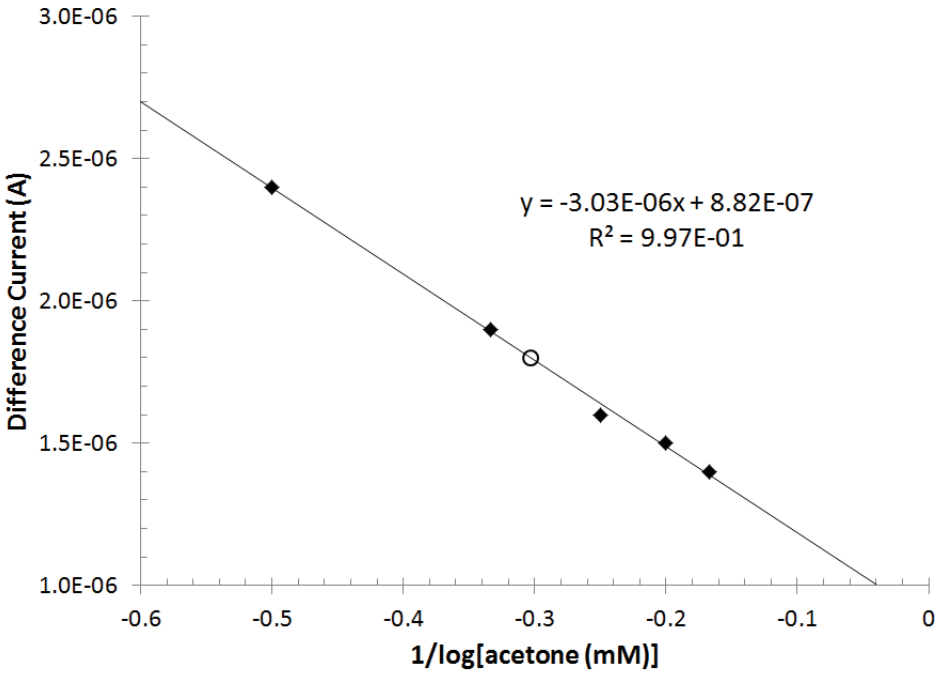


Figure 5: Calibration curve (solid diamonds) for headspace acetone and breath sample (open circle) analysis. The breath sample correlates to ~0.5 mM acetone.

Breath Sample

The breath sample was introduced as above and evaluated cyclic voltammetrically in 0.5 $M$ $H_2SO_4$ as consistent with the calibrants. $\Delta i$ for the breath sample is shown in Table II as

1.80 $\mu$A. From the calibration line, the breath sample correlates to an acetone concentration of 0.46 mM, a value well within the calibrant set and well within the physiological range anticipated for a fasting human.

To roughly estimate the acetone levels for the subject, a Ketostix urine test strip was used to verify ketosis and correlate urine measurements of acac acid to breath acetone. Urine analysis determined the subject was in a mild ketotic state and fell between 5-15 $mg/dL$ acac. Assuming a median value of 10 $mg/dL$, this converts to a value of 1 $mM$ for a range of 0.5 to 1.5 mM. Although the sticks measure acac and not acetone directly, the two compounds are in equilibrium with each other at a ratio of 1:7 (acetone:acac) in a non-ketoacidotic state. The corresponding acetone value for urine analysis is then $\sim$0.14 mM for a range of 0.07 to 0.21 mM The breath measurement and urine estimation are comparable given the uncertainties in the urine measurement and associated estimations.

## Conclusions

Several conclusions are drawn from this preliminary study. First, phenomenologically derived calibration curves of acetone concentration adsorbed onto platinum from the vapor phase can be derived in acidic solution using cyclic voltammetry. Measurement is performed by observing electrolysis of adsorbed acetone in the voltammetric butterfly region. Second, Breath acetone sampled on human breath yields a value in the physiological range and roughly consistent with a urine test strip. Finally, the protocol described here requires further validation and refinement.

## Acknowledgments

This work was funded by a sponsored research agreement between GM Nameplate (Seattle, WA) and the University of Iowa Research Foundation.

## References

1. Laffel, L. *Diabetes Metab Res Rev* **1999,** *15,* 412-426.

2. Kalapos, M. P. *Biochimica et Biophysica Acta* **2003,** *1621,* 122-139.

3. Phillips, M. *Analytical Biochemistry* **1997,** *247,* 272-278.

4. Wieckowski, A.; Zelenay, P.; Szklarczyk, M.; Sobkowski, J. *Journal of Electroanalytical Chemistry and Interfacial Electrochemistry* **1982,** *135,* 285-299.

5. Bänsch, B.; Härtung, T.; Baltruschat, H.; Heitbaum, J. *Journal of Electroanalytical Chemistry and Interfacial Electrochemistry* **1989,** *259,* 207-215.

6. de Hemptinne, X.; Schunck, K. *Transactions of the Faraday Society* **1969,** *65,* 591-597.