

Fast Degradation of Hydrogen Peroxide by Immobilized Catalase to Enable the Use of Biosensors in Extraterrestrial Bodies

Paige A. Reed,^{1,*} Bryan A. Lagasse,^{1,2,*} and Carlos D. Garcia¹

Abstract

Hydrogen peroxide has been postulated to be present on the surface of Europa and Enceladus. While it could represent a potential source of energy for possible life-forms, H₂O₂ may also interfere with a number of current detection technologies, including biosensors. To take advantage of the selectivity and portability of these devices, simple and reliable routes to degrade the potential H₂O₂ present should be developed and implemented to prepare for this possibility. Unfortunately, most of the current approaches for removing H₂O₂ are slow, may affect the sample, or could interfere with the performance of biosensors. To address these limitations, catalase was immobilized onto silica particles and used as a means to selectively decompose H₂O₂ prior to the analysis of common biomarkers with a biosensor. For these experiments, glucose, L-leucine, and lactic acid were used as representative examples of biomolecules such as carbohydrates, amino acids, and organic acids, respectively, which could be used as biomarkers on extraterrestrial bodies. While the decomposition reaction between catalase and H₂O₂ is well known, to our knowledge this is the first instance where catalase has been used in combination with a microfluidic paper-based analytical device (μPAD) to implement selective sample pretreatment. Key Words: Peroxide—Catalase—Biosensor—μPAD. *Astrobiology* 21, xxx–xxx.

1. Introduction

SINCE THE PRESENCE of a liquid environment is considered a requirement for the development of life (as we know it), there is a growing interest in the exploration of extraterrestrial oceans. Among these so-called Ocean Worlds, Europa, a moon of Jupiter, and Enceladus, a moon of Saturn, are of particular importance. Both of these moons are thought to harbor a subsurface ocean potentially capable of supporting some form of biological life associated with the use of H₂O₂ as an energy source (Carlson *et al.*, 1999; Gomis *et al.*, 2004). Following this hypothesis, a number of reports have proposed that, if H₂O₂ is present below the icy shell and can be circulated through the subsurface oceans, it could indicate the presence of a radiation-driven ecosystem (Chyba, 2000; Dartnell, 2011; Ball and Brindley, 2015, 2019) similar to processes that are posited to have existed on Earth prior to photosynthesis (Haqq-Misra *et al.*, 2011). It is also important to mention that, while several papers have pointed to the potential presence of H₂O₂ in other extraterrestrial bodies such as Mars (Encrenaz *et al.*, 2012), many other groups have

also provided alternative explanations for those observations and considered that neither H₂O₂ nor superoxide is required to explain the results collected by Viking LR and GEx probes (Houtkooper and Schulze-Makuch, 2007; Quinn *et al.*, 2013). Regardless of the concentration, the potential presence of H₂O₂ in these samples represents a particular challenge for the detection of biomarkers, including amino acids, organic acids, and carbohydrates (Georgiou *et al.*, 2016).

Historically, the standard technique applied for the detection of biomarkers on extraterrestrial bodies has been gas chromatography coupled to mass spectrometry (Pietrogrande, 2014). However, the environment believed to exist on Europa and Enceladus may also hinder the implementation of this approach because the H₂O₂ could interfere with the breakdown of organic molecules during the pyrolysis step (Benner *et al.*, 2000; Navarro-González *et al.*, 2006). To address this limitation, a number of liquid-based analytical techniques have been proposed (Blanco *et al.*, 2017; Ribette *et al.*, 2019). Out of those, immunoassays, electrochemical sensors (Thomson *et al.*, 2020), and microfluidic capillary electrophoresis technologies hold the most

¹Department of Chemistry, Clemson University, Clemson, South Carolina, USA.

²Department of Chemistry and Life Science, United States Military Academy, West Point, New York, USA.

*Authors contributed equally to the work.

promise in terms of power requirement, weight, sensitivity, and ability to detect biomarkers in model systems (Derveni *et al.*, 2012; Sims *et al.*, 2012; Willis *et al.*, 2012; Butterworth *et al.*, 2015; Mathies *et al.*, 2017, 2019; Moreno-Paz *et al.*, 2018; García-Descalzo *et al.*, 2019; Lezcano *et al.*, 2019). Unfortunately, even low concentrations of H_2O_2 present in the sample could interfere with all of these platforms because it may induce structural changes in the antibodies (required for selectivity in immunoassays) or through oxidizing the dyes used in fluorescence-based systems (Stockton *et al.*, 2013). Therefore, addressing the potential presence of H_2O_2 seems to be a requirement to implement current methods to detect biomarkers on Ocean Worlds. In this regard, some of the most common methods to degrade H_2O_2 in biological samples are based on the use of metal catalysts, such as the Fenton reaction (Goldstein *et al.*, 1993) or UV radiation (Gardner and Shama, 1998). While these methods are able to decompose H_2O_2 , the kinetics and efficiency of these reactions can be significantly affected by the sample composition and/or may lead to significant temperature changes (Wang, 2008). Furthermore, methods based on UV degradation could potentially affect the composition of the sample due to the poor selectivity of the hydroxyl radicals formed, leading to the degradation of the analytes (Gardner and Shama, 1998; Wang *et al.*, 2006; Ferreira Santos *et al.*, 2019).

Aiming to provide a fast, efficient, and selective method to degrade H_2O_2 , this manuscript describes the possibility of integrating immobilized catalase as a sample pretreatment step, enabling the analysis of biological samples via the use of oxidase enzymes on microfluidic paper-based analytical devices (μ PADs). This enzymatic approach was selected considering that catalase is not only selective but also features one of the highest turnover numbers in nature. Furthermore, dried catalase has been shown to maintain its enzymatic activity even in the presence of up to 3 Gy of γ radiation (Georgiou *et al.*, 2016), which makes this enzyme well suited for extraterrestrial sample analysis. Similarly, aqueous samples maintain their catalytic activity when exposed to γ radiation; however, there is a more significant decrease in activity as the dose, dose rate, and total exposure time increase (Cuba *et al.*, 2010). It is also important to note that the aqueous catalase samples exposed to γ radiation up to 50 Gy at a rate of 5.5 Gy h^{-1} were able to degrade 89% of the H_2O_2 in the sample at 27°C (Cuba *et al.*, 2010). Given both the efficiency of catalase and its ability to maintain catalytic activity after exposure to γ radiation, this simple hydrogen peroxide removal method could aid in the selection of samples potentially containing the selected biomarkers that are to be confirmed by other techniques.

2. Methods

Catalase from bovine liver (240 kDa, isoelectric point = 5.4) was purchased in aqueous solution, with an activity greater than 40,000 units per mg of protein (Worthington Biochemical Corporation, Lakewood, NJ, USA). Hydrogen peroxide was purchased as 35% w/w (VWR International, Radnor, PA, USA) and diluted with deionized water (Milli-Q, Millipore Water Systems, Billerica, MA, USA). Fresh dilutions were prepared daily to ensure that the concentration of H_2O_2 did not fluctuate due to thermal or photolytic

decomposition. Phosphate buffer solutions (PBS) were prepared by diluting sodium phosphate monobasic (Fisher Scientific, Pittsburgh, PA, USA) in deionized water to a final concentration of 100 mM. The pH of the solution was measured by using a glass electrode connected to a digital pH meter (Orion 420A+, Thermo, Waltham, MA, USA) and adjusted to 7.0 with 2 M NaOH (Sigma-Aldrich, Milwaukee, WI, USA). Lactic acid solution ($\leq 85\%$), glucose, and D/L-leucine were all purchased from Sigma-Aldrich (Milwaukee, WI, USA). Stock solutions of glucose, lactic acid, and L-leucine were prepared by using phosphate buffer (pH 7.0) and diluting to a concentration of 100 mM. Enzyme solutions containing horseradish peroxidase and either glucose oxidase (from *Aspergillus niger*), L-amino acid oxidase (from *Croatalus adamanteus*), or lactate oxidase (from *Pediococcus* sp.) were made by diluting the enzymatic powder in PBS to a value of 1 mg mL^{-1} .

The μ PADs used were fabricated by using Whatman 3MM chromatography paper (21427-395 VWR International, Radnor, PA, USA). To ensure uniform size, all μ PADs were patterned with a 30 W CO_2 laser engraver (Epilog Legend Mini Helix 24, Epilog Laser, Golden, CO, USA), set to 50% power and 50% speed (vector mode). The μ PAD design was created with Adobe Illustrator 2019 (Adobe Inc., San Jose, CA, USA). The final design featured three detection zones, each with a diameter of 7.62 mm, and was labeled for identification of the respective oxidase enzyme deposited in the detection zones (G = glucose, L = lactate, and A = leucine) by using the raster mode (60% speed, 50% power) on the laser engraver. The detection zones were connected with small channels, width of 1.63 mm and length of 8.25 mm, in order to prevent the enzymes from mixing when the liquid sample was introduced. A channel (1.63 mm wide by 7.29 mm long) at the bottom of the design allowed the device to be used as a “dipstick” or as a more classical μ PAD where sample solutions are pipetted directly onto the device.

Catalase was selected as a catalyst for the degradation of H_2O_2 in the original sample. Because even a small amount of the enzyme leaching to the detection zones would interfere with the analysis, the enzyme was immobilized onto silica particles, according to a previously reported method (Evans *et al.*, 2014). Briefly, 0.1 g of silica beads was added to 10 mL of a solution of 5% v/v (3-aminopropyl) triethoxy silane (APTES) in ethanol (99.9% pure, LC grade) and thoroughly mixed for 3 h. The functionalized silica beads were then centrifuged at 9000 RPM for 4 min, had the supernatant removed, and were rinsed with 2 mL of fresh methanol in order to remove any remaining APTES in solution. Next, the silica beads were rinsed twice in 2 mL aliquots of 100 mM PBS with centrifugation steps between each rinse. The silica beads were then placed in 1.5 mL of fresh 100 mM PBS with 0.5 mL of a stock solution containing catalase. This solution was agitated to form a uniform suspension and incubated at 2°C for 30 min. The silica beads were then washed with fresh 100 mM PBS and finally stored in 2 mL of 100 mM PBS at pH 7.0 for a maximum of 24 h.

H_2O_2 was quantified colorimetrically through the oxidation of either iodide (producing a red-brown color) or 3,3',5,5'-tetramethylbenzidine (TMB, to generate a blue color). In both cases, images were taken with a CanoScan

LiDE 7005 flatbed scanner (Canon Inc., Melville, NY, USA) after 15, 30, 45, and 60 min of exposure to the sample. Analysis of the color intensity and gradient was performed by Adobe Photoshop 2019 (Adobe Inc., San Jose, CA, USA) in a method similar to our previous work (McCann *et al.*, 2017). In all cases, 5.0 μ L of oxidase enzyme was pipetted onto the detection zone of the μ PAD and allowed to dry for 15 min under ambient conditions. Afterward, 3.0 μ L of 1% TMB in methanol was pipetted over the enzyme and allowed to dry for 15 min. An aliquot of 220.0 μ L of sample was placed into a well on a 96-well plate. Samples were diluted with 30.0 μ L of either fresh PBS (pH 7.0, control) or a suspension of catalase immobilized on silica beads (Cat-SiB). Once the μ PAD dried, the injection region was dipped into the sample well until the sample wicked throughout the entire device ($\sim$ 3 min); then the μ PAD was placed in a Petri dish to dry under ambient conditions.

Enthalpimetry experiments were conducted by placing 100 μ L of a 3.0% w/w H₂O₂ solution in one well of a 96-well microplate. An infrared camera (FLIRONE Pro iOS version, FLIR Systems Inc, Wilsonville, OR, USA) was attached to an iPhone X (Apple Tech Co., Cupertino, CA, USA) and positioned to observe the temperature change in the sample when catalase was introduced by focusing the camera in thermal MSX mode on the well with the peroxide sample (Lagasse *et al.*, 2020). Once the recording of the sample was initiated, 100 μ L of the catalase was injected into the sample well. The change in temperature was recorded by the IR camera, and 3 μ L aliquots of the sample were removed every 15 s and spotted on 8.38 mm diameter circles of chromatography paper pretreated with a 2.0 M KI solution dried on the surface. These circles were dried under ambient conditions, scanned, and evaluated by the method previously described. The change in temperature was compared to the change in the intensity of the color produced from the oxidation of iodide to determine the relationship between temperature change and reaction progression.

To test the ability of transition metals in the decomposition of H₂O₂ via the Fenton reaction, solutions containing Fe(II), Fe(III), Co(II), and Cu(II) were prepared. Copper (II) chloride dihydrate, copper (II) acetate monohydrate, cobalt (II) acetate tetrahydrate, iron (III) oxide, and potassium hexacyanoferrate (III) were purchased from Sigma-Aldrich (Milwaukee, WI, USA). With the exception of iron (III) oxide, all other metal solutions were diluted to a concentration of 1.0 M in deionized water (Barnstead Nanopure Diamond, Thermo-Fisher Scientific, Waltham, MA, USA). As iron (III) oxide is insoluble in water, a suspension was created by placing 0.5222 g of the powdered iron (III) oxide (<5 μ m particle size) in 1000 μ L of ethanol. In all transition metal experiments, 10 μ L of the solution was deposited on a central region of the μ PAD and allowed to dry under ambient conditions. A 5.0 μ L aliquot of 2.0 M KI was placed on the detection region of the μ PAD to determine whether the H₂O₂ in the sample was decomposed, similar to the colorimetric method described above. Finally, a 10.0 μ L sample containing the analyte and 3.0% w/w H₂O₂ was injected onto the μ PAD, and the region of the μ PAD with the transition metal was exposed to UV light at 365 or 405 nm by a Uvitron Skybeam LED Spot curing system (Uvitron International, West Springfield, MA, USA) with a 6 mm lens providing an intensity of 5980 mW/cm².

To monitor the decomposition of H₂O₂, samples were analyzed by UV/vis spectrometry using a Genesys10 UV/vis spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) at 240 nm. The decrease in absorbance due to H₂O₂ decomposition was monitored as a function of time. The volume of the silica particle suspension was varied to determine the relationship between the volume of suspension and the initial rate of peroxide decomposition.

3. Results

To provide a direct comparison with established methods, the degradation of H₂O₂ was initially investigated by using the Fenton reaction (Goldstein *et al.*, 1993) and UV radiation (Gardner and Shama, 1998). These initial experiments were performed with a solution containing 3% w/w H₂O₂, which mimics the maximum possible concentration expected on Mars, according to spectroscopy data (Encrenaz *et al.*, 2012). As can be observed in Fig. 1A, the addition of Fe₂O₃ and UV radiation to the sample containing H₂O₂ led to a significant increase in temperature, reaching an average of 114°C within 30 s (maximum temperature 167.7°C). Even under these conditions, residual amounts of peroxide were observed, evidencing the limitations of the approach. While a clear color change was observed in the detection region, which correlated with the oxidation of KI, it was not possible to quantify the amount of peroxide remaining in the sample due to the scorching of the μ PAD substrate (high temperatures produced). This further supported the preliminary conclusion that the Fenton reaction method would not be feasible for a μ PAD system. On the other hand, when catalase was added to the solution containing H₂O₂, a fast degradation of hydrogen peroxide was observed (2H₂O₂ $\rightarrow$ 2H₂O + O₂) along with only a mild increase in the temperature (+10°C, Fig. 1B). As can also be seen in Fig. 1B, only 30 s was required for catalase to decompose the initial H₂O₂, supporting the suitability of the proposed approach (signal corresponding to remaining H₂O₂ within noise values).

Next, the effect of the methodology selected to incorporate catalase into the μ PADs was investigated. As the simplest approach, catalase was immobilized on the sample injection pad by simple adsorption. In this case, 15 μ L of catalase was dispensed and allowed to dry for 15 min under ambient conditions. The efficiency of this approach was then investigated by dispensing 30 μ L of a solution containing 3% w/w H₂O₂ and 10–100 mM glucose on the pad, allowing the sample to wick through the device, and finally quantifying the concentration of glucose in the detection spot of the device. According to these experiments (data not shown), it was observed that the enzyme was capable of rapidly decomposing H₂O₂. However, a significant decrease in the signal measured in the detection region was also observed. This issue was attributed to the migration of catalase along the channel and into the detection region. This behavior can be related to the high structural stability of the enzyme, which prevents surface-induced changes in the structure on the protein that would otherwise favor the adsorption of the enzyme onto the surface of the substrate. Fig. 2A shows a representative example of the displacement of catalase (stained with Coomassie Blue) in a simple μ PAD, from the injection zone into the detection zone.

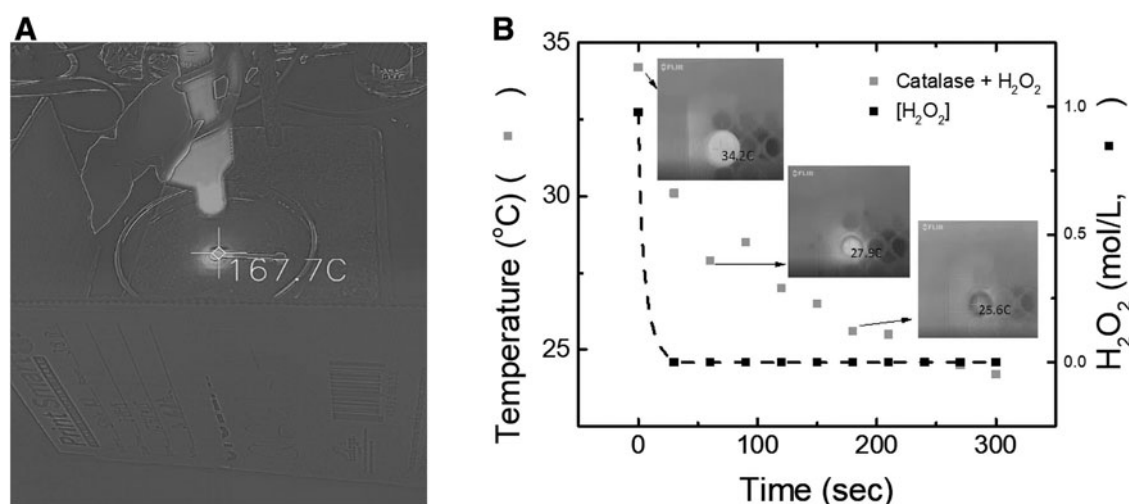


FIG. 1. (A) Thermal image of a solution (100.0 μL) containing 100 mM glucose and 3% w/w H_2O_2 upon the interaction with Fe_2O_3 and UV light (365 nm). (B) Dependence of both the temperature and remaining amount of H_2O_2 in a sample containing 100 mM glucose and 3% w/w H_2O_2 upon the interaction with catalase (100.0 μL containing 4100 units). Line included to guide the eye.

As can be observed in Fig. 2A, the free catalase was transported from the injection region with the sample as it wicked across the device by capillary action. This caused a significant decrease in the resulting signal. It is posited that this decreased signal is a result of the catalase decomposing the H_2O_2 produced by the oxidase enzymes and a competitive inhibition of the oxidase enzymes.

To prevent this issue, catalase was immobilized on a solid substrate. In this case, silica microbeads were selected due to their uniform size (20 μm , which prevents their migration

through the paper) and ability to be rapidly functionalized (Evans *et al.*, 2014). As catalase does not readily adsorb to silica beads at neutral pH values, particles modified with APTES were used as the support (Cat-SiB). As can be observed in Fig. 2B, this strategy maintains the enzyme in the selected location and prevents its interference with the oxidases used to detect the biomarkers. Figure 2C shows the results corresponding to calibration curves obtained for glucose in the presence of catalase immobilized by different methodologies. It should be noted that, when catalase is not

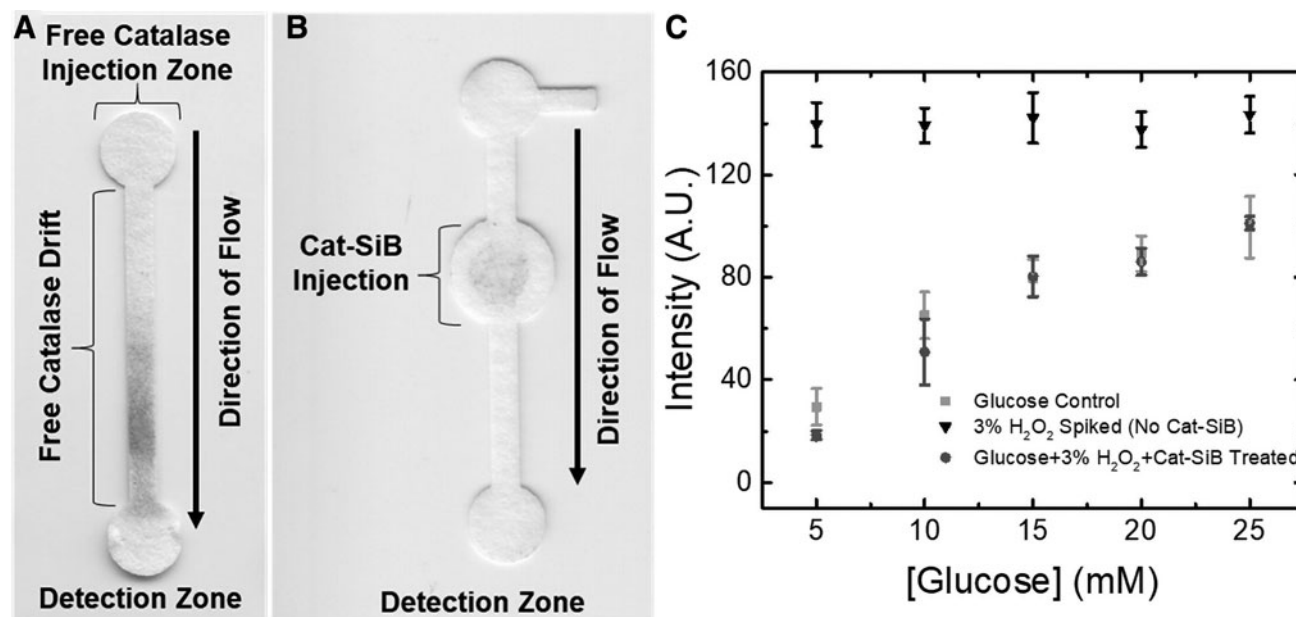


FIG. 2. (A) Photograph of a μPAD (prepared by adsorbing catalase on the paper) showing the location of the enzyme after injecting 20 μL of 100 mM PBS. (B) Photograph of a μPAD (prepared by immobilizing catalase on the silica beads) showing the location of the enzyme after injecting 20 μL of 100 mM PBS. (C) Color intensity produced at the detection spot upon the injection of a sample; all experiments had glucose oxidase and TMB in the detection region. Control experiments used samples with varying concentrations of glucose in PBS. H_2O_2 -spiked samples had the additions of 3% w/w H_2O_2 in the injected samples. Cat-SiB had 30.0 μL of Cat-SiB suspension in PBS injected into sample containing glucose at varying concentrations and 3% w/w H_2O_2 .

present, the initial H₂O₂ contained in the sample is able to reach the detection zone and react with the chromogenic agent, leading to a signal that is independent of the glucose concentration. On the contrary, when the sample containing glucose and H₂O₂ was treated with Cat-SiB, a trend matching that obtained with plain glucose was obtained. This indicates that catalase is not only active but also able to degrade the H₂O₂, avoiding the interference of both H₂O₂ (which would give a false positive) and catalase (which would give a false negative).

To study the kinetics of the Cat-SiB, the decomposition of peroxide was monitored at various volumes of the Cat-SiB suspension with UV spectroscopy. A comparison of the volume of Cat-SiB suspension to the total volume (v/v%) is shown in Fig. 3. It was found that a 7.5 v/v% of Cat-SiB to sample was adequate to fully decompose 65 mM H₂O₂ in less than 5 min. Additionally, due to the noncovalent bond between the APTES-coated silica beads and the catalase, it was found that an equilibrium process resulted in free catalase present in the buffer solution after approximately 24 h when the suspension was stored at 2°C. When the experiments were scaled down to the volumes for the microwell plates, it was found that the degradation of the 0.98 M H₂O₂ samples was completed within 5 min with no degradation of the analyte signal. Previous reports have defined one unit of catalase as the amount required to decompose 1 μmol of H₂O₂ per minute at pH 7.0 and 25°C. Based on the known volume of stock catalase used to prepare the Cat-SiB suspension and the rate of degradation, it was possible to determine the number of units of catalase required for this method (Felhofer *et al.*, 2010). It was calculated that at a ratio of 7.5% v/v Cat-SiB a minimum of 60 units of catalase were present to achieve the necessary initial H₂O₂ degradation rate.

To show that the inclusion of immobilized catalase had no detrimental effects on the detection of the selected analytes, calibration curves of glucose, L-leucine, and lactic acid were generated in the presence and absence of 7.5 v/v% of Cat-SiB. The sensitivity with and without catalase was not significantly different, and the method did not interfere

with analyte detection (94 ± 8% signal relative to the control). It was also found that the intensity of color that was produced decreased after 30 min. As such, 30 min post sample introduction was used as the scan time for the duration of the study. All three selected analytes (glucose, L-leucine, and lactic acid) were analyzed simultaneously, and the only color change observed was the one that corresponded to the analyte of interest (*i.e.*, L-leucine with L-amino acid oxidase). This final test confirmed that each respective oxidase enzyme allowed for detection of its associated analyte with no cross contamination.

4. Discussion

While the exact concentration of hydrogen peroxide on extraterrestrial bodies, such as Europa and Enceladus, remains a point of discussion and interpretation, some estimations have been given based on obtained spectroscopic information. For example, hydrogen peroxide was posited to be present in the leading atmosphere of Europa due to a 3.5 μm infrared adsorption band and inferred from ultraviolet reflectance spectra. Based off the Near-Infrared Mapping Spectrometer (NIMS) spectrum and comparison to laboratory samples of frozen hydrogen peroxide at different concentrations, Carlson *et al.* report a concentration of 0.13 ± 0.07% w/w (Carlson *et al.*, 1999). While this interpretation is considered the most extensive, it is not indisputable quantitative evidence. Additionally, more recent investigations suggest lower concentrations of hydrogen peroxide (~0.04% w/w) and argue that the original observations represent a local maximum (Hand and Brown, 2013). Similarly, a 3.5 μm IR band recorded on Enceladus by Cassini's Visual and Infrared Mapping Spectrometer (VIMS) has been contested as to whether or not it originated from hydrogen peroxide (Newman *et al.*, 2007; Loeffler and Baragiola, 2009). Regardless of the interpretation, even minute concentrations of hydrogen peroxide could interfere with analysis of biomarkers. In our experiments, samples that contained 1% w/w (0.33 M) and were not pretreated

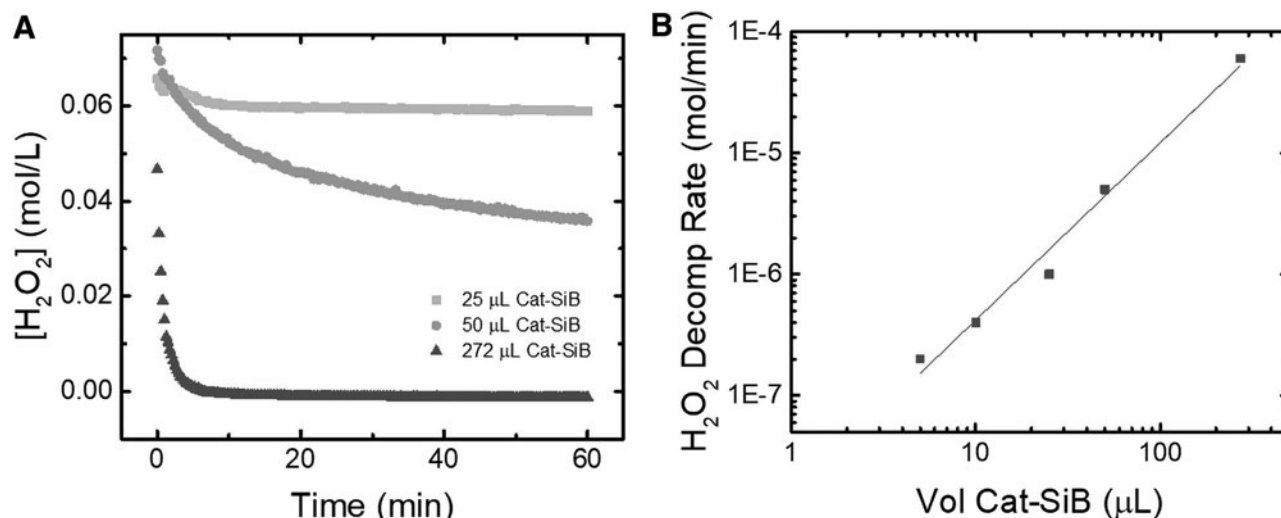


FIG. 3. (A) Measurement of absorbance from 65 mM H₂O₂ sample at $\lambda = 240$ nm via UV spectroscopy with different volumes of Cat-SiB causing degradation over time; the decrease in concentration of H₂O₂ is dependent on the volume of Cat-SiB suspension added to the sample. (B) Linear relationship between the initial rate of decomposition of H₂O₂ and the volume of the Cat-SiB suspension.

with Cat-SiB still displayed a strong oxidation of the TMB and KI used as the colorimetric dye. This suggests that, if H_2O_2 is present on extraterrestrial bodies, some form of sample pretreatment would aid in the identification of biomarkers.

It is well known that transition metals and ultraviolet irradiation catalyze the decomposition of hydrogen peroxide to form hydroxyl radicals (Weiss, 1935). This process is well established in environmental fields for the treatment of wastewaters as the hydroxyl radicals rapidly oxidize the natural organic compounds found therein (Wang *et al.*, 2006). While metal-catalyzed peroxide decomposition is effective, with rate constants ranging from $7.1 \times 10^2 \text{ s}^{-1}$ for platinum to $4.57 \times 10^{-4} \text{ s}^{-1}$ for iron III oxide (Yeremin, 1979; Lousada *et al.*, 2013), they present several drawbacks for biological samples including the decomposition of organic molecules. While the adsorption of the peroxide onto a solid metal catalyst has been found to be a relatively fast process, the decomposition of peroxide is a relatively slow process that lasts for hours. This large reaction time can increase radical formation due to stabilization of the adsorbed species on the oxide surface. These usually short-lived species can then self-terminate to reform H_2O_2 (Mancuso *et al.*, 1999; Lousada *et al.*, 2013). Additionally, these Fenton-like processes are optimized for acidic media (Huang *et al.*, 2001), which is in contrast to the neutral or alkaline conditions proposed on Mars, Europa, and Enceladus (Soffen, 1976; Kempe and Kazmierczak, 2002; Glein *et al.*, 2015). Not only would lowering the pH of samples be labor intensive, but it could also potentially negatively affect any organic molecules in the sample. Additionally, it was found that iron III oxide immobilized on chromatography paper in combination with a 365 nm high-intensity light source significantly increased the temperature to a maximum of 167°C . This not only burned the μPAD substrate but also led to a potential loss of analyte by exothermic means. Thus, despite the widespread use of transition metal oxides and UV light for peroxide decomposition, Fenton-type reactions and UV decomposition methods were deemed incompatible for biosensors.

Alternatively, enthalpimetry experiments proved that free catalase could decompose 3% w/w H_2O_2 at a neutral pH, in under 30 s, with only a 10°C temperature increase. While small volumes ($100 \mu\text{L}$) of free catalase were capable of rapidly decomposing H_2O_2 , a significant decrease in the signal in the detection region was observed when free catalase was applied to μPADs . It was determined that the catalase was transported via capillary action into the detection region, thereby decomposing the H_2O_2 produced by the analyte-enzyme reaction and weakening the resulting signal. By immobilizing the enzyme on silica microbeads, it is possible to eliminate this signal degradation. While the efficiency of the catalase immobilization process is relatively low (approximately 90% of units lost) due to the reversible nature of electrostatic interactions, the method was capable of decomposing 3% w/w H_2O_2 in under 5 min with no negative impact on the biomolecule analytes. Furthermore, the immobilization onto a solid substrate allows compatibility with common liquid sampling techniques and possible integration into current spaceflight technologies. A main advantage of the Cat-SiB method is that it converts the homogeneous catalyst of catalase solution into a heterogeneous catalyst, which can be removed from samples.

5. Conclusions

The proposed method of eliminating H_2O_2 that is present in a sample by utilizing silica particle immobilized catalase was shown to be effective. It is first necessary to immobilize the catalase on the surface of silica beads, which are then placed in a suspension and injected into the sample. The method degrades up to 3% w/w H_2O_2 in a sample with no adverse effects on the analytes. This concentration of H_2O_2 is more than double the potential concentration on extraterrestrial environments, which makes the enzymatic degradation of H_2O_2 by catalase a potential method for the identification of biologically relevant molecules.

Acknowledgments

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Address correspondence to:
 Carlos D. Garcia
 Department of Chemistry
 Clemson University
 211 S. Palmetto Blvd
 Clemson, SC 29634
 USA

E-mail: cdgarci@clemson.edu

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Abbreviations Used

APTES = (3-aminopropyl) triethoxy silane
 Cat-SiB = catalase immobilized on silica beads
 μPAD = microfluidic paper based analytical device
 PBS = phosphate buffer solution
 TMB = 3,3',5,5'-tetramethylbenzidine