Non-Instrumented Nucleic Acid Amplification

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Introduction

Needs and Problem

- In both developed and developing nations, early and accurate diagnosis is critical to the health of the individual and the general populace as a whole [1].
- Current diagnostic devices implemented in developing countries suffer from sensitivity and accuracy issues [2]. There is also a long list of diseases with no field-adapted diagnostic tool.
- Lack of an accurate and suitable diagnostic test for use in the field means that identification of disease is based solely on clinical symptoms.

Problem

- An accurate method of infectious disease diagnosis is the use of nucleic acid probes in conjunction with nucleic acid amplification [3].
- The most commonly used nucleic acid amplification method is polymerase chain reaction (PCR) shown in Figure 1.
- Current PCR thermocyclers are large and expensive involving a need for highly trained clinicians. Even portable thermocycler can only run for a couple hours at most using a portable electricity source [4].

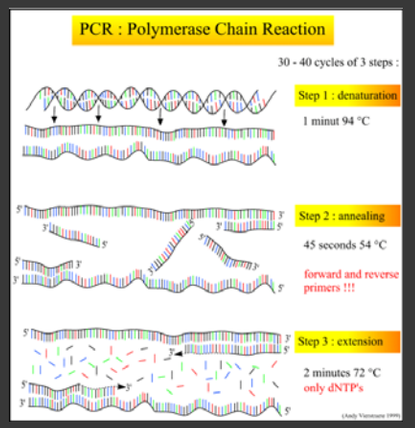

Figure 1: Steps and temperatures of PCR [6].

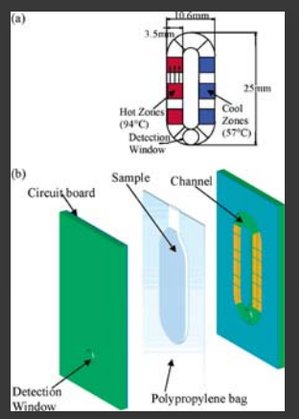

Figure 2: Convectively driven PCR loop Proposed by Wheeler et al [5]

Solution

- Use a convectively driven PCR loop for a more energy efficient execution of PCR while maximizing the speed of each thermocycle [5]. An example is shown in Figure 2
- To eliminate the dependence on electricity, an exothermic chemical reactions will be used as a heat source. However, exothermic reactions rarely have stable temperature control.
- A phase change material (PCM) will be used to stabilize the temperature at the desired temperature range.

Goals

Project Objectives

- Determine ideal PCM criteria
- Investigate and characterize possible PCM candidates
- Design a PCM device that mimics the specifications of a convection dependent PCR device.
- Test each PCM using a protocol that tests the amount of thermal stabilization each PCM could provide.

PCM Selection

Ideal PCM Properties

- Thermal properties include: a melting temperature near 94°C, tight melting band, high enthalpy of fusion, and high thermal conductivity.
- Physical properties include: low change in density over phase change, high density to maximize thermal storage per unit volume, and no subcooling.
- Chemical properties include: stable, minimum phase separation, chemically compatible with device, non-corrosive, non-toxic, non-flammable, and non-polluting.

PCM Characterization

Each PCM was thermochemically characterized to analyze which material would be most appropriate for application in this device. Differential scanning calorimetry was used measure the material's melting point and the heat of fusion for the PCM. Results are summarized in Table 1.

Table 1: Selected PCM's and their relevant tested properties

Chemical Name	Type	Tm (C)	Hf per Vol (J/cm3)
Sorbitol	Organic Compound	91.0	118.796
Magnesium Nitrate Hexahydrate	Hydrated Salt	90.5	252.434
Cerroshield (Low 203)	Bismuth, Lead, Tin Alloy	96.8	337.212
Rubitherm RT100	Paraffin wax	86.4	236.316
IGI 8526A	Synthetic wax	88.1	223.769
IGI 8728A	Synthetic Wax	88.5	223.951

PCM Testing

Device Design

Test device was based on the convectively driven PCR device designed by Wheeler et al. Dimensions and flow rate in the device matched the specification mentioned by Wheeler et al. The device was fashioned as a multilayered polymeric laminate device for rapid prototyping. The fluid channel made of polyethylene terephthalate (PET) film was sandwiched between two PCM layers made of acrylic containing a totally of 3.6 mL of PCM. Between the fluid channel and the PCM layer was a heat conductive aluminum section to optimize heat transfer. Outside of the heating zone, sections of PET were used to minimize heat conduction outside of the heating zone. 3M 9471 pressure sensitive adhesive was used between each layer. Temperature was measured with a thermocouple embedded in the fluid channel.

Test Protocol

Resistance heaters were used to represent the exothermic reaction. The testing protocol involved an initially heating phase where the device was heated at a constant 24V until the temperature reach 99°C. At this point, the heaters were disconnected allowing the device to cool passively. Any temperature stabilization was due entirely to the presence of the PCM. A relevant temperature zone of 93-99°C was determined to be a range where DNA melting occurs [7]. The amount of time each device spent between 93-99°C was measured.

Test Results

Each device was tested three times. The average temperature measured within the fluid channel for each device is shown in Figure . The average time each device stayed within 93-99°C is shown in Table 2. The wax devices expanded upon melting causing leakage of the PCM. Only the IGI 8728a is reported here.

Table 2: Average time each device stayed within 93-99°C.

Material	Time (sec)
Control	387 ± 33 sec
Magnesium Nitrate Hexahydrate	274 ± 5 sec
Sorbitol	360 ± 15 sec
Cerroshield	736 ± 28 sec
IGI 8728a	314 ± 44 sec

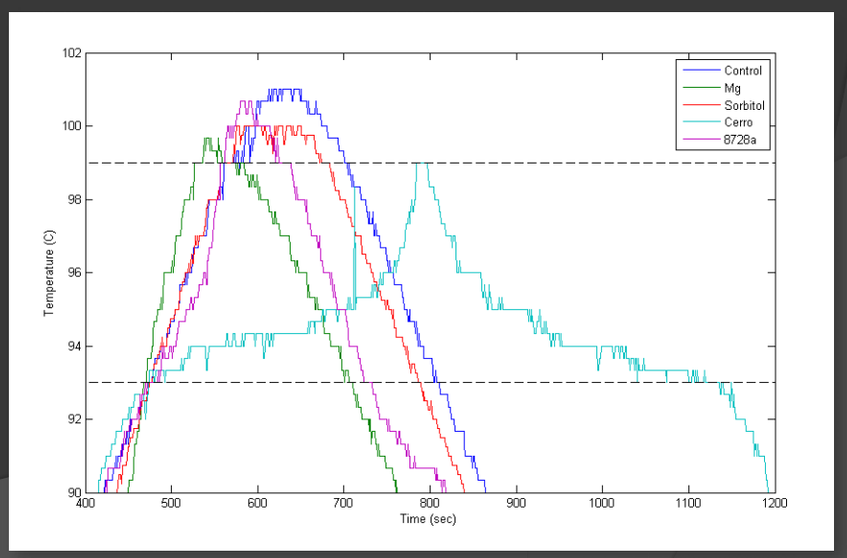

Figure 3: Display of the average temperature profile measured in the heating zone of each device. Each curve is displayed such that they enter the DNA melting temperature range (93-99°C) at the same time.

Discussion and Conclusions

- Cerroshield successfully has a two fold stabilization over the control for temperature stabilization between 93-99°C for a total of 12.3 min.
- Successfully showed that PCMs could be used to thermally stabilize in a miniature device.
- Results were highly repeatable
- Waxes could not be testing in a rigid device due to the high expansion over the phase change
- Sorbitol and the Magnesium Nitrate Hexahydrate did not show a phase change stabilization in these small volumes making them a poor choice for this device

Future Work

- Replace resistive heaters with an exothermic chemical reaction
- Use the combination of an exothermic reaction as well as the PCM to generate a convectively driven loop

References

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