REMUNOM
ISSN 2178-6925

Received: 20/01/2026 - **Accepted:** 01/02/2026
Vol: 02.01
DOI: 10.61164/gvsvce49
Pages: 1-17

TERMÔMETRO DIGITAL PARA DISPOSITIVOS DE ELETROFORMAÇÃO DE VESÍCULAS UNILAMELARES GIGANTES

DIGITAL THERMOMETER FOR GIANT UNILAMELLAR VESICLES ELECTROFORMATION DEVICES

TERMÓMETRO DIGITAL PARA DISPOSITIVOS DE ELECTROFORMACIÓN DE VESÍCULAS UNILAMELARES GIGANTES

Juracy Leandro dos Santos Júnior
Mestre em Engenharia de Computação
Discente do Programa de Pós-graduação em Engenharia Elétrica e de Computação da Universidade Federal de Goiás, Goiânia, Goiás, Brasil
E-mail: juracyleandro@ufg.br

Sebastião Antônio Mendanha
Doutor em Física,
Instituto de Física da Universidade Federal de Goiás, Goiânia, Goiás, Brasil
E-mail: sebastiao_mendanha@ufg.br

Marcus Carrião dos Santos
Doutor em Física
Instituto de Física da Universidade Federal de Goiás, Goiânia, Goiás, Brasil
E-mail: mscarriao@ufg.br

Sílvio Leão Vieira
Doutor em Física Aplicada à Medicina e Biologia
Instituto de Física da Universidade Federal de Goiás, Goiânia, Goiás, Brasil
E-mail: slvieira@ufg.br

Resumo

Este trabalho descreve o desenvolvimento de um termômetro digital multicanal para validar o controle de temperatura de um equipamento de eletroformação de vesículas unilamelares gigantes (GUVs). O protótipo apresentado foi construído com recursos convencionais e de baixo custo, como componentes e circuitos da plataforma de código aberto Arduino e termistores de coeficiente de temperatura negativo (NTC), resultando em um equipamento com boa precisão, baixo custo de produção e fácil manutenção. O dispositivo permite a medição simultânea de temperatura em quatro canais e registra os valores no computador para avaliação temporal. Este equipamento foi essencial no processo de criação de um dispositivo de eletroformação de GUVs discutido em outro trabalho, mas apresenta diversas possibilidades de aplicação na pesquisa e desenvolvimento de outras tecnologias dependentes de temperatura. Em particular, o dispositivo permitiu o desenvolvimento do

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protocolo de eletroformação de GUVs ao identificar o processo de atraso causado pelo posicionamento do termômetro fora da amostra estudada.

Palavras-chave: Medição de temperatura; Controle de temperatura; Biofísica; Biomembranas.

Abstract

This work describes the development of a multichannel digital thermometer to validate the temperature control of giant unilamellar vesicle (GUVs) electroformation equipment. The prototype presented was built with cheap and conventional resources, such as elements and circuits from the Arduino open-source platform and negative temperature coefficient (NTC) thermistors, resulting in equipment with good precision, low production cost and easy maintenance. The device allows temperature measurement in four channels simultaneously and records the values on the computer for temporal evaluation. This equipment was essential in the process of creating a GUV electroforming device discussed elsewhere, but there are several possibilities for application in the research and development of other temperature-dependent technologies. In particular, the device allowed the development of the GUV electroformation protocol by identifying the delay process caused by positioning the thermometer outside the studied sample.

Keywords: Temperature measurement, Temperature control, Biophysics, Biomembranes.

Resumen

Este trabajo describe el desarrollo de un termómetro digital multicanal para validar el control de temperatura de equipos de electroformación de vesículas unilamelares gigantes (GUV). El prototipo presentado se construyó con recursos económicos y convencionales, como elementos y circuitos de la plataforma de código abierto Arduino y termistores de coeficiente de temperatura negativo (NTC), lo que resultó en un equipo con buena precisión, bajo costo de producción y fácil mantenimiento. El dispositivo permite la medición de temperatura en cuatro canales simultáneamente y registra los valores en la computadora para su evaluación temporal. Este equipo fue esencial en el proceso de creación de un dispositivo de electroformación de GUV, discutido en otro lugar, pero existen diversas posibilidades de aplicación en la investigación y el desarrollo de otras tecnologías dependientes de la temperatura. En particular, el dispositivo permitió el desarrollo del protocolo de electroformación de GUV al identificar el proceso de retardo causado por la posición del termómetro fuera de la muestra estudiada.

Palabras clave: Medición de temperatura, Control de temperatura, Biofísica, Biomembranas.

1. Introduction

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In several technological applications, it is necessary to establish the thermal behavior of equipment and, in certain cases, ensure precise temperature control. These applications can be found in industrial processes or in household devices such as air conditioning and ovens, as well as in experimental setups such as the electroformation process (Ang; Chong; Li, 2005; Chakraborty *et al.*, 2018; Fraden, 2016; Levine, 2018; Ogata, 2009). Specifically, in biophysical experimentation, assessing or maintaining the temperature stability of solutions is essential. Maintaining the temperature at specific values is reported as one of the key factors to be controlled during the electroformation of giant unilamellar vesicles (GUVs)(Bagatolli; Needham, 2014; Li *et al.*, 2016; Politano *et al.*, 2010), which are important model systems to emulate cell membranes.

The development of device dedicated to the electroformation process has been described in the literature over the years (Bagatolli; Needham, 2014; Drabik; Doskocz; Przybyło, 2018; Li *et al.*, 2016; Politano *et al.*, 2010). They are primarily based on chambers where a phospholipid film is deposited on electrodes, which are then hydrated in pure aqueous solution or saline solutions. These biomembranes may exhibit different phases in their lipid layers depending on temperature: a rigid ordered gel phase, a more flexible phase, and a disordered fluid crystalline phase (Ghellab *et al.*, 2019; Mustonen; Kinnunen, 1991). Controlling the temperature of the medium in which the biomembrane is produced can determine which phase it presents, thus controlling the effect of membrane permeability, as permeability is influenced by membrane fluidity (Li *et al.*, 2016; Shimanouchi; Umakoshi; Kuboi, 2009). In GUVs formation, temperature changes cause an increase in their growth due to a decrease in the bending modulus and an increase in fluidity during the formation process. However, the measuring of temperature inside the chamber is very inconvenient, since the biomaterials used in these experiments are very sensitive and will demand extreme caution with cleanness. On the other hand, using the thermometer outside of the chamber requires a measurement validation. Thus, an essential step for the development of an electroformation chamber with temperature control is needed.

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In this work we present a low-cost multichannel device to measure and log temperatures. The prototype was used to validate the thermometer used to assess and control temperature in a GUVs electroformation device described elsewhere (Dos Santos *et al.*, 2019; Santos Junior; Vieira, 2022). This multichannel device was developed by customizing the circuit of the Arduino open-source platform, composed of an ATMEGA328P® microcontroller and discrete commercial components. In this way, it was possible to design a device that utilizes easily accessible electronic components and is economically viable compared to pre-existing thermometers. Here, we propose a commercial multichannel thermometer and scientific data acquisition platform (e.g., NI DAQ-9212; approximately US$2,900) as an available option. Its originality lies in the development and validation of a customized, relatively low-cost system (approximately US$73) for thermal monitoring in electroforming protocols of giant unilamellar vesicles (GUVs). The practical advantage of the system lies in its ability to accurately quantify the thermal delay between the control sensor and the internal sample. This capability is absent from generic solutions. This capability enables adjustment of experimental parameters, significantly improving protocol reproducibility. Thus, the system presents itself as a viable, accessible alternative to high-cost scientific solutions without sacrificing quality. Such types of solutions can be helpful in research laboratories with limited resources for investing in complex proprietary systems. Therefore, the system offers a viable alternative to commercial products and expensive industrial platforms, and it can be adapted for other applications requiring synchronized thermal monitoring.

3. Methodology

For electronic temperature measurement, we can find two main types of thermoelements frequently used as temperature sensors: thermocouples and thermoresistors. The proposed circuit uses a Thermally Sensitive Resistor, or thermistor, to measure temperature. The thermistor used has a negative temperature coefficient (NTC) and is an epoxy-coated NTC sensor with a resistance of 10,000 Ω at 25 °C, of the insulated wire type. The NTC thermistor has several advantages when

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compared to other temperature sensors as it has a small volume, high sensitivity, wide range of nominal resistance and low cost, being widely used in several areas, such as medicine, household appliances, automotive, military, aerospace, among others (Chakraborty *et al.*, 2018; Liu *et al.*, 2024). The NTC is a resistive device in which the passage of current causes voltage drops. That is, any change in its resistance due to temperature changes can be converted into a voltage change. This change is measured using the thermistor as part of a voltage divider circuit. A stabilized and constant power supply voltage is applied to the circuit in series with the thermistor, whose voltage is monitored. This supply is made with a programmable precision voltage reference, using the TL431 integrated circuit, programmed to provide 4092 mV. The combined system uncertainty was estimated based on the NTC sensor tolerance (1%), the TL431 voltage reference accuracy (0.5%), and the ADC 10-bit resolution. The calculated total expanded uncertainty results in approximately ±0.5 °C, which is consistent with the experimentally observed standard deviations (between 0.21 °C and 0.40 °C) presented in Table I.

Figure 1(a) shows the diagram of the circuit assembled as a voltage source and the NTC in the voltage divider, detailing the electronic components and input and output connections, as described in the component captions. This voltage divider circuit operates as a resistance-to-voltage converter. In this project, the magnitude of the output voltage is read by a 10-bit analog-to-digital converter (ADC) of the ATMEGA328P® microcontroller. From the value of the NTC resistance, we can obtain the temperature value at the sensor. For this, we will use an equation based on the β parameter of the NTC sensor, which is derived from the interpolation function found by Steinhart and Hart (Ebrahimi-Darkhaneh, 2019; LIU *et al.*, 2020). The expected error in the temperature can be estimated considering the error in the NTC sensor resistance (LIU *et al.*, 2020; Temperature Sensing with Thermistors Billions of people every day use technology that has, 2020), which is 1%. This means, for example, that the sensor, being at 25 °C, the resistance value can be between 10,100 Ω and 9,900 Ω. Therefore, a 1% error means approximately ±0.25 °C. The error of the 10-bit ADC converter used can represent an error in the reading of each bit of about 50 Ω of the

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resistance value. This represents an error of ±0.1 °C, which is smaller than the error of the NTC sensor.

2.1 Stabilized voltage source

The TL431 integrated circuit is a three-terminal adjustable shunt regulator with thermal stability. The output voltage can be adjusted with two external resistors to any value between 2.5 V and 36 V. The tolerance at 25 °C is 0.5%, and the low output deviation with respect to temperature ensures good stability across the temperature range. A variable resistor fixed at 4092 mV was used to adjust the output value. This stabilized and constant voltage was used as the analog reference for the ADC converter and as the voltage source for the resistance-to-voltage conversion circuit, as shown in the circuit diagram in Figure 1(a).

2.2 Resistors

The current through the NTC sensor should be kept as low as possible to avoid probe heating. Self-heating is a phenomenon that occurs when a current flows through the NTC thermistor, dissipating this energy as heat and warming the thermistor core, affecting measurement accuracy. However, as the bias current decreases, the sensor sensitivity also decreases, resulting in noisy measurements. To maintain a balance between sensitivity gain and bias current, the value of the series resistor should be sufficiently large to limit the bias current and minimize power dissipation (Steinhart; Hart, 1968; Temperature Sensing with Thermistors Billions of people every day use technology that has, 2020). The series resistor chosen for an NTC with a resistance of 10 kΩ at 25 °C and using a voltage source less than 5 Volts will have a resistance of 10 kΩ. With this configuration, the magnitude of the output voltage at the base temperature of 25 °C will be half the supply voltage. When there is a change in the NTC resistance due to temperature changes, the fraction of the supply voltage that passes through the NTC also changes, producing an output voltage proportional to the fraction of the total resistance in series between the output terminals. This potential divider circuit operates as a resistance-to-voltage converter, where the NTC resistance is controlled by temperature, with the output voltage produced being proportional to temperature. The hotter the NTC becomes, the lower

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the output voltage. The series resistors used were chosen with sufficiently close values and were used as an input parameter in the control algorithm.

2.3 Sensors

The four temperature sensors used are NTC thermistors (Fraden, 2016). Three sensors were installed inside the chambers that receive the electroformation solution, while one monitors the room temperature. Recording the ambient temperature allows us to check if external temperature variations affect the temperature control of the electroformation chambers. The expression to obtain the temperature value T involves a calibration temperature T0, for which we adopt the ambient temperature, the β coefficient of the NTC thermistor, and the resistance R0, obtained from the calibration temperature. To ensure that the four sensors are measuring temperatures as close as possible, adjustments were made to the β and R0 values provided by the manufacturer, considering that these values have a 1% error margin. The four sensors were submerged in water at different temperatures to check for discrepancies in the temperature values measured by them. By repeating this process after incrementing and decrementing the β and R0 values, we obtained differences between the probes on the order of 0.1%.

The system has inherent limitations due to its configuration, the operating range of the negative coefficient thermistor (NTC) sensors, and the 10-bit resolution of the analog-to-digital converter (ADC). To mitigate the influence of possible external noise sources and abrupt fluctuations, a layered signal processing algorithm was implemented: initially, for each acquisition port, the arithmetic mean of ten sequential readings with a 200 μs interval is performed; subsequently, a ten-point moving average filter is applied independently to each temperature value calculated for each sensor, this entire process takes a maximum of 10 ms to complete. We guarantee synchronization between the sensors at a 1 s sampling rate. This algorithm minimizes the vulnerability associated with low-cost electronics. Additionally, the strategic inclusion of a dedicated channel for monitoring ambient temperature provides a reference for distinguishing external variations, enabling the evaluation and compensation of any interference in the chamber’s-controlled microenvironment. The

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number of channels is limited by the ATMEGA328P's multiplexed ADC pins, which support up to 6 channels.

2.4 Custom Printed Circuit Board

The printed circuit board developed for the digital thermometer features stackable 2.54 mm connectors, positioned to fit and secure to the Arduino platform board. On the board, we have precision voltage reference, the resistors of the voltage divider circuit along with connectors for the NTC probes, as well as connectors for possible digital signal inputs and outputs. In Figure 1(b), we have the design of the digital thermometer's printed circuit board.

2.5 Sensor Placement and Thermal Delay

The sensor integrated into the GUV electroformation device is placed externally to the cuvettes that will receive the solution. This intermediate position between the heat source and the chamber causes the temperature measured by this sensor to lag the temperature of the solution inside the chamber. This delay arises from the sensor being closer to the source that supplies or removes heat. However, as explained earlier, the sensor was positioned externally to avoid contamination and misuse issues.

By collecting temperature data, it is possible to measure the stabilization time between the temperature of the solution inside the chamber and the temperature read by the sensor fixed on the aluminum support on the chamber's side base. Additionally, it allows us to verify if there are temperature differences among the aqueous solutions in the three chambers.

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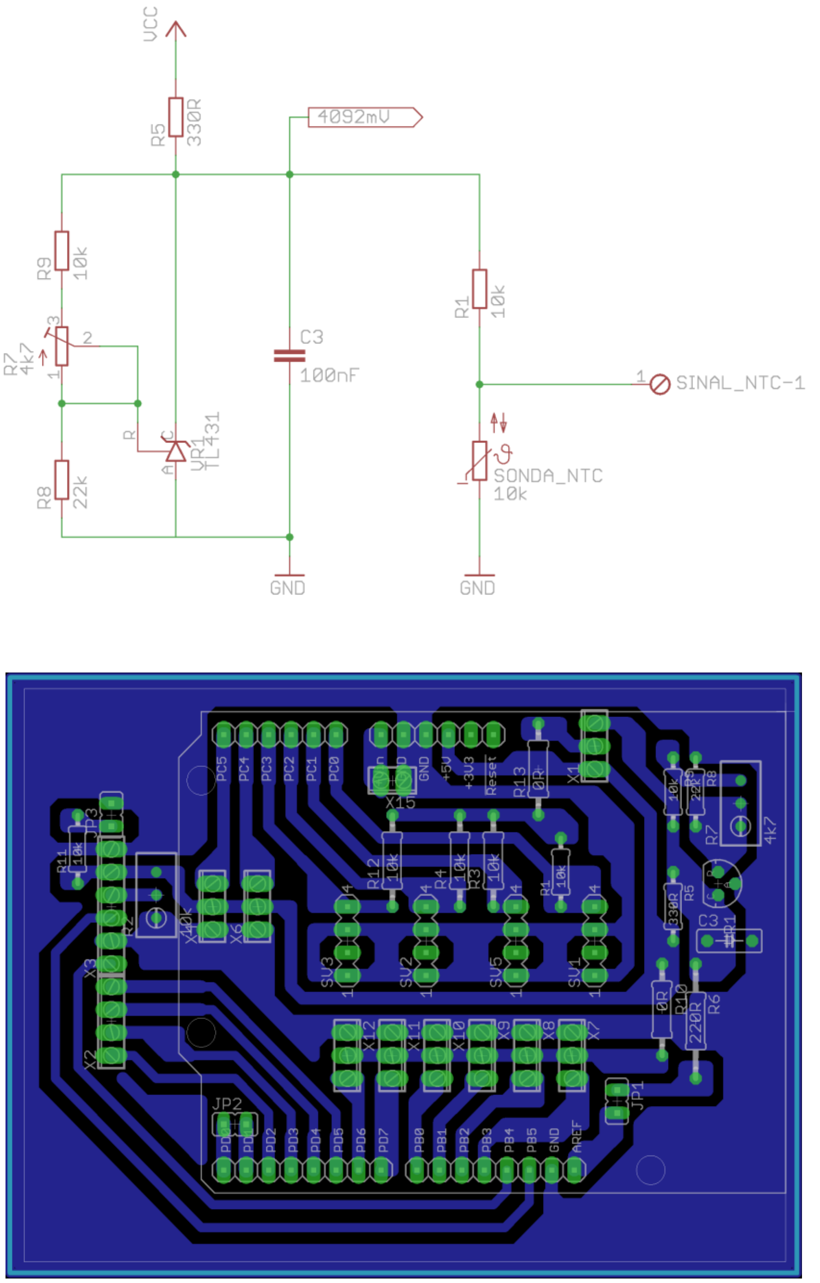

Fig. 1. (a) Schematic design of the precision source and voltage divider circuit for the NTC. (b) Image detailing the printed circuit board developed with the electronic components and external connections of the digital thermometer.

2.6 Reading algorithm

The microcontroller used features a 10-bit successive approximation multichannel ADC converter with ±2 accuracy in the least significant bit (LSB). It can map input

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voltages between 0 and the reference voltage. The reference voltage used provides 4092 mV, resulting in a resolution between readings of approximately 4 mV per unit. The system has up to 6 multiplexed ADC input channels selectable through internal register configurations. The various voltage signals are digitized one by one after channel selection. The analog input signal takes about 200 µs to be digitized. To reduce noise in the measurements, ten readings are averaged before converting the values into temperature. The algorithm writes the temperature values of each sensor into a ten-element array. After filling in the array elements, the first recorded point is discarded, and the remaining points are shifted to register the most current value. This entire process takes no more than 10 ms to complete. Every second, the serial communication requests the transmission of the temperature value from the four probes. This request receives, in response, the average of the ten points from each corresponding array for each temperature probe. This process attempts to efficiently describe the temperature behavior every second. The diagram of the control algorithm's functioning process can be seen in Figure 2.

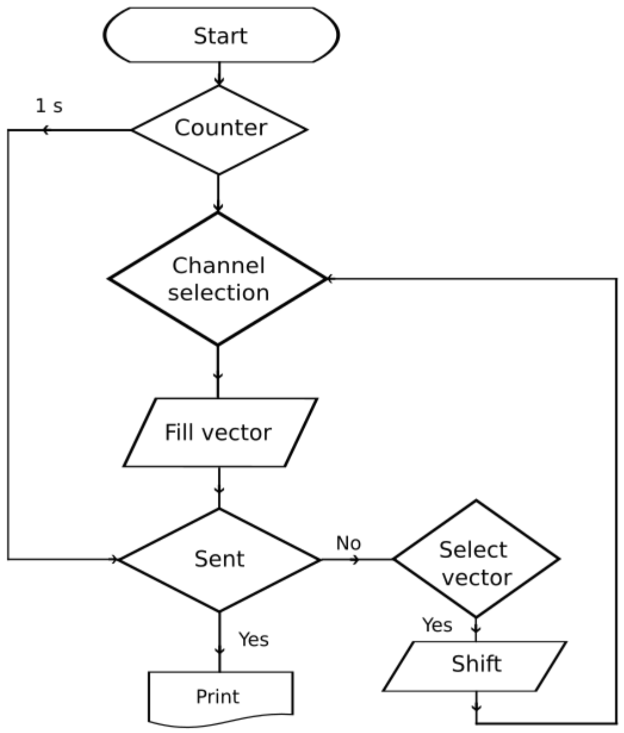

Fig. 2. Flowchart of the algorithm for recording temperature values.

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2.7 Experimental procedure

The internal temperatures of the electroformation chambers were collected from the NTC sensors submerged in 525 µL of aqueous solution. The arrangement where the aqueous sample is deposited was constructed from polytetrafluoroethylene (PTFE), a material chosen for its highly non-reactive nature. The sensor external to the aqueous solution is fixed in a hole in the aluminum base surrounding the chambers. The ambient temperature sensor is located less than 10 cm from the chambers. Data acquisition was carried out for 150 minutes, and the temperature values from the sensors were collected and recorded every second. Figure 3 shows a schematic of the experimental design and a photograph of the real system.

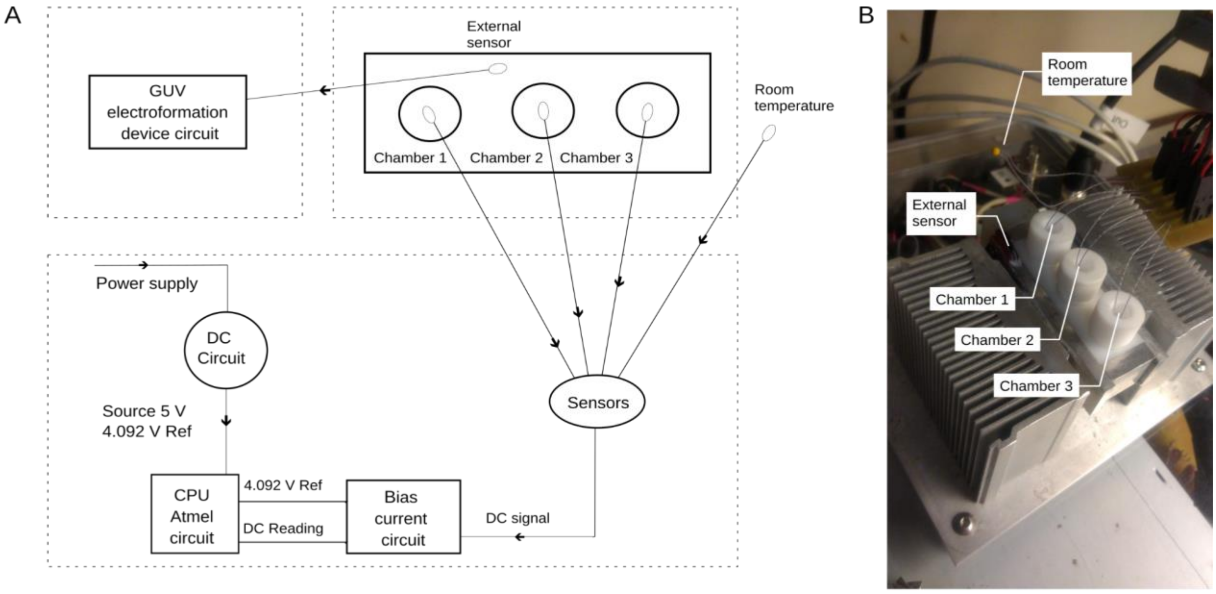

Fig. 3 (A) Schematic design of temperature acquisition and comparison experimental protocol. (B) Photograph of the proposed design.

3. Results and Discussion

The temperature of the solution in which the biomembrane is formed determines the phases of its lipid layers. The process of GUVs electroformation needs to be initiated in the disordered fluid phase, so maintaining the temperature with minimal variation in this range ensures the success of the process. Additionally, it is important to

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Received: 20/01/2026 - **Accepted:** 01/02/2026
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estimate the time required for the solution inside the cuvettes to reach the same temperature as the external sensor, that is, the temperature set by the device operator. Based on this information, a protocol for using the equipment will take this delay into account.

The temperature used in the procedure is around 60 °C, and the goal was to evaluate the temperature of the three chambers, the difference in temperature of the sensor on the external surface, and the influence of ambient temperature on this process. Figure 4 illustrates the behavior of temperature values measured with the external fixed sensor, with the ambient sensor, and with the three sensors immersed in pure water, one in each chamber.

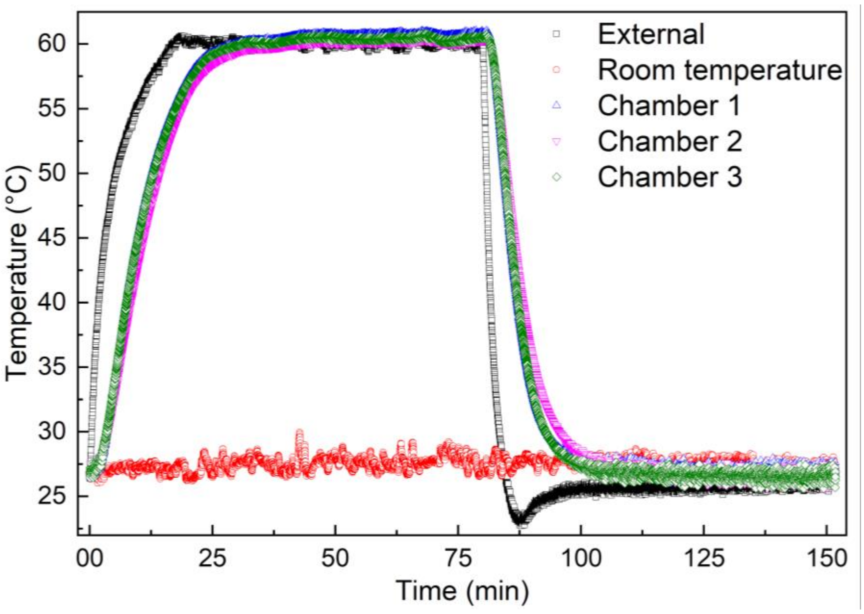

Fig. 4 Temperature control response using the external sensor together with the temperature reading of the three chambers plus the ambient temperature. The square symbol represents the temperature of the sensor on the aluminum support on the side base, the circle represents the ambient temperature, the triangle, the inverted triangle and the diamond represent the temperatures of the aqueous solutions in the electroforming chambers.

REMUNOM
ISSN 2178-6925

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The external sensor is used for closed-loop temperature control of the electroformation chamber, showing the smallest deviation in temperature value. The first 25 minutes represent the heating ramp, and after 75 minutes, we have the cooling ramp. After the heating ramp, the temperature stabilizes at the settled value where the three submerged sensors show a stable trend compared to the value of 60 °C, with an average deviation of 0.35 °C. The externally fixed sensor records a shorter response time for heat exchange, as it is closer to the heat exchanger, with a difference to the temperature inside the chambers of approximately 8 minutes.

As can be seen, the digitization and processing of signals corresponding to temperature values for four sensors by the microcontroller ATMEGA328P®, using the described parameters, proved to be very effective. The internal temperature stability was confirmed, as well as the equivalence in temperature reading with the external sensor after waiting for a difference in response time. With this information, it was found necessary to wait a minimum of 8 minutes after stabilizing the external sensor to initiate the electroformation process. The temperature reading circuit resolution ensures a standard deviation in the measured temperature value of less than 0.5 °C. A comparison of the statistical values obtained for the set point of 60 °C is presented in Table I.

TABLE I. Temperature parameters obtained by sensors in the different study regions. N represents the number of samples; Mean, arithmetic mean value; SD, standard deviation; Min, the minimum value and Max.

Sensor	N	Mean (°C)	SD	Min (°C)	Max (°C)
External	3001	59.99	0.21	59.39	60.55
Chamber 1	3001	60.52	0.35	59.27	60.03
Chamber 2	3001	59.95	0.40	58.42	60.13
Chamber 3	3001	60.26	0.29	59.16	60.36

The thermometer prototype can, besides verifying fluctuations and differences in heat exchange response time, be a viable alternative to commercial systems, as it presents a low cost for acquisition, just over 70 US dollars currently. The total cost of

REMUNOM
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the prototype along with the price of some key components for its manufacture can be seen in Table II.

TABLE II. Cost in US dollars of the main electronic components and the summed cost of the other various components necessary to assemble the AC field control prototype.

Component	Quantity	Cost per unit (US dollar)	Total cost (US dollar)
Trimpots	1	2.60	2.60
Resistors	10	0.15	1.5
NTC	4	1.40	5.6
Printed circuits	1	2.21	2.21
Capacitors	1	0.46	0.46
ATMEGA328P	1	19	19
Others	1	15.0	15
Total			**73.07**

4. Conclusion

The digital thermometer system developed proved to be effective in measuring the temperature inside the electroformation chambers. Using the ATMEGA328P® microcontroller, it was possible to digitize the temperature values from four sensors and send these values for logging on to a computer. Since temperature is one of the key factors for the electroformation process described in the literature, this prototype can be an alternative to more expensive commercial systems. The use of discrete commercial components did not affect efficiency, as deviations of less than 0.5 °C occurred. Checking the temperature directly in the electroformation solution contributed to correcting the startup time of the electroformation process, validating the temperature measured by the external thermometer embedded in the electroformation device. With this proposed digital thermometer, it was possible to obtain the difference in response time between the external temperature and the

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internal temperature of the solution. For a set value of 60 °C, the internal temperature took about 8 minutes to reach the desired value. Therefore, the developed digital multichannel thermometer is a cheap and effective alternative, costing less than 70 US dollars at current values, and can be useful for both the giant unilamellar vesicle electroformation processes involving temperature measuring and control.

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REMUNOM
ISSN 2178-6925

Received: 20/01/2026 - **Accepted:** 01/02/2026
Vol: 02.01
DOI: 10.61164/gvsvce49
Pages: 1-17

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