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A Noncontact Temperature Measurement Method in Polymerase Chain Reaction Reactors

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Abstract—A new noncontact method for measuring temperatures of liquids, which is based on the fluorescent probes, is proposed. The method is intended for measuring temperatures of reaction media in reactors of devices for polymerase chain reactions in real time and can be used for determining dynamic temperature parameters.

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Continuous measurement of temperature in small volumes of liquid medium is a nontrivial problem. The use of sensors, such as thermocouples, is not always possible for two reasons. First, this measurement method is characterized by inertness, determined by the mass of the measuring element and equalization rate of its temperature, when the temperature of the medium is changed. When the mass of the sensor is large compared to the mass of the evaluated medium, its presence will create substantial distortions in the dynamics of the temperature changes of the medium. As a result, the measurements become uninformative in principle. Second, the sensor must be in contact with the measured medium. This is not always possible technologically, e.g., if the medium must be located in the hermetically closed reactor. In addition, the use of the sensors does not allow one to analyze the space distribution of the temperature in the presence of temperature nonuniformity of the medium, being highly probable in the course of heating or cooling of the studied object.

Noncontact temperature measurement methods are free of the above-mentioned drawbacks. One method used is measurement of the thermal self-radiation of a studied object using an infrared sensor or thermal imager. Another noncontact temperature measurement method is based on the use of fluorescent probes that are sensitive to the temperature of the medium [1]. This method is suitable, since it allows one to study processes in closed capacities, including microliter volumes, which are made of transparent (for the visible light) materials [2]. The measurements are performed using different fluorometer detectors.

Thus, it has been proposed to use the fluorescent dye rhodamine B as a probe for measurements [3]. Its fluorescence quantum yield depends on the temperature by reason of the mobility of amides as a part of a molecule. The coefficient of the temperature dependence of its fluorescence in a range of measured temperatures was 2.3% K^{-1}. This allowed the authors to perform measurements with an error of ± 1.4 K (95% confidence interval). Sulforhodamine B with a maximum temperature dependence of 1.55% K^{-1} at an exciting light wavelength of 560 nm was applied for recording temperatures in [4]. At the wavelengths of 470–483 nm used in the aforementioned work for measuring temperatures, the temperature dependence of the fluorescence of sulforhodamine B was 1.19% K^{-1} [4].

The purpose of our work is to design a noncontact temperature measurement method in reactors of devices for conducting the real-time polymerase chain reaction (RT-PCR). The RT-PCR is one of the most widespread methods of molecular-genetic analysis, used in medicine, agriculture, criminalistics, and many other areas. To conduct the PCR, it is necessary to ensure cyclic heating and cooling of the reaction medium between different specified temperature levels in a range of 60–95°C. The apparatus used for the RT-PCR is a combination of a controlled thermostat and fluorometer detector for measuring concentrations of reaction products. Since, for practical application of the analysis, it is important to complete it in a minimal time, efforts have been made to reduce the duration of transient processes in the course of thermocycling [5]. For this purpose, the manufacturers of devices aim at increasing the heating and cooling rate

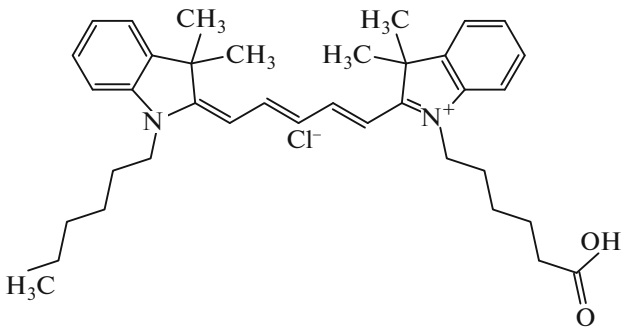

Fig. 1. Structural formula of the Cy5 fluorescent probe (1-[6-hexyl]-1'-[6-hexane acid]-3,3,3',3'-tetramethylindodicarbocyanin chloride).

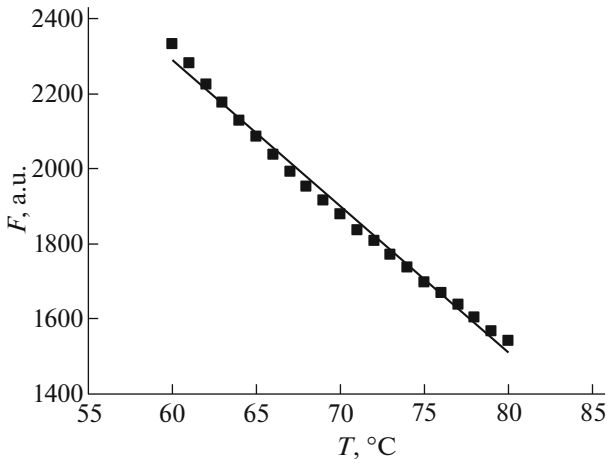

Fig. 2. Temperature dependence of the fluorescence of the Cy5 probe.

of the thermostat. However, the *thermostat–reactor–reaction mixture* system possesses a substantial inertia, since the thermal contact between the walls of the thermostat and reactor is imperfect, mechanical agitation is absent in the mixture volume, and the temperature is equalized predominantly owing to convection. It is known from practice that the time constant for thermal transitions inside the reactor is about 10 s. It is known that the temperature equalization rate in reactors substantially differs in different devices for the RT-PCR [5, 6]. The error of the RT-PCR-based quantitative analysis substantially depends on the rate of this process and dynamic temperature uniformity, understood as a minimal deviation of rate constants for individual reactors from the average value [7, 8]. In reactors with a slower temperature equalization the efficiency of the PCR drops, since in this case the time for the complete reaction behavior is insufficient.

For experimental analysis of thermal processes in the reactors of the RT-PCR devices, we adapted the noncontact temperature measurement method, which is based on fluorescent probes. As a probe, indodicarbocyanin fluorescent dye (Cy5) was used (Fig. 1). It is used in molecular biology as a long-wavelength fluorophore (the excitation wavelength is 645 nm, and the emission wavelength is 670 nm). The fluorescence of these dyes is determined by a chain of coupled double bonds connecting two indole nuclei. In this case, the fluorescence wavelength is proportional to the length of this chain. This system is very mobile, determining, seemingly, the sensitivity of its fluorescence to the temperature.

The measurements were performed at a reaction mixture volume of 25 μL, which contains a 0.2-μM aqueous solution of the Cy5 fluorescent probe. As a reactor, standard microvials for the PCR with a volume of 0.2 μL were used. The dependence of the fluorescence on the temperature in the studied temperature range is sufficiently linear (Fig. 2), and the deviation from the linearity at the ends of the range does not exceed 0.3 K. The temperature dependence coefficient was 2.1% K^{-1}, being substantially better than in [4] and close to the result obtained in [3]. The random temperature measurement error at a measurement interval of 0.5 s per dot was ±0.33 K (95% confidence interval).

This procedure allowed us to determine the rate of transient thermal processes in the reactors of the devices for the ANK-family RT-PCR, designed at the Institute for Analytical Instrumentation (St. Petersburg). Figure 3 shows an example of the recoding results of transient processes in two reactors (thin lines) and the thermal unit of the thermostat. As an example, the reactors most differing in the transient process rate were selected. The temperature in intervals of *1*, *2*, and *3* was 60, 80, and 60°C, respectively. The thermostat temperature variation rate was 1.7°C/s. The values shown in Fig. 3 are normalized to the difference of intensities between the first and second intervals. For quantitative analysis of the temperature dynamics of the reaction medium, the fluorescence values in the interval of 20–66 s were approximated by the exponential dependence.

The influence of the clamp of the thermostat cover on the quality of the thermal contact between the walls of the thermostat and the reactor, which is characterized by a medium heating rate and uniformity in reactors of the device for the RT-PCR, was studied using the above procedure. For this purpose, the fluorescence in the reactors was measured and the time constant of the exponential dependence approximating the dependence of the fluorescence on the time was evaluated. Next, the pressure applied to the thermostat cover was increased and, again, the time constant of the fluorescence variation was evaluated. The obtained values before and after intensification of pressure were 7.5 ± 0.5 and 7.0 ± 0.4 s, respectively, Thus, the thermal contact quality can be raised by increasing the clamp effort of the thermostat cover within the known limits. It was also determined that

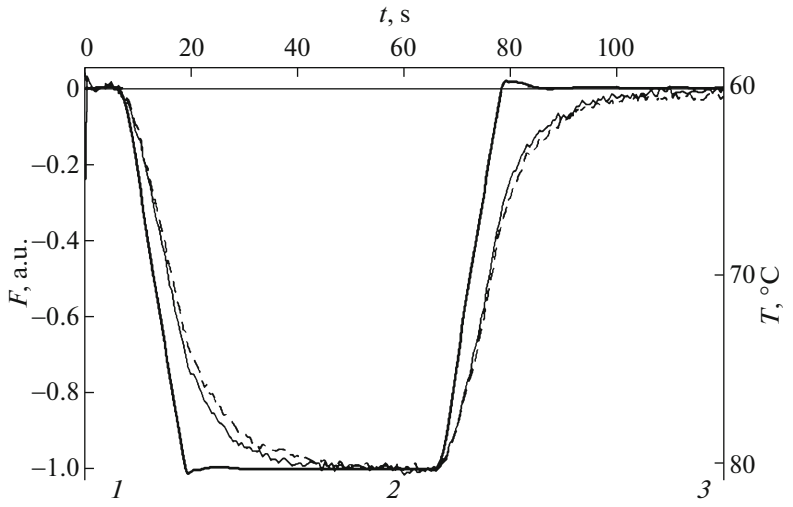

Fig. 3. Recording of the fluorescence in two reactors of the ANK RT-PCR device. The thin lines are the inverse normalized time dependence of the fluorescence. The thick line is the time dependence of the temperature of the thermostat.

the thermal contact is improved, if the system is preliminarily heated up to 95°C within several minutes. This fact is confirmed by the decrease in the time constant to 6.0 ± 0.5 s. This effect is likely to be the result of the reactor deformation, leading to its denser shrinkage in the cell of the thermal unit.

The obtained results show that the proposed noncontact temperature measurement method is an efficient tool for studying thermal processes in reactors of the RT-PCR devices. These studies play an important role in designing new devices with improved thermal characteristics.

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