Design of a thermocycler based on light and air having optimal heat capacity

Pramod Upadhyay

This report describes the design of a 'fast' thermocycler for polymerase chain reactions (PCR). In this design of the thermocycler, the sample holders were placed over a copper foil made by electroforming, having an optimal heat capacity, and showing exact fitting of the sample holders. While an electric bulb was used as the source of heat, a muffin fan was used for cooling. A matched copper-wire resistance thermometer was used to monitor the temperature. Conventional poly-propylene tubes or ordinary glass capillaries were used as sample holders for DNA amplification. For exercising control over various timed events necessary for conducting PCR, the apparatus made use of the printer adapter of an IBM personal computer. The 'fast' thermocycler was evaluated for its response time, temperature profiles generated, and efficiency in amplifying DNA in the laboratory. Cost of the materials, excluding a PC, of this thermocycler works out around Rs 3000 only.

The polymerase chain reaction (PCR) technique has emerged as one of the most widely used techniques in molecular biology in the recent years[1]. The increase in the popularity of this technique has been accompanied by corresponding improvements in the apparatus used for performing it. A variety of engineering approaches to control temperature have been investigated for their applicability to the design of a PCR apparatus, including resistive heating and cooling by means of a refrigerant[2]; temperature cycling, using the Peltier effect[3], through the use of heated and chilled air-streams[4,5], and most recently, in a continuous flow manner[6].

In most of these designs the sample holder is placed inside a temperature-controlled metallic block, often weighing more than 500 g. Assuming that the specific heat of the material of metallic block is similar to that of aluminium, its heat capacity would be around 450 JK^{-1} which would be rather too high to heat or cool, for example, 10 samples, each of 50 µl, having heat capacity of 2.1 JK^{-1} only. The high heat capacity of the metallic block often limits the rate at which it could be heated or cooled. In thermocyclers based on heated and chilled air-streams, while the heat capacity of medium is quite small due to the low thermal conductivity of air, an air cyclone would be required to achieve uniformity of temperature.

In this report a thermocycler having a unique design of copper foil that holds sample holders having approximately 10 JK^{-1} heat capacity is described. In this design, copper foil is heated by placing it close to a halogen lamp, and subsequently cooling it by blowing a gentle stream of air over it. Owing to the optimal heat capacity of the copper foil, it can be heated and cooled quite rapidly. Furthermore, the high thermal conductivity of copper (unlike air) assures uniformity of temperature. A thermally matched temperature sensor is used to take the full advantage of fast temperature cycling of copper foil.

Features of the thermocycler

In this thermocycler samples can either be placed inside conventional poly-propylene tubes or else placed inside capillary tubes, and then placed on a blackened foil made by electroforming copper over poly-propylene tubes or capillary tubes. In the electroformed copper foil, tight fitting of the sample holder, and uniform and controlled thickness is ensured. A muffin fan blows air of ambient temperature over this copper foil. The temperature of the copper foil is maintained by controlling the light energy emitted by a 500 W halogen lamp. A 'matched' temperature transducer is used for monitoring temperature changes and communicating with the controller. The control is built around the parallel printer adapter of an IBM PC. Attaining of fast temperature cycling becomes possible with this thermocycler due to low heat capacity of the copper foil. For the incorporation of the above-outlined features in the design proposed here, the following rationale and overview of advantages were considered.

Optimal heat capacity of platform for sample holder

For the optimal heat capacity of the platform for sample holder, the heat capacity of such a platform should be nearly ten times the heat capacity of the sample holders to ensure heat transfer from the platform to the sample holders without any significant temperature change. A foil made by electroforming copper over poly-propylene tubes or capillary tubes served as a platform for sample holders. Thus by using an electroformed foil, the fitting of poly-propylene tubes or capillary tubes was observed to be exact, resulting thereby in maximum heat transfer. Furthermore, we observed that while a foil made by conventional machining process required highly specialized tools, the foil made by electroforming copper gave uniform deposition of copper with thickness (mass) that could be controlled.

Using light energy for heating

An electric bulb has a very fast response compared to nichrome wire heaters used in most designs. Faster ramp rate with practically zero 'dead time' was attained by heating the copper foil by the light of a 500 W halogen lamp. Phase control of AC mains was used to control the light intensity of the bulb, and thus the temperature of the sample holder.

Using an ordinary fan for cooling

An industry standard 'muffin fan' of 3 1/8" size and having an air blowing rate of 35 cubic feet per minute (CFM) (the cooling fan used in most electronic equipment) was employed for blowing air over the sample holder for cooling. This obviated the need for a specially designed fan.

Using a thermally matched copper-wire resistance thermometer as a temperature sensor

A thermally matched temperature sensor was developed. An insulated copper wire was packed inside the capillary and its resistance was used as a measure of temperature. The weight of the copper wire inserted inside the capillary was such that the heat capacity of the inserted wire became equal to the heat capacity of 20 µl (volume of the reaction mix) of the sample. This is a major simplification, since a temperature sensor of any desired heat capacity can be made very easily by this method.

Choosing a PC printer adapter for control

The parallel printer adapter of an IBM PC was used as it is fairly ubiquitous and well standardized. It is well suited for moderate data transfer rates. Furthermore, it required minimal hardware support, and very simple programming.

Using commonly available materials and electronic components

All electronic components used were 'Commercial' grade, and could be obtained from a hobby store.

Intelligible circuit and program

A very simple circuit with minimum components was designed. The accompanying program, written in Power Basic, is a popular version of BASIC. It is a fairly straightforward program with no special algorithms.

Design and fabrication of thermocycler

Copper foil

Poly-propylene tubes or glass capillaries (outer diameter 1.5 mm and inner diameter 1.0 mm, Top Syringes, India), employed in our laboratory for obtaining blood samples from rabbits by retro-orbital puncture, were glued on an acrylic sheet. Electrically conducting silver paint was applied on the surface on which copper was to be electroformed and was connected to a power supply as the cathode. The anode was made from a sheet of copper. Electroforming was carried out in 20% copper sulphate solution in 5% sulphuric acid at 1 A/dm^2. The temperature was maintained at 55°C. As the current efficiency of electrolysis was not 100%, it was therefore desirable to periodically weigh and find out the amount of copper deposited. Approximately 30 g copper was deposited on the surface to make a platform for 16 sample holders. After electroforming, the copper foil was removed from the acrylic sheet by subjecting it to hot and cold water a couple of times.

The assembly

A schematic diagram of the fast temperature cycler is shown in Figure 1. The copper foil made by electroforming was mounted on a frame. One of the depressions was used to place the temperature sensor. The foil was blackened by permanent marker ink. A 500 W halogen lamp (Philips, India) was mounted below at a height of 5 cm, and a muffin fan (Rexnord, India, 12 \ DC; 0.2 A) was mounted over the fc a height of 5 cm.

The sensor

In order to make a matched temper: sensor, for example, for a 20 µl sar first the heat capacity of 20 µl sa was calculated. Assuming that the cific heat and density of the sample the same as for water (4.18 Jg^{-1} K^{-1} 1 gml^{-1}, respectively) the heat cap would be:

$$20\ \mu l/1000\ \mu l\ g^{-1} \times 4.18\ Jg^{-1}\ K^{-1} = 0.0836\ JK^{-1}.$$

Given the specific heat of coppe 0.384 Jg^{-1} K^{-1}, for a length of copper to have 0.836 JK^{-1} heat capacit should weigh 0.0836 JK^{-1}/0.384 K^{-1} = 0.217 g.

Thus the heat capacity of 217 n copper wire and 20 µl sample was f to be similar.

This weight (217 mg) of thin lated copper wire was therefore pa inside a capillary and its ends sealed with minimum amount of silicone adhesive, such that both ends of copper wire protruded out o capillary. These ends were meant t connected to the circuit board.

In order to calibrate the sensor, it held at a series of temperature valu

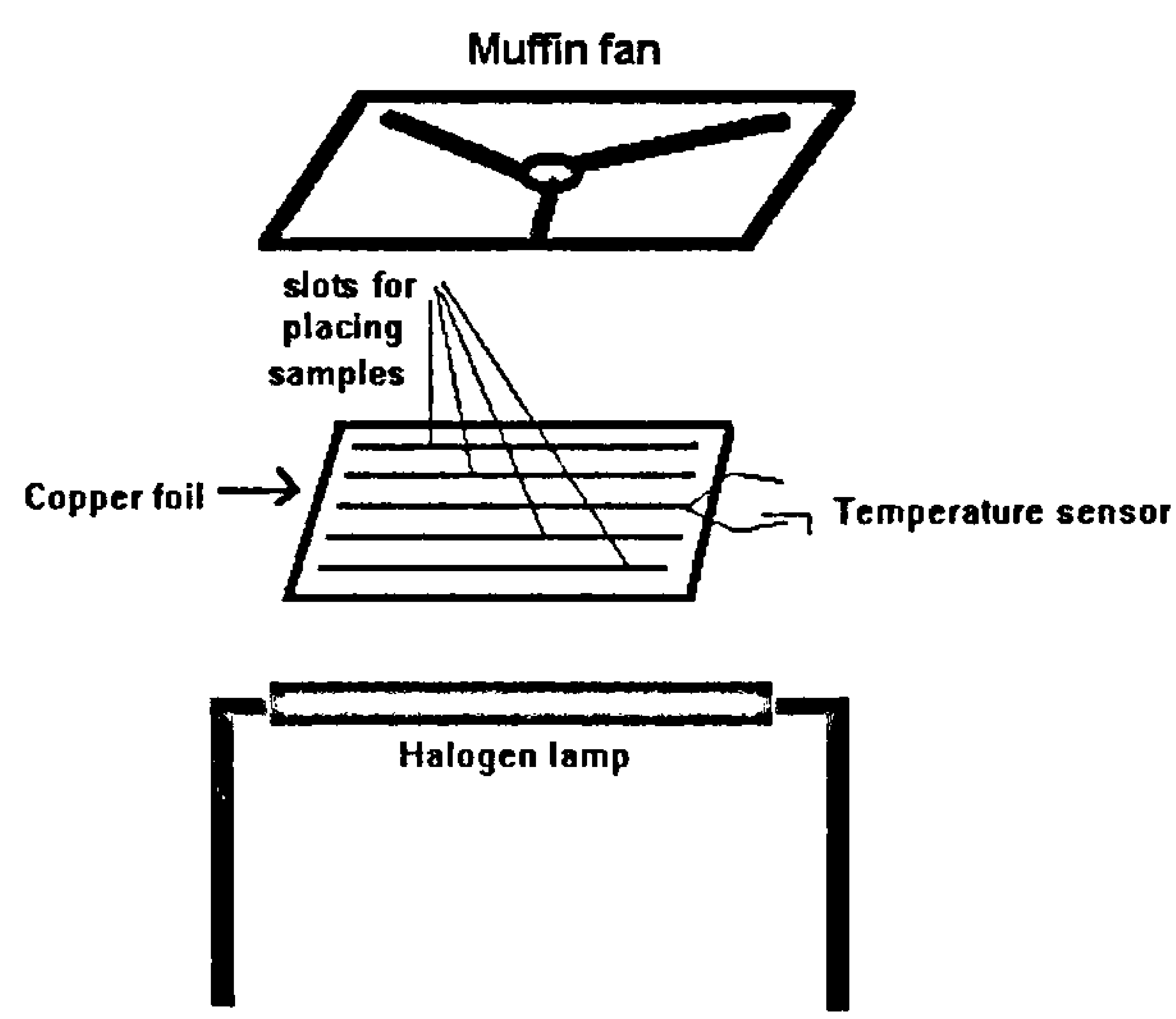


Figure 1. Schematic diagram of the light and air thermocycler.

read by a mercury thermometer, and the number presented by the analog to digital converter in response to each value was recorded (see *data multiplexer* below). A calibration equation was constructed using these numerical values of temperature and response.

In order to compare the temperature–time profiles of the sensor and the sample, another sensor was constructed to record the temperature of the PCR mix. In such a sensor, the electrical conductance of this sealed PCR mix was recorded at different temperatures and a calibration curve was plotted. Both the sensors, the 'PCR mix' temperature sensor and the 'copper wire' temperature sensor, were placed on the copper foil kept under the lamp.

The controller

Construction of the circuit: All the components were purchased over the counter from entertainment electronics stores. The circuit was wired on a 'solder breadboard' and instructions given in ref. 7 were followed. The circuit comprised of 3 parts: the data multiplexer, the timer and the power supply.

The data multiplexer: The block diagram of the data multiplexer, and its working are shown in (Figure 2). Four bits of the control port were decoded into 16-control lines, and an eight-bit data bus was fed to three transparent octal latches. A 12-bits error signal, calculated from the set point, and temperature of the sensor, was written on a latch. The error signal was transferred from layer 1 by the data transfer pulse and loaded to counters by the load pulse. At the end of the countdown, the carry out 'fired' the triac. Two layers of latches were used as a 12-bit word that was to be transferred in one go[8].

To read the temperature of the sensor, the resistance of the copper wire was converted into voltage, scaled, and was amplified. This voltage was fed to a sample and hold, and then to a 12-bit analog digital converter (ADC). By using a 2 × 4 bits tristate, the lower 8 bits of the converted number were read. The higher 4 bits were selected by one of the bits of the latch and were read in a similar manner.

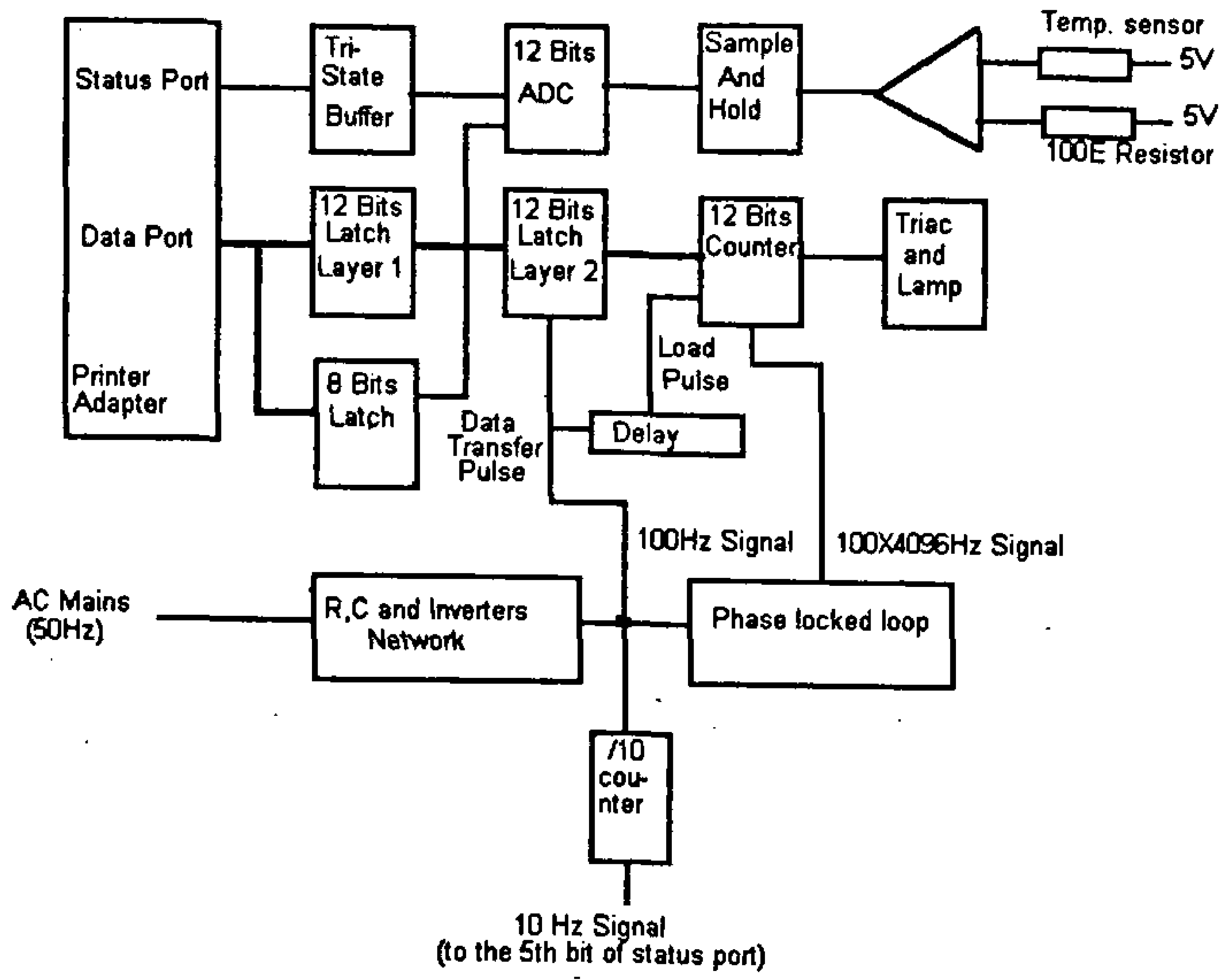


Figure 2. Block diagram of the circuit of the multiplexer and time

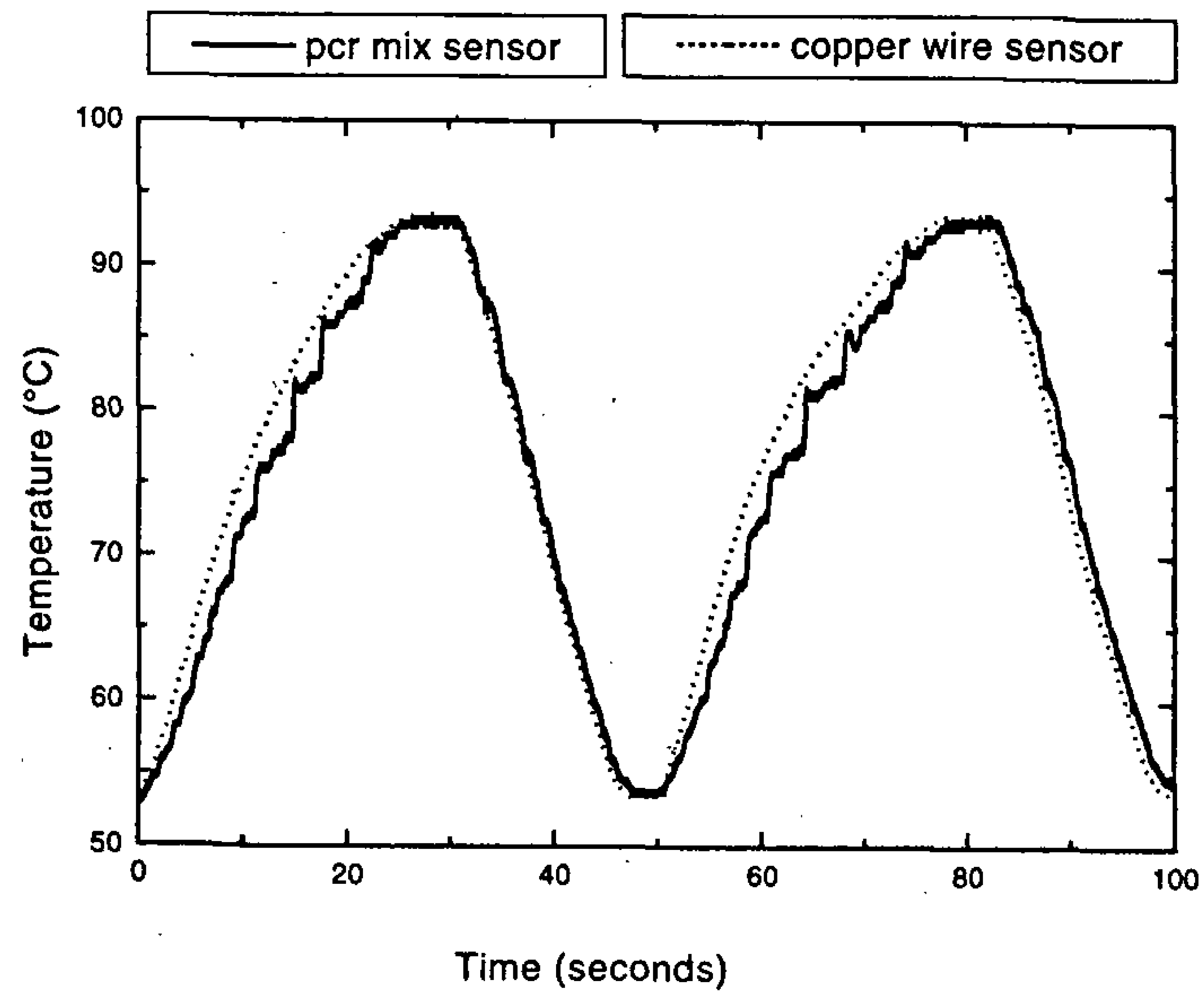


Figure 3. Temperature–time profiles of two different sensors. a PCR-mi ture sensor, and a copper-wire temperature sensor.

The timer: The block diagramme of the timer is shown in Figure 2. Since a 12-bits error signal was calculated, every half cycle of the AC mains was divided into 4096 points. AC-mains-synchronous 100 Hz was multiplied by 4096 by the phase-locked loop. The AC-mains-synchronous 100 Hz signal was used to transfer data from latch layer 2 to latch layer 1. The computer was interrupted at 10 Hz, also derived from AC-mains-synchronous 100 Hz, by the fifth bit of the status port.

Power supply: A conventic pin-regulator-based power s made to power the circuit.

The software

The interface described above to run by a program written Basic'. An 'assembly routine data from the ADC. The prog lates the error signal, 'proportional and integral' and sends it to the respective l

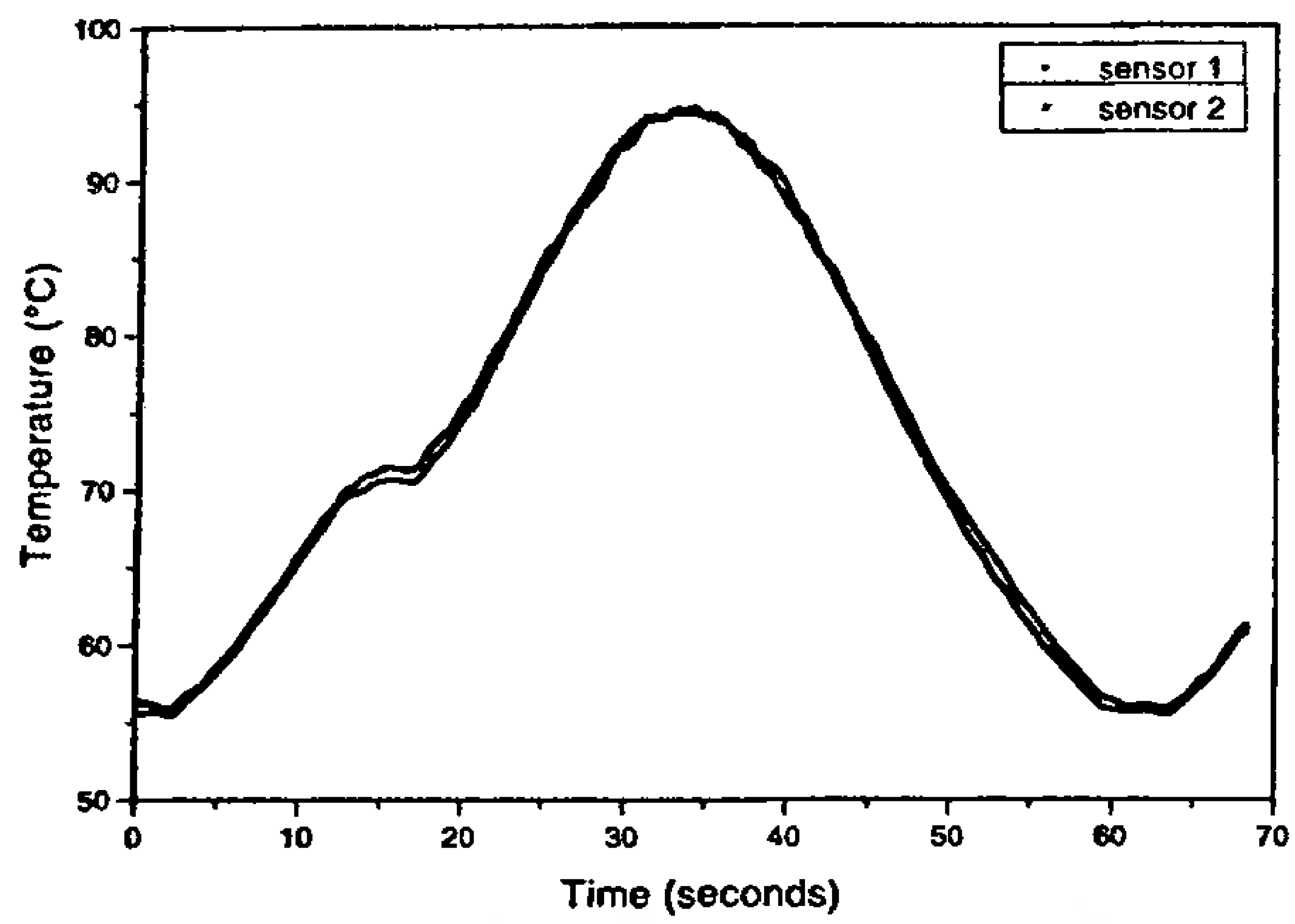
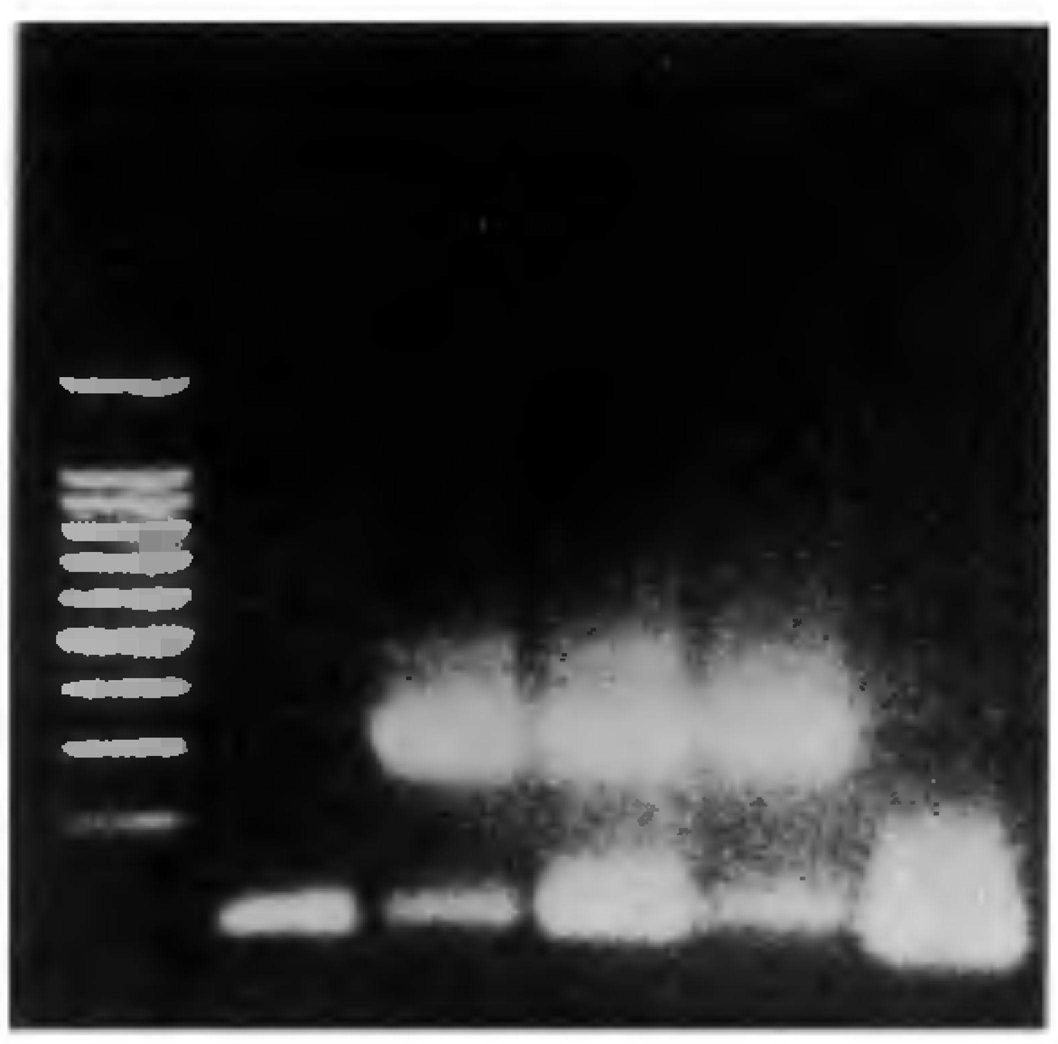


Figure 4. Temperature–time profiles at two different locations, as measured by the copper-wire temperature sensors.



1 2 3 4 5 6

Figure 5. Gel electrophoresis of amplified DNA. A 100-bp ladder is shown in lane 1. Lanes 2 and 6 show negative control with conventional cycling and fast cycling, respectively. Lane 3 shows amplification with conventional timings. Lanes 4 and 5 show amplification under fast timing. Fifty microliter of the reaction mixture (3 µl, 25 mM $MgCl_2$; 4 µl, 2.5 mM dNTP; 5 µl, 10× buffer; and 0.2 µl taq polymerase and primers; DNA and water) was used for amplification, and 5 µl of amplified product was loaded on ethidium bromide-stained gel for electrophoresis.

DNA amplification

For testing the efficiency of the instrument in the amplification of a 307-bp amplicon for the diagnosis of Hepatitis B virus[10], fifty microliter of the reaction mixture (3 µl, 25 mM $MgCl_2$; 4 µl, 2.5 mM dNTP; 5 µl, 10× buffer; and 0.2 µl taq polymerase and primers; DNA and water) was loaded in polypropylene tubes and was subjected to either of the two protocols: (i) conventional cycling, i.e. 1 min for denaturing DNA, 1 min for annealing, and 2 min for extension, for 30 cycles and, (ii) fast cycling with 10 s denaturing, 30 s annealing, and 30 s extension for 30 cycles.

Results and discussion

Assembly

Space constraints must necessarily limit the details of assembling and operating the thermocycler reported here. It is nevertheless believed that sufficient information is contained in this report to enable the construction of such a thermocycler[11].

Evaluation of the temperature sensor

Figure 3 shows a comparison of the temperature–time profiles generated by the PCR-mix sensor and copper-wire sensor. The temperature of the PCR-mix sensor did not show a change as smoothly as that for the copper wire sensor, and the maximum time lag was of the order of 5 s. Thus we conclude that the duration of the time for which the PCR samples were to be maintained at a particular temperature should not be less than 5 s. The maximum rate of temperature change in the temperature–time profiles shown in Figure 3 was of the order of 3°C/s.

Uniformity of the temperature

Two additional temperature sensors were kept at the two extreme corners on the copper foil, and their temperatures recorded. Figure 4 shows the difference between the temperatures recorded by the two sensors as less than 1°C. It was therefore concluded that the apparatus achieved a uniformity of temperature distribution of the order of 1°C across the samples.

DNA amplification

Figure 5 shows the results of the amplification experiment. Lane 1 shows a 100-bp ladder. In lanes 2 and 6, negative controls with conventional cycling and fast cycling, respectively, are shown. Positive controls are shown in lanes 3, 4 and 5. Lane 3 shows amplification with conventional cycling. And in the lanes 4 and 5, amplification with fast cycling, carried out at two different times at different locations, are shown. Comparing these with the yield of the PCR product when conventional timings were used, it is obvious that it is more than sufficient for visualizing the amplified product on the ethidium bromide-stained gel.

Conclusions

A unique design thermocycler to carry out polymerase chain reaction has been presented in this paper. The thermocycler is designed with locally available materials and is evaluated for its uniformity of temperature, temperature ramp rate, and efficiency of amplifications. Due to faster temperature ramp rate, the time required for amplification could be reduced to 45 min, which is one third of conventional time. Cost of the materials, excluding a PC, of this thermocycler works out around Rs 3000 only.

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10. Details of the primers to be published later.
11. We are planning to hold a training workshop to disseminate the knowhow of this thermocycler, in October 1999. Readers, who are keen on participating in this workshop, should write to the author.

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Pramod Upadhyay is in the National Institute of Immunology, Aruna Asaf Ali Marg, New Delhi 110 067, India