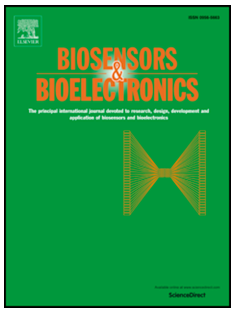

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CRediT authorship contribution statement

Stefano Cinti: Conceptualization, Data curation, Formal analysis, Methodology, Writing - review & editing.

Roberta Marrone: Conceptualization, Data curation, Formal analysis, Methodology, Writing - review & editing.

Vincenzo Mazzaracchio: Conceptualization, Data curation, Formal analysis, Methodology, Writing - review & editing.

Danila Moscone: Conceptualization, Data curation, Formal analysis, Methodology, Writing - review & editing.

Fabiana Arduini: Conceptualization, Data curation, Formal analysis, Methodology, Writing - review & editing.

Novel bio-lab-on-a-tip for electrochemical glucose sensing in commercial beverages

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Abstract

The development of portable and user-friendly sensing platforms is a hot topic in the field of analytical chemistry. Among others, electroanalytical approaches exhibit a high amenability for reaching this purpose, i.e. the commercial strips for diabetes care are an obvious success. However, providing fully-integrated and reagent-free methods is always a leitmotiv. In this work, we evaluated the use of a disposable pipette tip, opportunely configured to demonstrate the first example of an electrochemical biosystem in a pipette tip, namely bio-lab-on-a-tip. The combination of a pipette tip, wire electrodes, enzyme, and cotton wool filter, allows the fabrication of a novel electroanalytical platform that does not need expertise-required tasks. To demonstrate the feasibility of this novel method, glucose is detected in beverages by means of chronoamperometry. The experimental setup, entirely built inside the pipette tip, is able to 1) block impurities/interferences from matrix, 2) load/release reagents for the bio-assay, 3) reduce the operating task to zero, and 4) perform electrochemical detection. With optimized experimental parameters, the bio-lab-on-a-tip is able to detect glucose linearly up to 10 mM with a detection limit of 170 μM. The effectiveness of the platform was confirmed by testing commercial beverages, e.g. Coca-Cola and Coca-Cola Zero, with high accuracy. In addition, the shelf-life of the novel device was evaluated, highlighting the role of cotton wool filter for providing a suitable environment for glucose oxidase stability. The novel concept can be easily generalized for further applications in the field of non-invasive clinical diagnostics and in-situ environmental monitoring.

Keywords: Electroanalysis, Biosensor, Lab-on-a-tip, Glucose, Beverages, Miniaturization

1. Introduction

The need for smart analytical devices is of great importance for several fields including biomedical, environmental, security and agri-food ones. The world's most popular example is still relevant after *ca*. 50 years, as the glucose strip for diabetes patients should be considered a masterclass for everyone working in the field (Turner, 2013). Indeed, the early diagnosis of unknown diseases, the preservation of environmental equilibrium, and the rapid assessment of food quality are only some of the examples on how portable tools can boost the life of non-specialists (Feingold, 1977). The task to develop smart and accurate devices can be accomplished by the exploitation of several disciplines, including analytical chemistry, biology, engineering, biomimetics, material science, rational design, chemometrics, and microfabrication (Weston and Hood, 2004; Wu et al., 2017; Avoundjian et al., 2017; Quesada-González and Merkoçi, 2018; Arduini et al., 2020) by using a cooperative multidisciplinary approach, which make us able to reach unprecedented results. However, although the efforts in the biomedical fields appear to be very attractive, the agri/industry-food field is mainly bounded to conventional techniques (e.g. chromatography, spectrophotometry, electrophoresis, titration) (Poms et al., 2004; Nollet and Toldrà, 2012; Lara et

al., 2016). However, currents methods do not allow for monitoring at the point of need. Regarding food and beverages, both for producers and consumers, keywords like quality, freshness, allergens, authenticity and adulteration have significant relevance (Regattieri et al., 2007; Cucu et al., 2013; da Silva et al., 2016; Hong et al., 2017). All the steps of the entire process in food and drink industries (e.g. choice of raw matter, treatment, production, quality tests, safety) up to consumers, need rapid, reliable, and low-cost methods to monitor the presence of certain expected and/or unexpected compounds (e.g. by-products). Traceability of raw materials represents an important information into their value chains: developing methods that provides a traceable fingerprint from producer to retailer is highly needed (Hamburg, 2011; Wognum et al., 2011; Kassal et al., 2018). Plenty of commercial products display the caption "gluten-free, alcohol-free, sugar-free, lactose-free, etc" in their label. However, requirement for diets, intolerances and allergies are not satisfied just by reading a label. In certain cases, the amount of a certain molecule can be lower than a significant threshold, but still nonzero. This issue could be improved and simplified by the adoption of portable tools, lowering requirements, and improving answers. Sensors and biosensors, in their widest architectures, are suitable candidates to match this objective assessment: research around the field of portable devices, miniaturization, matrix treatment, showed and is showing plenty of promising approaches within all the design such as electrochemical, colorimetric, fluorometric, pressure-based (Cinti, 2019a; Vashist et al., 2015; Arduini et al., 2017b; Kates et al., 2001; Bagheri et al., 2019; Yu et al., 2019; Zeng et al., 2018). In particular, those based on electrochemical methods display convincing features that make them an ideal option: they are portable, low-cost, battery-powered, unaffected by the color of matrices, user-friendly and sustainable. In particular, the last feature "sustainable" is currently leading the development of novel analytical approaches. Sustainability is often only associated to economics and environment. However, a great significance of this term should be focused on its social implications. For instance, the development of analytical tools that allows everyone to evaluate and monitor particular conditions, without the need of specialists and instruments, enhances the social sustainability. A desirable outcome in providing effective solutions involves enabling citizens, especially those living in remote areas, to be actively involved in societal care. In this effort, bio-analysis contains many examples that go beyond the state of the art in the agri-food sector: in particular, the combination with paper-based substrates as novel platforms produced innovative tools as exemplified by Paper-based electrochemical biosensor for detection of alcohol in commercial beers, Paper-based ELISA to rapidly detect Escherichia coli to monitor food and water quality, Paper-based chromatographic chemiluminescence chip for the detection of dichlorvos in vegetables and 3D paper-based origami device for different types of pesticides in waters (Cinti et al., 2017; Shih et al., 2015; Liu et al., 2014; Arduini et al., 2019). As reported by research from our group and others, the role of paper combined with biological elements, nanomaterials and microfabrication, has emerged to be a hot topic in the field of decentralized monitoring: the adoption of new protocol, concepts and technologies, demonstrated the success of innovative point of need platforms in terms of improving sensitivity, minimizing the instruments and making detection more user-friendly (Mahato et al., 2017; Arduini et al., 2017a; Martinez et al., 2010; Cinti et al., 2019b; Yu et al., 2018).

However, there is space for novel configurations in the smart bio-analytical field, as exemplified by a novel concept presented in this work. For the first time, a commonly known micropipette tip is configured to contain 1) a biological recognition system, 2) a substrate for matrix pretreatment, 3) reagents, and 4) an electrochemical transducer. The benefit of cotton filter has been consolidated into a pipette tip, which served to test environmental samples (Cinti et al., 2018b). The present configuration offers an innovative perspective for the design of future low-cost diagnostics. It is the only existing example of an analytical biosystem that is entirely integrated into a tip. It allows the end-user a unique "sample and analyze" process, without ulterior procedures. The cotton filter inside the tip is responsible for four tasks that are entirely self-supported: the cotton filter 1) loads the reagents, 2) filters gross impurities that can be contained in the matrix, 3) releases the reagents and 4) provides a biocompatible environment for enzyme entrapment. Enzymes are usually

immobilized through the use of cross-linking, self-assembled monolayers and polymeric network (Minteer, 2017). For instance, Talarico and colleagues (2016) adopted chitosan film to provide a biocompatible and favorable microenvironment for acetylcholinesterase enzyme immobilization to detect pesticides, while chitosan hydrogel beads have been used to immobilize Candida rugosa lipase by physical adsorption and covalent cross-linking (Kim et al., 2017). Loaiza et al. (2015) have covalently stabilized lactate oxidase onto printed electrodes through a combination of polyethyleneimine and glutaraldehyde, forming a dense cross-linked mesh. Other approaches include the use of chromatographic paper for hosting glucose oxidase for diabetes monitoring, layer-by-layer assembly of PDDA-P450 enzyme onto magnetics beads to convert aflatoxin B1, and even the development of a bioreactor based on polymeric ionic liquids laccase membrane for decolorization of the anthraquinonic dye (Cinti et al., 2018a; Malla et al., 2018; Haj Kacem et al., 2020). However, the use of organic solvents, time-consuming and risk-associated procedures, laboratory equipment, are just some of the limitations associated with the above-reported approaches. The adoption of cotton filter as an innovative factor in developing integrated bioplatforms is also oriented toward sustainable development goals since 1) it does not require chemicals to be added by the end-user, 2) it is affordable and abundant and 3) it is easily disposable. As a case study, the detection of glucose has been chosen as an analyte. The electrochemical detection is based on the consumption of the oxygen during reaction. Briefly, when glucose is oxidized by the glucose oxidase, the amount of oxygen decreases. It is consistent with a decrease of the cathodic current generated at the gold electrode as a consequence of the oxygen reduction (Zhao et al., 2006). The more glucose present, the more oxygen is consumed, thus the cathodic current recorded is lower. In comparison with the existing technologies, three main motivations justified the innovation within the bio-lab-on-a-tip. 1) The system is obtained by using common materials such as pipette tip, cotton filter, wires. It is in line with the sustainable development goals, because it opens to everyone the possibility in developing diagnostics by utilizing sources that are sometimes unconsidered for this use. 2) It meets the ASSURED criteria established by WHO, regarding the use of affordable, user-friendly, deliverable devices for diagnostics. The use of a biosensor within a tip, avoid the step of sampling that sometimes is a leading cause of error: for the first time, within this configuration, the sampling and analysis are carried out simultaneously. 3) The construction of this bio-lab-on-a-tip is easier than screen-printed electrodes (SPE): to produce SPE one needs non-aqueous conductive inks, screen-printer machine, mask, oven to treat the inks. Instead, the approach here reported opens to possibility for novel concept of fabrication and application. In addition the use of strategic materials like cotton filter, is commercially available everywhere. During the (hand-made) fabrication step, the cotton filter is loaded with an enzyme, i.e. glucose oxidase, which specifically reacts with glucose contained in soft drinks. The filtering due to the cotton filter, the release of preloaded reagents and the by-production of an electroactive compound, made the quantification of glucose easy (unique step) and effective (discrimination between beverages). Everything is embedded into the tip of a micropipette, from the biological reagent to the readout. The innovation of incorporating a biosystem into a tip, with application in the agri-food field, has been applied towards the detection of glucose in Coca-Cola and Coca-Cola Zero. With the increased awareness of health among the population and the possible introduction of sugar taxes (e.g. in Italy) to limit sugar consumption, this approach could be used for instance by customs authorities, industries, and consumers for establishing the sugar content of products. In addition, the employment of an enzyme in this tip-based configuration, strengthened by its satisfying shelf life in the cotton environment, opens to other possibilities in the field of user-friendly biosensing, including nucleic acids–based sensors and immunosensors.

2. Experimental section

2.1. Chemicals and apparatus

Potassium chloride (KCl), Sulfuric acid (H_2SO_4), Potassium phosphate monobasic (K_2HPO_4), Potassium phosphate dibasic (KH_2PO_4), Hydrogen peroxide 30% w/w (H_2O_2), D-(+)-glucose ($C_6H_{12}O_6$), Glucose oxidase from Aspergillus niger Type X-S, Citric acid ($C_6H_8O_7$), L-Ascorbic acid ($C_6H_8O_6$), Sodium bicarbonate ($NaHCO_3$) were obtained from Sigma Aldrich (USA). Coca-Cola ® and Coca-Cola ® Zero (The Coca-Cola Company, USA) were purchased in local store. The cotton wool filters (cigarette filters) from the brand Rizla (Ireland) were purchased in local store. Cyclic voltammetry (CV) and chronoamperometry were carried out using as the potentiostat an EmStat^3 (PalmSens, Netherlands) connected to a laptop.

2.2. Electrochemical configuration

A three-electrode system was fabricated by using wires (Figure 1). In particular: 1) the working electrode was realized with a gold wire (diameter of 0.03 mm) purchased from A-M Systems, (USA), 2) the reference electrode was realized with a silver wire (diameter of 0.25 mm) purchased from Goodfellow (UK) and 3) the counter electrode was realized with a stainless steel wire (diameter of 0.3 mm) purchased from Goodfellow (UK). All the wires were used with a length of 3 cm and all were insulated with the use of shrinkable tubing (PTFE, HS Sub-Lite-Wall®) around the body of the wires, gently provided by Zeus Industrial Products (Ireland).

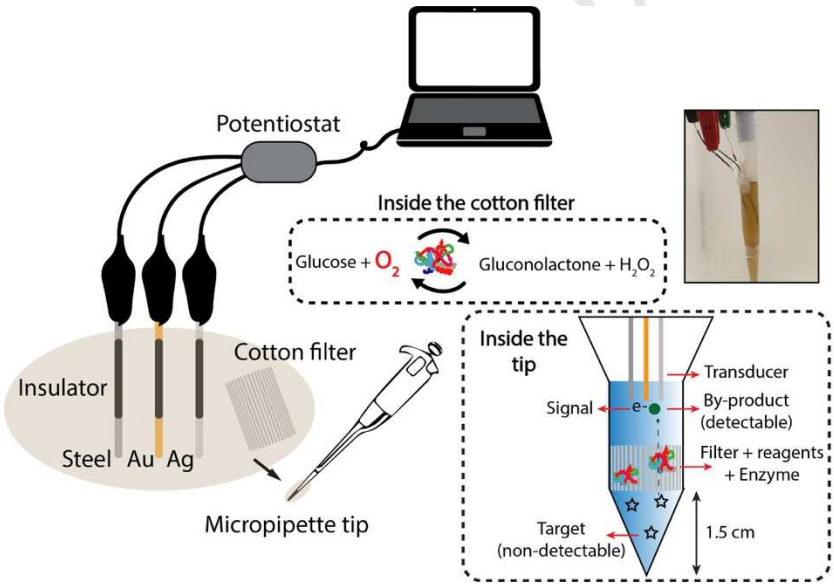

Figure 1. Schematic representation of the bio-lab-on-a-tip system for glucose detection.

2.3. Bio-Lab-on-a-Tip fabrication

A D1000 tip (Diamond Tip, Pipetman, Gilson) of an automatic pipette (Gilson, Italy) was used as the container of the electrochemical cell. Inside the tip, a small piece of cotton filter (3-mm height, 4-mm diameter) with a selected weight of 0.045 ± 0.002 g, was placed. The untreated commercial cotton filter was placed 1.5 cm far from the hole of tip: this position was dependent by the internal diameter of the pipette tip, and in addition it allowed to evaluate the similarity among different setups. Before of being placed into the pipette tip, the cotton filter was impregnated with a solution containing glucose oxidase (50 U/mL) dissolved in phosphate buffer 0.05M + KCl 0.1 M, pH=7.4. All the experiments were carried out at room temperature, *ca*. 25°C. Successively, three holes were made on upper part of the tip to allow the wires electrodes being inserted, successively sealed with an epoxy paint, to avoid air going inside the tip. Additional pictures of the experimental setup have been reported in the supporting information file, Figure S1.

2.4. Glucose determination

Subsequent to the construction of the bio-lab-on-a-tip, 500 µL of sample was taken by means of a pipette. When sample goes into the tip, it passes through the cotton filter that will release the enzyme and the other reagents that have been previously loaded. As reported in Figure 1, the filter is previously loaded with the enzyme and the reagents (50 mM phosphate buffer (pH=7.4) and a supporting electrolyte, i.e. 0.1 M potassium chloride). What happens is the following: the sample containing the target analyte enters the pipette tip. Initially, sample contains a non-detectable target, i.e. glucose, that is converted through the passage into the cotton filter, where it is oxidized by the enzyme. The oxidation involves the consumption of molecular oxygen that is consistent with a lower cathodic current recorded at the wire electrodes into the tip. The physical adsorption of the enzyme, avoids possible denaturation of enzyme, allowing glucose oxidase converting the substrate in the entire electrochemical cell, i.e. cotton filter and pipette tip. One minute is waited to allow the enzymatic reaction to occur, then a chronoamperometric detection is performed, recording a cathodic current deriving by the oxygen reduction.

3. Results and discussion

3.1. Setting-up tip configuration

For this study, chronoamperometric detection of simulant (a releasing-oxygen specie), i.e. hydrogen peroxide, using an applied potential of -0.5 V (vs. Ag) was performed, in accordance with literature (Wadhawan et al., 2003). The working potential has been chosen as the optimal compromise between sensitivity and repeatability, and the cyclic voltammetry studies in presence of a O_2-simulant have been reported in the supporting information file (Figure S2). In order to evaluate the suitability of a pipette tip being used as the detection system, a comparison between tip and out-tip has been carried out without the use of enzyme. The reason of this comparison is due to the presence of cotton filter, that could be responsible of affecting the diffusion of analyte within the tip. As reported in Figure 2, the two configurations have been compared.

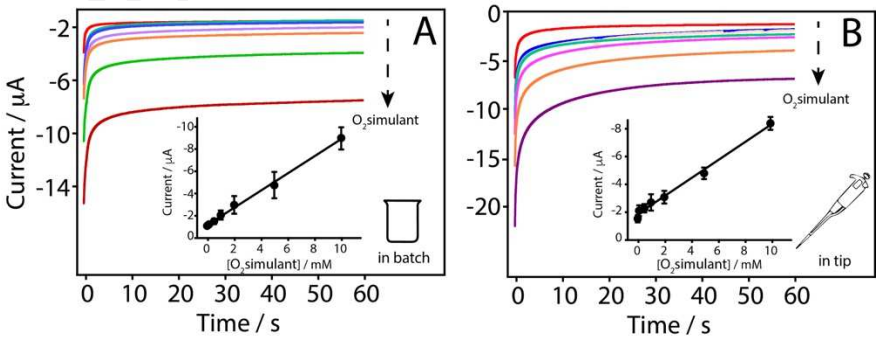

Figure 2. Calibration curve of oxygen releasing simulant, i.e. hydrogen peroxide, analyzing 0.1, 0.5, 1, 2, 5 and 10 mM obtained by using A) “in batch” and B) ”in tip” approaches. All the concentrations have been analyzed in triplicate and -0.5 V (vs. Ag) has been applied for 1 min during the choronoamperometric detection.

The two set of experiments have displayed a good degree of accordance. In fact, Figure 2A, related to the use of a classical electrochemical measurement in a beaker, namely “in batch”, showed a linear correlation in the range between 0.1 and 10 mM described by the following equation: $y = (2.03 \pm 0.05) + (0.51 \pm 0.01)\ x$, with a R^2 of 0.990. From the other side, the configuration named “in

tip" has showed a linear correlation, in the same concentration range (0.1-10 mM), expressed by the equation y = (2.57 ± 0.10) + (0.47 ± 0.02) x, with a R^2 of 0.970. The main motivation of this study was about the evaluation of the effect of cotton filter on the sampled solution: due to the filter porosity and structure, entrapments phenomena could appear during measurements. By comparing the sensitivity achieved by using the tip (with a bare cotton filter inside) with the measurements obtained in batch, it can be observed that no significant differences are observed within the experimental errors (<5%). Therefore, the tip configuration does not affect the electrochemical response at the wire electrodes, highlighting its suitability in being used to develop the ultimate biosystem.

3.2. Glucose detection with the bio-lab-on-a-tip

As previously reported in the experimental section, an enzyme-based biosystem was developed to specifically determine glucose. The reaction that has been taken into account is the oxidation of glucose by the glucose oxidase, which consumes the oxygen present in the solution as a co-factor. Experimentally, the anodic current around -0.5 V, relative to oxygen reduction, is monitored. For further development of this novel platform, the presence of the cotton filter appears indispensable as it will constitute the "reactor": herein glucose oxidase is adsorbed, in order to provide a device as easy as possible for end-users. The parameters that have been optimized are the following: concentration of glucose oxidase in the filter and enzyme-substrate reaction time.

3.2.1. Optimization of glucose biosensing

In order to determine the optimal enzyme concentration to be used, the experiments have been carried out by using different concentrations of glucose oxidase, namely 10, 50 and 100 U/mL. A volume of 20 µL has been previously selected as the optimal volume to be loaded by the filter: this choice avoided the enzyme solution leaking out the cotton filter (not shown). Regarding this optimization, all the enzyme levels has been tested in triplicate, using a brand-new cotton filter for each measurement and by measuring a 2 mM glucose. The measurements have been carried out by the chronoamperometry technique, applying a potential difference of -0.5 V (vs. Ag). The results of the study are reported in Figure 3A.

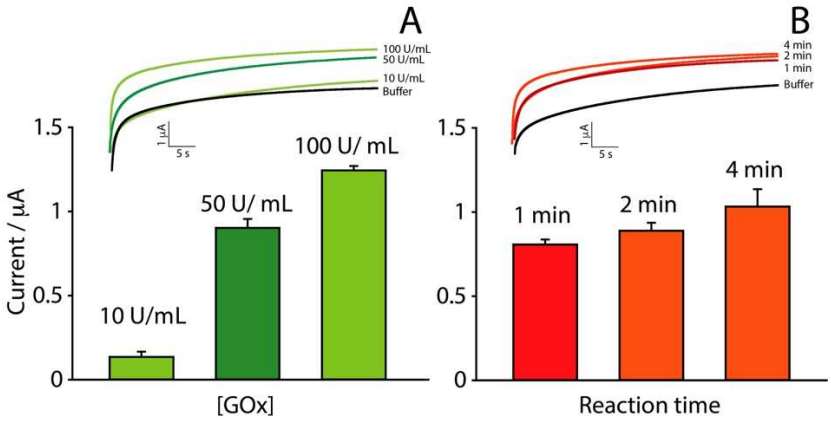

Figure 3. Optimization of the experimental conditions for the chronoamperometric detection of 2 mM glucose by using the bio-lab-on-a-tip configuration. A) Glucose oxidase units study, from 10 to 100 U/mL with a reaction time of 1 min; B) Reaction time study, from 1 to 4 min with a [GOx] = 50 U/mL. Current response has been recorded after 60 s with an applied potential of -0.5 V (vs. Ag).

For the further development of the bio-platform, 50-U/mL concentration of glucose oxidase has been selected. By observing the histograms, it is obvious to note how a concentration of 10 U/mL does not allow obtaining a good sensitivity, while the use of 100 U/mL does not reflect into a significant signal increase for justifying the doubling of the enzyme concentration. The choice of 50 U/mL thus represented the best compromise between the signal variation and the glucose oxidase amount used. In addition to this, the optimization of the reaction time has been also interrogated, i.e. the time in which the enzyme converts the substrates in by-products. Reaction times ranging from 1-4 minutes have been evaluated. The cotton filter was modified with 20 µL of 50 U/mL of glucose oxidase, and 2 mM glucose have been analyzed. As reported in Figure 3B, the optimal reaction time under the experimental conditions has been chosen equal to 1 minute. This has allowed to obtain good sensitivity values reducing the duration of the experiments.

3.2.2. Glucose detection in standard solutions

Following the optimization of the biosystem integrated into the pipette tip, different glucose concentrations in potassium chloride solutions have been analyzed. The previous optimized parameters have been adopted and glucose have been measured in the range comprised between 0.5 and 10 mM. The measurements have been performed in triplicate and the results are shown in Figure 4.

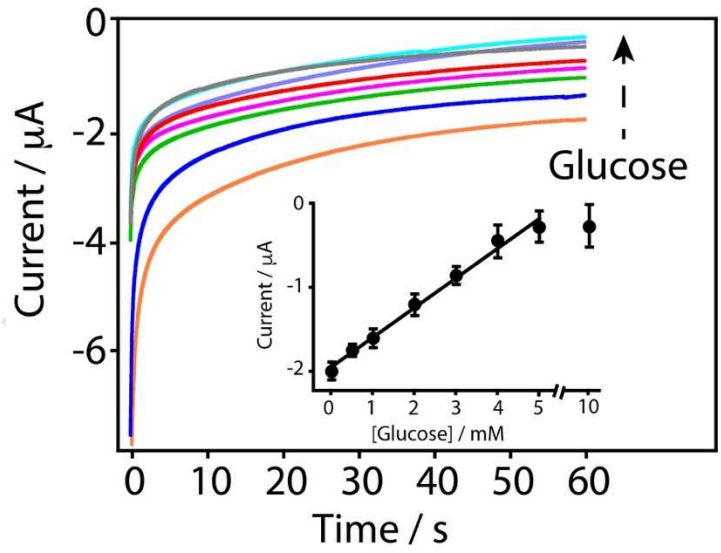

Figure 4. Chronoamperometric curves obtained analyzing: 0, 0.5, 1, 2, 3, 4, 5 and 10 mM of glucose. Inset: Calibration curve obtained by analyzing glucose in the range comprised between 0 and 10 mM. Experimental conditions are: applied potential of -0.5 V (vs. Ag), acquisition time of 1 min, [GOx] of 50 U/mL and reaction time of 1 min.

As it can be observed, the absolute value of signal (current difference) increases as the glucose concentration increases. It has been possible to obtain a good correlation of up to almost 5 mM of glucose, described by the equation $y = (2.03 \pm 0.06) + (0.35 \pm 0.02)x$ with a correlation coefficient R^2 equal to 0.960.

The main analytical parameters have been evaluated, such as the limit of detection (LOD) and limit of quantification (LOQ). The LOD has been calculated as the ratio of standard deviation of the blank and the sensitivity, multiplied by 3, and resulted ca. 0.17 mM. Following this approach, LOQ, calculated as 3.3 *LOQ, resulted equal to 0.56 mM.

The repeatability of the platform has been also estimated, calculated as the relative standard deviation (RSD) for three measurements using a concentration of 2 mM: a value of 12% has been obtained. It should be noted the significative value of this result, considering that three different bio-lab-on-a-tip have been used and that the entire fabrication steps has been manual performed. Considering the entirely produced prototype, starting from raw materials such as micropipette, tip, cotton filters, wires, it would be certainly improved by utilizing a mass scale production. In addition, the shelf life has been evaluated: for establishing this, cotton filters have been modified with 20 µL of glucose oxidase 50 U/mL and the responses have been evaluated in presence of 2 mM glucose up to 7 days. The modified cotton filters have been modified on the same day and kept in the refrigerator (4° C) until the day of measurement. Figure S3, Supporting Information, highlights how the recorded signal displays a 50%-drop after 7 days of storage at 4°C compared to the measurements carried on the fabrication time (day 0). In addition, it should be noted how the present method contains innovation in sensing glucose compared to the other main electrochemical platforms: in fact, the biosystem integrated into a pipette tip allows the users to reduce all the analytical procedures and treatments to zero. As reported in Table S1, bio-lab-on-a-tip configuration avoids sample treatment, chemicals addition and adjustment of experimental conditions, e.g. pH, ionic strength.

3.3. Real application of bio-lab-on-a-tip to detect glucose in soft drinks

3.3.1. Study of the interferences

A useful application of this platform could consist in evaluating the occurrence of glucose in soft drinks. In this work, glucose measurement has been carried out in Coca-Cola® and Coca-Cola® Zero samples. However, a study on possible interferents should have been carried out prior to analyze complex matrices. According to the Coca-Cola® and Coca-Cola® Zero labels, it was chosen to verify how the presence of electroactive species such as citric acid, ascorbic acid, and sodium bicarbonate could interfere with the determination of glucose. It should be noted that these species, being electroactive, could interfere with the measurements even in absence of enzyme. In addition, the high specificity of glucose oxidase, as widely reported in literature, does not allow oxidizing mono- and di-saccharides, e.g. fructose, mannose, galactose, sucrose, and in addition, these sugars are not electroactive within the experimental conditions (Yang et al., 2003; Wu et al., 2004; Su et al., 2017). The optimized platform was applied by measuring the signals deriving from solutions containing the interferent in the presence of a fixed concentration of glucose, as shown in Supporting Information, Figure S4. The measurements, carried out in triplicate, have not evidenced signal difference between the analysis of 2 mM glucose and a solution containing 2 mM glucose + 2 mM of the interferent compounds, namely citric acid, ascorbic acid, and sodium bicarbonate. Within this experimental conditions, no significative interferences have been highlighted.

3.3.2. Glucose detection in Coca-Cola® and Coca-Cola® Zero

From the values reported on the label, Coca-Cola® contains an amount of sugars equal to 10.6 g, without however detailing the different types of sugars that can be contained. As previously shown, the linearity interval of the platform has reached an upper limit of 5 mM, therefore a measurement of raw Coca-Cola® has not been suitable. The dilution of Coca-Cola® was necessary when evaluating the effect of the matrix, in order to have a glucose concentration within the linearity range. Coca-Cola® was diluted using the following ratios: 1/100; 1/150 and 1/200. All the diluted solutions and those with fortifications in the 1-2 mM glucose range have been analyzed. The 1/100

dilution is the one that has enabled the achievement of best results in terms of sensitivity within the linearity of the biosystem, and in Figure 5A some results have been displayed.

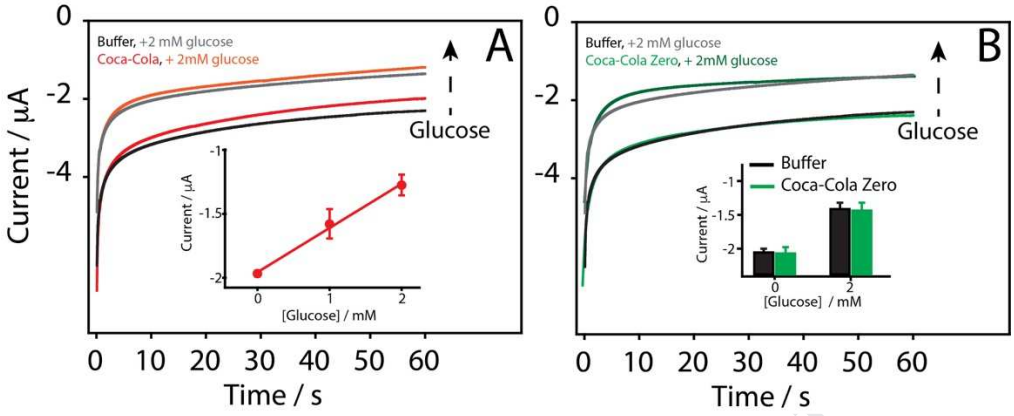

Figure 5. Effect of the cotton filter on the real matrix, namely A) Coca-Cola and B) Coca-Cola Zero. Evaluation of the matrix effect by performing the analysis in samples spiked with glucose in at level of 1 and 2 mM. The experimental conditions are those reported in caption of Figure 4.

Looking at the chronoamperograms, it is obvious how the analysis of Coca-Cola® has produced a current value differing from that observed by analyzing a blank solution. This behavior depends both on the glucose present and on the matrix. By spiking the sample with two glucose levels, a small difference in sensitivity observed compared to the currents recorded in standard solutions was observed: red curves represent measurement in Coca-Cola®, and they appear slightly different from those obtained in buffer solution, black curves. By exploiting the addition method, a concentration of glucose in the Coca-Cola® resulted equal to 64 mM. Successively, Coca-Cola® Zero has been taken into account. In this case, a relevant glucose concentration is not expected, but the effect of the matrix could have an influence, thus different dilutions have been evaluated: the sample has been analyzed in its raw form and by diluting by 10- and 100-fold. Looking at the results, it was decided to continue with the measurement of undiluted Coca-Cola® Zero, because the signal obtained in standard solution appeared similar to the one in the soft drink, as shown in Figure 5B. In addition to this, spiking Coca-Cola® Zero with 2 mM glucose resulted in a sensitivity similar to that obtained in measurements carried out in standard solutions. According to the label, the bio-lab-in-a-tip has confirmed the absence of glucose in Coca-Cola® Zero. In addition, after having established the absence of glucose at the detection limit of the system in the matrix, the accuracy of the method was evaluated through recovery experiments. Coca-Cola® Zero was spiked with 1 and 2 mM glucose, and through the standard addition method the calculated percentage recoveries were equal to 98 ± 10, and 101 ± 10 %, respectively.

4. Conclusion

For the first time an electrochemical biosystem, entirely integrated into a micropipette tip, has been developed and successful applied towards the detection of glucose in commercial soft drinks. The bio-lab-on-a-tip has exploited the use of common materials such as a micropipette tip (served as the electrochemical cell), cotton filter (from cigarettes) and conductive wires. The experimental setup, entirely built inside the micropipette tip, has been capable to 1) block gross impurities from matrix, 2) load/release reagents for the bio-assay, 3) reduce the operating task to zero, and 4) perform electrochemical detection. In particular, the cotton filter inside the tip has been responsible for four fully self-supported tasks: 1) loading the reagents, 2) filtering gross impurities that can be contained

in the matrix, 3) releasing the reagents and 4) providing a biocompatible environment for enzyme entrapment. A satisfactory sensitivity in the determination of glucose in standard solutions in an extended range up to 5 mM, with a calculated detection limit equal to 0.17 mM has been obtained. The bio-lab-on-a-tip has displayed effectiveness to verify the presence and absence of glucose, respectively in Coca-Cola® and Coca-Cola® zero, demonstrating that the end-user is able to detect glucose in unique step: sampling/catalysis/detection.

The presence of some interferents such as ascorbic acid, citric acid and sodium bicarbonate have not significantly affected the measurement of glucose. The present concept could inspire novel conceiving of bioplatforms, being this the first example of an electrochemical biosystem integrated into a tip. In the future, this configuration can be used for the detection of other compounds using different enzymatic reactions, or for the development of devices useful for environmental or medical-clinical analyzes. Thanks to the use of 3D printers, it could be possible to print pipette tips capable of providing the optimal positioning for the wire electrodes depending on the space available inside the tip. Furthermore, future directions will be aimed at detecting larger molecules, such as proteins and nucleic acids. Thanks to the use of techniques such as screen-printing, a future step could be the development of fully printed and disposable devices.

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Highlights

- Experimental setup for sustainable measurements
- A bioreactor integrated into a common cotton wool filter
- Glucose detection in a single step into a micropipette tip
- Bio-lab-on-a-tip as the starting point for novel biosystems

Declaration of interests

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: