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Portable method of measuring gaseous acetone concentrations

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ABSTRACT

Measurement of acetone in human breath samples has been previously shown to provide significant non-invasive diagnostic insight into the control of a patient's diabetic condition. In patients with diabetes mellitus, the body produces excess amounts of ketones such as acetone, which are then exhaled during respiration. Using various breath analysis methods has allowed for the accurate determination of acetone concentrations in exhaled breath. However, many of these methods require instrumentation and pre-concentration steps not suitable for point-of-care use. We have found that by immobilizing resorcinol reagent into a perfluorosulfonic acid polymer membrane, a controlled organic synthesis reaction occurs with acetone in a dry carrier gas. The immobilized, highly selective product of this reaction (a flavan) is found to produce a visible spectrum color change which could measure acetone concentrations to less than ppm. We here demonstrate how this approach can be used to produce a portable optical sensing device for real-time, non-invasive acetone analysis.

1. Introduction

Acetone is an exhaled volatile organic compound that has been shown to act as a biomarker for metabolic conditions in the bloodstream. In cases of fasting, exercise, or diabetes mellitus, the liver produces ketones, to act as an additional energy source, which are metabolized into acetone and other ketone bodies [1,2]. The acetone produced travels through the blood and is excreted through either urine or exhaled breath [1]. In the case of exhaled excretion, a partition coefficient has been found to be 330 parts in the blood for every one part that leaves with expired air [3]. Using breath analysis techniques, acetone concentrations in exhaled breath have been previously shown to correlate strongly with acetone concentrations in the blood as well as with other ketones such as β-hydroxybutyrate [4,5]. In addition, recent studies have found that a possible correlation exists between blood glucose levels and volatile organic compounds such as acetone [6–9]. Measurement of acetone from breath has been shown to allow better diagnostic control of a patient's diabetic condition than through the use of blood glucose measurements alone [10].

Among the most successful breath analysis methods previously reported are selected ion flow tube mass spectroscopy [11] (SIFT-MS) and cavity ringdown spectroscopy [6] (CRDS), which have been shown to accurately measure concentrations of acetone in breath samples. While these methods are effective, they can function only in a hospital or outpatient capacity due to the extensive equipment and training required for their use. In addition, these methods require significant amounts of time to run which limits their use as real-time diagnostic tools. Recently, an interesting optical approach has been demonstrated using the solvatochromatic response of metallo-porphyrins to acetone [12,13]. The color response depends on generic correlations to ligand polarizability [14] and is thus susceptible to numerous interferences. Consequently, arrays of sensing elements are required to ensure specificity to a particular agent of interest and relatively large instrumentation and frequent human intervention are needed for definitive analysis. Evolution of non-invasive exhaled breath analysis toward POC use along the lines of existing invasive blood sampling techniques requires the development of lightweight, low cost devices capable of real-time testing. Recent advances in photo-physics have produced visible light emitting diodes (LEDs) and photodiodes sufficiently small to incorporate into palm-sized devices that make an optical approach attractive for POC use if it can be made sufficiently selective.

We have recently demonstrated [15] that the optical response arising from chemical reaction between a dye molecule immobilized within a polymer membrane catalyst and volatized toxins can be used for very sensitive (sub-ppb) real-time detection. The specific sensing mechanism involves the acid-catalyzed Fridel–Crafts acylation of ambient toxic anhydrides (trimellitic, phthalic, and maleic) with immobilized resorcinol to produce quinones with highly selective optical characteristics in the visible region of the electromagnetic spectrum. Nafion® 1100 was used as the membrane catalyst. Perfluorsulfonic acid (PSA) polymers such as

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commercial Nafion® have been used for many years as matrix supports for immobilized dyes in optical sensing applications because: 1) they are highly transparent in the UV–vis–NIR region; 2) their amphiphilic nature, arising from their fluorocarbon backbone and sulfonate groups, is capable of both trapping lipophilic ligands and exchanging ionic species; and 3) they have excellent chemical, thermal, and mechanical stability. Prior to our work, optical sensing utilizing these membranes resulted from the complexation or protonation/deprotonation of immobilized dye molecules through interaction with diffusing ion species yielding highly sensitive but, typically, non-specific color responses [16]. The ability of perfluorosulfonic acid groups in protonated perfluorosulfonic ionomers (PFSIs) to catalyze numerous chemical reactions is well known but had never been previously exploited to produce highly selective optodes. Selectivity arises due to the unique spectroscopic products that are produced by the bond breaking, and formation that occurs through chemical reactions of immobilized dyes with agents of interest. In this article, we demonstrate how this unique optical sensing approach may be extended to acetone detection in exhaled breath.

2. Materials and methods:

Nafion® membrane (Sigma-Aldrich, Nafion® 117, protonated, 0.007 in. thickness) was used as received and immersed in a 12 g/L solution of resorcinol (Acros Organics, 98%) in ethanol (Acros Organics, ACS spectroscopic grade, $>95\%$ purity) for 31 min as described in further detail in our previous work [15]. The concentration of resorcinol present in the samples was maintained well in excess of the acetone exposure levels, to ensure that it would not limit the reaction. Samples were then rinsed with deionized water and allowed to air dry. After drying, the membrane was suspended in a nitrogen purged flask maintained at 60 °C with a water bath. A known concentration of acetone (Acros Organics, ACS spectroscopic grade, 99% purity) was injected into the flask, the flask sealed, and the acetone allowed to vaporize. After 15 min, the membrane was removed and the signal response was measured ex situ using a UV/visible spectrometer (Ocean Optics HR 2000+ CG-UV–NIR High Resolution Spectrometer).

The in-situ studies were performed using an acetone bubbling system, which consisted of a mass flow controller feeding a known amount of nitrogen into a glass aerator filled with acetone. The outlet stream was connected to a dilution gas stream to allow gas feeds to reach low ppmv concentrations of acetone in the combined stream. The gas mixture was fed into a sealed sample gas collector maintained at ambient pressure, where it would be opened into the in-situ sensing system. The acetone concentrations present for a given flow rate of nitrogen were determined by collecting samples of the gas mixture and exposing them to the resorcinol-imbibed membranes. The response of this could be measured and the concentration determined from the calibration plot described in the following section. This process provides an accurate controlled inlet stream for the in situ experiments.

To identify reaction product, membrane samples were soaked in known volumes of ethanol for 6–24 h, making sure that there was no observed absorbance of either resorcinol or product remaining in the membrane. The extracted solution was then concentrated and the identity of the product molecule was determined using electrospray mass spectroscopy (Micromass Q-TOF-2).

3. Results and discussion

The acid-catalyzed reaction of acetone with resorcinol immobilized in protonated PFSIs was initially hypothesized to yield an immobilized flavan compound as the condensation product (4-(3,4-Dihydro-7-hydroxy-2,4,4-trimethyl-2H-1-benzopyran-2-yl)-1,3-benzenediol) according to the reaction scheme shown in Fig. 1 [17,18].

Upon exposure to acetone, the membranes were shown to change from transparent to bright yellow with the intensity of the color increasing with increased concentration of acetone in the system. The exposure concentrations for this study were targeted at the nominal acetone concentrations found in the exhaled breath of diabetics [6,19]. As the resorcinol-imbibed membrane is exposed to varying concentrations of acetone there is a very noticeable increase in absorbance at the near-UV to visible range (380–430 nm) which accounts for the visible shift in the membranes as seen in Fig. 2.

A strong visible response was recorded at 400.69 nm, which was attributed to the formation of the hypothesized product. The visible response at 400.69 nm also showed a strong correlation ($R^2=0.99$) to the measured concentration of acetone added to the system as demonstrated in Fig. 3.

The hypothesized reaction product of Fig. 1 was confirmed using electrospray mass spectroscopy. The flavan product, $C_{18}O_4H_{20}$, has a molar mass of 300.1362. The electrospray measurement includes an additional H^+ ion with 1.0078 m/z. Thus, the high resolution signal of 301.1440 m/z observed in Fig. 4 is precisely associated with the product. The molecular fragments associated with bond cleaving are also observed in the figure. A loss of 110.0368 m/z is observed in our data, associated with cleaving of the resorcinol group from the flavan ring structure. In addition, opening of the ring at the ester and loss of the attached resorcinol, the ester, and part of the ring structure would account for the loss of 152.0474 m/z, yielding the most prominent feature observed in Fig. 4.

In order to gain a better understanding of the kinetics of this reaction, we built an in-situ flow cell system to allow monitoring of the UV/vis spectrum during exposure of the membrane. To achieve this, the membrane was placed in a holder inside a sealed quartz cuvette maintained at 60 °C. Varying concentrations of acetone vapor were then flowed continuously through the quartz chamber allowing for a constant concentration exposure to the membrane. UV/Vis was acquired over the course of the exposure at 30 s intervals as can be seen in Fig. 5. Fig. 5 shows the evolution of the visible region as 0.64 ppmv of acetone is exposed to the membrane for 15 min. Taking a cross-section of this data at 400 nm allows for observation of the kinetics of this reaction as shown in Fig. 6.

As the objective was to develop a fast and inexpensive method for acetone detection, we devised a new sensor system by which the absorbance of the exposed membrane can be measured instantaneously in a compact and portable system, as shown in Fig. 7. The prototype uses a series of light emitting diodes (LEDs) and a photodiode array set in parallel to a heated sample holder into which the membrane can be secured. The three LEDs inside the apparatus provide wavelengths of the red, green, and blue range of the visible spectrum (though for this experiment only the response in the blue region was being observed, with the peak wavelength for the blue LED residing at 430 nm). The sample Nafion® membrane is placed into the test apparatus and a

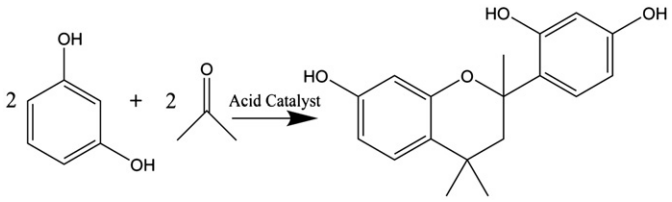

Fig. 1. Reaction scheme for condensation reaction of acetone and resorcinol.

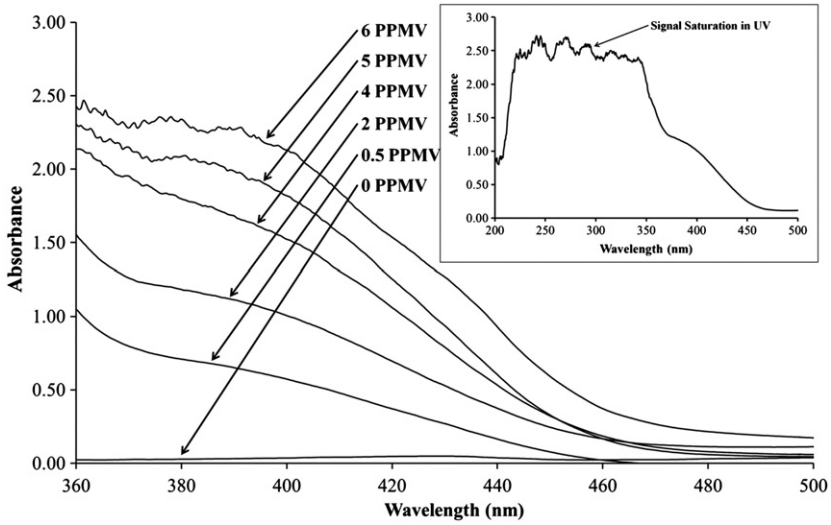

Fig. 2. Ultraviolet and visible spectrum of resorcinol imbibed Nafion® membranes exposed to varying concentrations of acetone (as indicated) in a sealed flask. Inset shows full spectrum for 2 ppmv.

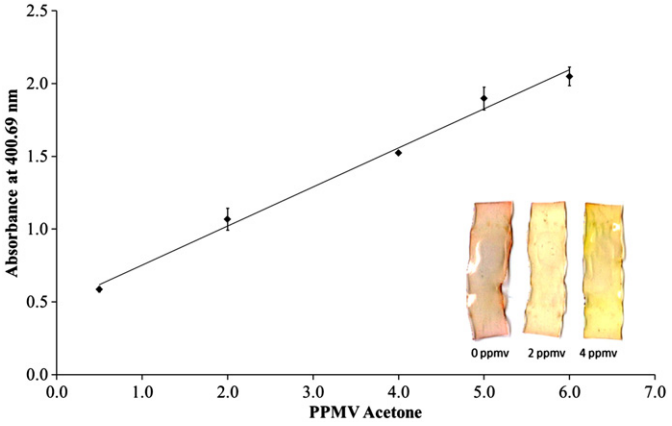

Fig. 3. Ex-situ absorbance response of resorcinol-imbibed membranes at 400.69 nm after exposure to acetone. Error bars indicate ± one standard deviation from the data collected. The inset shows sample membrane images taken prior to (0 ppmv) and shortly after exposure to acetone.

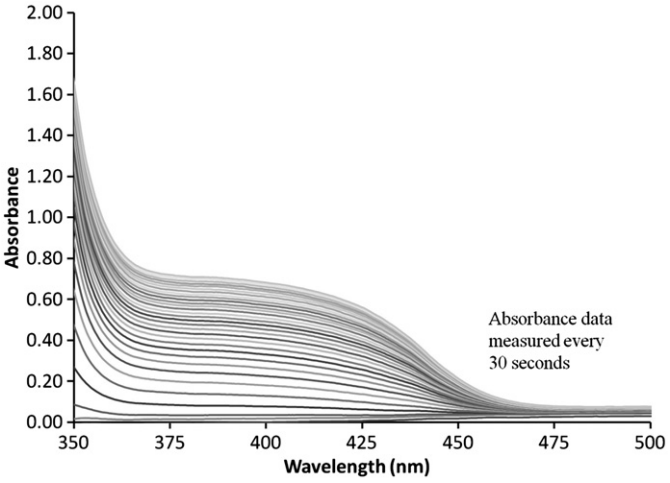

Fig. 5. Time elapsed absorbance spectra for membrane exposed to 0.64 ppmv acetone in in-situ flow cell system. Samples were taken in 30 s intervals for 15 min.

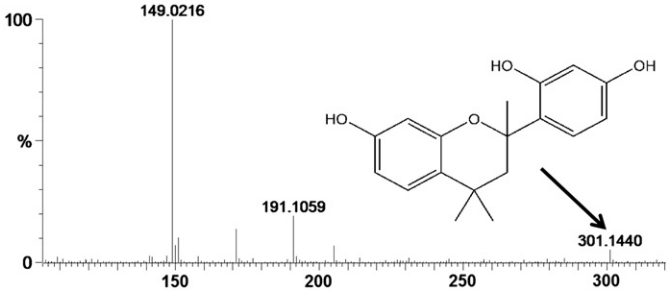

Fig. 4. Partial electrospray mass spectrum of extracted product from acetone-exposed Nafion® membrane.

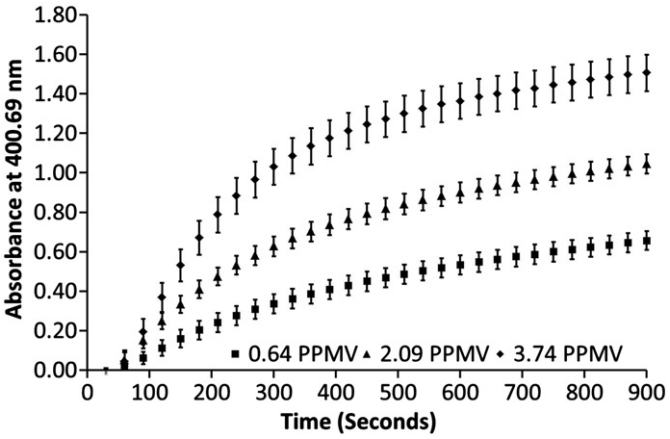

Fig. 6. Time dependent absorbance data at 400 nm for three concentrations of acetone produced using in-situ flow cell. Error bars indicate ± one standard deviation from the data collected.

background is taken for the system after a purge of nitrogen gas through the chamber. A fixed volume of the sample gas is released into the sealed chamber, and it is warmed to the desired temperature for the reaction by a ceramic heater and diffuses into the membrane where it reacts with the resorcinol immobilized in the membrane. This reaction with the resorcinol dye produces an

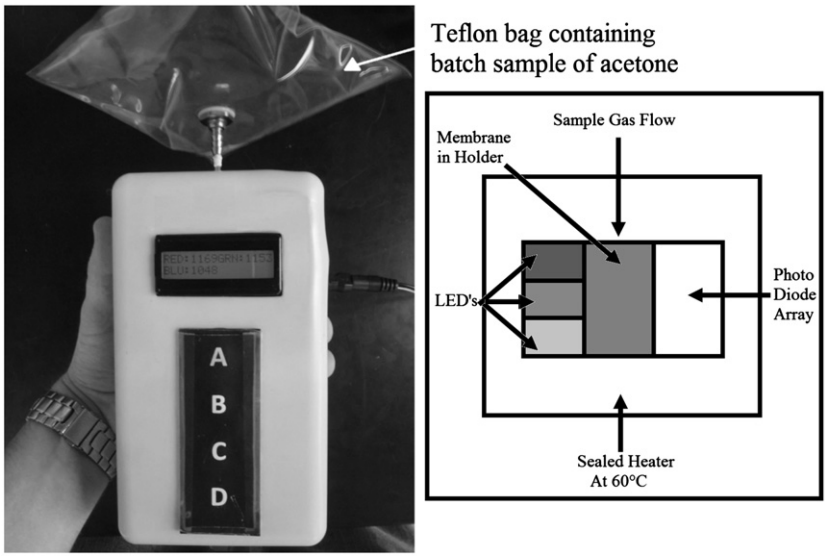

Fig. 7. Prototype for in situ optical detection and schematic of operation. Also shown is the Teflon bag attached to prototype and containing known concentrations of acetone.

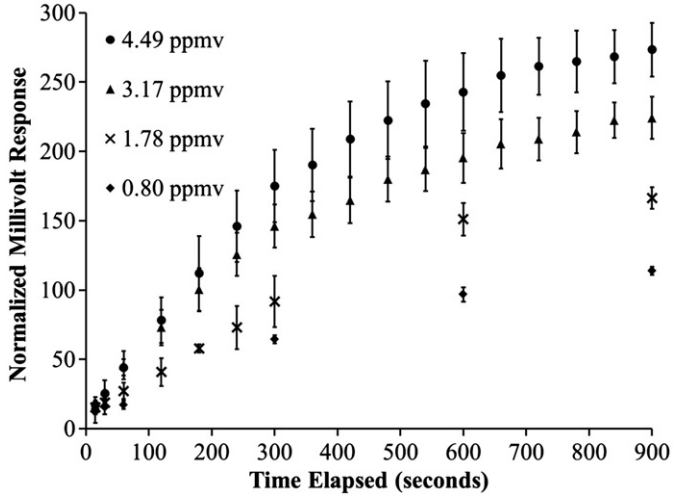

Fig. 8. In-situ time dependent response of prototype containing Nafion membranes to various concentrations of acetone. Error bars indicate ± one standard deviation from the data collected.

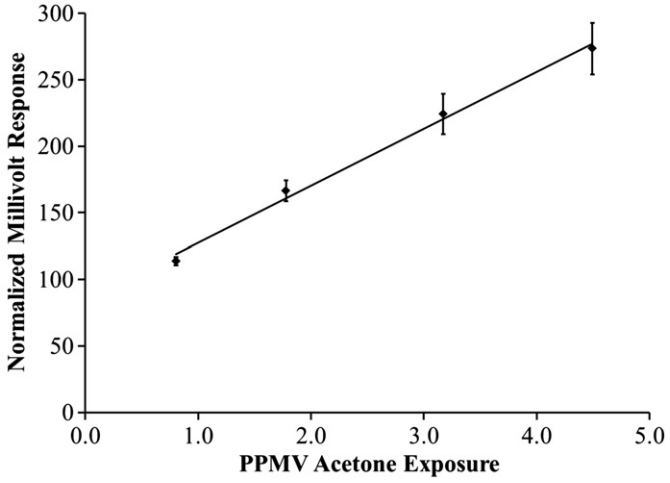

Fig. 9. Comparison of absorbance data for in situ prototype response after 15 min of various acetone concentrations on resorcinol containing Nafion® membranes and UV/vis data for the same samples. Error bars indicate ± one standard deviation from the data collected.

observable color change which is measured through a voltage change in the photodiode array. The system is set to output a scale of millivolt readings from the photodiode array which enables the same capabilities as those of a standard spectrometer.

The observed change in response is recorded at various times during the exposure as shown in Fig. 8. After 15 min of exposure, it was found that the response leveled off for all concentrations present in the system. Each exposure concentration levels off at a different absorbance, indicating either that all of the acetone in the system has been reacted with the resorcinol imbibed membrane or that partition equilibrium in membrane versus ambient conditions has been attained. Fig. 9 shows a correlation of absorbance response from the blue LED on the photodiode array and exposure concentration for the same samples after 15 min of exposure. The acetone concentrations for this figure were determined measuring the absorbance of the membranes ex situ after completion of the sensor system experiment.

Interestingly, a larger variance at higher acetone concentrations is observed both in the spectrophotometer data (Fig. 6) and also in the photodiode potentials of our LED-based prototype (Fig. 8). Such an increase is likely due to the fact that the samples for both types of measurement were prepared using a bubbler to saturate a nitrogen carrier, and then diluting this carrier with additional nitrogen, as previously described. Thus, the bubbler stream contributes successively larger fractions to the overall measurement error as the acetone concentration is increased. Such consistency between the two analyses provides further validation of the LED-based sensing approach.

With this portable breath analysis system, the concentration of acetone present can be determined in future clinical breath tests without the need for bulky equipment or off-site laboratory testing. This method has the potential to reduce the work necessary for clinical studies that attempt to further validate breath analysis techniques as non-invasive surrogates for blood testing. This portable breath analysis system could also be used to provide further insight into not only the diagnostics and control of

diabetes but also in control of weight loss and in monitoring of dietary based seizure treatments in epileptic patients [20].

In addition to acetone, other volatile organics are present in human breath in significant quantities such as methyl-, ethyl-, and propyl-alcohol [10,21] and may present potential interferences. We have determined that our system does not undergo a colorimetric response to these compounds under the same exposure conditions as those of acetone. This observation is consistent with the fact that the alcohols are unlikely to react with resorcinol under these conditions. Ketones other than acetone are present in human breath in much smaller proportions (<30 ppb levels relative to ppm levels) and include butanone and 2-propanone [21,22]. We have therefore not attempted to calibrate their response.

Our future work will focus on identifying the immobilized reagents necessary for the detection and measurement of other organic compounds in human breath at ppb levels as well as to screen whether such compounds interfere with the quantification of acetone levels using this approach. Such multi-gas detection capability is necessary to de-convolute the complex metabolic processes that are responsible for their generation. Furthermore, the presence of water in sufficient quantities can have a significant influence on the observed response. The relative humidity in a human breath sample (originally at 100%) collected at ambient temperatures will be reduced to about 15% at 60 °C temperature of the sensing reaction. Even at this level, we have determined that water can affect the optical response. Such behavior is due to the presence of water as a side-product of the reaction scheme depicted in Fig. 1 as well as de-protonation and consequent de-activation of the catalytic perluorosulfonic acid centers within the membrane. As noted in the experimental detail, the in-situ measurements and associated calibrations reported in this paper were performed in nitrogen. Thus, they would apply only to breath samples from which water has been condensed out or otherwise removed. We currently have work on-going to mitigate the impact of this requirement.

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