Development of a measuring device for the study of thermal processes during the polymerase chain reaction

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Abstract. In this paper, we consider the problem of studying transient temperature processes in thermal blocks of analyzers of nucleic acids based on real-time polymerase chain reaction, in particular, the ANK-32 device. Characteristics of transient temperature processes were obtained using sensors built into the ANK-32. The result of the work is an original measuring device that is able to clarify the characteristics is obtained.

1. Introduction
Currently, devices based on the polymerase chain reaction (PCR) have been actively distributed in medicine, science, health care and forensic science. This is due to the fact that studies carried out with their help, in contrast to NMR spectrometers and other various optical meters and sensors, provide the necessary information in a shorter time [1-10]. In addition, the use of PCR allows to obtain a series of data that cannot be obtained by other methods [8-14].

Polymerase chain reaction is a method based on multiple selective copying of the necessary for the study of the DNA segment under the influence of various enzymes in artificial conditions. In figure 1 shows a diagram of the formation of the necessary DNA for research. On each subsequent amplification cycle, previously synthesized DNA fragments are again copied using DNA polymerases. As a result, the number of specific DNA fragments increases many times, which in turn greatly simplifies the subsequent analysis.

\textbf{Figure 1.} Polymerase chain reaction scheme.
The formation process consists in the fact that on each subsequent amplification cycle, the previously synthesized DNA fragments are again copied using DNA polymerases. As a result, the number of specific DNA fragments increases many times, which in turn greatly simplifies the subsequent analysis. And also increases its accuracy and reliability compared with other optical methods [5, 7, 9-16].

For carrying out the polymerase chain reaction, the reaction mixture is used, consisting of the following components: primers, Taq - to decipher DNA polymerases, mixtures of deoxynucleotide triphosphates (dNTPs), buffer, and also the sample under study. The reaction mixture passes several phases of heating and cooling sequentially during PCR. As a result, when carrying out PCR, it becomes necessary to have an accurate idea of the change in temperature of the reaction mixture placed in a test tube [17]. The data on the value of T is necessary for the subsequent identification of DNA chains (comparing them with test samples). In our work is considered one of the solution to this problem.

2. The design of measuring device and its principles of operation

The temperature sensors developed at the present time either do not satisfy the accuracy of measuring T, or cannot be placed in a test tube to the sample under study. In devices, which are in operation for implementation of PCR, it is possible to obtain data on the temperature of the amplifier (precision thermostat) [18, 19], using the built-in temperature sensor. However, this temperature characterizes the state of the outer wall of the tube, which, in turn, does not allow to draw accurate conclusions about the temperature of the reaction mixture inside the tube. In figure 2 shows the dependence of temperature change measured by these sensors.

![Figure 2. Data received from the device ANK-32.](image-url)
The analysis presented in figure 2 dependences show that it is necessary in some cases to specify the results using this measuring device in order to reproduce the temperature measurements of the reaction mixture itself. This creates additional difficulties. Therefore, the development of a measuring device for measuring the temperature of the reaction mixture itself is very relevant.

As a result of the research, the parameters of the developed measuring device were determined. The size of the temperature sensor should not exceed the dimensions of the test tube with the reaction mixture, which are 21 mm in height and 6 mm in diameter, temperature measurement should be implemented with an accuracy of 0.01 K, the time resolution should be at least 0.01 s.

Most modern measuring devices are implemented as ready-made printed circuit boards using various microchips. This fact dictates the need to implement the developed measuring device in this form.

In this case, it is more expedient to use a thermistor as a temperature sensor, due to its small size, a significantly higher signal than that of a thermocouple, as well as a higher accuracy of measurement.

The choice of a thermistor as a temperature sensor determines the principle of construction of the concept of the measuring device, namely the presence of analog and digital parts. The main problem in measuring the temperature in the sensors on the thermoelectric effect is the compensation of the parasitic resistance of the connecting wires. Especially when measuring T with high accuracy. In figure 3 shows the connection diagram of the thermistor.

![Figure 3. Thermistor connection diagram using the Kelvin method.](image)

In this scheme (fig. 3), we proposed a new solution to the problem of measuring the current flowing through a thermistor using a resistive voltage divider using a resistor with a very low value of the temperature coefficient of resistance (<50 ppm). The voltage applied to the divider is distributed between the resistor with a low temperature coefficient of resistance and the thermistor is proportional to the values of their resistances. Since the voltage divider is used, the current on both resistors will be the same, which will allow determining the resistance of the thermistor at the time of measurement with high accuracy. This made it possible to keep the dependence of resistance RTD on the ambient temperature of the thermistor close to linear. In figure 4 this dependence for used sensor is presented.
Figure 4. The dependency $R_{ref}$ from temperature $T$ of thermal sensor.

This allows you to use the following dependence for measurements:

$$R_{ref} = R_0 \left(1 + A(T - 273,15) + B(T - 273,15)^2\right)$$  \hspace{1cm} (1)

Where, $R_{ref}$ is the resistance value of the thermistor in ohms at a certain value of temperature, measured in degrees Kelvin; $R_0$ is the resistance value of the thermistor at ambient temperature components of 0 K; A and B constants of value:

$$A = 3,81 \times 10^{-3} \text{ K}^{-1}$$ \hspace{1cm} (2)

$$B = -6,02 \times 10^{-7} \text{ K}^{-2}$$ \hspace{1cm} (3)

The use of an analog signal implies its further conversion to a digital code. As a result, the need arises to use an analog-to-digital converter (ADC).

The need for accurate temperature measurements implies the use of low-noise and highly stable elements. As a consequence, the sigma-delta ADC was chosen - AD7714. The presence of two measuring channels in it allows to realize the input circuit of the measuring device in the form of a resistive voltage divider.

Temperature measurements are carried out as follows: the first channel is used to measure the voltage across a resistor with a constant resistance value, which in turn allows determining the current flowing in the circuit, and the second channel is necessary to measure the voltage drop across the thermistor. As a result, this allows to obtain the exact value of the resistance of the thermistor at the
time of the measurement. Next, the temperature of the reactive mixture is determined by converting
the obtained resistance value in accordance with the technical documentation of the thermistor.

To visualize the received data, it is necessary to implement on a printed circuit board a device that
would be able to communicate with a computer. A microcontroller of the ARM STM32F303VCT6
family was chosen as such a device. The choice of this particular microcontroller is explained by its
high speed, 32-bit resolution, as well as the presence of the necessary interfaces for working with
peripheral devices.

Data transfer from the microcontroller is implemented through the UART, through the CP2102
chip, which was chosen due to its high stability, as well as low current consumption.

The principle of operation of the selected microcontroller is based on interruptions of the system
timer called every 10 milliseconds. During each of the interruptions, the values of the voltages on the
resistive voltage divider are digitized, their further mathematical processing and sending the obtained
temperature value of the reaction mixture in text form.

Next, the information goes to a data processor written in Python 3 versions, which visualizes
temperature characteristics using the serial, matplotlib, and drawnow libraries. In figure 5 shows the
appearance of the measuring device developed by us.

3. Conclusion

The developed measuring device will allow to obtain the temperature characteristics of the
transients of the reaction mixture in a test tube with a given accuracy, which will optimize the
performance of devices based on PCR. The temperature measurement error is no more than 0.01 K.
The measurement time for one temperature measurement is no more than 0.01 s.

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