Electronic Supplementary Material (ESI) for Analyst.

Electricity-free, chemical heater for isothermal nucleic acid amplification, with applications to COVID-19 home testing

Supplemental Information

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S1. Materials.

Mg/Fe powder was obtained in the form of Flameless Ration Heaters (also called self-heating MREs, *Meals-Ready-To-Eat*) packets. The metal powder is comprised of Mg particles, ranging in diameter from 0.3 mm to 0.5 mm mechanically alloyed with smaller particulates of Fe, and mixed with ~1% NaCl by mass. This formulation reflects the electrochemical nature of the aqueous exothermic reaction between the magnesium anode and atmospheric oxygen cathode [S1]. The iron protects the magnesium against corrosion. The NaCl increases the electrolyte conductivity and has been reported to significantly affect reaction kinetics [S2-S3]. Since we obtain the powder in small quantities, it is difficult to assess the cost of the powder purchased in bulk, but a conservative estimate is much less than $0.10 per gram.

Many types of materials in powder form can function as a phase change material (PCM) with desired phase transition temperatures. These include paraffin waxes of various molecular masses, polyethylene glycols, fatty acids such as palmitic acid, and various proprietary PCMs. Here we use the granular, wax-like PureTemp™ 63 (PureTemp, previously Entropy Solutions,

Fig. S1: The magnesium alloy and PCM before (left) and after (right) mixing.

Inc., (Minneapolis, MN) that undergoes phase transition in the temperature range between 61 °C and 65 °C and has, latent heat of 206 J/g, a solid specific mass of 0.84 g/cm^3, and is comprised of roughly spherical particles varying in diameter from 0.4 to 0.6 mm. This material is derived from agricultural products, is biodegradable and non-toxic, and costs in small lots approximately $0.025 per gram.

We place weighted amounts of the Mg/Fe powder (.45 grams) and PCM material (10.5 grams) in a 5 ml tube, and thoroughly mix for a few minutes (FISHER brand® Vortex Genie 2^{TM}12-812) to form a homogeneous mixture with approximately 20 mL total (**Fig. S1**).

S2. Experimental Set-up

To determine time-temperature heating curves, a 200-μl tube was filled with 30 microliters of water and inserted into the chemical heater. A type-K thermocouple (0.5-mm diameter, plastic coated) is inserted through a hole drilled in the tube's lid, and then sealed with acrylonitrile cement, assuring that the thermocouple remains submerged in the water. A second thermocouple is inserted in the powered Mg/Fe-PC material mixture, to directly monitor the EPCM temperature. This latter measurement depends on the position (depth) of the thermocouple in the powder. Both temperatures were recorded every 30 seconds with an Arduino MEGA 2560 microcontrollers and MAX 31856 thermocouple module; sent through the microcontroller serial port to a PC with Parallax™ PLX DAQ data acquisition software; and saved into a Microsoft Excel™ spreadsheet. A few of the experiments were carried out in an environmental chamber (Benchmark myTemp™ digital incubator, Model H2200-HC) to assess the effects of various ambient temperatures (10 to 40 °C) on system's performance.

The real-time fluorescence emission was detected with a Dino-Lite (AM411ST-GFBW) USB digital fluorescent microscope with built-in 375-nm UV LEDs for exciting fluorescence and a 510-nm filter to detect the green fluorescence from DNA-intercalating dyes added to the reaction mixture (Fig. S1). The 1.3 Mega (1280 x 1024) pixel images were captured with a CMOS camera and saved in JPG format. An image was captured every 30 seconds. The captured images were processed with the MATLAB™ image

Fig. S2: Chemical heater with real time fluorescent monitoring

processing software. Several green pixel intensities were selected and averaged for each capture time to generate a fluorescence intensity vs time curve.

S3. LAMP Reaction and Dye materials and protocol:

A LAMP reaction of 20 μl total volume comprised:

- 10 μl WarmStart® Colorimetric LAMP 2X Master Mix (for DNA and RNA) from New England Biolabs (Ipswich, MA, USA)
- Set of Six primers for amplifying COVID-19 gene N3 [25]

Primer name	**Sequence (5' to 3')**	**Concentration (μM)**
F3	TGGCTACTACCGAAGAGCT	0.2
B3	TGCAGCATTGTTAGCAGGAT	0.2
FIP	TCTGGCCCAGTTCCTAGGTAGTCCAGACGAATTCGTGGTGG	1.6
BIP	AGACGGCATCATATGGGTTGCACGGGTGCCAATGTGATCT	1.6
Loop F	GGACTGAGATCTTTCATTTTACCGT	0.8
Loop B	ACTGAGGGAGCCTTGAATACA	0.8

- 0.8 μl Evagreen® green, fluorescent nucleic acid binding dye (20x dilution) (Biotium, Freemont, CA, USA).
- Target: 1.4 μl isolated COVID-19 RNA diluted 100x in human saliva
- H_2O 6.8 μl

The reaction time was 30 minutes for end-point detection.

S3. 3D-printed cup

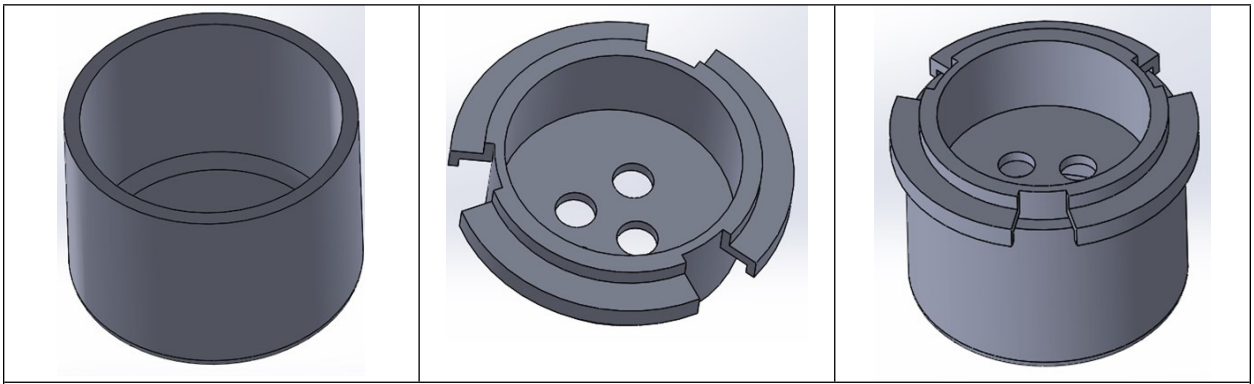

Fig. S3: Chemical heater device: (Left) cup, (Center) lid, (Right) assembled for test, (**d**) top view photograph of Chemical heater with four 200-μl PCR tubes.

S4. Detection of reaction products with LCV dye

A mixture of Leuco crystal violet (LCV) with sodium sulfite is added to the reaction mix. In the absence of amplicons, the compound LCV-sodium sulfite is nearly colorless. In the presence of dsDNA, LCV binds to the double-stranded DNA and changes from colorless to violet [S4]. Therefore, positive test is indicated by color change from colorless to violet (**Fig. S4**).

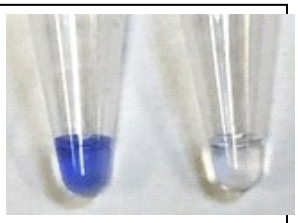

Fig. S4: Amplicon detection with LCV. (left) positive test. (right) negative test

S5. Benchtop LAMP experiments

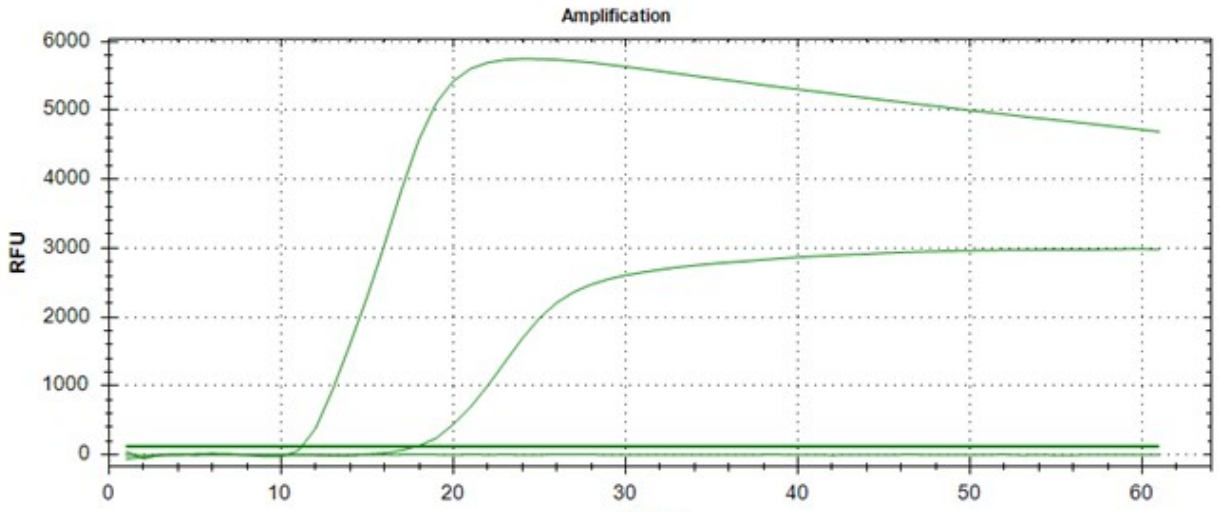

Fig. S5: Amplification curves (fluorescent mission intensity as a function of time, min) for the same samples that were tested with our chemical heater. Samples diluted ten-fold and hundred-fold produce amplification curves. The sample diluted thousand-fold does not present an amplification curve, suggesting that the benchtop instrument and the chemical heater have similar limits of detection.

Supplement references

S1. Lauren E. Oleksyk, Donald Pickard, Robert Trottier, 1993, DEVELOPMENT OF THE FLAMELESS RATION HEATER FOR THE MEAL, READY-TO-EAT, UNITED STATES ARMY NATICK RESEARCH, DEVELOPMENT AND ENGINEERING CENTER NATICK, MASSACHUSETTS, FOOD ENGINEERING DIRECTORATE, TECHNICAL REPORT NATICK/TR-93/030, https://apps.dtic.mil/dtic/tr/fulltext/u2/a265693.pdf

S2. KG Shah, D Guelig, S Diesburg, J Buser, R Burton, P LaBarre, R Richards-Kortum, and B Weigl, “Design of a new type of compact chemical heater for isothermal nucleic acid amplification” *PLOS One* (2015) **10**,1.

S3. JP Goertz, KM Colvin, AB Lippe, JL Daristotle, P Kofinas, and IM White, “Multi-stage chemical heating for instrument-free biosensing” *ACS Applied Materials & Interfaces* **(2018)** *DOI: 10.1021/acsami.8b11611*.
S4. Shigehiko Miyamoto, Sotaro Sano, Koji Takahashi, Takaaki Jikihara, 2015, Method for colorimetric detection of double-stranded nucleic acid using leuco triphenylmethane dyes, Analytical Biochemistry, 473, 28-33. https://doi.org/10.1016/j.ab.2014.12.016