MINIATURIZED CAPSULE SYSTEM FOR HYDROGEN SULFIDE DETECTION IN THE GASTROINTESTINAL TRACT

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ABSTRACT

This work presents an ingestible capsule platform for real-time, wireless detection of hydrogen sulfide (H_2S) as a biomarker of inflammation in the gastrointestinal (GI) tract. The design integrates an analog front-end (AFE) potentiostat and Bluetooth Low Energy (BLE) microcontroller on a flex-rigid printed circuit board (PCB) with a miniaturized electrochemical H_2S sensor to perform amperometric measurements with the desired capsule form factor. Packaged within a 3D-printed shell, the capsule successfully monitors physiological H_2S concentrations within a linear range of 3.7–12 ppm with sensitivity of 0.17 µA/ppm, providing a framework for non-invasive, on-site monitoring of gaseous biomarkers in the GI tract.

KEYWORDS

Ingestible Capsule, Hydrogen Sulfide, Gastrointestinal Tract

INTRODUCTION

Digestive diseases, such as inflammatory bowel disease (IBD), have been intimately linked with alterations of the gut microbiome and are a chronic source of intestinal inflammation; however, the underlying etiology is unclear [1]. Understanding the interactions between intestinal microbiota and epithelial tissue dysregulation would inform disease diagnosis and treatment. Transient gaseous molecules, such as H_2S, nitric oxide (NO), and hydrogen peroxide (H_2O_2), are key mediators of inflammation within the GI tract, and have been implicated as potential biomarkers for disease screening [2]. These biomarkers are short-lived, making it difficult to accurately detect their concentration in the body [3]. Thus, non-invasive approaches to accurately determine the role of the gut microbiome in the development of GI disorders are needed.

Of the inflammatory markers mentioned, endogenous H_2S – produced by sulfate-reducing bacteria (SRB) in the intestinal microbiome – is involved in both pro- and anti-inflammatory processes, providing a non-specific marker to diagnose GI inflammation [4]. However, current methods for monitoring gut microbial H_2S production in real-time are lacking. Previous studies have measured H_2S concentrations in flatus, which can be highly invasive, and via stool analysis, which lack the temporal and spatial resolution to accurately represent the current state of the intestines [5]. Breath testing also is not sufficient, as little gut microbial-produced H_2S reaches the breath due to its short circulating half-life and measurements are susceptible to interference from H_2S produced by oral microbes, which are indistinguishable [6]. Therefore, miniaturized systems capable of real-time and localized monitoring of bacteria-derived inflammatory markers *in situ* are essential to detect transient markers.

Existing endoscopic surveillance methods rely on direct visualization of the mucosa and are unable to detect molecular biomarkers of microscopic disease [7]. Recently, endoscopic capsules, such as the PillCam SB [8] and Atmo Capsule [9], have been utilized to non-invasively access the small bowel, enabling visual inspection of the GI tract and local measurement of physiological analytes (e.g., Atmo Capsule: oxygen, temperature, hydrogen, and carbon dioxide). However, there are currently no reported ingestible capsules that detect H_2S. We previously reported both electrochemical H_2S sensing and bioimpedance sensing on the benchtop using existing commercial off-the-shelf (COTS) components at MicroTAS 2021 [10], highlighting their potential utility to monitor H_2S as a marker of inflammation.

In this work, we have significantly advanced the PCB electronics design and modified the COTS H_2S sensor from [10] to enable integration into a miniaturized, wireless capsule platform. Furthermore, a 3D-printed package was made using digital light processing (DLP) to achieve a capsule form factor of 15 x 34 mm, which is compatible for studies in porcine animal models. The current capsule system was deployed in a custom gas testing set-up for evaluation of H_2S concentration. Overall, this work progresses the current state-of-the-art for wireless, ingestible capsule technologies and has the potential to improve the precision of localized sensing approaches for GI diagnostics.

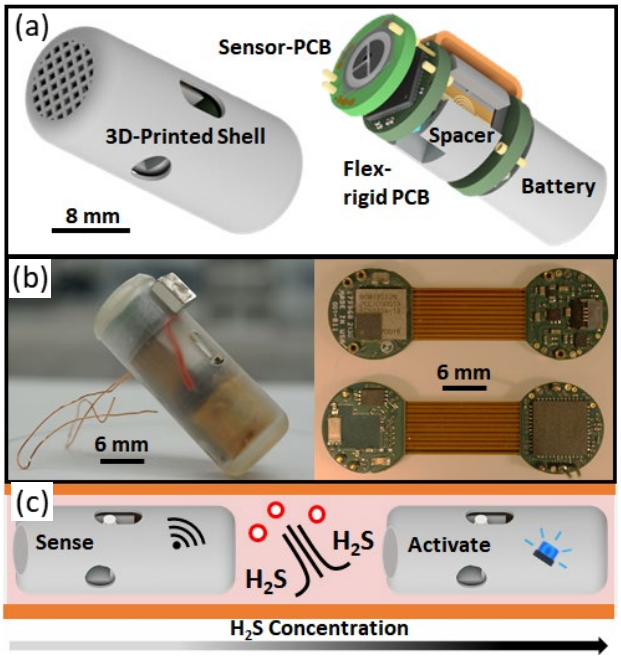

Figure 1: Overview of the ingestible capsule platform for real-time detection of H_2S. (a) Rendered image and (b) photographs showing the flex-rigid PCB encapsulated inside a DLP 3D-printed shell (dimensions: 15 x 34 mm). (c) Operational scheme of the capsule, where increasing H_2S concentration will activate a prompt (blue LED) if threshold H_2S level is reached.

DESIGN OF CAPSULE SYSTEM

Figure 1a shows a schematic of the capsule system comprised of a flex-rigid PCB, a modified electrochemical H_2S sensor-PCB, and a 3D-printed spacer encapsulated in a DLP 3D-printed shell. Similarly to [11], a combination of rigid and flexible substrates

978-1-940470-04-7/HH2022/$25©2022TRF
DOI 10.31438/trf.hh2022.18

Solid-State Sensors, Actuators, and Microsystems Workshop
Hilton Head Island, South Carolina, June 5-9, 2022

allows the electronics to bend with the battery (Figure 1b). The system is designed to continuously perform electrochemical measurements to monitor for elevated H_2S concentrations based on a predetermined threshold (Figure 1c).

Capsule Electronics

The PCB layout was designed using EAGLE (Autodesk, San Rafael, CA) to create two separate electronics boards: a circular 12 mm Sensor-PCB with an 8 mm cutout for inserting the H_2S and pin headers for interfacing with the AFE, and a flex-rigid PCB, containing the AFE circuitry and BLE microcontroller (MCU-BLE). An overview of the flex-rigid PCB utilized for the capsule electronics is shown in Figure 2a. Specifically, the flex-rigid PCB incorporates several key COTS components: (1) an AFE potentiostat IC (AD5941, Analog Devices) for generating sensor excitation signals, as well as to amplify the output current of the sensor and digitize the signal using an onboard analog-to-digital converter, (2) an MCU-BLE (BGM13S, Silicon Labs) paired with an external 2.45 GHz antenna (WLA.01, Taoglas) for wireless data acquisition (signal power: 0 – +18 dBm) and energy management, and (3) a 3.3 V voltage regulator, (TPS610981, Texas Instruments), paired with a 3.0 V lithium manganese coin cell battery (2L76, Energizer) and a magnetic reed switch (HSR-502RT, Hermetic Switch) to power the electronics.

The flex-rigid PCB was commercially fabricated on a double-sided 6-layer FR-4 ceramic substrate with embedded polyimide flex regions (15 mm in length) connecting the traces between two rigid boards (Sierra Circuits, Sunnyvale, CA), thus allowing the PCB to bend. When bending the flex regions (bend radius: 1.2 mm) no significant changes to signal noise was observed. As shown in Figure 2b, all traces between the MCU and AFE (across the flex region) connected digital inputs and outputs, as well as VDD and GND. Analog signals were processed directly between the Sensor-PCB and AFE, implying the dominant source of system noise was from the absence of filtering at the AFE sensor inputs/outputs. The Sensor-PCB and AFE on the flex-rigid PCB were attached using three gold header pins (22-gauge), connecting the working (WE), counter (CE), and reference (RE) electrodes. The coin cell battery (170 mAh) was connected to the flex-rigid PCB using 30-gauge threaded wire fixed with copper and Kapton tapes, while the magnetic reed switch was soldered between the (+) battery terminal and the voltage regulator. The assembled stacked electronics were inserted into the DLP 3D-printed shell (microfluidic resin, CadWorks3D).

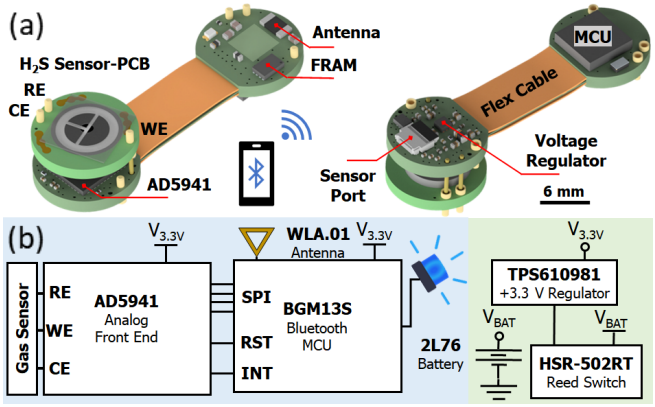

Figure 2: (a) CAD Rendering of the PCB electronics: sensor encapsulated inside a DLP 3D-printed shell and the flex-rigid PCB. (b) Circuit diagram of the PCB. Power consumption: 5 mA (idle, BLE) and 10 mA (active, BLE).

Integration of the modified H_2S sensor

The H_2S sensor is comprised of three bare carbon electrodes screen-printed on a permeable membrane, which were extracted from an existing COTS H_2S sensor (3SP-H2S-50, SPEC Sensors). Each sensor dye is submerged into a liquid electrolyte reservoir and attached to a plastic laminate backing (2 mm cutout) with WE, CE, and RE electrodes connected to separate conductive carbon tape pads (15 x 15 mm). This construction allows H_2S to readily access the sensor surface while electrolyte can diffuse through the permeable membrane to the bottom-side of the sensor, isolating the conductive pads.

The modified electrochemical H_2S sensor is depicted in Figure 3. An electrolyte reservoir (6 mm inner diameter) was 3D-printed, using fused filament fabrication (FFF) (MK3S+, Prusa), with biocompatible polylactic acid (PLA, Hatchbox). The sensor-pad dye was trimmed to roughly 10 mm in diameter and placed on top of the reservoir, encapsulating 10 µL of 0.5 M acetic acid as the electrolyte. The sensor was sealed with epoxy about the sensor-well interface (carbon pads exposed). The assembled sensor was inserted into the Sensor-PCB cutout and electrode connections between the copper pads were made using 36-gauge wires and silver epoxy (MG Chemicals). Gas permeable Teflon tape was cut to size (10 x 10 mm) and fixed on top of the H_2S sensor. This assembly method was employed to rapidly integrate a H_2S sensor within a capsule form factor and highlight the modular integration of the Sensor-PCB with the flex-rigid PCB.

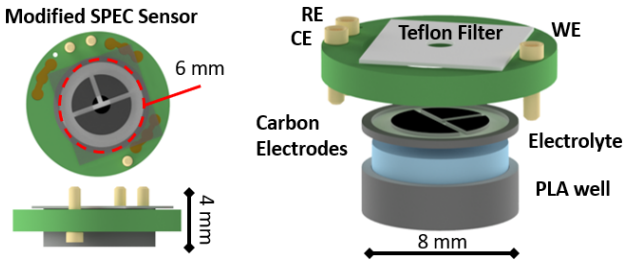

Figure 3: Modified electrochemical H_2S sensor assembly: shown as the top-down, cross-sectional, and exploded view of the H_2S sensor and Sensor-PCB attachment.

OPERATION OF CAPSULE PLATFORM

The capsule prototype was controlled wirelessly using a custom phone app. This allowed remote initiation of amperometric measurements and the ability to change the energy modes of the onboard electronics to minimize power consumption. Known challenges associated with wireless ingestible capsules include a lack of sufficient power to transmit signals through the body and limited operational lifetime due to restrictions on battery size, resulting from excess current consumption from idle components and wireless communication [12]. By default, the system operates in idle mode consuming 5 mA of current. When an amperometric measurement is initiated, the AFE is placed in active mode (10 mA with BLE), while sequentially exciting the electrochemical sensor and recording the readout signal. The MCU then periodically interrogates the AFE (100 ms sampling rate) and transfers data to be wirelessly transmitted to the phone until a predetermined number of measurements is obtained, returning the capsule to idle mode. As shown in Figure 4a, a magnetic reed switch allows the electronics to be turned on and off depending on proximity to an external magnetic field. Here, a small permanent magnet (1 cm^2) when placed within ~2 cm of the reed switch is sufficient to disconnect the system and turn of the blue indicator light emitting diode (LED). This allows the capsule to consume no current between packaging and being used.

The capsule has been designed to operate based on H_2S-driven feedback control of other downstream capsule functions. As a proof of concept, this was demonstrated by lighting an LED when the H_2S sensor detected gas at or above a set threshold (~8 ppm) (Figure 4b). This system design would enable biofeedback-driven control and provide alerts to users of patterns of inflammation throughout the GI tract based on the inflammatory marker of interest. Furthermore, in a realistic environment it is expected that sensor drift would need to be accounted for when setting a threshold concentration, therefore considerations for progressively updating the threshold value would be required.

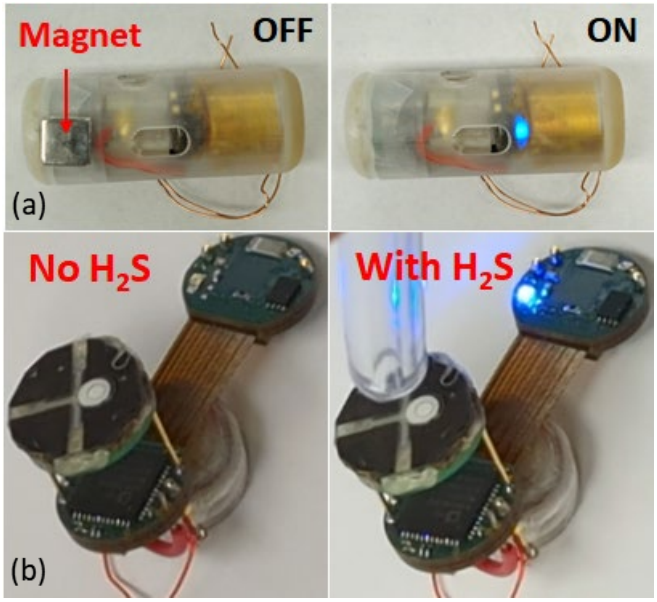

Figure 4: Photograph of assembled capsule demonstrating (a) the on/off state of the capsule prototype in the proximity of a small magnet (~2 cm) and (b) initializing an LED sequence once a threshold concentration of H_2S (8 ppm) is reached.

EXPERIMENTAL SETUP

The sensor-equipped capsules were evaluated in a custom-made gas set-up, providing a controllable environment for sensor assessment. A schematic detailing the design of the gas venting set-up is shown in Figure 5. To generate various gas concentrations, pulses of H_2S were applied by controllably venting 50 ppm of non-flammable nitrogen-diluted H_2S gas (Gasco, Huntington Beach, CA) into a plastic chamber (364 mL) until a desired ppm was reached. A flow regulator (Gasco) with a shut-off valve was attached to each gas tank, providing a constant flow rate of 0.2 SLPM (standard liter per minute) into the chamber. The open-close state of the flow regulator was toggled remotely using a bi-polar stepper motor (Nema-17, Stepper Online) and driver (A4988, Pololu). The duration of the gas pulse (H_2S ppm in chamber) was controlled by a two-way solenoid air valve (gas valve) connected to each gas tank, creating an interlock to resist leaking. The chamber was returned to a 0-ppm state by opening a motorized ball valve (US Solid) and forcibly purging air via a 12-V vacuum pump (Karlsson Robotics) into the chamber over a 10 s interval. The state of all valves, motors, and pumps was controlled via an Arduino development kit (Mega2560, Adafruit). Though the setup can replicate the proper ratios of gases found in the GI tract, due to the size of the setup and safety considerations (flammability), high ppm H_2S gas composition was not tested.

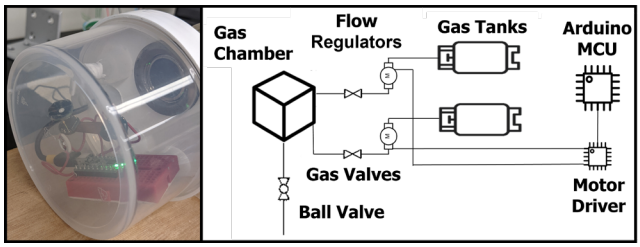

Figure 5: Photograph and schematic of the gas testing set-up.

CHARACTERIZATION OF MINIATURIZED CAPSULE SENSING SYSTEM

Physiological concentrations of H_2S in healthy adults within the GI tract ranges between 10.2–100 ppm and can deviate depending on the state of the gut, particularly in patients with ulcerative colitis [13]. Utilizing the gas testing setup, concentrations of H_2S, ranging from 3.7–15.6 ppm, were generated by gradually increasing the gas infill time into the chamber. An infill time of 2 s resulted in an H_2S concentration of 3.7 ppm, where increasing infill time at 2-s intervals resulted in the desired peak of 15.6 ppm H_2S. After each infill period the chamber was kept closed for 30 s, holding the ppm of H_2S constant for evaluation with the miniaturized capsule system, followed by air venting. Gas concentrations were independently validated using unmodified 3SP-H2S-50 SPEC sensors connected to a benchtop potentiostat (CHI Instruments) and AD5941 development kit (Analog Devices) [9].

Figure 6a shows the wireless amperometric gas measurements performed by the sensor-integrated capsule with an excitation voltage of 0 V (vs. C) over 2.5 hours at a 100 ms sampling rate. Increasing H_2S concentrations were measured over several time-controlled H_2S pulses, separated by a 6-minute interval, which were recorded continuously with the capsule platform then wirelessly transmitted to the phone app. Three consecutive pulses were applied for each H_2S concentration. A current range of 0.25 – 2.2 µA was observed corresponding to 3.7–15.6 ppm H_2S, which were well within the sensing range of the capsule electronics. The resulting calibration plot, as shown in Figure 6b, was found by correlating the calculated ppm of H_2S in the chamber to the averaged peak current response (N = 3 pulses), displaying a linear current response between 3.7–12 ppm (correlation coefficient R2 = 0.998) with a sensitivity of 0.17 µA/ppm.

This result exhibits an excellent sensitivity, although the linear range found here is lower than desired to target physiological GI concentrations. It was found that at higher gas concentrations, beyond 12 ppm, a slight drift in the sensor response was observed, which deviated from its expected linear behavior. This drift may be caused by the eventual drying of the liquid electrolyte over time due to repeated air purging cycles during prolonged monitoring experiments (over 2 hours). The addition of humidity control in the chamber would be a suitable resolution to increase the sensor's linear range and operational lifetime. Furthermore, the GI tract is composed of a mixture of gases: nitrogen, oxygen, methane, hydrogen, and carbon dioxide [13]. Most notably hydrogen is readily abundant (50%) and a known interferent of electrochemical measurements of H_2S, therefore thorough assessment of the selectivity of any H_2S sensor against known interferents is needed. The authors also recognize further characterization of the wireless signal propagation is necessary to ensure a robust Bluetooth link in the GI environment under non-ideal conditions. Overall, the results indicate reliable and rapid response to dynamically changing H_2S concentrations and demonstrate a proof-of-concept capsule with sensor-driven control.

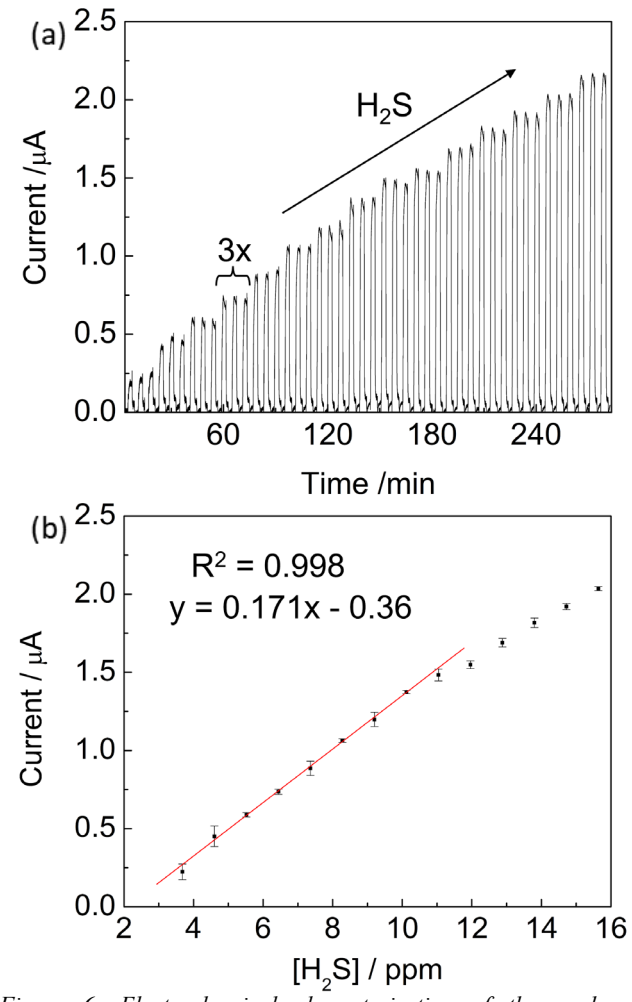

Figure 6: Electrochemical characterization of the packaged capsule: (a) amperometric measurements are recorded wirelessly in the gas-testing chamber (100 ms sampling rate). H_2S gas is pulsed at a ~6 min interval for increasing infill times to obtain gas concentrations between 3.7-15.6 ppm. (b) A resulting calibration curve was observed (N = 3) and displayed a linear response and sensitivity of 0.171 μA/ppm.

CONCLUSIONS

This work demonstrates one of the first capsule-based approaches for wireless detection of H_2S towards an ingestible diagnostic tool for locally identifying GI inflammation. The developed system integrates a modified COTS H_2S sensor-PCB with PCB electronics into a clear resin shell, providing a platform for studying the dynamics of transient gaseous inflammatory markers in the GI tract. When assembled, the capsule achieves a miniaturized form factor (15 × 34 mm) and successful continuous monitoring of H_2S at sub-physiological concentrations, realizing the goals of our previously reported work.

Future work will aim to thoroughly assess the selectivity of the H_2S sensor for known interferents, such as methane, hydrogen, and carbon dioxide at expected ratios in the GI tract, as well as perform bioimpedance measurements to confirm the intestinal tissue state. Overall, this platform demonstrates reliable sensor performance, and provides insight on the challenges and systems integration steps required to develop miniaturized capsules for understanding the relation between the gut microbiome and inflammatory diseases within the GI tract.

ACKNOWLEDGEMENTS

This work was supported by the National Science Foundation ECCS Program under Award #1939236. The authors acknowledge support from the Clark Doctoral Fellows Program.

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