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PAPER IN FOREFRONT

Direct electrochemical biosensing in gastrointestinal fluids

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Abstract

Edible electrochemical biosensors with remarkable prolonged resistance to extreme acidic conditions are described for direct glucose sensing in gastrointestinal (GI) fluids of different pH ranges and compositions. Such direct and stable glucose monitoring is realized using carbon-paste biosensors prepared from edible materials, such as olive oil and activated charcoal, shown to protect the activity of the embedded glucose oxidase (GOx) enzyme from strongly acidic conditions. The enzymatic resistance to low-pH deactivation allowed performing direct glucose monitoring in strong acidic environments (pH 1.5) over a 90-min period, while the response of conventional screen-printed (SP) biosensors decreased significantly following 10-min incubation in the same fluid. The developed edible biosensor displayed a linear response between 2 and 10 mM glucose with sensitivity depending on the pH of the corresponding GI fluid. In addition, coating the electrode surface with pH-responsive enteric coatings (Eudragit® L100 and Eudragit® E PO), of different types and densities, allows tuning the sensor activation in gastric and intestinal fluids at specific predetermined times. The attractive characteristics and sensing performance of these edible electrochemical biosensors, along with their pH-responsive actuation, hold considerable promise for the development of ingestible devices towards the biosensing of diverse target analytes after prolonged incubation in challenging body fluids.

Keywords Edible electrode · Biosensor · Gastrointestinal fluids · Acid resistant surfaces · Selective activation

Introduction

Disorders in the gastrointestinal (GI) tract affect the majority of the worldwide population. An efficient monitoring of relevant biomarkers in the GI environment plays a decisive role in the early diagnosis and efficient treatment of such disorders [1, 2]. Innovative technologies are required to perform reliable diagnosis of such diseases, involving the development of biocompatible and high sensitive devices able to detect selectively important biomarkers in specific segments of the GI tract [3].

During recent years, electrochemical biosensors have demonstrated their utility for continuous on-body and non-invasive monitoring of diverse analytes and biomarkers circulating in different biofluids [4–7]. However, the tremendous variability of pH and composition (e.g., bacteria, lipases, proteases) along different sections of the GI tract represents a major challenge for such direct biosensing. In particular, the strong acidic environment of the gastric fluid may compromise the stability of the biocatalytic enzymes and the protein-rich media accelerates the biofouling of electrode surfaces. These issues greatly compromise the stability and overall biosensor performance [4, 8–10]. This lack of biosensor stability hinders greatly its practical utility in harsh environments, as the GI tract. One approach to protect enzymatic biosensors from extreme conditions, such as pH or temperature, is to use carbon paste electrodes [10, 11]. Such remarkable protection of enzymes from extreme conditions has been attributed to the confinement of the biocatalysts in the non-polar environment of the mineral oil pasting-liquid binder which

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minimizes the proteins' mobility and their denaturation. These attractive advantages of carbon paste electrodes, combined with the ability to retain oxygen in the paste, have facilitated their integration into microneedle type biosensors [12, 13] and in implantable self-powered sensors [14]. Moreover, carbon paste-based sensors offer several additional advantages, including low background currents, ease of modification, renewability, and low cost [15]. While carbon paste biosensors demonstrated dramatic enhancement of the enzyme stability following prolonged storage conditions of high temperatures or acidic environments [10, 11], the performance of these biosensors was assayed in PBS pH 7.4 and not in the strong acid media [10]. Ideally, the biosensors should display prolonged stability while performing in situ analysis directly in the GI tract (without treating the media). Moreover, to ensure their safe in vivo operation, these biosensors should be fabricated with fully biocompatible and/or biodegradable materials [16, 17]. Designing "green" biocompatible sensing devices for the direct and prolonged biosensing in biofluids of such harsh conditions remains a major challenge. Within this context, the incorporation of biodegradable stimuli-responsive materials, such as pH-responsive enteric coatings, would facilitate the protection and selective activation of such diagnostic devices both in specific locations and at desired times.

An attractive and safe approach to monitor target analytes in different sections of the GI tract is to use ingestible devices, which allow direct access to the gut surroundings during their passage through the GI tract [18]. Such ingestible biomedical devices offer considerable potential improving the diagnosis and treatment of diseases associated with the GI tract [18]. Edible devices, derived from natural foods and foodstuffs [18], represent a very attractive route for creating ingestible devices for direct monitoring of important biomarkers of each gut segment, including electrolytes, enzymes, hormones, and other chemical byproducts produced by the gut microbiome, which are in continuous transfer/exchange motion provided by the gut mucosal membrane [19]. An example of such promising application is the in situ monitoring of electrolytes and glucose levels in the stomach for early detection of ischemia [20]. Recent efforts have led to edible electrochemical sensors, based on carbon paste made of different food materials, which demonstrated excellent conductivity and electrochemical performance [17]. However, the application of such edible carbon paste-based electrochemical sensors for selective activation and prolonged operation in strongly acidic environments, such as GI fluids, has not been demonstrated.

This paper reports the development of remarkably acid resistant food-based edible electrochemical biosensors and their prolonged operation in GI fluids of different pH and compositions, along with attractive performance towards the monitoring of glucose. Activated charcoal and olive oil, used as the edible sensor conductor and binder, respectively, serve to protect the GOx enzyme from the harsh conditions present in some GI fluids, such as the strong stomach acid. These biosensors can directly measure glucose levels in strong acidic conditions (e.g., gastric fluid, pH 1.5) and be further protected with pH-responsive enteric coatings towards a controlled activation in specific GI fluids at desired times. As the enteric coating dissolves gradually, the edible carbon paste provides additional protection of the enzyme, holding tremendous potential for sensing capsules passing through the several parts of the GI tract.

Using glucose as a model analyte, we demonstrate below the attractive performance of these edible glucose biosensors after prolonged exposure in simulated gastric (pH 1.5) and intestinal (pH 6.5) fluids. The operational stability of the edible biosensors in both GI fluids is evaluated, demonstrating no loss in sensitivity up to 90 min. The attractive stability of these edible electrochemical biosensors—based on two levels of protection (the paste environment and the enteric coating)—along with their favorable analytical performance offers considerable promise for the development of ingestible diagnostic devices towards physiological controlled biosensing of important target analytes in challenging body fluids.

Materials and methods

Apparatus

Chronoamperometric measurements were performed at room temperature with a CHI1230A potentiostat (CH Instruments, Austin, TX) controlled by a CHI1230A software. A Maxi-Mix (Type 16,700 Mixer) vortex, pH meter (Seven Easy, Mettler-Toledo, Switzerland), and semiautomatic MMP-SPM printer (Speedline Technologies, Franklin, MA) were also used.

Reagents and solutions

All reagents used were of the highest available grade. Edible activated charcoal (Nature's Way Products, Inc., Green Bay, WI) and extra virgin olive oil (Filippo Berio, Europe) were purchased from a local grocery store. Simulated gastric and intestinal fluids (both free of enzymes), glucose oxidase (GOx) from *Aspergillus niger*, Type X-S (EC 1.1.3.4), bovine serum albumin (BSA), chitosan, D(+)-glucose, and phosphate buffer solution (1.0 M, pH 7.4) were obtained from Sigma-Aldrich (St. Louis, MO). The pH-responsive commercial polymers (Eudragit® L100 and E PO) were obtained from Evonik Industries (Germany). Ethanol and 2-propanol were obtained from Fisher Scientific. Sodium hydroxide (NaOH) pellets were obtained from Mallinckrodt Chemicals. Hydrochloric acid (HCl) and acetic acid were obtained from EMD Chemicals Inc. (Gibbstown, NJ). Conductive carbon

ink (E3449) and silver/silver chloride ink (E2414) were obtained from Ercon Inc. (Wareham, MA).

All aqueous solutions were prepared with deionized water obtained from a Millipore Milli-Q purification system (18.2 MΩ cm at 25 °C). Solutions prepared included: 0.1 M phosphate buffer (PBS) solution, pH 7.4; 2 or 4% (*w*/*v*) Eudragit® L100 polymer in isopropanol supplemented with 0.05% (*w*/*v*) SDS; 4 or 8% (*w*/*v*) Eudragit® E PO polymer in ethanol supplemented with 0.05% (*w*/*v*) SDS; 0.5% (*w*/*v*) chitosan solution (in 0.1 M acetic acid); 1 M glucose in PBS, pH 7.4; Gastric fluid (pH 1.5 or 5.0) and intestinal fluid (pH 6.5) were prepared following the commercial specifications in deionized water.

Preparation of biosensors on edible or conventional screen-printed electrodes

The edible electrodes for glucose determination were prepared by using edible activated charcoal as the conductive filler material and edible olive oil as a binder. Edible paste with the enzyme was prepared by hand-mixing thoroughly 17 mg of GOx (227,553 U/g), 100 mg of activated charcoal, and 100 μL of olive oil using a mortar and a pestle. A portion of the resulting mixed paste was packed into plastic tubes (2.8 mm diameter and 4 cm length). For electrochemical measurements, electrical contacts were made by inserting a conductive stainless steel wire into the top side of the packed paste while the bottom side was smoothened using a wax paper to give a flat electrode surface.

Conventional glucose biosensors were prepared using screen-printed carbon electrodes (SPCEs) as electrochemical transducers and immobilizing the enzyme using chitosan. The printing and enzyme immobilization steps used here are similar to those described in our previous works [21, 22], using a customized electrode sensor template, of stainless steel, developed using AutoCAD software (Autodesk, San Rafael, CA) and produced by Metal Etch Services (San Marcos, CA). In the printing process, a sequence of Ag/AgCl conductive ink was used to print the conductive current collector, and then conductive carbon ink was used to print the working and counter electrodes. Finally, the printed layers were cured at 85 °C for 20 min after each printing step. For the preparation of the glucose biosensors, the working electrode surfaces were modified by drop casting 3 μL of a GOx solution (40 mg mL^{-1} containing 10 mg mL^{-1} BSA stabilizer in PBS 0.1 M, pH 6.5) mixed in a 1:1 (*v*/*v*) ratio with a chitosan solution (0.5% (*w*/*v*) in 0.1 M acetic acid).

Biosensor modification with the enteric coatings

In order to control the activation of the edible biosensors in the different parts of the GI tract, the bottom side of the packed tube was coated with the polymers Eudragit® E PO (which dissolves at pH ≤ 5.0) or Eudragit® L100 (which dissolves at pH ≥ 6.0) for measurement in gastric or intestinal fluids, respectively. A single layer (3 μL of 2, 4 or 8% (*w*/*v*)) of the polymeric solutions (methacrylate-based polymers in the precursor solution) was drop casted onto the electrodes surfaces and the isopropanol or ethanol were evaporated at room temperature.

Enteric coating dissolution experiments

After coating the sensor surfaces, the dissolution of the coatings was evaluated at different times and at different pH conditions by the incubation of the different biosensors in simulated gastric (pH 1.5) and intestinal (pH 6.5) fluids. The pH value of these solutions (lower than 5.0 or higher than 6.5, respectively) ensured the complete dissolution of the polymeric coatings and the consequent activation of the edible biosensors at specific times.

Amperometric measurements

Chronoamperometric measurements were used to monitor the glucose levels in 0.1 M PBS (pH 7.4) and simulated gastric fluid (pH 1.5 and 5.0) or intestinal fluid (pH 6.5). The chronoamperometric responses were recorded at room temperature in the sample solution, applying a potential of + 0.6 V (vs. Ag/AgCl) for 60 s.

Results and discussion

In this article, we demonstrate the direct and prolonged operation of edible electrochemical biosensors in GI fluids of different pH and compositions, using glucose as a model analyte. The new biosensing approach relies on using completely edible materials, such as olive oil and activated charcoal, which serve to protect the GOx enzyme from the harsh acidic conditions of some GI fluids. Such edible materials were homogeneously mixed with the GOx enzyme and firmly packed into plastic tubes (Fig. 1a, steps 1–2). These biosensors present different paths of use: direct measurement in the GI tract (Fig. 1a, step 3), or controlled activation by protecting the biosensor surfaces with different types of pH-responsive enteric coatings (Fig. 1a, steps 3′–5′). In this second path, the biosensor is activated by the pH-triggered dissolution of the enteric coating in the GI fluid of interest at desired times. In this work, the pH-responsive polymers Eudragit® E PO and Eudragit® L100, which dissolve below pH 5.0 and above pH 6.0, respectively, were selected to demonstrate the selective activation in simulated gastric and intestinal fluids [23, 24]. These biocompatible methacrylate-based coatings, which gradually dissolve in the respective fluids, help to minimize the non-specific adsorptions in these complex media, and lead

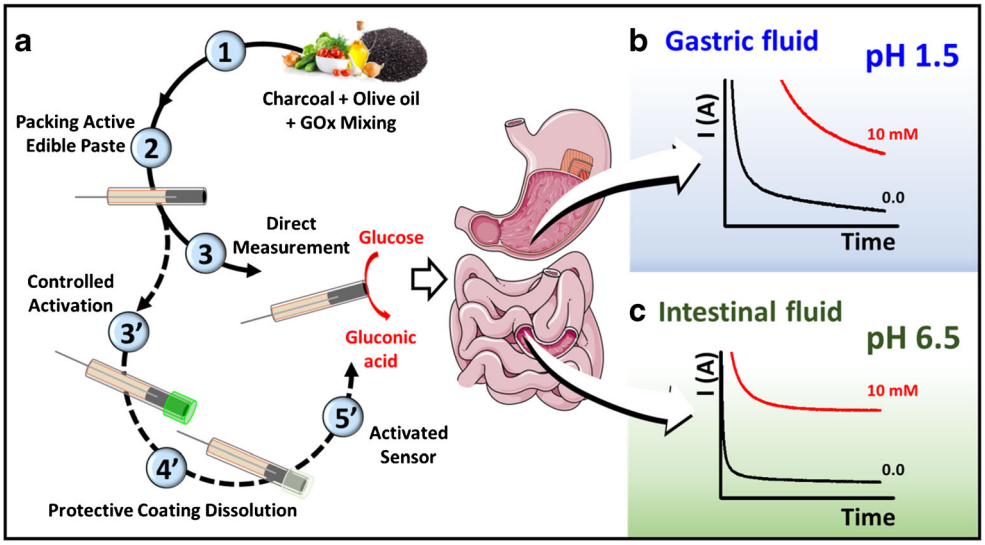

Fig. 1 Direct and controlled sensing in the GI tract using edible biosensors. (a) Schematic of the edible biosensor preparation (steps 1 and 2), and its use for direct measurement (step 3), or its protection with pH-responsive enteric coating for controlled activation in the GI tract (steps 3′–5′). Chronoamperograms obtained in gastric fluid pH 1.5 (b) or intestinal fluid pH 6.5 (c) before (black lines) and after (red lines) spiking 10 mM glucose. E_{app} = + 0.6 V (vs. Ag/AgCl), 60 s

to delayed exposure of the fresh biosensor surface at particular measuring times [25].

The biosensing performance of the presented edible biosensors has been demonstrated by chronoamperometric measurements to monitor the glucose levels in both gastric (pH 1.5) and intestinal (pH 6.5) fluids (Fig. 1b, c, red chronoamperograms corresponding to gastric and intestinal fluids, respectively, spiked with 10 mM glucose). The operational stability towards glucose biosensing after prolonged incubation in these complex media has been also compared to that offered by conventional GOx-modified SPEs.

Different experiments were performed aiming to demonstrate the effective performance of the edible electrochemical biosensors in both GI fluids, their excellent acid stability, and the controlled activation by using different enteric coatings. Initially, the response of the sensors to the presence of different glucose concentrations was compared in different GI fluids. Figure 2a displays the calibration curves constructed for glucose in various fluids, including PBS (pH 7.4), intestinal fluid (pH 6.5), and gastric fluid (the latter tested at two pH values, 5.0 and 1.5). A linear relationship between the measured oxidation current and the glucose concentration was found over the 2–10 mM range for all the tested fluids. Completely identical slope values were observed for PBS and intestinal fluid (20.3 ± 0.3 ($R^2 = 0.997$) and 19.6 ± 0.8 ($R^2 = 0.994$) nA mM^{-1}, respectively) with no apparent change in the sensitivity (overlapped black and red curves, respectively). However, the slope drastically decreased when using gastric fluid at the two pH values tested (10.5 ± 0.4 ($R^2 = 0.997$) and 6.9 ± 0.5 ($R^2 = 0.994$) nA mM^{-1}, for pH 5.0 and 1.5, respectively). This might be attributed to a possible matrix effect due to the different composition of these commercial GI fluid simulants. Despite such difference, increasing levels of glucose were readily detected in all these fluids. The corresponding chronoamperograms obtained in intestinal fluid pH 6.5 and gastric fluid pH 1.5 before (dotted lines) and after (solid lines) spiking glucose (2–10 mM) show increased oxidation currents, proportional to the glucose concentration (Fig. 2b, c). A slightly loss of enzymatic activity was observed for the measurements obtained in gastric fluid reflected in the increased time required for the current signal stabilization when compared with the response in intestinal fluid (60 s vs. 20 s, respectively). Nevertheless, the sensitivity and linearity demonstrated by the sensor in the gastric fluid has shown to be effective for glucose detection. Although an exhaustive analytical characterization of the edible biosensor was beyond the

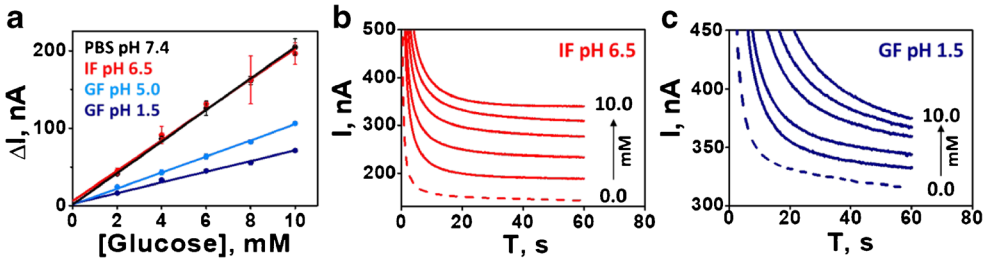

Fig. 2 Glucose biosensing in GI fluids using edible biosensors. **a** Calibration curves constructed for glucose in PBS pH 7.4 (black), intestinal fluid (IF) pH 6.5 (red), gastric fluid (GF) pH 5.0 (light blue) and pH 1.5 (dark blue). **b** Chronoamperograms obtained in IF pH 6.5 (**b**) or GF pH 1.5 (**c**) before (dotted lines) and after (solid lines) spiking glucose (2–10 mM). E_{app} = +0.6 V (vs. Ag/AgCl) for 60 s. Error bars estimated as triple of the standard deviation ($n = 3$)

scope of this study, the data presented in Fig. 2 clearly demonstrate LODs below 1 mM in both GI fluids (slightly lower in the gastric fluid at both pH values than in the intestinal fluid due to the higher sensitivity demonstrated by the biosensor in the later media). These preliminary results illustrate the possibility of measuring glucose in different GI fluids of diverse composition and pH range.

The great improvement of the stability of the edible biosensors observed for continuous glucose monitoring in low-pH media—both gastric and intestinal fluids—has been assessed by comparing their performance with that of conventional biosensors consisting of SPCEs modified with GOx through chitosan (see Materials and methods section). In this study, both types of the biosensors were immersed in gastric (pH 1.5) and intestinal (pH 6.5) fluids, and their response to glucose was measured and compared at different times. The conventional SPCE biosensor displayed a rapid loss of 68% of its initial biocatalytic activity within 10-min immersion in the gastric fluid (pH 1.5), as demonstrated by the chronoamperograms and corresponding sensor response percentages shown in Fig. 3a, b (in green, curve 1), respectively. Remarkably, the edible paste-based biosensor displayed an outstanding 19-times improvement in sensitivity in the same gastric fluid when compared with the SPCE (slopes of 6.9 vs. 0.37 nA mM^{-1}, for edible and conventional sensors, respectively).

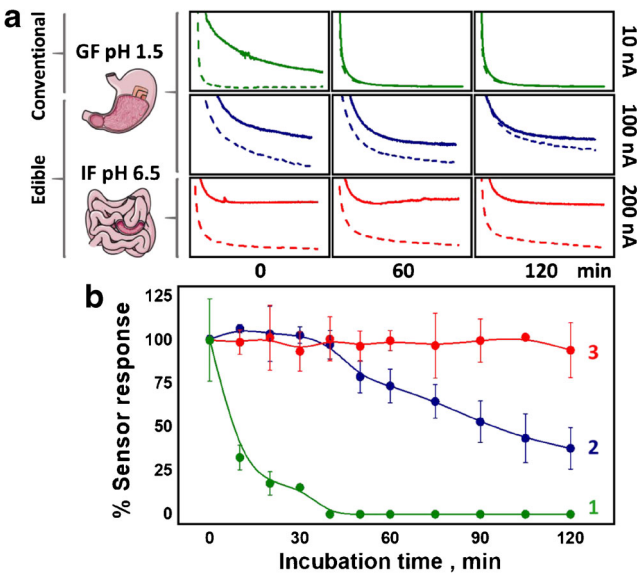

Fig. 3 Operational stability for continuous glucose monitoring in GI fluids and comparison of the biosensing performance of edible and conventional biosensors. **a** Chronoamperograms obtained: in gastric fluid (GF) pH 1.5 using conventional or edible biosensors (green and blue, respectively), or in intestinal fluid (IF) pH 6.5 using edible biosensors (red), before (dotted lines) and after (solid lines) spiking 5 mM glucose at 0-, 60-, and 120-min incubation times. **b** Comparison of the (%) response of conventional and edible biosensors in GF pH 1.5 (1 and 2) or IF pH 6.5 (3) at specific incubation times (monitoring every 10 min). E_{app} = +0.6 V (vs. Ag/AgCl) for 60 s. Error bars estimated as triple of the standard deviation (n = 3)

In addition, the edible biosensors displayed high stability in connection with the different incubation times, retaining ~ 50% of their initial response after prolonged 90-min incubation in the same gastric fluid (Fig. 3b, curve 2, in blue), which confirms the protection that the edible materials impart to the GOx enzyme. Moreover, the response of the edible biosensor was found to be fully stable in intestinal fluid pH 6.5 (curve 3, in red), displaying a ~ 94% response after 2-h continuous incubation in that fluid. Overall, these results confirm the excellent stability of the edible biosensors in both gastric and intestinal fluids and the feasibility to perform the accurate determination of glucose (and other relevant analytes) after prolonged incubation in such complex biofluids of extreme pH.

Finally, with the intend of simulating the activation of the edible biosensor in different locations of the GI tract, the controlled activation of these biosensors in different GI fluids was examined by protecting the working biosensor surface with different enteric polymeric coatings, Eudragit® E PO or Eudragit® L100, which dissolve below pH 5.0 and above pH 6.0, respectively. The effect of the density of the polymeric coatings upon the sensor activation time was evaluated by covering the sensor working electrode surfaces with enteric coatings of different densities (polymer percentages of 2, 4, and 8%). The coating dissolution was thus evaluated at different times and at different pH values by incubating in both GI fluids. Figure 4a displays the relative (%) response of edible biosensors coated with the Eudragit® E PO polymer in gastric fluid pH 1.5 (cyan and blue curves), and intestinal fluid pH 6.5 (green curve), at different incubation times. As expected, the coating gradually dissolved in gastric fluid pH 1.5 (cyan and blue curves), showing sensor activation times dependent on the coating density (15 and 30 min, for the 4 and 8% coatings, respectively). However, the same Eudragit® E PO (4%) coating remained stable in intestinal fluid pH 6.5 (green curve) even after an overnight incubation, indicating the effective and specific pH actuation of this polymeric coating for prolonged measurement in the gastric environment. Similarly, Eudragit® L100 was tested towards a controlled activation of edible biosensors in intestinal fluid pH 6.5 (Fig. 4b, dark and light green curves). This polymeric coating demonstrated dissolution in intestinal fluid pH 6.5 but stability in gastric fluid pH 1.5 (Fig. 4b, blue curve) even after overnight contact with the biofluid. The edible biosensor response was also dependent on the Eudragit® L100 coating density, displaying activation times of ~ 10 and ~ 20 min when using 2 and 4% coatings, respectively. Additionally, the use of such pH-responsive enteric coatings serves to minimize non-specific adsorption effects in these complex media. Overall, the results displayed in Fig. 4 demonstrate the possibility of precisely tailor the activation of the edible biosensor in specific GI fluids at controlled times by using different percentages of enteric coatings with different pH responses.

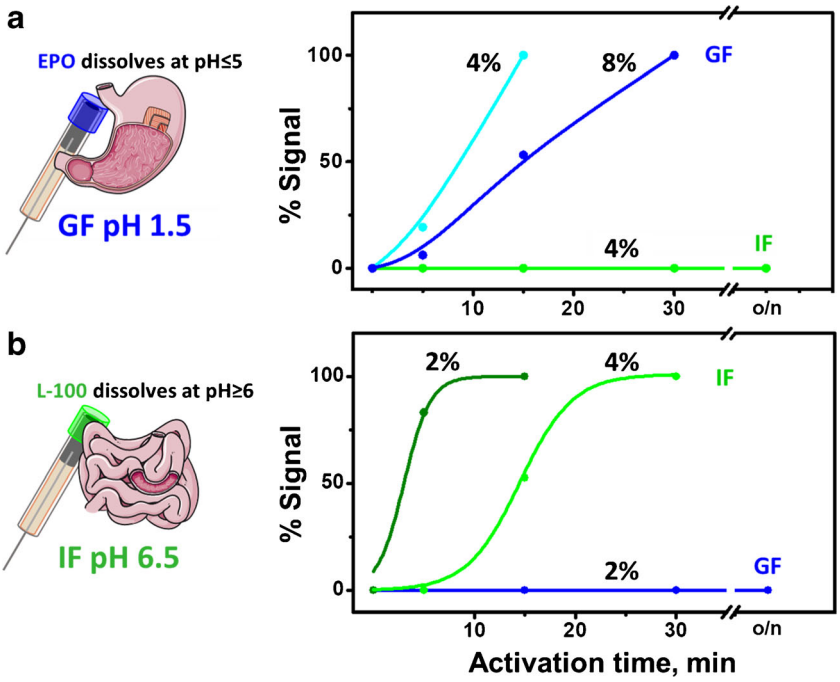

Fig. 4 Controlled activation of the edible biosensor in the GI tract by using pH-responsive enteric coatings. Relative (%) response of edible biosensors coated with the commercial enteric coating Eudragit® E PO (**a**) or Eudragit® L100 (**b**) in gastric fluid (GF) pH 1.5 and intestinal fluid (IF) pH 6.5 at specific incubation times using different polymer percentages. Coating density, 2, 4, or 8%. Glucose concentration, 5 mM

Conclusions

In summary, we have presented the fabrication of edible electrochemical biosensors with remarkable acid resistance and their direct and prolonged operation in GI fluids of different pH and compositions. The edible materials used for the fabrication of the sensor, olive oil and activated charcoal, protect the GOx enzyme from the harsh acidic conditions of some GI fluids, such as the gastric fluid. These edible biosensors can be used for direct measurement in GI fluids (e.g., glucose detection in gastric fluid pH 1.5), or be protected further with pH-responsive biocompatible coatings that allow performing a controlled and specific activation after prolonged exposure to gastric and intestinal fluids. Data presented demonstrated that the edible biosensor developed offered linear ranges between 2 and 10 mM glucose with LODs below 1 mM in both gastric fluids, and operated efficiently in strong acidic conditions (pH 1.5) after 90-min incubation. This combination of enteric coatings and pH-resistant enzyme systems provides two levels of protection and stabilization, and is promising also for improving treatments involving delivery of active enzymes to the GI tract, such as the one applied in severe chronic pancreatitis. Alternative edible food materials containing natural mediators or biocatalytic horseradish activity could be incorporated in future designs to develop edible biosensors with improved selectivity (by operating at lower potentials) and sensitivity (by coupling GOx/peroxidase activities). Such remarkable acid-resistant edible carbon-based biosensing surfaces, with enhanced ability against low-pH environments, have potential applications for developing ingestible capsules for real-time in vivo monitoring, of important analytes, directly in the GI tract.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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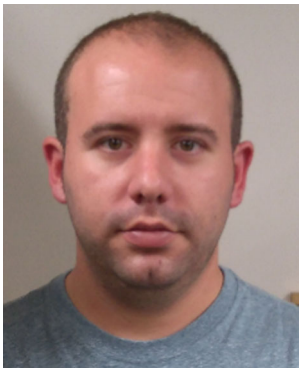

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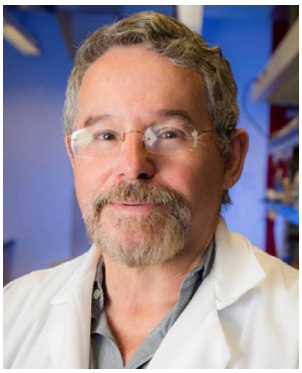

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