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REVIEW

Interferences from oxygen reduction reactions in bioelectroanalytical measurements: the case study of nitrate and nitrite biosensors

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Abstract Bioelectroanalytical procedures based on cathodic processes are often subject to interference from dissolved oxygen. At the potentials applied for analyte detection, oxygen reduction may occur directly at the electrode or may be catalyzed by the electron mediators or the sensing enzyme of the biosensor. These processes affect the background current and may thus result in erroneous analyte quantification. In this review, current strategies to circumvent these oxygen interferences are presented and critically assessed with respect to their compatibility for on-site monitoring with amperometric biosensing devices operating at low potential. The main strategies consist in (1) use of oxygen scavenging systems to remove dissolved oxygen from the sample, (2) design of bioelectroanalytical approaches to shift the applied potential for analyte detection to more positive values, and (3) development of electrode materials to increase the overpotential for the oxygen reduction reaction. The latest developments in these approaches have recently led to the first biosensing devices based on reductases fully compatible with on-site monitoring requirements and this opens up possibilities for their widespread application.

Keywords Oxygen reduction reaction · Oxygen scavengers · Overpotential · Amperometric biosensors · Reductase · Cathodic processes · Electron mediator

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Introduction

Point-of-care tests and on-site monitoring devices are designed to provide analytical results directly on measurement at the spot where the sample was taken, for instance, at home for health diagnostics or in the field for testing of environmental samples [1]. These tests should be based on use of handheld devices and inexpensive disposable chips that require only elementary instructions for use. The main challenges for point-of-care and field tests reside in low sample volume, complexity of the sample matrix, and impracticality of sample pretreatment. Thus, the analytical approach must be highly selective for the analyte. Among point-of-care methods, the bioelectroanalytical approach for glucose sensing is the market leader in volume, with glucose test strips for home use being manufactured on a scale of 10^{10}/year. The key for this success is the combination of the high selectivity inherent to the biorecognition element, which allows the quantification of a specific analyte from a complicated matrix, with the simple electrochemical method for signal transduction, which is based on low-cost, simple instrumentation.

Beside the glucose biosensor, which takes advantage of the low-cost and robust glucose oxidase as a biorecognition element, many other electrochemical biosensors for field analytical chemistry and point-of-care applications have been developed. However, amperometric biosensors functioning under both cathodic and anodic conditions may be prone to limitations related to the oxygen dissolved in the sample.

Biosensors based on oxidases as biorecognition elements are sensitive to oxygen, since the latter is an electron acceptor in biocatalytic processes involving these enzymes. The strategies to circumvent this particular issue rely on fast direct or mediated electron transfer from the oxidase to the electrode to short-circuit the electron transfer from the oxidase to oxygen. The oxygen dependence of oxidase-based amperometric biosensors has been mostly solved and as a

result, these biosensors have become useful for medical [2–5] and environmental [6–8] applications. This topic was surveyed elsewhere [2] and will not be considered in this review.

On the other hand, oxygen interference still strongly impedes biosensing based on reductases as biorecognition elements. Indeed, the presence of dissolved oxygen is often deleterious because of the cathodic oxygen reduction to hydrogen peroxide and/or water [9] (Eqs. 1, 2).

$$O_2(g) + 2H^+ + 2e^- \rightleftharpoons H_2O_2(aq) \quad (1)$$

$$O_2(g) + 4H^+ + 4e^- \rightleftharpoons 2H_2O \quad (2)$$

The analyte reduction usually occurs at a potential more negative than these cathodic oxygen reduction processes which generate high background current and reactive species that may interfere with the analytical process of interest. Moreover, biosensors are often based on mediated electrochemistry, and the reduced forms of many mediators react with oxygen [10]. Thus, biosensors based on reductase or cathodic processes are especially prone to oxygen interference.

Nitrate and nitrite biosensors, which are potentially very valuable for on-site determination of nitrate in water, soil, or plant samples with respect to economic, environmental and health issues [11–21], are examples of amperometric biosensors that, until recently, were limited in their field applications because of the interference of oxygen. A large variety of other analytes, such as perchlorate [22], chromate [23], glutathione disulfide [24, 25], trinitrotoluene (TNT) [26], hydrogen [27], and dimethyl sulfoxide (DMSO) [28, 29], are also conveniently quantified via electrochemical biosensors working in the cathodic potential range and are affected by dissolved oxygen.

Many approaches [30] have been developed for solution deoxygenation, such as vacuum degassing and inert gas purging. However, these oxygen-removal methods rely on laboratory-based equipment and are not easily applicable for on-site monitoring.

In this review, the current strategies to circumvent the interferences due to oxygen in field bioelectroanalytical methods involving amperometric biosensing devices based on reductive procedures will be presented and critically assessed. A wide spectrum of strategies have been reported and they can be classified in three main categories: (1) oxygen scavenging systems, (2) analyte detection at more positive potentials, and (3) increase in the overpotential for the oxygen reduction reaction.

Oxygen removal

In many branches of analytical chemistry, dissolved oxygen needs to be removed from the sample [30]. For oxygen removal in bioelectrochemical measurements, inspiration may be found from the existing procedures; however, direct application of the latter is not always possible. Indeed, in the particular case of field bioelectroanalytical methods, the requirements for a generally applicable oxygen scavenger system are as follows:

1. A high concentration of the oxygen-reducing compound must be possible to maintain anaerobic conditions for a prolonged period of time in small open volumes.
2. Components, reaction intermediates, and final products of the oxygen-scavenging system must not interfere with the electrochemical process.
3. Components, reaction intermediates, and final products of the oxygen-scavenging system must not interfere with the biorecognition element or the mediators involved in the analyte quantification.
4. The kinetics of oxygen reduction must be fast, to minimize the waiting time necessary to achieve anaerobic conditions.

Chemical oxygen scavengers such as sodium sulfite are applied on a large scale for food preservation or in analytical procedures such as phosphorescence experiments [31]. Sodium sulfite [32] as well as thiosulfites and ascorbic acid [33, 34], at low concentrations, are suitable for the elimination of the interference from oxygen in electroanalytical measurements. Recently, the use of sodium sulfite as an oxygen scavenger was extended to a field nitrate amperometric biosensor based on recombinant eukaryotic nitrate reductase [35]. Oxygen reacts with the sulfite ions, which produce the inert sulfate anions (Eq. 3), and the biosensing devices can be applied for the field determination of nitrate under ambient air.

$$O_2 + 2SO_3^- \rightarrow 2SO_4^- \quad (3)$$

However, even at neutral pH, sulfites may be electroactive in the electrochemical window for analyte detection. Therefore, the maximum sulfite concentration which does not affect the analysis is limited to a few millimoles per liter [35]. This is sufficient to reduce the oxygen initially present in the sample, since the concentration of dissolved oxygen in air-saturated aqueous electrolytes is below 0.4 mM [36]. However, the oxygen diffusing in the electrolyte during the sample preparation and during the measurement may deplete the full amount of the reducing agent. Thus, this approach requires large volumes or closed systems to limit the diffusion of oxygen from air into the sample and thus maintain the anaerobic conditions for a sufficient period of time. However, for

on-site measurements, only small sample volumes (less than 100 μL) are often available, and the handling of an open system is more straightforward. Under these conditions, it is not possible to maintain anaerobic conditions with sulfite for the time required for the electrochemical measurements.

Moreover, the kinetics of the reaction between sulfite and oxygen is rather slow at neutral pH and in the absence of metal ions [32]. The use of acidic or basic media and the addition of metal ions as catalysts are not always possible, since they may be detrimental to both the activity of the sensing enzyme and the electrochemical window available for the measurement.

To overcome the interference related to the electroactivity of the reducing agent and to increase the kinetics of the scavenging process, biocatalyzed oxygen reduction with a very mild reducing agent may be exploited. Several enzymatic oxygen-scavenging systems are suitable for various polarographic [37], biochemical [38, 39], and fluorescence [40–42] applications. In particular, glucose oxidase in the presence of catalase has been shown to be an effective catalyst for oxygen reduction [37, 42–44]. In this approach, oxygen is reduced by catalytic oxidation of glucose by glucose oxidase (Eq. 4). Then, in a second step, the hydrogen peroxide resulting from this reaction is dismutated by the catalase into water and O_2 (Eq. 5). Oxygen molecules regenerated by the catalase are further reduced by the oxidase. In the net reaction, two glucose molecules are oxidized for the reduction of one oxygen molecule (Eq. 6). This enzymatic system is efficient to achieve and sustain strict anaerobic conditions.

$$O_2 + C_6H_{12}O_6 \rightarrow C_6H_{10}O_6 + H_2O_2 \ (\text{oxidase reaction}) \quad (4)$$

$$H_2O_2 \rightarrow {}^1/_2O_2 + H_2O (\text{catalase reaction}) \quad (5)$$

$$O_2 + 2C_6H_{12}O_6 \rightarrow 2C_6H_{10}O_6 + 2H_2O (\text{net reaction}) \quad (6)$$

The use of the electro-inactive and relatively inert glucose molecule for the reduction of oxygen opens up the possibility to introduce high concentrations of the oxygen scavenger. Thus, oxygen depletion in a low volume for extended time periods under ambient air becomes accessible without affecting the biorecognition component of the biosensor and without interfering with the electrochemical process. Moreover, the oxidase as a biocatalyst ensures fast reduction of the dissolved oxygen.

This enzymatic oxygen scavenging system was exploited in DMSO biosensing with DMSO reductase as the biorecognition element either retained on the electrode surface in solution behind a dialysis membrane [28] or immobilized via bovine serum albumin/glutaraldehyde cross-linking [29]. In the latter sensor design, DMSO sensing in a sample volume as low as 200 μL was possible. Both DMSO biosensor devices are, however, not fully suitable for field applications since they rely on fully closed systems, which may be inconvenient to use and too complex to build as single-use sensors.

Recently, a similar glucose oxidase–catalase–glucose oxygen-removal system was incorporated in a biosensor based on nitrate reductase for on-site nitrate sensing in plant extract samples. In this case, the analytical approach relies on disposable screen-printed electrochemical cells with a sample volume of 200 μL [45, 46]. The measurement is performed directly with a droplet of sample exposed to the ambient air. To compensate for the large amount of oxygen diffusing from the ambient air into the sample during the sample preparation and measurement, glucose is used at high concentration (50 mM). Although glucose does not affect nitrate biosensing, the limitation lies in the formation of its oxidation product. Indeed, with glucose oxidase as a catalyst for the oxidation of the hemiacetal in β-D-glucose, D-glucono-1,5-lactone is produced and is quickly hydrolyzed to D-gluconic acid (Fig. 1). This acid production leads to a continuous pH drop over time in low-volume open cells [40], hence affecting the activity of the sensing enzyme. In the special case of this nitrate biosensor, quantification of nitrate in an open electrochemical cell was still possible by applying a high buffer concentration to compensate for gluconic acid production and to maintain the pH at the optimum value for the biorecognition element.

Still, the acid production associated with the glucose oxidase based system impedes its broader application in on-site monitoring and point-of-care bioelectroanalytical methods for two reasons:

1. The high ionic strength related to the increased buffer strength may not be suitable for all biorecognition elements.

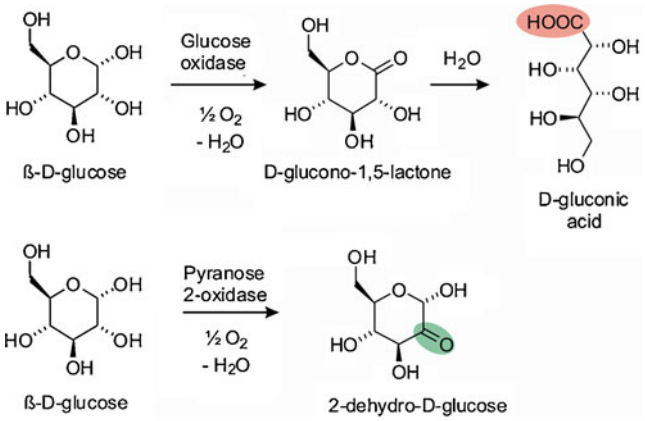

Fig. 1 Glucose oxidase and pyranose 2-oxidase catalyzed reactions starting from β-D-glucose [45]

2. The time available to perform the measurement is defined by the time over which the buffer can maintain the pH at the optimum value required for the sensing enzyme.

The issue of electrolyte acidification was addressed with another oxidase that circumvents the production of gluconic acid on oxygen reduction. Pyranose 2-oxidase catalyzes the oxidation of a secondary alcohol of glucose to the respective ketone [47] (Fig. 1). This product is pH-neutral and does not affect the activity of nitrate reductase [45]. The time period over which the anaerobic conditions are maintained is only limited by the maximum glucose concentration that can be used. Moreover, the absence of acidification over time gives significantly more freedom in the choice of buffer conditions, which can thus be adjusted to the requirements of the sensing enzyme [40, 45].

This makes the pyranose 2-oxidase based system most promising for oxygen removal for general applications of electrochemical biosensors based on a wide range of biorecognition elements, cell geometries, and sample volumes. Beside nitrate sensing, this oxygen-scavenging strategy is anticipated to be especially valuable for nitrite biosensors for implementation in on-site monitoring [48].

The main drawback of oxygen scavengers, including enzymatic systems, is the necessity to add a reagent to the sample. Although the oxidase and the catalase may be immobilized on the electrode surface by retaining these two enzymes behind a dialysis membrane [28], glucose still needs to be added to the sample.

An entirely different strategy to achieve reagentless removal of oxygen from the sample consists in the cathodic oxygen reduction at a large and porous electrode placed in front of the sensing electrode [49]. Such devices have been described in the electrochemical sensing of N_2O. However, these sensors often have a short lifetime and are more difficult to build [50]. Moreover, their major drawback is that OH^- is produced from the cathodic oxygen reduction reaction. The increase in local pH at the sensing electrode [51] may affect the activity of the biorecognition element. This may be the reason why no report involving bioelectroanalysis using such an electrochemical oxygen scrubber has been published to date.

Analyte detection at more positive potentials

In biosensors based on cathodic processes, the working potential for analyte detection is usually more negative than the potential at which oxygen reduction occurs and therefore oxygen interference occurs. The need to apply low working potentials is often a consequence of unoptimized biosensor design with respect to the thermodynamic potential for analyte reduction. This is illustrated by nitrate and nitrite, which are two important analytes [14] for which amperometric sensing is impeded by dissolved oxygen. The standard thermodynamic potential for the NO_3^-/NO_2^- redox couple (Eq. 7) is 0.845 V versus the standard hydrogen electrode (SHE) at pH0; thus, at pH7, the reversible reduction of nitrate to nitrite would occur at 0.431 V. Similarly, the redox potentials for nitrite reduction to ammonium (Eq. 8) are 0.892 V and 0.340 V versus SHE at pH0 and pH7.0, respectively.

$$NO_3^- + 2H^+ + 2e^- \rightleftharpoons NO_2^- + H_2O \quad (7)$$

$$NO_2^- + 8H^+ + 6e^- \rightleftharpoons NH_4^+ + 2H_2O \quad (8)$$

These potentials are positive enough to avoid interference from the oxygen reduction reaction at most electrode surfaces. Still, for most nitrate and nitrite biosensors, oxygen must be removed from the sample because the working potentials for analyte detection are negative enough for cathodic oxygen reduction [11, 15, 35, 45, 52]. It is therefore essential to target a working electrode potential as near as possible to the thermodynamic potential for the analyte reduction in the design of bioelectroanalytical strategies.

Electron mediator

Although the enzymatic electrocatalytic reaction may itself occur at a significant overpotential, a significant source for additional overpotential often results from the choice of electron mediators with excessively negative redox potentials. For example, methyl viologen, with a standard redox potential for the first reversible one-electron reduction of −0.44 V versus SHE, is usually applied as an electron mediator in nitrate biosensors based on nitrate reductase [11, 15, 35, 45, 52]. The reduced methyl viologen donates its electron to the heme iron center in nitrate reductase. The standard redox potential of the heme iron is typically between −0.123 and −0.174 V [53–55]. The difference of about 0.3 V between the mediator and the heme redox center in the enzyme ensures a thermodynamic driving force for the electron transfer. However, at the potential required for the reduction of methyl viologen, cathodic reduction of oxygen occurs at most electrodes. In addition, oxygen may react directly with the reduced methyl viologen [56]. Thus, to avoid or limit oxygen interference, a possible strategy consists in selecting electron mediators with a standard redox potential near to the one of the electron-accepting center of the biorecognition element.

One example that fully exploits this strategy was reported for a nitrite biosensor based on a cytochrome cd_1 nitrite reductase. This enzyme catalyzes the one-electron reduction of nitrite to nitric oxide (Eq. 9). The site of electron entry in

the protein is a *c*-type heme with a formal potential of about 0.25 V versus SHE at neutral pH [57–59].

$$NO_2^- + 2H^+ + e^- \rightleftharpoons NO + H_2O \quad (9)$$

Cytochrome *c*-552, a small homodimeric heme protein with a formal potential of 0.254 V versus SHE at pH6.3, was used to shuttle electrons between the electrode and nitrite reductase. Both proteins were co-immobilized in a cross-linked poly(vinyl alcohol) matrix. Cytochrome *c*-552 proved to be a particularly good mediator in the nitrite reductase based biosensor. The high values and good match of the redox potentials of the electron-mediating protein and the sensing enzyme made it possible to apply a potential for nitrite sensing that was high enough to avoid the cathodic oxygen reduction [19].

Still, interference from oxygen was observed in this particular case owing to the ability of cytochrome cd_1 nitrite reductase to catalyze the four-electron reduction of molecular oxygen to water [60]. Interference-free nitrite sensing based on this mediated bioelectrochemical device in the presence of oxygen was eventually achieved by taking advantage of a complementary strategy (see later).

Artificial electron mediators for nitrate reductase were also screened with the goal to avoid oxygen interference in nitrate biosensors [61]. This is more challenging than for nitrite biosensors, since the formal potential of the active site for nitrate reduction in nitrate reductase is typically more negative than in nitrite reductases. In nitrate reductase, nitrate is reduced to nitrite at a molybdenum center with standard redox potentials for both Mo^{VI}/Mo^{V} and Mo^{V}/Mo^{IV} couples typically around 0 V versus SHE [53–55]. Moreover, the two possible electron acceptor centers in nitrate reductase, an FAD and a heme group, display standard redox potentials around −0.28 V and −0.16 V, respectively [53–55]. Therefore, from a thermodynamic point of view, nitrate reduction at significant rates is not expected at potentials more positive than −0.16 V. At this potential, the onset of the oxygen reduction reaction at the electrode may already be observed [45]. Thus, to limit oxygen interference, the redox potential of the electron mediator must be especially near to the redox potential of the electron acceptor center of the nitrate reductase. Several mediators that fulfill this requirement, such as phenothiazine dyes, azure A, thionin, and patent blue, are efficient electron mediators for nitrate reductase [61]. Still, an oxygen-insensitive nitrate sensing device for field sensing based on this strategy has not been reported yet.

Direct electron transfer

To achieve a working potential that is more positive than the oxygen reduction and also to avoid any possible reaction between the mediator and oxygen [10], a direct electron transfer between the redox enzyme and the electrode may be beneficial.

Almeida et al. [62] reported on a cytochrome *c* nitrite reductase that displays direct electron transfer properties on pyrolytic graphite electrodes. This enzyme performs the six-electron reduction of nitrite to ammonia (Eq. 8) and was applied as a biorecognition element in a nitrite biosensor [20].

The highest catalytic currents were obtained at very negative potentials (as negative as −0.68 V vs SHE), thanks to the large driving force for the direct electron transfer. However, under these conditions, the interferences from cathodic reduction, in particular of oxygen, are increased. On the other hand, at working potentials as high as -0.280 V, although the linear range was limited, nitrite quantification was possible. Although measurements under ambient air have not been attempted, the working potential of the proposed biosensor may make it possible to avoid the oxygen reduction reaction.

Protein engineering

Another strategy to further shift the catalytic process to more positive potentials in direct bioelectrochemistry of redox enzymes relies on genetic engineering. In the case of nitrate reductase, the two possible electron acceptor centers are the FAD and the heme group (redox potentials around −0.28 V and −0.16 V, respectively). Thus, to limit oxygen interference in nitrate biosensors, direct electron transfer to the heme would be desired. To make the heme group more accessible, a nitrate reductase lacking the FAD domain was engineered [63]. The simplified nitrate reductase fragment was capable of selective nitrate reduction when in contact with an electron donor. Although the bioelectronanalytical applications of this approach are still to be demonstrated, this represents a pilot step for bioelectrocatalysis at reduced overpotentials.

Bienzyme system

A completely different approach to shift the potential required for analyte detection in a potential range free of electrochemical interference consists in using a bienzyme system: Cui et al. [12] reported a biosensor based on nitrate reductase and salicylate hydroxylase for electrochemical determination of nitrate under ambient air without suffering from oxygen interferences. The nitrate reductase reduces nitrate to nitrite in the presence of NADH, which is converted to NAD^+. In parallel, NADH together with oxygen is used by a salicylate hydroxylase to irreversibly decarboxylate salicylic acid, which produces catechol. The latter is detected upon anodic oxidation at high potentials, thus avoiding the cathodic reduction of oxygen. Since nitrate reductase competes with salicylate hydroxylase for the NADH, the catechol production and the anodic current

intensity decrease with increasing nitrate concentration. The sensor is fully insensitive to oxygen; however, the drawback of the method is that the expensive NADH molecule is needed at high concentrations in the solution to ensure a broad detection range for nitrate sensing.

Increased overpotential for oxygen reduction

Instead of targeting a potential for analyte detection as positive as possible, oxygen interferences may also be circumvented by increasing the overpotential for the cathodic oxygen reduction, or, in other words, by shifting the latter to potentials as negative as possible.

Electrode material

The standard redox potentials for the two-electron reduction of oxygen to hydrogen peroxide (Eq. 1) and the four-electron reduction of oxygen to H_2O (Eq. 2) are $E°(O_2/H_2O_2)$=0.695 V and $E°(O_2/H_2O)$=1.23 V versus SHE (at pH0) [9]. Thus, the redox potentials for the O_2/H_2O_2 and O_2/H_2O couples at pH7.0 are 0.281 V and 0.815 V versus SHE, respectively. At most electrode materials used in (bio) electroanalytical applications, such as gold and carbon, the oxygen reduction occurs at significant overpotentials, which already limits its deleterious effect. By selecting appropriate materials for fabrication of the electrode to further increase the overpotential, one may fully avoid oxygen interferences.

The basal plane of highly oriented pyrolytic graphite (HOPG) electrodes and boron-doped diamond (BDD) electrodes display extreme overpotentials for oxygen reduction reactions [64–67]. The inhibition of oxygen reduction is caused by the lack of active sites for dioxygen binding on both the basal plane of HOPG and the BDD surface consisting of sp^3 carbon. On the other hand, for outer-sphere-type redox couples, including various electron mediators for redox enzymes, the oxidation–reduction processes have been reported to be close to reversible [68]. Thus, the insensitivity to oxygen and the good electrode kinetics for outer-sphere redox couples of these electrode materials may be a useful feature for field electroanalytical methods.

The main limitation resides in the cost of these materials, which are not yet compatible with disposable electrodes. Moreover, the extreme overpotentials for oxygen reduction are only observed at impurity-free BDD or HOPG electrodes. Upon electrode modification for enzyme and mediator immobilization, increased oxygen sensitivity may be observed.

Experimental timescale

The oxygen interference may be circumvented by tuning the timescale of the measurement. If the kinetics of the catalytic process is significantly faster than the kinetics of oxygen reduction, the latter may be suppressed by decreasing the timescale of the experiment to a value at which the bioelectrocatalytic process is still observed. For example, if cyclic voltammetry is used for signal detection, by sufficiently increasing the scan rate, one can exclude oxygen reduction at the potentials at which analyte detection is performed. This was exploited in the aforementioned example of a nitrite biosensor based on direct electron transfer between a nitrite reductase and an electrode. The nitrite reductase catalyzes both nitrite reduction and oxygen reduction reactions. However, the latter occurs at rates lower than the former. An increase of the scan rate from 5 to 50 mVs^{-1} made it possible to exclude the oxygen reduction reaction without significantly affecting the catalytic current due to nitrite reduction. Under these conditions, it was possible to quantify nitrite without previously removing the dissolved oxygen from the samples [19].

Since the cathodic oxygen reduction in aqueous conditions is an electrochemically irreversible process, the same strategy may also be used in cases where the oxygen reduction reaction at the electrode is significantly slower than the bioelectrocatalytic process involved in the analyte detection.

Adjusting the pH of the electrolyte

For both two-electron reduction (Eq. 1) and four-electron reduction (Eq. 2), the redox potential for oxygen reduction will shift by −0.059 V (at 298 K) when changing the electrolyte pH by 1 unit as anticipated from the Nernst equation. Thus, if the biorecognition element displays a broad pH window or several pH optima for maximal enzyme activity, the most basic conditions should be chosen to extend the potential range for O_2-insensitive measurements. This may of course only be applied if the redox potential for analyte detection is independent of pH.

Conclusion and outlook

The latest generation of enzymatic oxygen-scavenging systems has nearly fulfilled all the requirements for field bioelectroanalysis and overcome the issue of oxygen interference in important biosensor classes. The major advantage is that the method can be applied independently of the reductase used as the biorecognition element. The need to add the reducing agent to the sample may be seen as a drawback with respect to the ease of operating the sensing device. However, with the advent of low-cost microfluidic chips [69], all components of the oxygen scavenger may be incorporated in the sensing chip, and thus appear as fully reagentless to the user. The only real drawback may be the impracticality to apply very small volumes (1–5 μL) owing

to the overwhelming diffusion of oxygen from air into the small sample volume. When such low volumes are required, the strategies that allow measurements in the presence of dissolved oxygen are the primary choice. In particular, the applied potential for analyte detection may be shifted to more positive values to circumvent the cathodic oxygen reduction reaction. However, this strategy may seem costly, since it needs to be developed for the biosensing of each new analyte. It has also proved to be extremely challenging for some systems, such as nitrate biosensors. It is, however, when possible, the most productive approach, since it also avoids interferences from other electroactive species, beside oxygen, that may be present in the sample matrix.

Developing electrode materials with increased overpotential for the oxygen reduction reaction would allow their implementation in biosensors for all types of analytes and for measurements in the presence of dissolved oxygen. However, the issue that still remains to be solved is how to modify these electrode surfaces without increasing the sensitivity towards oxygen. Eventually, these materials may be applied unmodified with the enzyme and mediator noncovalently attached to their surface. Nevertheless, the reaction of oxygen with the mediator or the enzyme may also limit the application of this approach.

Strategies involving the experiment timescale or the pH of the electrolyte may be used to complement the other approaches in order to suppress the residual oxygen interference.

Although significant progress in strategies to circumvent oxygen interference in field bioelectroanalysis has been made only recently, their rapid development has already provided a full toolbox that promises oxygen-insensitive bioelectroanalysis for most types of analytes soon.

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