Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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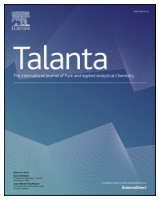

Label-free and portable field-effect sensor for monitoring RT-LAMP products to detect SARS-CoV-2 in wastewater

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ABSTRACT

The COVID-19 pandemic caused by the coronavirus SARS-CoV-2 has proven the need for developing reliable and affordable technologies to detect pathogens. Particularly, the detecting the genome in wastewater could be an indicator of the transmission rate to alert on new outbreaks. However, wastewater-based epidemiology remains a technological challenge to develop affordable technologies for sensing pathogens. In this work, we introduce a label-free and portable field-effect transistor (FET)-based sensor to detect N and ORF1ab genes of the SARS-CoV-2 genome. Our sensor integrates the reverse transcription loop-mediated isothermal amplification (RT-LAMP) reaction as a cost-effective molecular detection exhibiting high specificity. The detection relies upon pH changes, due to the RT-LAMP reaction products, which are detected through a simple, but effective, extended-gate FET sensor (EGFET). We evaluate the proposed device by measuring real wastewater samples to detect the presence of SARS-CoV-2 genome, achieving a limit of detection of 0.31×10^{-3} ng/μL for end-point measurement. Moreover, we find the ability of the sensor to perform real-time-like analysis, showing that the RT-LAMP reaction provides a good response after 15 min for concentrations as low as 0.37 ng/μL. Hence, we show that our EGFET sensor offers a powerful tool to detect the presence of the SARS-CoV-2 genome with a naked-eye method, in a straightforward way than the conventional molecular methods for wastewater analysis.

1. Introduction

Since 2019, the COVID-19 pandemic caused by the novel coronavirus SARS-CoV-2 has been a public health and social problem [1]. Currently, it is well-known that SARS-CoV-2 is transmitted in two forms, by direct or indirect contact [2]. Thereby, a challenging task is to detect SARS-CoV-2 at an early stage to hold the transmission.

Research efforts have been devoted to develop several methods for detecting and quantifying the SARS-CoV-2, mainly focused on clinical environments. One can find rapid detection methods, such as rapid antigen detection (RAD) [3] or rapid detection of antibodies (RDA) [4], and nucleic-acids detection [5]. This latter, due to its specificity, is regarded as the most reliable to detect the presence of SARS-CoV-2 [6,7].

Furthermore, detecting the presence of SARS-CoV-2 genome in wastewater is a fundamental tool for epidemiological surveillance and slow rate transmission of the virus [8,9]. The main molecular method used to detect the genetic material of SARS-CoV-2 in wastewater is the reverse transcription-quantitative polymerase chain reaction (RT-qPCR) [10–12]. Nevertheless, the main disadvantage of such method is that, it requires ex-pensive infrastructure, qualified personal and advanced scientific techniques to realize the testing. Therefore, rapid and accurate detection tools should be developed to strengthen the wastewater-based epidemiology using molecular methods in limited-resources conditions [13].

Regarding nucleic-acid detection methods, isothermal amplification is an alternative technique used to reduce the cost and speed up the process of pathogens [14]. Particularly, loop-mediated isothermal amplification with simultaneous reverse-transcription (RT-LAMP) is a powerful tool due to its high sensitivity, simplicity, and fast response [15]. Recently, several works have developed innovative tools to detect SARS-CoV-2 based on the RT-LAMP reaction, with cost-effective and

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straightforward sensing setups. For instance, using a fluorescence detection scheme [16], a colorimetric assay [17], and an electrochemical sensor [13], are the most recent advances in novel sensing technologies. Nonetheless, they still requiring bench-top instruments or lack automated measurements protocols.

Among the wide variety of biosensors, sensing based on field-effect transistor (FET) is a promising technique due to its advantages such as low-cost fabrication, miniaturization, reliability, sensitivity, and label-free detection [18,19]. In general, FET biosensors can have different structures, acting as sensitive layers, mainly based on complex structures [20,21] or advanced materials [22,23]. On the other hand, the extended-gate FET (EGFET) devices are cost-effective and straightforward alternatives to develop FET-based sensors [24]. The main advantage of an EGFET sensor is that, the sensitive element is isolated from the transistor *per se*, so it can be interchangeable, demonstrating its ability to develop attractive sensing platforms [25]. Despite technological advances of biosensors based on EGFET devices, they still limited in its applicability for its us-age in portable equipment to perform *in-situ* measurements. This limitation is mainly due to several facts. i) It is not straightforward to integrate the reference and measurement electrodes in a single structure [26]. ii) A labeled receptor into the sensing surface is required to provide specificity [27–29]. iii) The easy-to-integrate process of the electronic measurement setup for FET-based sensors within a small-sized device for portable applications [30,31].

Hence, we hypothesize that detecting nucleic-acids is possible using an EFGET device without electrode modification. That is, a simple device could able to detect SARS-CoV-2 genome through quantifying physicochemical phenomena without a labeled receptor. This could be possible by sensing pH changes due to the isothermal amplification process. As previously shown in Refs. [32–34], an RT-LAMP reaction is an amplification method, which can be detected through pH changes in the reaction mixture [35]. On the other hand, EGFET-based sensors can selectivity measure pH depending on the electrodes configuration. That is, a change in the pH value modifies the electrical current and the electrical potential due to physicochemical phenomena occurring at the surface of the sensitive electrode [36,37]. Hence, by means of an EGFET-based sensor, one could be able to track the pH changes due to the RT-LAMP. An EGFET-based sensor coupled with the RT-LAMP reaction is a promising naked-eye technology to straightforwardly track the genetic amplification process, similarly to colorimetric assays [38]. Moreover, our EGFET-based sensor has the following main advantages: i) it retrieves quantitative results, ii) minimizes the reaction volume compared with classical eppendorf-based tubes, and iii) is devised for fast, reliable, and *in-situ*.

Inspired by the current trend on wastewater-based epidemiology and low-cost molecular detection methods, it worth to develop affordable devices for *in-situ* pathogen detection or in water quality laboratories [39,40]. However, to the best of our knowledge, there is scarce work around affordable sensors for wastewater epidemiological surveillance, as it requires specific molecular detection methods. For this purpose, herein, we introduce an attractive device for detecting SARS-CoV-2 genome in wastewater based on an EGFET sensor and the RT-LAMP reaction. The main contribution of this work relies upon the development a fully integrated EGFET device, directly coupled with an RT-LAMP reaction. Hence, the FET-based sensor is able to measure the amplification products, through pH changes, with high specificity provided by the RT-LAMP primers. Finally, the sensor demonstrates its reliability and robustness to detect SARS-CoV-2 in real wastewater samples, exhibiting promising results towards automated and affordable devices to detect the SARS-CoV-2 genome in resource-limited settings.

2. Experimental section

The experimental protocol for measuring SARS-CoV-2 genome with the proposed FET sensor is show in Fig. 1. Firstly, the samples are collected from wastewater treatment plants (WTTP). Then, the genetic material is extracted and concentrated using a custom and highly-sensitive method described in Ref. [9]. Subsequently, the RNA is mixed with the RT-LAMP primers (see Table S2), from which a 50 μL drop is placed over the surface of electrodes of the EGFET. The RT-LAMP reaction occurs, at the electrode surface, by controlling its temperature at 63 °C for approximately 30 min. Finally, by monitoring the drain-to-source current change of the FET device, the RT-LAMP products can be monitored due to pH changes as the reaction progress. Additionally, to verify the results, we performed electrophoresis in agarose gel to observe the RT-LAMP products.

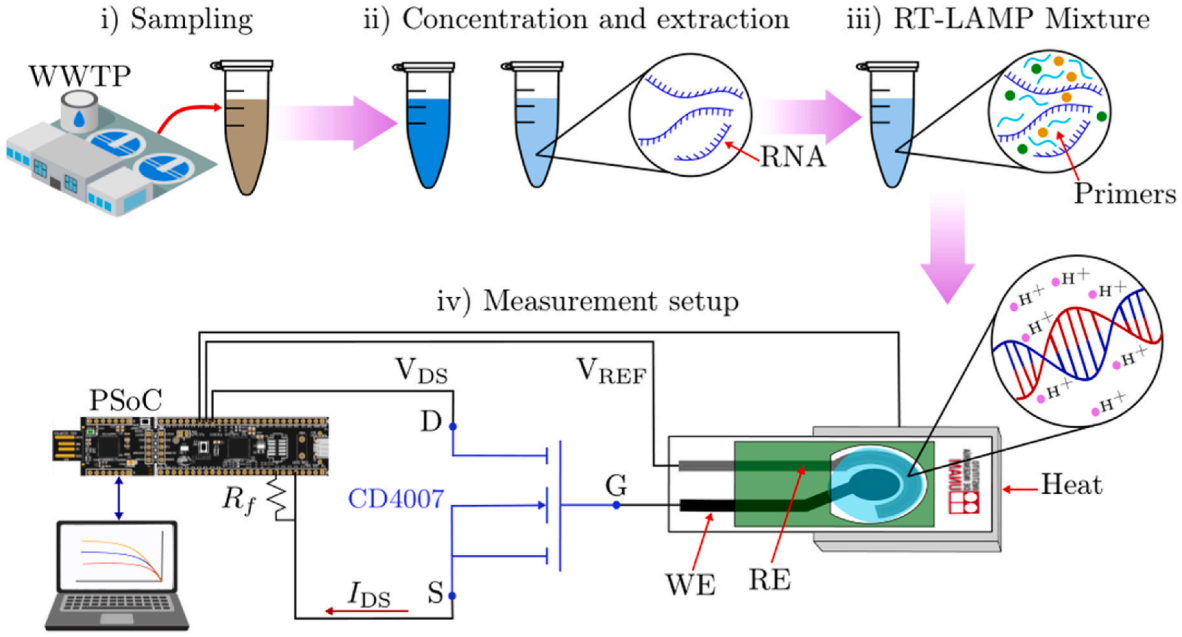

Fig. 1. Schematic diagram of the EGFET-based sensor to detect SARS-CoV-2 genome in wastewater samples. i) Wastewater sampling from the wastewater treatment plant (WTTP). ii) Concentration and extraction of the nucleic-acids. iii) RT-LAMP mixture for isothermal amplification reaction. iv) Measurement setup to monitor the RT-LAMP products through pH changes with EGFET-based sensor.

2.1. Wastewater samples collection

Located in the metropolitan area of the Querétaro State in Mexico, one can find two wastewater treatment plants, South and Santa Rosa plants, where the influent was sampled during the middle of 2021 (see Table S1). Samples of 500 mL volume were stored and kept at 4 °C until their use.

2.2. Concentration and extraction of RNA

After the sampling process succeeded, the samples were concentrated the same day, using the electronegative membrane method due to its high detection limit for SARS-CoV-2 genes [9]. In summary, the pH of the sample was adjusted to 3.5 with 2 N HCl, and the samples were filtered through a negatively charged nitrocellulose membrane with pores of 0.45 μm in diameter (Millipore, Netherlands). Following the instructions provided by the manufacturer, the membranes were cut and used in an RNA extraction kit, Power kit RNeasy Power Microbiome kit (Qiagen, Germany). After extraction, the samples were refrigerated to preserve them until their use at −20 °C.

2.3. RT-LAMP assay

Firstly, we obtained the reverse transcription for 5 mL of RNA, using a QuantiTect Reverse Transcription Kit (Qiagen, Germany), following the manufacturer's instructions. Then, we prepared the RT-LAMP reaction of 50 μL, where each reaction contained: 3 μL $MgSO_4$ (NEB), 5 μL Buffer Bst (NEB), 5 μL of 2 mM dNTPs (Thermo Scientific), 2 μL of 10X primer's core mix, 2 μL of 10X primer's loop mix, 10 U of Bst 2.0 DNA polymerase (NEB), 4 μL cDNA and nuclease free water, which was added to complete the total volume of 50 μL. We designed the specific primers for the SARS-CoV-2 N and ORF1ab genes (see Table S2), as shown in Ref. [41]. The destined primers were validated using the tool Primer-Explorer V5 (https://primerexplorer.jp/e/). The RT-LAMP reactions were verified using standard electrophoresis in 1% agarose gel with SYBR Safe DNA Gel Stain to verify the correctness of the amplification products. Lastly, the RT-LAMP amplicons were validated through sequencing (w https://www.ncbi.nlm.nih.gov/nuccore/om522662).

2.4. Operational principle of the EGFET sensor

Monitoring pH with EGFET-based sensors has been proposed due to its low-cost and versatility [24]. The EGFET devices are classified as potentiometric sensors when they used only the reference (RE) and working (WE) electrode. This is due to the electrical potential changes on the surface of the working electrode, which modify the characteristic curves of the underlying MOSFET device [42]. Thus, following our hypothesis that, pH changes due to the RT-LAMP reaction can be measured using an EGFET-based sensor, it is necessary to define some working parameters of the proposed device. The drain-to- source current, I_{DS}, of an N-channel MOSFET, in the linear region, is defined as

$$I_{DS} = \mu C_{OX} \frac{W}{L}\left[\left(V_{REF} - V_{th}^{*}\right)V_{DS} - \frac{1}{2}V_{DS}^{2}\right], \quad (1)$$

where μ is the ion mobility, C_{OX} is the gate oxide capacitance per unit area, W and L are the width and length of the N-channel, respectively, V_{REF} is the reference voltage that polarizes the reference electrode RE, and V_{DS} is the drain-to-source voltage. The reaction between the analyte and the electrode establishes the behavior of our sensor, causing changes in the threshold voltage V_{th}^{*} as follows [24]

$$V_{th}^{*} = V_{th} + E_{REF} + \chi_0 - \frac{U_M}{q} - \varphi, \quad (2)$$

where V_{th} is the threshold voltage of the MOSFET, E_{REF} is the polarization potential of the reference electrode, χ_0 is the superficial dipole potential of the analyte, U_M the work function of the reference electrode, q is the electron charge and φ is the potential of the surface at the analyte. Furthermore, E_{REF} can be expressed using the Nernst equation

$$E_{REF} = E_0 - \left(\frac{RT}{nF}\right)pH, \quad (3)$$

where the standard potential E_0, of an Ag/AgCl electrode, typically is 0.22 V at room temperature, R is the ideal gas constant, T is the absolute temperature, n is the number of electrons involved in the reaction, F is the Faraday constant, and pH expresses the hydrogen ion concentration as $pH = -\log_{10}[H^+]$. Finally, the potential φ can be described by [43]

$$\varphi = 2.303\frac{kT}{q}\frac{\beta}{\beta+1}(pH_{PZC} - pH), \quad (4)$$

with k the Boltzmann constant, the buffer capacity β defined as an underlying property of the working electrode material, and pH_{PZC} is the pH value considering that the surface change is zero.

Thus, by following the above parameters, there is a straightforward relationship among the current I_{DS} and changes in pH; which in turn, are promoted by the RT-LAMP reaction. For this aim, the main task is to experimentally obtain the output characteristic curves of the FET-based sensor to detect the RT-LAMP products.

2.5. Measurement setup

To develop a portable measurement devices for the EGFET sensor, we overcome the limitations of the conventional setups [37,44]. For this purpose, we designed a custom system based on a programmable system-on-chip (PSoC) family 5LP (Cypress Semiconductor), with a 32-bits ARM Cortex-M3 CPU working at 80 MHz, integrating hardware stages and software processes within a single device.

The proposed measurement setup is shown in Fig. 1(iv). The EGFET comprises a commercial N-channel MOSFET model CD4007 (Texas Instruments™), which is coupled with a screen-printed electrode system (SPEs). This latter comprises a reference RE and working WE electrode, in a flexible substrate with an area of 330 mm^2. The RE was made of Ag/AgCl ink; whereas, the WE was fabricated using carbon paste. Experimentally, we used a different SPEs for each measurement, and placed a drop of 50 μL volume, for each measurement, where we took care that the analyte covered the surface of the RE and WE. The WE is connected to the gate (G) terminal of the MOSFET, and the RE is polarized with the reference voltage V_{REF}; whereas the drain (D) terminal is connected at drain-to-source voltage V_{DS}. Both voltages, V_{REF} and V_{DS}, are generated by the PSoC using two 8-bit digital-to-analog converters (DAC). The drain-to-source current, I_{DS}, is measured at the source (S) terminal using a transimpedance amplifier (TIA) and external resistor R_f. These measurements are digitized by a 16-bit analog-to-digital converter (ADC), within the PSoC, to generate the output curves, current I_{DS} as a function of voltage V_{DS}. Therein, the amplitude of the current depends on the pH changes as the RT-LAMP reaction progresses. For this aim, under the electrodes, a resistive heater is placed to raise the temperature up to 63 °C using a pulse width modulation (PWM) signal generated by the PSoC. The temperature was controlled using a digital PID control with a steady-state error of ±0.5 °C for 30 min, until the RT-LAMP reaction finishes. Thereby, we propose an attractive measurement system for EGFET sensors that, due to its characteristics, has the potentiality to develop point-of-collection devices, ideal for field testing and *in-situ* experiments.

3. Results and discussion

3.1. Specificity and concentration

The first experiment was designed to confirm the successful operation of our FET sensor to detect and quantify RT-LAMP products through

the pH changes. Firstly, we know that the sensor can detect changes in pH in a range where the pH of negative and positive samples is located (see Fig. 4). Then, based on our hypothesis, we considered the pH value of the negative template control (NTC) sample as a baseline value, thus, in the positive samples one can see the pH changes due to the isothermal amplification. Thus, we performed the sensor characterization using a SARS-CoV-2 positive sample at seven concentrations made by serial dilutions. Fig. 2(a) shows the output curve of the EGFET, which was obtained for $C_1 = 0.01$, $C_2 = 0.1$, $C_3 = 1$, $C_4 = 10$, $C_5 = 100$, $C_6 = 1000$ and $C_7 = 10{,}000 \times 10^{-3}$ ng/μL. In Fig. 2(a), one can notice how the amplitude of the I_{DS} increases proportional to the concentration, measured at a fixed value of $V_{REF} = 2.5$ V, which corresponds to the MOSFET saturation zone. Therein, the vertical dashed line indicates the useful set of I_{DS} values useful to characterize the sensor's response. Following this idea, Fig. 2(b) shows the calibration curve of the EGFET sensor given by I_{DS} as a function of the sample concentration. The plot shows the experimental data (black dots), the uncertainty of the measurements (vertical lines), and the best fitted model (solid line). From there, one can see that the output of the EGFET exhibits a linear increment as the concentration grows-up. Thus, the sensor sensitivity is 56.2 (μA /log (ng /μL)), a determination coefficient r^2 of 0.9917, which determines a highly linear behavior, and the limit-of-detection (LoD) of 0.31×10^{-3} ng/μL for the concentration range from 0.01 to $10{,}000 \times 10^{-3}$ ng/μL. Interestingly, the obtained LoD is in the same order of magnitude as in similar works [45], but keeping in mind that our sensor is label-free, and work without electrode modification.

3.2. Detection of RT-LAMP amplicons for SARS-CoV-2 genome

Subsequently, we proved the ability of our sensor to detect pH changes due to RT- LAMP amplification, after 30 min of reaction, for three positive samples labeled as BP, BQ and BR (see Table S1). The output characteristic curves of the proposed sensor are depicted in Fig. 3 (a); wherein, we measured the current I_{DS} as a function of voltage V_{DS}, when V_{DS} was swept from 0 to 4 V, with a fixed value of $V_{REF} = 3.0$ V. From Fig. 3(a), one can notice that the amplitude, I_{DS}, is larger for positive samples than the amplitude for the negative template control (NTC) sample. This makes sense, because the amplitude of I_{DS} depends on the pH of the sample [46]. That is, in positive samples, the pH value decreases after the RT-LAMP reaction succeeds [35]. Furthermore, one can deduce this relationship by following equations (3) and (4); and, in turn,

The relationship between I_{DS} and pH value allows detecting, indirectly and qualitatively, the presence of SARS-CoV-2 genome using RT-LAMP products in wastewater samples. For this purpose, the measurements were done by taking $V_{DS} = 3.5$ V, in the saturation zone of the EGFET. Therefore, using the sensor's model shown in Fig. 2(b), the concentration for the three samples was of approximately 6.3, 6.6, and 6.1 ng/μL for samples BP, BQ and BR, respectively. To quantify the difference between positive and negative samples, we computed the change in the amplitude of the measured current I_{DS} as

$$\Delta I_{DS} = I_{DSm} - I_{DS0}\ , \tag{5}$$

where I_{DSm} and I_{DS0} are the measured current of the positive and NTC samples, respectively. For this purpose, we took the value of the I_{DS} from each sample, at $V_{DS} = 3.5$ V and $V_{REF} = 3.0$ V. Fig. 3(b) shows the value of ΔI_{DS}, where the three positive samples exhibit a similar increment value.

Furthermore, according to our setup, the RT-LAMP reaction was able to detect ORF1ab and N genes, showing that our sensor retrieves a readable signal, in the same order of magnitude, for both genes. Finally, to validate that the RT-LAMP reaction was carried out in the samples, we visualized RT-LAMP products in a 1% agarose gel. Fig. 3(c) shows characteristic RT-LAMP profiles in positive samples, and no amplification in the NTC sample. With these findings, we have demonstrated that our sensor can obtain accurate results to differentiate between a positive or negative sample, using a fast and reliable EGFET-based method.

3.3. Time monitoring of the RT-LAMP reaction

In the last experiment, we monitored the electrical signal provided by the EGFET sensor and the time course of the RT-LAMP reaction. Experimentally, we used an arbitrary positive sample to specifically detect the gen N of the SARS-CoV-2 genome. Before the RT-LAMP reaction, five dilutions were prepared at different concentrations ($BP_1 = 251.8$, $BP_2 = 25.18$, $BP_3 = 2.518$, $BP_4 = 0.2518$ and $BP_5 = 0.02518 \times 10^{-3}$ ng/μL). Thus, we monitored the RT-LAMP reaction by measuring the I_{DS} current, every 5 min up to 30 min for each solution. At each time instant, we obtained the output of the FET sensor, at a fixed $V_{REF} = 3.5$ V, to see changes in the amplitude of the I_{DS}.

Fig. 4(a) shows the output curves of the FET sensor with, $V_{REF} = 3.5$ V and $V_{DS} = 3.0$ V, for testing the BP_1 sample. Interestingly, one can see how the amplitude of I_{DS} increases with an acceptable resolution, due to a decrease on the pH value, as RT-LAMP reaction progresses for 30 min. Thus, this experiment was successful to determine that the detection of RT-LAMP products could be finished in less than 30 min. This was an interesting finding owing that, by using RT-LAMP, it reduces the testing time because the isothermal amplification has a fast response [47]; and, hence, 30 min are enough to complete the reaction with a readable signal.

Thereby, with established values for V_{REF}, V_{DS}, we obtained a relationship between the current I_{DS} and the time course of RT-LAMP for each sample. As shown in Fig. 4(b), the amplitude of I_{DS} increases as the

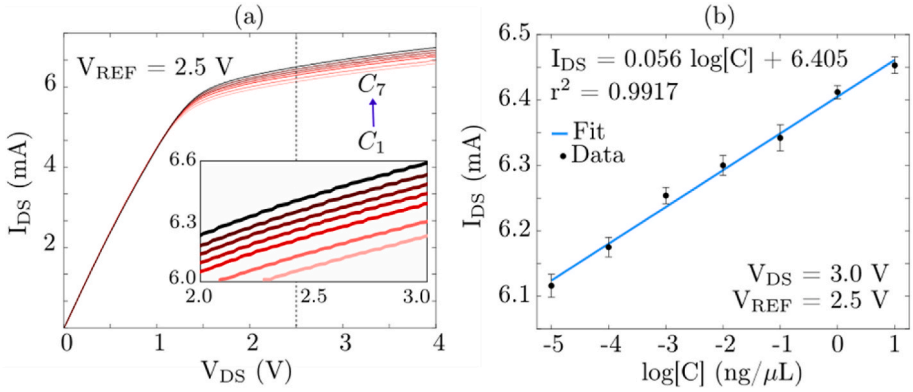

Fig. 2. Calibration of the EGFET-based sensor for detecting RT-LAMP amplicons in wastewater. (a) Output curves, current I_{DS} as a function of the voltage V_{DS} for different concentration, at a fixed $V_{REF} = 2.5$ V. (b) Calibration curve of the sensor, the output current I_{DS} as a function of the concentration C in a logarithmic scale. they are reflected on the amplitude of I_{DS} (see equations (1) and (2)).

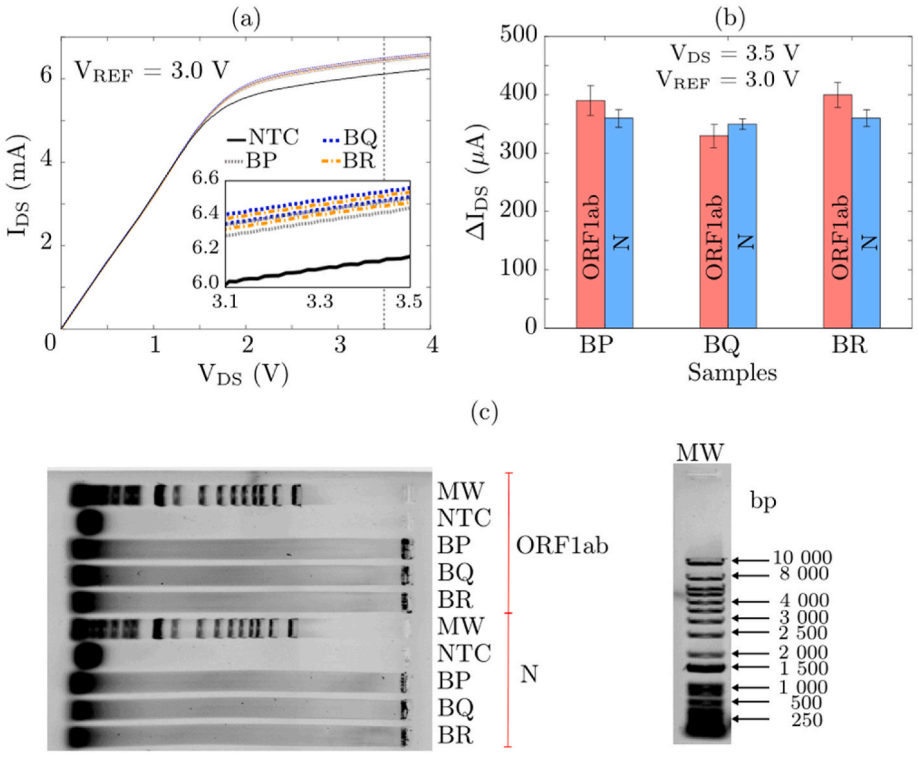

Fig. 3. End-point measurements to detect RT-LAMP products of the ORF1ab and N genes. (a) Output characteristic curves, I_{DS} as a function of V_{DS} for positive (BP, BQ and BR) and negative template control (NTC) samples at a fixed V_{REF} = 3.0 V. (b) Selectivity of the EGFET-based sensor using the current difference ΔI_{DS}. (c) Profiles of the RT-LAMP products on 1% agarose gel electrophoresis for positive and negative samples.

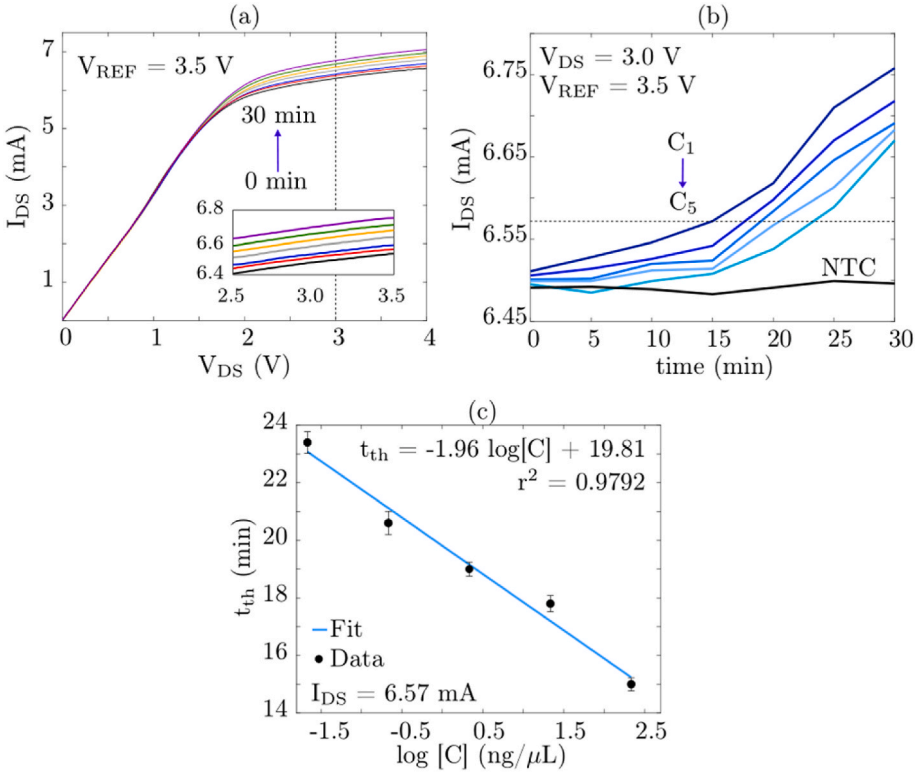

Fig. 4. RT-LAMP time analysis. (a) Output characteristic curves, I_{DS} as a function of V_{DS}, at a fixed V_{REF} = 3.5 V, for 30 min of monitoring RT-LAMP reaction. (b) Correlation between the current I_{DS} and RT-LAMP time course for different concentrations. (c) Calibration curve of the threshold time t_{th} as a function of the concentration C in a logarithmic scale.

pH value decreases when the measurement time progresses. With these result, one can deduce the following remarks. First, the EGFET sensor can monitor the course of RT-LAMP reaction for SARS-CoV-2 genome in wastewater samples through pH changes. Secondly, the reaction time depends on the sample concentration. Ultimately, one can establish a threshold time t_{th}, from which, the amplitude of I_{DS} significantly increases. The parameter I_{DS} is highlighted in Fig. 4(b) with a horizontal dashed line, located at 6.57 mA. From it, one can see that the required RT- LAMP reaction time is in the range from 15 to 25 minutes, for the lowest and the largest concentrations, respectively. Therefore, in this concentration range, our EGFET-sensor is faster than the conventional molecular methods without losing specificity.

Finally, Fig. 4(c) depicts the calibration curve for the time-dependent monitoring. Therein, it holds an inverse relationship between the t_{th} and the concentration C in a logarithmic scale, with a sensitivity of 1.96 $(\mu A / \log (ng / \mu L))$, and high linearity close to 99%. Furthermore, the LoD of the sensor reaches a value of 0.37 ng/μL, exhibiting that it can detect concentrations for diluted samples, without needing more time to retrieve enough amplifications products. Based on these findings, the proposed FET sensor is a promising tool to detect SARS-CoV-2 or other pathogens in real wastewater samples. Moreover, as can be seen in Fig. 4, the time course monitoring of RT-LAMP for SARS-CoV-2 can be regarded as real-time experiment as in PCR experiments. Nonetheless, this requires further validation to determine the final amount of base pair copies, as well as a large number of experiments to assesses the number of false positives retrieved by the sensors. The FET sensor, however, at this time provides the ability to control and monitor the RT-LAMP reaction for *in-situ* detection in limited-resources settings. Hence, the proposed solution is great advance towards affordable devices, for wastewater-based epidemiology to overcome public health problems.

4. Conclusions

A label-free, low-cost, and portable FET sensor coupled with RT-LAMP reaction, for detecting SARS-CoV-2 genome in real wastewater samples, was developed. The proposal integrates all the RT-LAMP and the EGFET transduction stages into a single device, to provide an affordable solution for in-situ experiments. The sensor platform could generate and monitor the RT-LAMP reaction to detect the presence of ORF1ab and N genes from SARS-CoV-2 through pH changes, showing high specificity. We showed how the sensor could measure RT-LAMP products with a limit-of-detection of 0.31 × 10-3 ng/μL, to differentiate among negative and positive samples to SARS-CoV-2 in real wastewater samples. Moreover, the FET-based sensor also allowed to supervise the time course of the RT-LAMP reaction within 30 min, which represents an advance for rapid molecular tests. Owing the findings on the label-free FET-based sensor, it can be considered as an attractive tool to detect SARS-CoV-2 in limited-resource environments for surveillance of the pandemic spread through wastewater-based epidemiology. Finally, proposed the sensor can be easily customized and reconfigured to detect other pathogens in environmental applications.

Credit authors statement

Bryan E. Alvarez-Serna: Conceptualization, Methodology, Software, Writing-original draft. **Roberto G. Ramírez-Chavarría:** Investigation, Methodology, Funding acquisition, Writing-original draft. **Elizabeth Castillo-Villanueva:** Investigation, Methodology, Writing-review & editing. **Julián Carrillo-Reyes:** Investigation, Methodology, Validation, Writing-review & editing. **Rosa María Ramírez-Zamora:** Writing-review & editing, Validation, Resources. **Germán Buitrón:** Writing-review & editing, Validation, Resources. **Luis Alvarez-Icaza:** Writing-review & editing, Validation, Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors do not have permission to share data.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.talanta.2022.124060.

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