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Electrochemical studies of galactose oxidase

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Abstract

One of the most interesting redox biocatalysts is galactose oxidase, a redox enzyme that catalyzes the oxidation of D-galactose to 1,6-D-galactodialdose in a single copper redox site. Its relevance for the development of galactose electrochemical biosensors and its potential for biosynthetic reactions due to its affinity for other substrates have increased the research focused on this enzyme and its possibilities. In this work, we review the literature on galactose oxidase studies related to electrochemical devices, electron transfer examples, immobilization strategies, and their applications.

KEYWORDS

bioelectrochemistry, biosensor, electrochemistry, galactose, galactose oxidase, sensor

1 | INTRODUCTION

Galactose oxidase (GOase; E.C. 1.1.3.9) is a copper metalloenzyme found mainly in the fungi *Gibberella fujikuroi*, *Fusarium graminearum*, and the most extensively characterized *Dactylium dendroides*.^[1] GOase is categorized as a mononuclear type II copper-containing enzyme, and it is composed of a single polypeptide with a molecular mass of ca. 68 kDa.^[2] It catalyzes the oxidation of D-isomers of a broad range of primary alcohols, such as D-galactose, dihydroxyacetone (DHA),^[3] as well as substituted benzyl alcohols,^[4] to their corresponding aldehyde, coupling with the reduction of dioxygen to hydrogen peroxide.^[5]

The crystallographic structure of GOase was successfully determined in 1991 by X-ray diffraction with a 1.7 Å resolution. It revealed an interesting feature of the active site copper ion coordinated in a nearly square pyramidal geometry.^[6] The enzyme structure was completely elucidated; three different domains, composed predominantly by β -structure with short turns, were structurally and functionally distinguished (Figure 1A). The copper at the active site is coordinated by Tyr495 as an axial ligand,

His496, His581, Tyr272, and a solvent molecule a weakly coordinated water molecule (pH 7) as an equatorial ligand (Figure 1B). The authors also concluded that there is a second organic cofactor post-translationally derived from the covalent bond between Tyr272 and Cys228. The thioether bond that links the two residues affects both the structure and reactivity of the protein.^[7] This feature has attracted considerable interest, resulting in detailed spectroscopic studies,^[8–12] mainly electron paramagnetic resonance,^[5,13,14] X-ray crystallography,^[6,7,15] and study of site-directed mutations,^[16–19] which have led to a reasonable comprehension of the catalytic mechanism.

The Cu and the Tyr272 of the GOase active site can exist in the following three distinct oxidation states: the catalytically active one with Cu (II) and tyrosyl radical (GOase^{ox}), the intermediate state with Cu (II) and tyrosine (GOase^{semi}), and the lowest state with Cu (I) and tyrosine (GOase^{red}).^[20] The catalytic mechanism proposed by Whittaker, considers as the first step the substrate binding to the equatorial copper position, displacing the water ligand, then, a proton is transferred from the alcohol to the axial Tyr495. After, in a step elucidated from isotope substitution experiments, a hydrogen atom is transferred from

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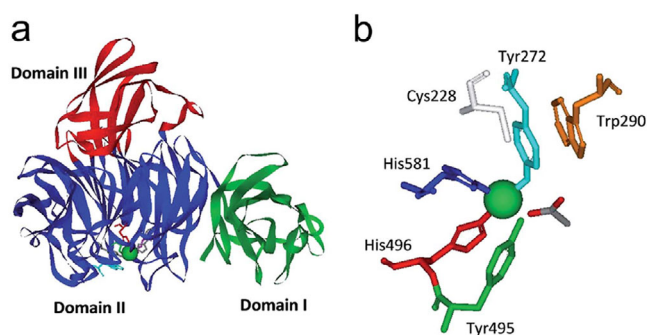


FIGURE 1 Structures of galactose oxidase from *Dactylium dendroides*. (a) Three-dimensional structural representation of the enzyme with the three domains noted. (b) The detailed structure of the catalytic active site. (Adapted from [5] with permission)

the substrate to the tyrosyl radical. The resulting substrate ketyl radical is then oxidized through electron transfer to the copper center, yielding Cu (I) and aldehyde. In the end, Cu (I) and tyrosine are re-oxidized by molecular oxygen, regenerating Cu (II) and tyrosyl while producing as sub-product hydrogen peroxide. [21]

The immobilization of redox metalloenzymes to electrodes is of interest for studying their catalytic properties by electrochemical methods, as well as for applications in bioelectrochemical devices, such as biosensors. In this article we will review the most relevant works for electrochemical studies of galactose oxidase, focusing on the evolution of the bioelectrochemical systems up to the state of the art and their applications for the development of galactose biosensors.

2 | DIRECT ELECTRON TRANSFER STUDIES OF GOASE

When trying to make redox enzymes to couple their natural activity to an electrode, achieving the direct electron transfer (DET) between the enzyme's active site and the electrode surface is often a great challenge. Redox metalloenzymes that have redox centers near the protein surface, such as hydrogenases and multicopper oxidases have shown very efficient DET with electrodes. [22,23] However,

DET of GOase with electrodes is quite elusive despite having its Cu redox site near to the protein surface (approximately at 8 Å). There are few reports in the literature compared with other copper-containing proteins, such as azurins, laccases or bilirubin oxidases. [23] Two decades ago Tkac et al. claimed indirect evidence of direct electron transfer of GOase to graphite electrodes by observing activation of the enzyme when an applied redox potential above 150 mV vs. SCE, coinciding approximately with that of the tyrosine radical of its active site (Table 1), was established. [24] When the electrode surface was covered with a cellulose acetate membrane to isolate it from GOase, then the activation effect by the applied redox potential disappeared. However, in these electrochemical experiments, the graphite surface had been previously modified with adsorbed ferrocene, thus mediated electron transfer of GOase cannot be ruled out in that case.

DET of GOase has been studied by depositing GOase on gold electrodes with different self-assembled monolayers (SAM) of thiols. [23] In the case of short-chain hydrophilic thiols, clear peaks due to adsorbed GOase were observed by cyclic voltammetry (CV). Nevertheless, the redox signals were very unstable and no bioelectrocatalytic effect in presence of galactose could be associated with them, suggesting that they corresponded to the denatured enzyme. The modification of the gold surface with longer chain thiols, which lead to a more compact SAM, suppressed the detection of peaks by CV but using the more sensitive differential pulse voltammetry (DPV) did allow detecting two redox processes at potentials similar to those of the active site's Cu and Tyr of GOase in solution (Table 1). Nevertheless, bioelectrocatalytic currents were also not observed in this case. [23] More recently, Wayu et al. have reported CV of GOase deposited over Au electrodes modified with either positively or negatively charged thiol SAMs in order to favor electrostatic interactions with the enzyme. More defined voltammetric peaks were observed with the thiol SAM having carboxylates as end groups, in which a single broad quasi-reversible wave centered at 160 mV vs. Ag/AgCl was measured. This redox process was attributed to the convolution of the two unresolved redox processes of the GOase active site (Cu^{2+/1+} and Tyr·/Tyr) due to the sluggish kinetics of DET. [25]

TABLE 1 GOase redox signals measured by direct electron transfer

Electrode	Technique	Redox Potential (mV vs. NHE)	Reference
Au with GOase in solution	Spectroelectrochemical titration	+410 (Tyr·/Tyr) and +159 (Cu ^{2+/1+})	[23]
Au/SAM/GOase	DPV	+440 to +460 and +130 to +200	[23]
Au/SAM/AuNP-GOase	CV	+440 and +265	[27]
GCE/RGO/AuNP/GOase	CV	+107	[28]
Au/SAM/CNT/GOase	CV	+350 to +390	[25]
FTO/TiO ₂ /GOase	CV	+300	[29]

The use of nanostructured electrodes can offer also advantages in shape-coupling between the enzyme and the electrode, yielding an optimal electron transfer by DET, as has been demonstrated for another copper enzyme, laccase.^[26] In order to improve the kinetics of heterogeneous electron transfer of immobilized GOase, wiring of the enzyme with different nanostructures has been studied by several research groups. An outstanding work by Abad *et al.* achieved plugging a small Au nanoparticle of 1.6 nm to the active site pocket of GOase by specific coordination of a linker to the enzyme's Cu, as shown by high-quality HAADF-STEM images (Figure 2). Upon attachment of the Au nanoparticle/enzyme hybrid to a SAM-modified Au electrode, the two redox processes of the active site could be resolved at 196 ± 5 and -21 ± 5 mV vs SCE (Figure 3). Although the immobilized GOase was able to electrocatalyze O_2 reduction at the potential of the $Cu^{2+/1+}$ wave, no electrocatalytic process was measured in presence of galactose in absence of oxygen. This latter result was attributed to the steric impedance of the Au nanoparticle preventing the substrate to bind at the GOase active site.^[27]

Entrapment of GOase in a composite film of reduced graphene oxide and poly(Lactide)-capped Au nanoparticles deposited on glassy carbon electrodes has been studied for revealing DET. A symmetrical quasi-reversible redox process was detected by cyclic voltammetry with a formal potential of -137 mV vs. SCE. This potential value is quite low compared to the expected ones for the redox centers of the GOase active site (Table 1). Furthermore, no oxidative electrocatalytic currents in presence of galactose that could be associated with the redox process detected by CV were shown. Instead, increased reduction currents upon galactose addition were measured by chronoamperometry at -0.42 V vs SCE in O_2 -saturated buffer.^[28] No explanation was given for this unexpected result, because GOase consumes O_2 during its catalytic cycle. In consequence, the cathodic current due to direct reduction of O_2 at the electrode should decrease in presence of galactose if the immobilized GOase is active.

Wayu *et al.* studied the effect of covalent attachment of different types of carbon nanotubes (CNTs) to Au electrodes modified with thiol SAMs on the DET of GOase adsorbed on top. The best result was obtained with single-walled CNTs functionalized with carboxylic groups. However, no significant improvement of the coverage and electron transfer rate of enzyme establishing DET, measured from the CVs in absence of substrate, was observed compared to the electrodes not modified with CNTs.^[25] In this work it was shown that there was active immobilized GOase for galactose oxidation by amperometric detection at 0.6 V (vs. Ag/AgCl) of the produced hydrogen peroxide during the catalytic cycle, thus the enzymatic elec-

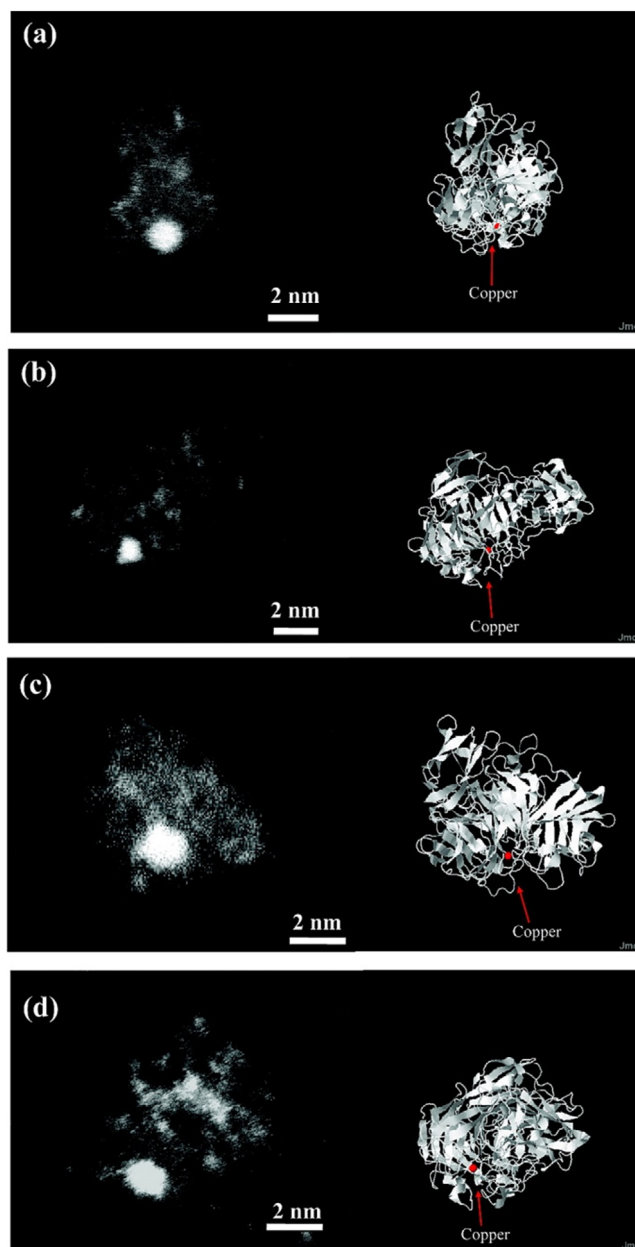


FIGURE 2 HAADF-STEM images for GOase/AuNP hybrid systems. 3D molecular displays of the protein in different orientations are also shown. The Au cluster is present in the area of the pocket with the Cu centre, as indicated by the arrows in each image. Reprinted with permission from Abad *et al.*, *J. Am. Chem. Soc.* **2009**, 131, 10229. Copyright 2009 American Chemical Society

trode was valid as a first-generation biosensor. In addition, very small electrocatalytic currents in the range of nA for galactose oxidation were measured by chronoamperometry under strict anaerobiosis. Therefore, the first indication of DET-based electrocatalysis of GOase was reported, suggesting a possible application as a third-generation biosensor.^[25] However, these very small electrocatalytic currents of galactose oxidation indicate that

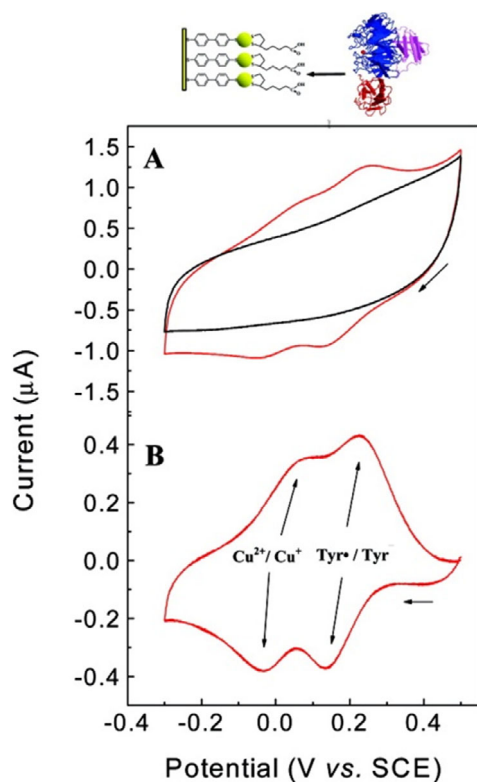


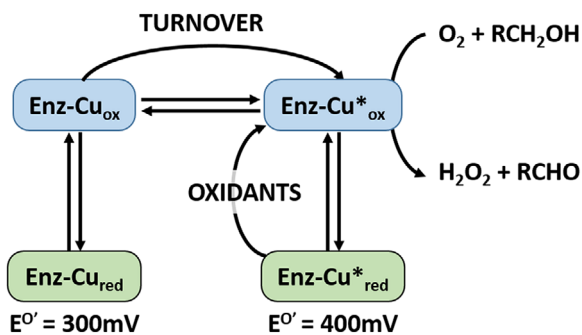
FIGURE 3 (A) CV of an Au electrode modified with a SAM consisting of biphenyl-4,4'-dithiol and carboxylate-Au clusters before (background, black line) and after incubation in GOase solution (red line). (B) CV after background subtraction. Measurements were carried out in nitrogen-saturated 20 mM MES buffer, pH 7.5. Scan rate was 20 mV/s. Reprinted with permission from Abad *et al.*, *J. Am. Chem. Soc.* **2009**, 131, 10229. Copyright 2009 American Chemical Society

only a minimal part of the immobilized enzyme that gives a non-turnover CV signal by DET is catalytically active. Higher DET-based electrocatalytic currents (up to 4 μA) of galactose oxidation have been reported more recently by Yin *et al.* with GOase immobilized on a photoelectrode. The enzyme was mixed with a 5% nafion dispersion and deposited on F-doped tin oxide (FTO) electrodes modified with TiO_2 nanorod arrays. The CV measurements showed an anodic peak at approximately +200 mV and a cathodic one at 0 mV (vs. Ag/AgCl reference electrode) when the GOase was immobilized. Chronoamperometric measurements at +200 mV showed that the photocurrents produced upon irradiation with UV light had a small increase when galactose was added to the solution, which was proportional to its concentration in the 0.01–1 mM range.^[29] However, a negative control of the photoelectrochemical measurements performed in the absence of immobilized GOase is lacking in this work. Therefore, direct oxidation of galactose at the photoexcited TiO_2 nanorods cannot be excluded.

3 | MEDIATED ELECTRON TRANSFER-BASED GOASE BIOELECTROCHEMICAL PLATFORMS

As DET between GOase and electrodes is very challenging, the use of redox mediators to transfer the electrons is a much more studied strategy. GOase ability to interact with redox species other than its natural substrates was presented several decades ago. In the late 1970s, an assay for galactose in blood serum and urine was developed relying on the amperometric measurement of oxygen consumption.^[30] The consumption of O_2 by galactose oxidase to form H_2O_2 was monitored with a membrane oxygen electrode able to determine the rate of oxygen depletion, which was assumed to be proportional to the concentration of galactose. As an advantage, the sample did not need severe treatment, as it was not needed to remove the protein nor incubation nor extraction. Even more, the analysis could be performed in less than 60 s. The authors found that they could take advantage of an already proven activation of galactose oxidase by the addition of ferricyanide,^[31] which accelerates its reaction when the concentration is in the millimolar range. The effect of such addition meant that the O_2 proportional to the galactose presence was consumed twice faster than in its absence, meaning the catalytic effect of the iron complex. As H_2O_2 induces an inhibiting effect iodide and molybdate were added to remove it from the solution. Another effect detected by the authors was that a mild shift in the pH from 7 to 8 did not alter the galactose oxidase-mediated reaction rate. The amperometric sensor showed a linear range for detection of galactose from 1.67 mM to 11.1 mM using 20 units of enzyme and 30 μL of the sample, although the authors claim that their detection limit could have been easily improved with a higher amount of sample or enzyme. After an analysis of potential interferences, only lactose yielded an 8% of GOase activity against galactose.

Besides attempting the analysis of galactose oxidase in solution, the next objective was to improve the analysis by immobilizing the enzyme on the surface of the electrode where the redox activity of the redox mediator was tuned while adding a secondary platinum working electrode to monitor the reduction of the H_2O_2 produced by the enzymatic reaction.^[32] The authors used this approach to measure the standard potential of GOase against the substrates galactose and dihydroxyacetone by means of several mediators: ferri/ferrocyanide, tetracyanomonomophenanthroline ferrate/ferrite, and cobalt terpyridine. The purpose was to check if the mediators caused an effect in the standard potential of the galactose oxidase's active site. The authors found out that the nature of the mediator barely affected the enzyme potential. This preliminary analysis of the mediators' activity allowed further analysis of the



SCHEME 1 Stable states of galactose oxidase proposed by Johnson et al. Only one of the four possible states is active, $\text{Enz-Cu}^*_{\text{ox}}$. The authors did not rule out the existence of other transient states that may appear during the turnover cycle. (Adapted from [32])

enzyme selectivity against many substrates, setting galactose itself as a reference substrate. Interestingly it was found there are other compounds with several primary alcohol substituents which oxidation to aldehyde was even more active than galactose itself, as stachyose (1.44 more active than galactose) and dihydroxyacetone (4.31 times more active than galactose). After a careful analysis of the enzyme performance and the possible redox states of its copper active site, distribution of stabilized redox states was proposed (Scheme 1) upon external redox potential.

Ferrocene carboxylic acid was also demonstrated as a good mediator for GOase electron transfer to freshly polished glassy carbon or gold working electrodes.^[33] GOase in solution managed to use the ferrocene carboxylic acid (0.2 mM) to transfer the electrons from galactose (50 mM) oxidation, yielding a 3-fold increase in the electrochemical current. The authors tested also other ferrocene derivatives obtaining a positive response, whereas other mediators like Meldola's blue, 1-methoxyphenazine methosulfate, or 1,4-benzoquinone did not. This work hinted that galactose oxidase was more efficient using iron-containing redox mediators than other only aromatic derivatives, which may be due to their low redox potential or inability to access the active site of galactose oxidase. Another interesting finding was that the enzyme did not show the same pH activity affinity for O_2 as for ferrocenes, which did not give biocatalysis at O_2 maximum pH 6–7. Instead the couple galactose oxidase-ferrocene monocarboxylic acid worked better at pH 9, which was attributed to the lower electrostatic interaction between ferrocene monocarboxylic acid and basic amino acids. This work was expanded to other ferrocene derivatives like ferrocene dimethanol, yielding a ca. 4-fold electrochemical increase.^[33] Even more, a deeper analysis of the standard potentials of several redox probes, their pseudo-first-order rate constants, and their dependence on pH values was examined. The authors found

that positively charged ferrocene derivatives with a redox potential slightly more positive than that of galactose oxidase were the best performing mediators; they also confirmed the pH dependence shift related to the local acidity in the access pocket of the enzyme's active site.

Another way to reveal GOase activity on an electrode is the classic coupling with a peroxidase. The addition of horseradish peroxidase (HRP) allows to reduce the hydrogen peroxide that appears as a by-product of galactose oxidase reaction, and to oxidize a typical redox mediator, that is, ferrocene.^[34] The electrode was prepared from a graphite rod inserted in a Teflon tube and connected to a brass rod for electric contact while covering the working area with a dialysis membrane fixed with epoxy resin and an O-ring. Prior to this latter topping, the electrodes were modified with ferrocene by adsorption incubating in a paraffin solvent containing the redox probe. The electrodes were also treated with a dextran derivative loaded with the enzymes, aiming to increase their storage stability. Being a cascade system with two enzymatic reactions coupled, a pH analysis was performed, showing that pH 7.85 is the optimum for the tandem reaction. This means that the enzyme limiting the process was HRP, as 7.85 matches with its optimal activity whereas GOase maximum is at 6.6.

A step further in improving MET-based GOase electrodes consisted on including polymers able to host the mediators, thus skipping the mass transfer limitations. This is the case of a galactose oxidase immobilized covalently to a platinum electrode modified with poly(glycidyl methacrylate-co-vinylferrocene).^[35] The resulting electrode included the mediator and an epoxy group to immobilize the enzyme through its lysine external residues, showing a fast amperometric response to additions of galactose (Figure 4). Other polymers based on pyrrole polymers have also been tested.^[36] Pyrrole was modified with epichlorhydrin to favor further covalent immobilization of galactose oxidase after the electropolymerization. The pH was optimized for this specific electrode configuration to take in account any possible effect due to the presence of pyrrole, finding a maximum of 7.5, one unit more basic than the polymethacrylate derivatives.

We mentioned earlier that GOase is not a very selective enzyme and can oxidize polyalcohol substrates other than galactose to aldehydes, offering many advantages in synthetic chemistry.^[37] The authors developed a bioelectrosynthetic enzymatic cascade, combining an applied potential from an electrode where the galactose oxidase is immobilized and working together with catalase to regenerate the oxygen and a ferrocene derivative ($\text{FcCH}_2\text{NMe}_3$) as redox mediator. Many alcohols were tested and their conversion recorded, but also the possibility provide only one stereoisomer from a pro-chiral substrate is remarkable.

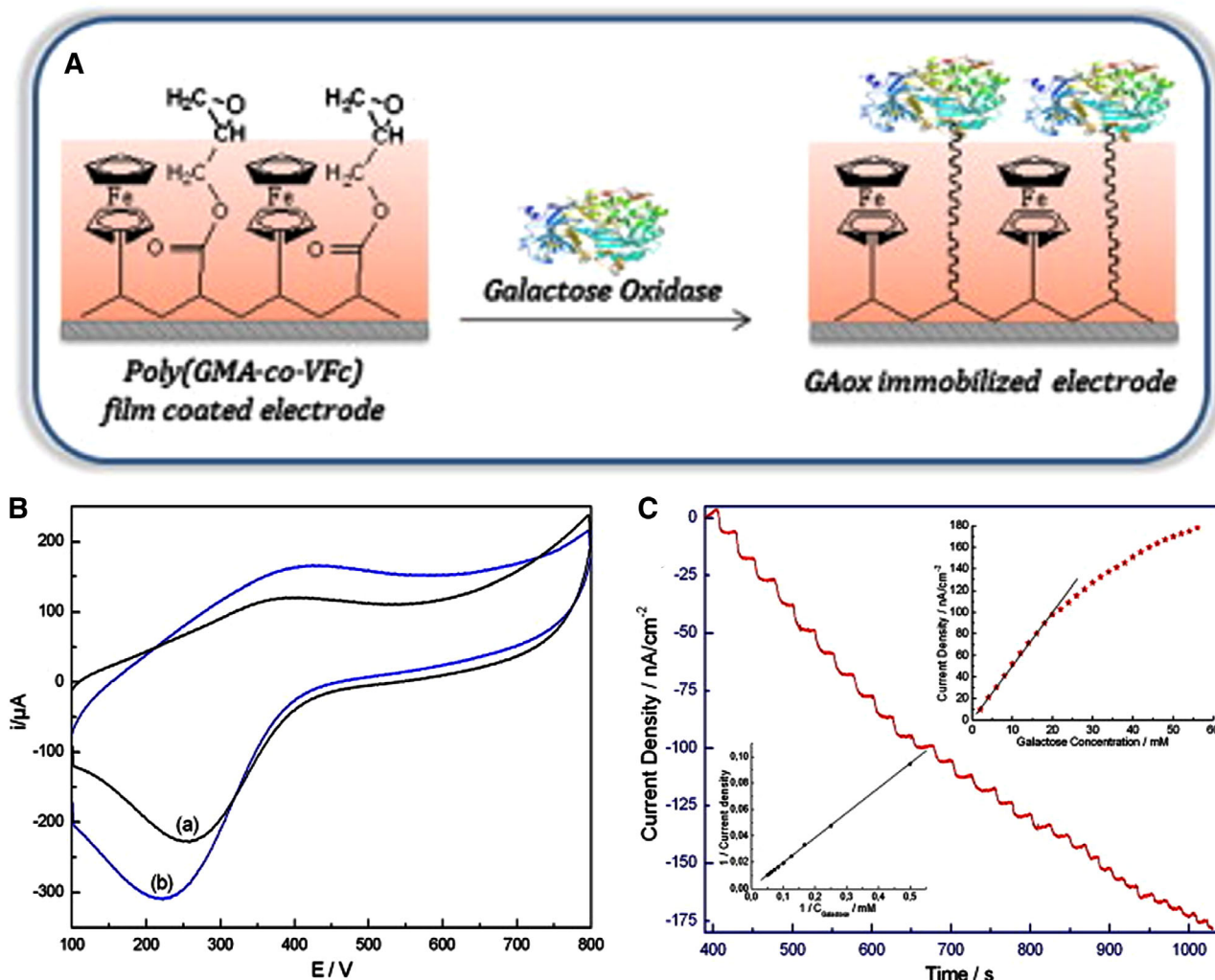


FIGURE 4 (a) Scheme of the redox polymer prepared for immobilization of galactose oxidase. (B) Cyclic Voltammograms of the Galactose oxidase electrode in phosphate buffer, pH 7.4 in (a) absence and (b) presence of 10 mM galactose. (C) Current-time responses were obtained with enzyme electrode at an applied potential +0.35 V to successive galactose injections in a stirred 10 mM PBS, pH 6.5. The Lineweaver-Burk plot and calibration curve are shown inset. Figures reproduced with RightsLink Permission from [35]

For example, galactose oxidase is able to use the pro-chiral 2-ethynylglycerol and selectively oxidize only one of the alcohols to aldehyde, yielding the chiral molecule (R)-2-ethynylglyceraldehyde. The electrodes were not used also as platform for immobilizing the GOase but only as electron acceptor to regenerate the ferrocenylmethyl derivative. The aldehyde yield reported was 46% and the conversion 67% for the oxidation of 2-ethynylglycerol. However, the electrochemical support was not immune to by-products. Different applied voltages ranging from 0.75 to 1 V showed that the potential increase elevated the overall conversion but also favored the appearance of the acid derivative and even formic acid. Additionally, there was demonstrated an influence of the redox mediator, as $\text{FcCH}_2\text{NMe}_3$ was the best among other ferrocene derivatives, ABTS or other organic mediators.

4 | APPLICATIONS OF GOASE ELECTRODES AS GALACTOSE BIOSENSORS

The monosaccharide galactose is a non-essential energy source for humans; however, it is an important nutrient for newborn infants and young children. Cells can utilize galactose both as an energy-providing nutrient and as a building block for the biosynthesis of many macromolecules in the body. [38] Galactose is an important constituent of complex oligo and polysaccharides, cell glycoconjugates, key components of immunological determinants, hormones, cell membrane structures and endogenous lectins. [39] It can also be incorporated into galactolipids, which are extremely important structural components of the central nervous system. [40]

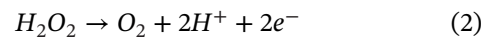
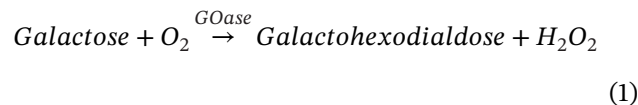
The principal diet sources of galactose are lactose-containing products, mainly milk and its derivatives. The lactose content in human breast milk is about 7 g/dL while in cow's milk the concentration is approximately 5 g/dL. Lactose may provide 40% of the caloric intake to breastfed babies whereas for adults it represents only 3–4% due to proportionally lower milk intake.^[41] Lactose is hydrolyzed into galactose and glucose by the enzyme β -galactosidase (lactase) in the small intestine. Both monosaccharides are absorbed by an active transport mechanism across the gut wall to finally reach the blood circulatory system.^[42] Malfunction in galactose metabolism may result in galactosemia,^[40] a disease that causes a variety of clinical symptoms. Accordingly, a deficiency of the enzymes participating in the metabolism of galactose can lead to health issues such as hepatosplenomegaly, bleeding disorders, Escherichia coli sepsis, cataracts and sometimes death.^[41,43,44] Therefore, an early determination of galactose levels is significant in the diagnosis, allowing for appropriate and timely treatment for preventing these life-threatening disorders. Despite this widespread implementation of galactosemia screening in newborns, more than 75% of galactosemic infants continue to decrease, therefore a faster and reliable assay is necessary to help reverse this fate.^[45]

Galactose determination has also great importance in the food and fermentation industries. There are two analytic methods for galactose detection in complex matrices such as food and biological samples. The first approach is either gas or liquid chromatography for a preliminary separation step to avoid interferences from other sugars, coupled to a variety of detectors (conductivity, refraction index, light polarization, dichroism).^[46,47] However, these methods usually are expensive, time-consuming, and often require specific equipment and skilled personnel to operate them.^[48,49] The second approach uses enzyme-based methods including galactose oxidase or galactose dehydrogenase in conjunction with electrochemical, spectrophotometric, polarimetric, or fluorometric detection of enzymatic products.^[3,45,47]

Biosensors using electrochemical detection methods offer a solution to some of these problems thanks to their high sensitivity, reproducibility, easy maintenance, relatively lower cost and skipping pre-separation/treatment procedures.^[50] Enzyme-based amperometric biosensors can generate an electrochemical current signal upon the analyte oxidation/ reduction, meaning an attractive solution for the point-of-care sugar testing as well as to play an important role in the continuous sugar monitoring.^[50,51]

The development of enzyme-galactose sensors has been burgeoning over the last few years to improve patient's life quality and to perform a better and quicker detection of

galactose in food industry (Table 2). In most of them the electrochemical response process is related to the oxidation of galactose using oxygen as co-substrate with production of H_2O_2 (Equation 1). The produced hydrogen peroxide is re-oxidized in the electrode providing an analytic response (Equation 2) that is directly proportional to the amount of the analyte present.^[52]



With the appearance of this first detection method, several doors were opened for the quantitative analysis of different types of sugars in distinct types of fluids. On an attempt to improve the quality of enzyme integration on a surface capable of translating the signal from its activity, the development of biosensors has been evident, namely in enzyme immobilization methods. Several enzyme-immobilization methods were studied where the enzyme can be either immobilized using cross-linkers (cross-linking), physical trapped generally by conducting polymers (entrapment), immobilized directly on the electrode (covalent binding), or physically absorbed (Scheme 2).^[53]

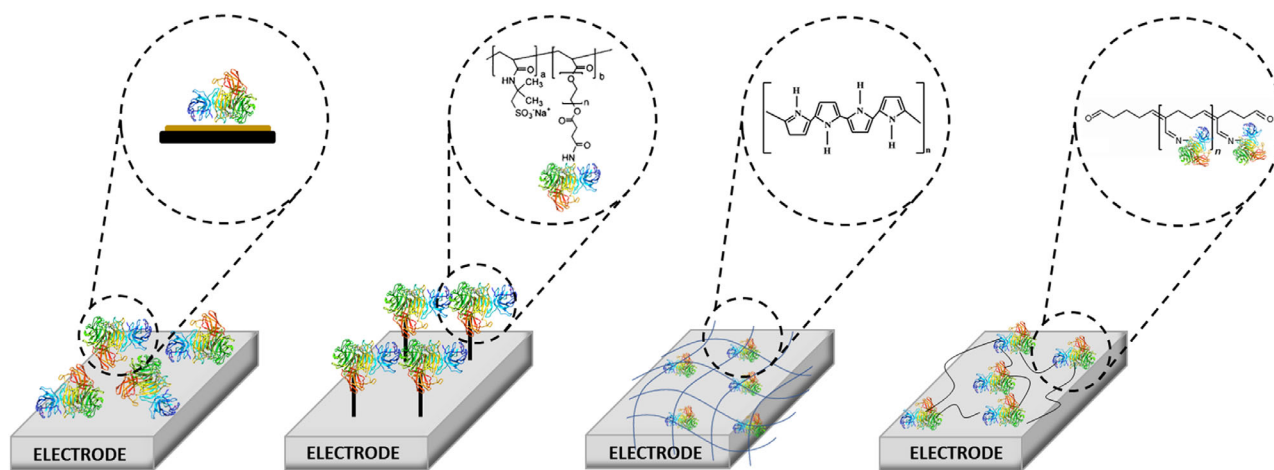
One strategy to immobilize enzymes on electroactive surfaces is the cross-link strategy. The cross-linking between enzymes uses free amino groups of lysine residues and make them react with a reagent such as glutaraldehyde (Glu). This immobilization approach improves the efficiency and stability, due to the highly strong and stable bonding. However, the utilization of cross-linking reagents can lead to an activity loss due to modification of the enzymes.^[54,55]

Taylor and co-workers described in 1977 an immobilization approach for the GOase, within a membrane. The authors optimized and evaluated the procedure using galactose in plasma and whole blood. The developed method incorporates GOase into glutaraldehyde (Glu), the optimized enzyme-membrane was defined as immobilization with 0.29 % by weight, glutaraldehyde at pH 5.8. Subsequently, the batch was casted onto a polycarbonate membrane (Pc). The linear range was between 0 to 500 mg % (28 mM) with a response time of 40s. In the end, of the 39 compounds screened, the only physiologically important interference found was dihydroxyacetone.^[56]

In the same context, Manowitz and his co-workers, developed a biosensor that employed a composite polymer able to prevent the interferences. The sensor was used with a flow injection analysis system for the detection of galactose in human plasma. The biosensor consisted of

TABLE 2 Characteristics of galactose sensors

Sensor design	Sensitivity	LOD (μM)	Linear Range (mM)	Response time (s)	Storage stability (d)	Sample	Reference
GalOx/Glu/PC/H ₂ O ₂ electrode	–	–	0.0–28.0	40	7	Plasma, Blood	[56]
GalOx/Collagen/H ₂ O ₂ electrode	1.0–3.0 mA/M	0.5	5.0×10^{-4} –0.6	60	~300	Serum	[65]
GalOx/Polypyrrole/Pt	$3.5\text{--}14.7 \text{ mA M}^{-1} \text{ cm}^{-2}$	500.0	5.0×10^{-4} –2.0	–	15	Blood	[59]
Glu/GalOx/Glu/1,3-DAB/Res/Pt	–	50.0	50×10^{-3} –6.0	18	30	Plasma	[57]
PC/GalOx/Ferrocinium/CPE	–	–	–	<120	–	–	[64]
PU/GalOx/Polyacrylamide/O ₂ /electrode	15.0–51.0 pA/mM	2.0	up to 1.5/10.0	0.5–5.0	30	–	[64]
GalOx/Ferrocene/Graphite	0.9 mA/M	2.8	5.6×10^{-3} –0.6	44	52	–	[34]
GalOx/Polypyrrole-[p(HEMA)]/Pt	937.0 $\mu\text{A/M}$	25.0	5.0×10^{-2} –10.0	70	270	Serum	[63]
GalOx/PVF/Pt	~1.0 $\mu\text{A/mM}$	–	up to 40.0	30–40	3	–	[47]
GalOx/Poly-GMA-Co-VFc/Pt	$23.0 \text{ nA mM}^{-1} \text{ cm}^{-2}$	100.0	2.0–20.0	5	~30	–	[35]
GalOx/PEP/Au	$1.8 \mu\text{A/mM}^{-1}$	25.0	2.0–16.0	5	~10	–	[36]
GalOx/CA/Co-SPCE	$7.0 \mu\text{A mM}^{-1} \text{ cm}^{-2}$	20.0	0.1–25.0	30	14	Serum	[45]
GalOx/Co ₃ O ₄ /Graphene/GCE	$6.6 \mu\text{A mM}^{-1} \text{ cm}^{-2}$	3.0	9.0×10^{-3} –0.6	15	~30	Serum	[58]
GalOx/Co ₃ O ₄ /MWCNTs/GCE	$10.4 \mu\text{A mM}^{-1} \text{ cm}^{-2}$	0.9	9.0×10^{-3} –1.0	20	~30	Serum	[58]



SCHEME 2 Types of enzyme immobilization strategies on surfaces: from left to right absorption, polymer cross-linking, entrapment, and covalent bonding.

galactose oxidase immobilized on a platinumized carbon electrode that had been modified with a composite polymer. First, the platinumized carbon electrode was modified using an electropolymerization process with 1,3-Diaminobenzene (1,3-DAB) and resorcinol (RES) monomers. Then, the modified electrodes were crosslinked to the enzyme by means of glutaraldehyde Schiff's base reaction. The

bioelectrode was further modified with a second layer of glutaraldehyde to form a sandwich format sensor (Glu-GOase-Glu-1,3-DAB/RES-Pt). The authors also reported that the composite polymer minimized the effect of possible interference from urate, ascorbate, and acetaminophen. This analytical biosensor system applied in human plasma, had a lower limit of detection of $50 \mu\text{M}$,

linearity up to 6 mM, a storage stability over 30 days at room temperature, and a high sample throughput (approx. 120 samples/h).^[57] Another example showing cross-linked enzymes included two bioelectrodes based on a) graphene (GR), Co₃O₄ nanoparticles and chitosan (CS) or b) multi-walled carbon nanotubes (MWCNTs), Co₃O₄ nanoparticles, CS. The electrodes presented two novel biosensing platforms for galactose determination. GOase was immobilized onto the electrode surfaces by crosslinking with Glu. Their performance was comparable, however the MWCNTs-based galactose biosensor provided 1.6-fold higher sensitivity than its graphene counterpart.^[58] Performance details is described on Table 2.

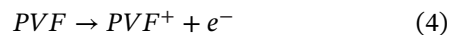
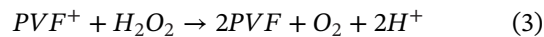
Another way to build the biosensors consists on entrapping the enzymes in a support, which may be fibre-based or a polymer membrane that allows the substrates and products to pass through while retains the enzyme. Entrapment can improve mechanical stability and minimize enzyme leaching. Since the enzyme do not have chemically interact with the polymer, denaturation is usually avoided.^[54,55]

A first example of a biosensor comprising GOase entrapped in a polymeric matrix on a platinum electrode surface. The biosensor was built by pyrrole electropolymerization in presence of the enzyme, including an optimization of the electrochemical properties to modulate the permeability and enzyme retention properties of the matrix. The current response increased linearly with increasing galactose concentration from 5×10^{-7} – 2×10^{-3} M with a sensitivity value in the range of 3.52 – 14.7 mA·M⁻¹ for the various polymers used.^[59]

Fortier et al. reported a biosensor based on the immobilization of the enzyme in Eastman AQ polymer coated with a layer of Nafion. The enzyme was mixed with the AQ polymer, the batch was casted and dried onto the surface of a platinum electrode. The film was then coated with Nafion to avoid dissolution of the AQ polymer film in the aqueous solution when the biosensor was under performance. The calibration curve obtained with the galactose oxidase was linear in the range of 1 to 20 mM. The steady-state response of the Pt/ GOase-AQ/Nafion electrode is reached after about 50 s.^[60]

Other reports presented the development of bioelectrodes for the determination of galactose by immobilization of the galactose oxidase in polyvinylferrocenium (PVF⁺ ClO₄⁻) matrix coated on a Pt electrode surface.^[47] This polymer had been shown to catalyze the electrooxidation and electroreduction of some organic species such as anthracenes in acetonitrile and the electrooxidation of H₂O₂ in aqueous solution.^[61] Additionally, the PVF⁺ ClO₄⁻ polymeric matrix can be used as a preconcentrating agent for the analysis of some inorganic anions.^[62] This galactose biosensor was optimized tuning the polymeric matrix thickness by means of the electric charge supplied, the electropolymerization time and potential. The chemi-

cal oxidation of the galactose (Equation 1 and 2) and subsequently oxidation of the H₂O₂ by the redox polymer occurs as follow:



Due to this unique feature of the redox polymer the currents measured were higher than the observed ones for glucose or sucrose. The response time was 30–40 s and the upper limit of the linear range was 40.0 mM galactose.

In another example a two-step protocol for constructing a galactose biosensor, entrapping the enzyme within a polymeric composite was developed.^[63] The composite material was polypyrrole with poly(2-hydroxyethyl methacrylate) hydrogel crosslinked with UV radiation. The enzyme-sensor showed a linear response range from 5.0×10^{-5} to 1.0×10^{-2} M, a response time of 70s, and a detection limit of 25 mM toward galactose.

Besides entrapment there are other examples exploring the covalent attachment of GOase onto a microelectrode Clark-type O₂ tip.^[64] The enzyme was immobilized on the electrode tip and covered with a polyacrylamide matrix and then coated with a polyurethane membrane. This micro biosensor presented a tip diameter of 15–40 μm, response times of 0.5-5 s, and a limit of detection of 2 μM of analyte. The linear range of response was dependent on the thickness of the polyurethane coating and extended up to 10 mM for galactose biosensors also presented a storage stability of up to 1 month and a sensitivity of 51×10^{-5} mA·M⁻¹.^[64]

Bertrand *et al.* developed in 1981 a biosensor where the GOase was covalent attached onto a collagen film. Films of highly polymerized insoluble collagen were activated, and the enzyme was linked by reaction with acyl azide by direct immersion of the activated membrane in the enzyme solution at alkaline pH. The enzyme/ collagen film was attached to Pt anode and the potential was fixed at +650 mV vs. Ag/AgCl reference electrode. A steady-state response was obtained after 5–6 min and a dynamic response in 1 min. The lowest galactose concentration detected was 5×10^{-7} M. The sensor exhibited calibration linearity from 5×10^{-6} – 6×10^{-4} M. Above the latter value, a response was still obtained but the current increase was no longer proportional to the galactose concentration. The sensitivity curve was in the range 1.0 - 3.0 mA·M⁻¹ depending on the membrane activity.^[65] Another examples of GOase covalently bonded to the electroactive surface were described earlier.^[35,36]

Some other biosensors rely on absorbed GOase modified electrodes. Physical absorption is a straight-forward immobilization strategy and may have a high commercial potential due to its simplicity, low cost activity preservation

as well as a relatively chemical-free enzyme binding.^[54,55] Buffone and co-workers reported a physically adsorbed GOase on a Clark-type electrode known as “Model 23A”. In this example the immobilized-enzyme method is suitable for monitoring the treatment of neonates suffering galactosemia, because it requires only a few minutes and 25 μL of serum.^[66]

An early attempt for mediated electron transfer amperometric biosensor consisted on the coating of a porous carbon electrode with a redox polymer loaded with [tris(2,2'-bipyridine)Os]^{2+/3+} for galactose measurement in human plasma. Under the optimum conditions the amperometric response of this sensor was linear over concentration ranges of 0.01–5.00 mmol dm^{-3} galactose.^[67] However, it was not until 1999 when the stability of absorption-based biosensors was improved by the co-immobilization of GOase and HRP drop-casted on the surface of a ferrocene-loaded graphite electrode. Dextran and inositol additives provided a high storage stability.^[34] This configuration carried a limitation, the oxidized form of the mediator is water soluble and leaked from the active layer, which was mitigated by using a high turnover HRP. Besides, as the enzymes are positively charged at the measuring pH, their electrostatic repulsion against ferricinium cation prevents to some extent its leakage through the external enzymatic layer. The biosensor offered a response time of 44 s for phosphate buffer and a low detection limit of 0.51 $\text{mg galactose L}^{-1}$.

GOase has also served as biocatalyst in a thin-layer flow-through injection analyzer biosensor coupled to an amperometric detector for monitoring the galactose content in several fermentation broths.^[68] The measuring construction consisted on galactose oxidase immobilized on pigs' small intestine membrane inside of the thin-layer enzyme cell; the detection relied on the electrochemical oxidation of the by-product H_2O_2 coming from the galactose oxidation reaction in a platinum electrode comprised in a two-electrode system. By using a biological membrane to immobilize the enzyme the results obtained for galactose oxidase biocatalytic performance differs from those where the enzyme is in solution or immobilized on the electrode surface. In this case, the optimization process resulted in a range of operational temperature from 32 to 45°C with a satisfactory linearity at 32°C; the flow rate yielded its optimal at 0.31 ml/min to reach the balance between the longer sample time in the cell versus the signal widening. The authors also found that the optimal pH was slightly above 6.5 and the best buffer concentration for the FIA analysis was 134 mM . Once optimized the authors performed an analysis of the effect of cations presence, finding that some of them (Mg^{2+} , Se^{2+}) improved the signals but most of them (Co^{2+} , Zn^{2+} , Cu^{2+} , Ca^{2+} , Mn^{2+}) decreased the signals detected. Fe^{3+} did not yield a significant effect.

However, the presence of Mg^{2+} and Se^{2+} cations in the buffer solution increased the reproducibility of the measurements. After the optimization the system was tested for several glucids, glycerol and dihydroxyacetone, finding some significant activity for raffinose (68%), lactose (16%) or glycerol (6%), whereas dihydroxyacetone yielded a 430% activity compared to that for galactose. Other sugars or similar compounds did not yield over a 5% of activity. The system kept up to 50% of initial activity over 6 weeks working time and 900 samples.^[68]

Screen printed electrodes (SPEs) have also been used for the development of point-of-care detection systems, as they are suitable for such portable and single-use applications. The structure of the SPEs and the single-use approach allows easier immobilization by mere deposition instead of covalent bonding, while allowing to include a mediator such as cobalt phthalocyanine.^[45] The SPE used had only a carbon printed working electrode and an Ag/AgCl reference electrode, using an external platinum counter electrode in the electrochemical cell. The working electrode printed with the cobalt phthalocyanine was firstly modified with carbon acetate and later with galactose oxidase. The carbon acetate plays the role of separating the enzyme from the electrode surface but allowing the H_2O_2 diffusion to the electrode surface. The H_2O_2 reached the electrode surface and suffered catalytic oxidation against Co^{2+} , which was regenerated by the bias potential. The use of 1% carbon acetate and 2 units of galactose oxidase was set as optimum for this electrode build, finding the best working conditions in 35°C and pH 7, which is coherent with the above-mentioned developments. In this case, the linear range covered by the electrode was 0.1 mM to 25 mM , with a detection limit of 0.1 mM and a sensitivity of 7 $\mu\text{A/mM}\cdot\text{cm}^2$. The electrode was also tested in presence of several interferences like urea, uric acid, paracetamol, or ascorbic acid. As these compounds were active the authors used a dummy biosensor to measure the interfering current and subtract it from the working electrode. Regarding the stability of the electrodes, it was checked up to 14 days, finding no decrease in its performance when stored in a desiccator at 4°C. The performance of the biosensor was as good as other conventional methods with the advantage of being performed with an electrode suitable for mass production at low cost.^[45]

5 | CONCLUSIONS

Galactose oxidase has proven itself as a very promising and interesting biocatalyst with future applications in several fields, not only in galactose biosensing but also for biosynthetic approaches. As a galactose transducer it has been proven its reliability and possibility to develop

point-of-care sensors for specific illnesses. Besides, its remarkable ability to yield aldehydes as products instead of carboxylic acids, which is the most usual activity of glycidase oxidases, should attract other industrial sectors in organic synthesis interested in sustainable synthetic routes. The recently Nobel-awarded strategies for customizing biocatalysts based on directed evolution may provide different strands of galactose oxidase for more accurate biocatalysts tuned for each of the specific applications where it may be used.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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REFERENCES

- M. M. Whittaker, J. W. Whittaker, *N. Biol. Chem* **1988**, *263*, 6074.
- R. Paukner, P. Staudigl, W. Choosri, D. Haltrich, C. Leitner, *Protein Expr. Purif* **2015**, *108*, 73.
- P. Kanyong, F. D. Krampa, Y. Aniweh, G. A. Awandare, *Mircrochim. Acta* **2017**, *184*, 3663.
- M. M. Whittaker, J. W. Whittaker, *Biochemistry* **2001**, *40*, 7140.
- H. Ikemoto, S. L. Mossin, J. Ulstrup, Q. Chi, *RSC Advances* **2014**, *4*, 21939.
- N. Ito, S. E. Phillips, C. Stevens, Z. B. Ogel, M. J. McPherson, J. N. Keen, K. D. Yadav, P. F. Knowles, *Nature* **1991**, *350*, 87.
- J. W. Whittaker, *Adv. Protein Chem* **2002**, *60*, 1.
- G. T. Babcock, M. K. El-Deeb, P. O. Sandusky, M. M. Whittaker, J. W. Whittaker, *J. Am. Chem. Soc* **1992**, *114*, 3727.
- S. E. Deacon, K. Mahmoud, R. K. Spooner, S. J. Firbank, P. F. Knowles, S. E. V. Phillips, M. J. McPherson, *Chembiochem* **2004**, *5*, 972.
- M. L. McGlashen, D. D. Eads, T. G. Spiro, J. W. Whittaker, *J. Phys. Chem* **1995**, *99*, 4918.
- J. W. Whittaker, *Methods Enzymol* **1995**, *258*, 262.
- M. M. Whittaker, J. W. Whittaker, *J. Biol. Chem* **1990**, *265*, 9610.
- F. Michel, F. Thomas, S. Hamman, C. Philouze, E. Saint-Aman, J. L. Pierre, *Eur. J. Inorg. Chem* **2006**, *18*, 3684.
- J. W. Whittaker, *Chem. Rev* **2003**, *103*, 2347.
- N. Ito, S. E. V. Phillips, K. D. S. Yadav, P. F. Knowles, *J. Mol. Biol* **1994**, *238*, 794.
- A. J. Baron, C. Stevens, C. Wilmot, K. D. Seneviratne, V. Blakeley, D. M. Dooley, S. E. Phillips, P. F. Knowles, M. J. McPherson, *J. Biol. Chem* **1994**, *269*, 25095.
- M. J. McPherson, C. Stevens, A. J. Baron, Z. B. Ogel, K. Seneviratne, C. Wilmot, N. Ito, I. Brocklebank, S. E. Phillips, P. F. Knowles, *Biochem. Soc. Trans* **1993**, *21*, 752.
- M. P. Reynolds, A. J. Baron, C. M. Wilmot, E. Vincombe, C. Stevens, S. E. V. Phillips, P. F. Knowles, M. J. McPherson, *J. Biol. Inorg. Chem* **1997**, *2*, 327.
- C. G. Sellsell, T. Barna, C. D. Borman, A. J. Baron, M. J. McPherson, A. G. Sykes, *J. Biol. Inorg. Chem* **1997**, *2*, 702.
- F. Himo, L. A. Eriksson, F. Maseras, P. E. M. Siegbahn, *J. Am. Chem. Soc* **2000**, *122*, 8031.
- M. M. Whittaker, J. W. Whittaker, *Biophys. J* **1993**, *64*, 762.
- K. A. Vincent, A. Parkin, F. A. Armstrong, *Chem. Rev* **2007**, *107*, 4366.
- S. Shleev, J. Tkac, A. Christenson, T. Ruzgas, A. I. Yaropolov, J. W. Whittaker, L. Gorton, *Biosens. Bioelectron* **2005**, *20*, 2517.
- J. Tkac, I. Vostiar, P. Gemeiner, E. Sturdik, *Bioelectrochemistry* **2002**, *56*, 23.
- M. B. Wayu, M. J. Pannell, N. Labban, W. S. Case, J. A. Pollock, M. C. Leopold, *Bioelectrochemistry* **2019**, *125*, 116.
- C. Gutierrez-Sanchez, M. Pita, C. Vaz-Dominguez, S. Shleev, A. L. De Lacey, *J. Am. Chem. Soc* **2012**, *134*, 17212.
- J. M. Abad, M. Gass, A. Bieloch, D. J. Schiffrin, *J. Am. Chem. Soc* **2009**, *131*, 10229.
- J. Xie, C. Chen, Y. Zhou, J. Fei, Y. Ding, J. Zhao, *Electroanalysis* **2016**, *28*, 183.
- Z. Yin, J. Zhi, *J. Photochem. Photobiol. A: Chem* **2020**, *397*, 112560.
- F. S. Cheng, G. D. Christian, *Anal. Chim. Acta* **1979**, *104*, 47.
- G. A. Hamilton, J. De Jersey, P. K. Adolf, in *Oxidase and Related Redox Systems (Proceedings of the Second International Symposium)* (Eds: T. E. King, H. S. Mason, M. Morrison), University Park Press, Baltimore, MD **1973**, pp. 103.
- J. M. Johnson, H. B. Halsall, W. R. Heinemann, *Biochemistry* **1985**, *24*, 1579.
- T. Yamaguchi, Yuji Murakami, Kenji Yokoyama, Hisateru Komura, Eiichi Tamiya, *Denki Kagaku Oyobi Kogyo Butsuri Kagaku* **1995**, *63*, 1179.
- J. Tkac, P. Gemeiner, E. Sturdik, *Biotechnol. Tech* **1999**, *13*, 931.
- E. Çevik, M. Senel, M. F. Abasiyanik, *Curr. Appl. Phys* **2010**, *10*, 1313.
- M. Senel, I. Bozgeyik, E. Çevik, M. F. Abasiyanik, *Synth. Met* **2011**, *161*, 440.
- S. Zhang, S. Rucolo, A. Fryszkowska, A. Klapars, N. Marshall, N. A. Strotman, *ACS Catal* **2021**, *11*, 7270.
- C. A. Williams, 'GALACTOSE', in *Encyclopedia of Food Sciences and Nutrition (Second Edition)* (Ed: B. Caballero), Academic Press, Oxford **2003**, pp. 2843.
- A. Flynn, 'Nutrition and Health: Galactosemia', in *Encyclopedia of Dairy Sciences (Second Edition)*, (Ed: J. W. Fuquay), Academic Press, San Diego **2011**, pp. 1051.
- A. I. Coelho, G. Berry, M. Rubio-Gozalbo, *Curr. Opin. Clin. Nutr. Metab. Care* **2015**, *18*, 422.
- P. S. Kishnani, Y.-T. Chen, 'Disorders of Carbohydrate Metabolism', in *Emery and Rimoin's Principles and Practice of Medical Genetics and Genomics (Seventh Edition)* (Eds: R. E. Pyeritz, B. R. Korf, W. W. Grody) **2013**.
- A. Abi-Hanna, J. M. Saavedra, 'Galactose', in *Encyclopedia of Human Nutrition (Second Edition)*, (Ed: B. Caballero), Elsevier, Oxford **1998**, p. 377.

43. A. M. Bosch, *J. Inherit. Metab. Dis* **2006**, *29*, 516.
44. G. T. Berry, 'Disorders of Galactose Metabolism', in *Rosenberg's Molecular and Genetic Basis of Neurological and Psychiatric Disease (Fifth Edition)* (Eds: R. N. Rosenberg, J. M. Pascual), Academic Press, Boston **2015**, p. 615.
45. P. Kanyong, R. M. Pemberton, S. K. Jackson, J. P. Hart, *Anal. Biochem* **2013**, *435*, 114.
46. K. S. Kurtz, S. R. Crouch, *Anal. Chim. Acta* **1991**, *254*, 201.
47. H. Gülce, İ. Ataman, A. Gülce, A. Yıldız, *Enzyme Microb. Technol* **2002**, *30*, 41.
48. J. A. Hamid, G. J. Moody, J. D. R. Thomas, *Analyst* **1989**, *114*, 1587.
49. Y. Xu, G. G. Guilbault, S. S. Kuan, *Enzyme Microb. Technol* **1990**, *12*, 104.
50. W. Putzbach, N. J. Ronkainen, *Sensors* **2013**, *13*, 4811.
51. J. Wang, *J. Pharm. Biomed. Anal* **1999**, *19*, 47.
52. L. C. Clark Jr., C. Lyons, *Ann. N. Y. Acad. Sci* **1962**, *102*, 29.
53. R. A. Sheldon, *Adv. Synth. Catal* **2007**, *349*, 1289.
54. H. H. Nguyen, S. H. Lee, U. J. Lee, C. D. Fermin, M. Kim, *Materials* **2019**, *12*, 121.
55. N. R. Mohamad, N. H. C. Marzuki, N. A. Buang, F. Huyop, R. A. Wahab, *Biotechnol. Biotechnol. Equip* **2015**, *29*, 205.
56. P. J. Taylor, Emil. Kmetec, J. M. Johnson, *Anal. Chem* **1977**, *49*, 789.
57. P. Manowitz, P. W. Stoecker, A. M. Yacynych, *Biosens. Bioelectron* **1995**, *10*, 359.
58. B. Dalkiran, P. E. Erden, E. Kiliç, *Anal. Bioanal. Chem* **2016**, *408*, 4329.
59. L. Coche-Guerente, S. Cosnier, C. Innocent, P. Mailley, *Anal. Chim. Acta* **1995**, *311*, 23.
60. G. Fortier, R. Béliveau, E. Leblond, D. Bélanger, *Anal. Lett* **1990**, *23*, 1607.
61. H. Gülce, H. Özyörük, A. Yıldız, *Berichte Bunsenges. Für Phys. Chem* **1994**, *98*, 228.
62. H. Gülce, H. Özyörük, A. Yıldız, *Electroanalysis* **1995**, *7*, 178.
63. S. I. Brahim, D. Maharajh, D. Narinesingh, A. Guiseppi-Elie, *Anal. Lett* **2002**, *35*, 797.
64. S. F. Peteu, D. Emerson, R. Mark Worden, *Biosens. Bioelectron* **1996**, *11*, 1059.
65. C. Bertrand, P. R. Coulet, D. C. Gautheron, *Anal. Chim. Acta* **1981**, *126*, 23.
66. G. J. Buffone, J. M. Johnson, S. A. Lewis, J. W. Sparks, *Clin. Chem* **1980**, *26*, 339.
67. J. Motonaka, K. Miyata, L. R. Faulkner, *Anal. Lett* **1994**, *27*, 1.
68. E. E. Szabó, N. Adányi, M. Váradi, *Biosens. Bioelectron* **1996**, *11*, 1051.

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