Advancing Electricity-free Molecular Diagnostics at the Point-of-care:

Optimizing the NINA platform for a malaria LAMP assay

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Abstract— **An increasing number of isothermal nucleic acid amplification test (NAAT) approaches are under development to liberate molecular diagnostics from the laboratory and enable efficient clinical treatment decision-making at the point-of-care (POC). Each of these approaches requires the optimization of individual reaction specifications for the most effective amplification of the target nucleic acids (NAs) including NA purity, primer/probe design and reagent composition, amplification reaction temperature window of performance, total amplification time to detect, and method of detection. As a result, significant variation exists in the critical specifications that need to be achieved. To provide better thermal management capability for NAAT POC use cases, we have improved the design of our previous NAAT enabling non-instrumented nucleic acid amplification (NINA) heater technology. Thermal modeling was used to define which specifications would have the greatest impact on overall system performance. As a result, we present an isothermal assay NINA heater platform with decreased warm-up time to assay temperature, increased thermal holdover time, and tighter adherence to the optimal assay target temperature for a malaria loop-mediated isothermal amplification assay.**

Keywords— Phase change material (PCM), thermal modeling, nucleic acid amplification test (NAAT), loop-mediated isothermal amplification (LAMP), malaria, Plasmodium falciparum

I. INTRODUCTION

In resource-limited settings, sparse centralized medical facilities compounded by the lack of decentralized molecular diagnostic testing present a critical barrier to timely diagnosis, treatment, and subsequent control and elimination of infectious diseases. Isothermal nucleic acid amplification methods are well suited for decentralized point-of-care (POC) or minimal infrastructure laboratory molecular testing since they significantly reduce the complexity of equipment and power requirements [1]. Despite reduced complexity, however, isothermal methods require a stable, constant heat source to enable nucleic acid amplification. This requirement poses significant challenges for laboratories or local health clinics in developing countries where electricity to power standard heat blocks is often unreliable or unavailable. To address this need, we previously developed a low-cost, reusable, electricity-free heater using an exothermic chemical reaction thermally coupled with a commercial phase change material (PCM) [2,3].

This heater achieved acceptable performance for a reusable device, but the inherent PCM thermal properties significantly constrained design options. Guided by thermal modeling of the system to understand which parameters had the greatest possible impact on design and fabrication, we overcame these limitations by developing a custom-engineered PCM with high enthalpy, narrow melt range, low hysteresis, and high thermal conductivity.

Many isothermal nucleic acid amplification techniques are under development and display a wide range of temperature profile specifications [4]. Of particular interest is the loop-mediated isothermal amplification (LAMP) detection of the 18S rRNA subunit of *Plasmodium falciparum* to facilitate low-resource setting (LRS) POC detection of malaria [5-9]. The recently published technical product profile for malaria elimination and eradication details use-case scenarios for which a malaria-LAMP assay executed in a suitable non-instrumented nucleic acid amplification (NINA) heater would be an ideal solution to enable LRS POC malaria test-and-treat efforts [10,11].

Initial testing showed that the existing reusable NINA prototype device, designed with a first-generation PCM (65PCM-1), was not able to maintain the specified amplification temperature, 64.5˚C +/-1.5˚C, for the required amplification time of 60 minutes. Furthermore, the warm-up time, or the time required for the system to heat from ambient temperature to the required amplification temperature, was excessively long. A short warm-up time is desired to improve usability and throughput.

We present these latest improvements as a critical development toward commercialization of the first reusable and first disposable electricity-free, infrastructure-independent nucleic acid amplification tests (NAATs). Furthermore, preliminary data for the performance of a

malaria-LAMP assay run in a reusable NINA heater configuration is presented (Table I).

Parameter	Specification	Description
Amplification Temperature ˚C	64.5˚C +/-1.5˚C	Ideal temperature for malaria loop-mediated isothermal amplification assay
Warm-Up Time	10 min	Time-to-amplification temperature after activating exothermic reaction
Amplification Time	60 min	Time from warm-up to end of the reaction (thermal holdover time must be longer than the assay amplification time)
Ambient Temperature	18˚–28˚C	Test environmental temperature range
Sample Size	Micro PCR tubes	Qiagen model #981005 0.2 mL
Number of Samples	5	Allows for assay controls and a dedicated thermocouple to monitor device functionality

TABLE I. These specifications for a malaria-LAMP assay push the functionality of the design by requiring a tighter temperature tolerance (+/- 1.5˚C) at a higher temperature (64.5˚C) than our previous assay.

This specific NINA prototype consists of four fundamental components: insulation, PCM, Sample tube and the exothermic reaction (Fig. 1).

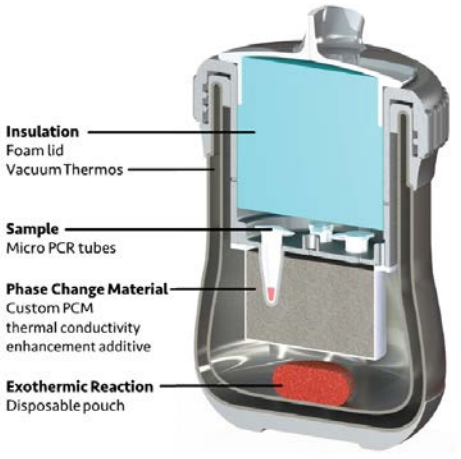

Fig. 1. Cross section of the prototype design shows the critical components of the exothermic-heated system.

II. Methods

A. Specifications and Critical Parameters

The target parameters for the malaria-LAMP assay of interest for detection of low parasitemias are shown in Table 2. Initial testing showed that the existing reusable NINA prototype device, designed with a first-generation PCM (65PCM-1), showed that the device was not able to maintain sufficient thermal holdover performance for the specified amplification temperature and time, 64.5˚C +/-1.5˚C for 60 minutes. Also, the warm-up time was excessively long. A short warm-up time is desired to improve usability and throughput for health care workers.

There are six key modifiable material properties associated with the thermal system (Table II).

Property	Variable	COMSOL Tested Values
1. System Thermal Resistance	Rsys	Original vacuum thermos design compared with extra 50-mm foam added radially on sides and top
2. Phase Chain Material (PCM) Volume	vPCM	28 ml and 39 ml
3. PCM Latent Heat	PCM J/g	200 J/g to 250 J/g
4. PCM Thermal Conductivity	PCM W/m K	0.8 W/m K to 2.0 W/m K
5. PCM Melt Range	PCM $\Delta T_{1\rightarrow 2}$	11˚C melt range to 2˚C melt range
6. Exothermic Reaction Profile	Reaction Formula	Simulated bolus reaction to ideal controlled reaction

TABLE II. Both COMSOL modeling and empirical testing (data not shown) were used to optimize the NINA prototype design to achieve desired thermal properties.

B. COMSOL Model

A 2D symmetrical, time-dependent COMSOL, finite element analysis (FEA) heat transfer model was developed using the integrated phase change function. This model was based on a 3D CAD SolidWorks rendering of the existing prototype and converted to a COMSOL model (Fig. 2).

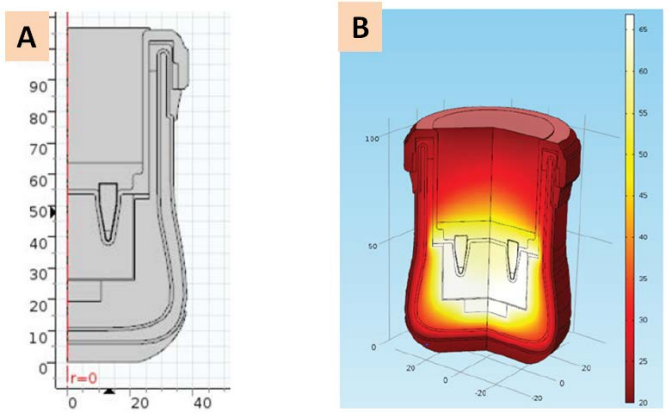

Fig. 2. CAD model converted into a functioning COMSOL model (A). The heat flow through the device can be observed in B) a graphic output of the COMSOL software.

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The heat input to the system was modeled as a step function power equation to match the experimentally observed temperature profile from the exothermic reaction (Fig. 3). The equation also allowed the total power input to be scaled for models that required more energy input. Typically, because the system pressure is equalized with ambient pressure, the exothermic reaction peak temperature is limited by the phase change of water at ~100°C.

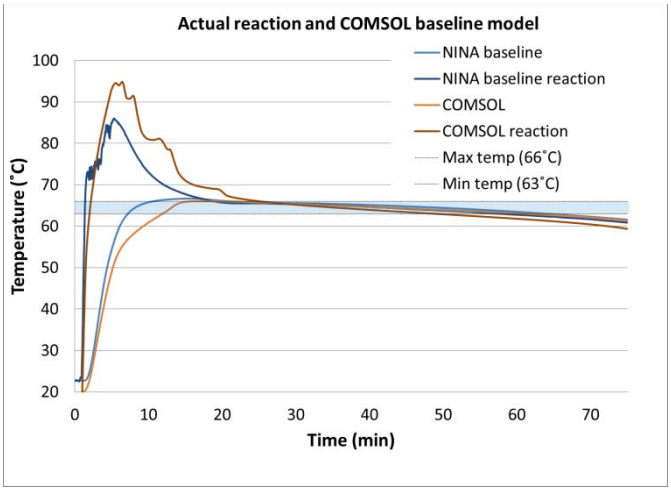

Fig. 3. Graph showing the simulated device temperature profile with an actual experimental run of the prototype. This data from the NINA device with the 65PCM-1 was used to calibrate the model.

III. Empirical Testing Methods

A. Operation of the Heater and Reference

The NINA heater is activated by placing a MgFe fuel pouch in the thermos and adding 5 mL (+/- 0.2 mL) of saline from the blow-fill-seal cartridge. The MgFe fuel begins to react immediately with the saline and produces heat. The tube holder is threaded onto the thermos, and the lid is then placed on top until the target amplification temperature is reached. After 10 minutes, when the heater has warmed to 61.5°C +/- 1.5°C, an isothermal assay in 0.2-ml PCR tubes can be inserted into the sample tube holders. After 60 minutes in the target temperature range, the tubes are removed for downstream processing using one of three methods, described below. After the test run, the exothermic reaction products contained in the pouch are discarded, while the thermos, insulation, PCM, and tube holders can be reused many times following a cool-down period.

B. Performance Monitoring

The thermal performance of the enhanced NINA heater design was evaluated using two Type T thermocouples (Omega Engineering, USA) and an NI 9211 (National Instruments, USA) data logger (Fig. 2). As a proxy of actual reaction temperature, one thermocouple was inserted through a 1.27-mm-diameter hole in the lid of a 0.2-mL PCR tube. The tube was filled with 25 µL of deionized water to mimic the amplification reaction volume. The second thermocouple was attached to the bottom of the aluminum cup to monitor the exothermic reaction temperature.

C. Differential Scanning Calorimeter Methods

A micro differential scanning calorimeter (DSC) (Setaram, USA) was used to measure the latent heat and melt range of the PCM. The DSC measures heat input into the sample to match a temperature profile, providing a plot of the melting and freezing cycle of a given material. Latent heat capacity is essential for the NINA device for energy storage during phase change. Melting range describes the temperatures during which phase change occurs. A narrow melt curve implies a discrete melting temperature, favorable for temperature control, while a wide curve indicates melting over a broader temperature interval. Samples were tested at a 0.5°C/min temperature ramp and brought through phase change and back to room temperature. The sample mass was 5 mg.

IV. DSC Results

A side-by-side DSC curve comparison of 1 and 2 PCM shows the improvements of both the latent heat and the narrow melt range (Fig. 4).

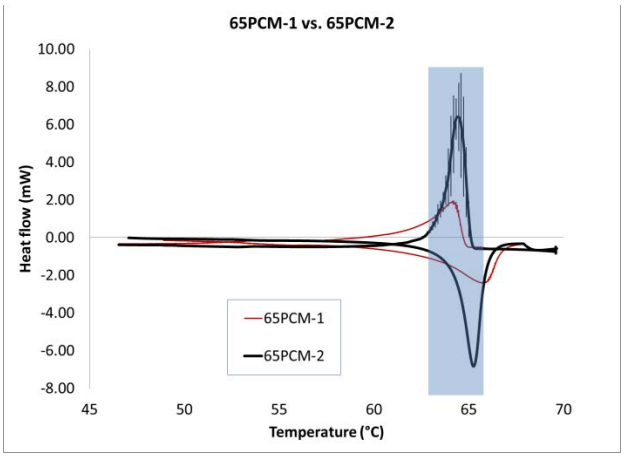

Fig. 4. Differential scanning calorimeter curves illustrate the narrower melt temperature range of 65PCM-2 over 65PCM-1. The narrower interval provides greater latent heat storage in the PCM over the required temperature specification range.

COMSOL Results

Parameter	Holdover Time (60-min target)	Warm-Up Time
65PCM-1 prototype	55	7
65PCM-1 COMSOL model	52	12
Increased thermal conductivity	48	9
Increased PCM volume	52	15
Additional insulation	55	12
Narrower melt range	**61***	**12**
Increased latent heat	**70***	**12**
Optimized exothermic reaction	75	12

TABLE III. The ability of the NINA heater to achieve the required temperature for the required time (target line) is predicted by the COMSOL model (as evidenced by the comparison of the baseline device made with the 65PCM-1 and the model).

A fundamental property of the NINA system is the ability to reach long stable holdover times by storing energy as latent heat in the PCM. Based on the COMSOL model and experimental validation, the key design parameters that impact the holdover time of the NINA heater are PCM melt range, PCM latent heat, and the exothermic reaction rate.

V. NINA Results

Based on initial testing and modeling, a new PCM (65PCM-2) was developed to optimize the performance of the NINA heater. The optimized device was tested under the same conditions as the previous version, and the holdover time of the device was significantly improved (Fig. 5). The new design showed 25 % improvement in warm-up and 100% improvement in holdover.

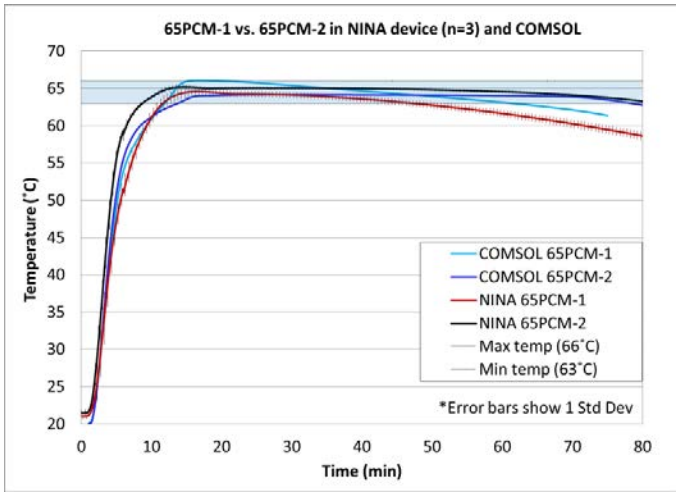

Fig. 5. This comparison graph illustrates our ability to predict performance of the NINA heater with the COMSOL model.

VI. Discussion

The six key device properties are discussed in further detail below:

A. Thermal Conductivity

The exact impact that the thermal conductivity of the PCM has on the system is difficult to quantify. The basic equation:

$q = -k\Delta T$

where:

q= Heat flux
k= Thermal conductivity of the material
ΔT= Temperature gradient

We modeled the system heat up, PCM melting, cooling, and freezing. Each of these steps is affected by the thermal conductivity and latent heat capacity of the PCM. A higher thermal conductivity reduces warm-up time and increases the effective volume of PCM contributing latent heat. This increases holdover time while decreasing warm-up time. However, the increased thermal conductivity of the PCM also reduces the overall resistance to heat loss causing the device to lose stored latent heat at a higher rate.
Significantly increasing the heat loss to an approximated theoretical maximum 2.0 W/m K did not improve the holdover time of the device. However, it did reduce the warm-up time.

B. PCM Volume

Initial non-FEA modeling suggested that adding PCM would improve the holdover time of the prototype by adding latent heat to the system. However, due to the geometry of the vacuum insulated housing, additional PCM can only be added to the bottom of the aluminum containment vessel. This added PCM was too far from the PCR tubes to access the additional latent heat. Both the COMSOL model and a modified prototype with extra PCM (data not shown) validated this concept—additional PCM does not always equate to extended amplification time. Increasing the PCM volume did significantly increase the warm-up time in the model due to the added distance the thermal energy needed to travel between the reaction and the sample with relatively poor thermal conductivity.

C. System Insulation

Increasing the insulation value of the system increased the holdover time in the model. However, this was a relatively modest improvement (6 min) for a significant amount of foam (50 mm) added radially to the top and the bottom. This added foam increased the overall volume of the device by approximately five times. The increase in size made this an inadequate solution to the problem. Warm-up time was not affected by the increased insulation.

D. PCM Melt Range

The melt range of the 65C-1 PCM is wide and extends outside the operating temperature of the LAMP assay. In order for the thermal system to fully use the latent heat potential in the PCM, it is critical that the melt range of the PCM fall within the specified temperature range for the isothermal assay. Both COMSOL modeling and empirical

data show that a narrower melt range can improve holdover and warm-up times. The improvement in the warm-up time was not observed in the model. However, it was significantly better in the 65C-2 PCM verses the original 65C-1 PCM.

E. PCM Latent Heat

Increasing the latent heat of the PCM is perhaps the most logical way to improve the holdover time of the prototype. However, there are natural limitations to what is realistic based on material properties. Similar to reducing the melt range, increasing the overall latent heat of the PCM has a significant impact on the holdover time of the modeled device. Both COMSOL modeling and empirical data demonstrate the significant impact that improving the latent heat of the PCM can have on the system. Improving the latent heat of the PCM did not significantly affect the warm-up time.

F. Exothermic Reaction Rate

Conceptually, a perfect exothermic reaction rate could extend the holdover time of the prototype indefinitely within a narrow ambient temperature range. By matching the heat loss of the system with the heat output of the reaction, the device would remain within specification as long as the reaction continues. Others have demonstrated designs aimed at optimized reaction rate [12-14]. Due to limitations with the existing form factor of the prototype, this parameter was not modified to address the holdover time.

VII. CONCLUSION

Understanding the design parameters of a chemical heater/PCM system is critical to optimize the device for each targeted isothermal nucleic acid amplification assay. COMSOL modeling is an effective tool to rapidly scan and evaluate the impact of relevant physical variables on system performance. COMSOL modeledchanges to independent thermal specification contributors effectively predicts which will have the most impact on the overall thermal performance (decreased melt range and increased latent heat).

The data described in Table 3 and Fig. 5 exemplifies the correlation between how model-predicted improvements in the design parameters impact the physical properties of the heater system.

The holdover time of the NINA design is vastly improved by modifying the melt range and the latent heat of the PCM. This advancement has allowed the PATH team to deliver functional prototypes for a malaria-LAMP assay and move forward with the next round of heater designs.

NEXT STEPS

PATH has multiple ongoing projects to integrate the NINA heater technology into a single-use disposable molecular diagnostic platform that includes sample collection, sample preparation, amplification, and detection. These platforms could significantly impact sensitivity, simplicity, and impact diagnostics at the POC.

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