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Oxygen-Enriched Triphasic Enzyme Electrodes on Superhydrophobic Carbon Cloth

Yichen Yu

King's College London Waterloo Campus, King's College London, London, United Kingdom
k23007420@kcl.ac.uk

Abstract. Electrochemical glucose sensors based on glucose oxidase (GOx) are often limited by oxygen availability, which constrains their sensitivity and linear range. Recent advancements in triphasic interface design, combining gas, liquid, and solid phases, have shown promise in overcoming this limitation. By using superhydrophobic carbon cloth (CC) as a substrate, these triphasic interfaces can sustain a stable interfacial air reservoir, facilitating continuous oxygen supply to the enzyme and enhancing sensor performance. Compared to traditional biphasic configurations, triphasic architectures have demonstrated significant improvements in sensitivity and linear detection range. This review summarizes the mechanisms behind triphasic interfaces, including their role in oxygen enrichment, and discusses various material systems (such as superhydrophobic carbon cloth) that have been explored to create such interfaces. Additionally, the challenges and strategies for optimizing these interfaces, including their use in biosensors, are reviewed. The potential for scaling this technology in low-power, flexible, and portable biosensing platforms is also highlighted. Overall, oxygen-enriched triphasic interfaces present a promising, passive solution to the oxygen limitation issue, extending the functional range of oxidase-based biosensors under ambient conditions without the need for additional hardware.

Keywords: Triphasic Interface, Oxygen Enrichment, Glucose Oxidase (GOx), Superhydrophobic Carbon Cloth, Electrochemical Biosensors

1. Introduction

Enzymatic electrochemical biosensors, particularly those utilizing glucose oxidase (GOx), are commonly constrained by oxygen transport limitations, which restrict their sensitivity and linear range [1]. In conventional liquid-solid configurations, oxygen must diffuse through the electrolyte, and its solubility and diffusion are limited. High analyte concentrations can rapidly deplete local oxygen, reducing the sensor's efficiency and accuracy [2]. This challenge has spurred considerable research into alternative solutions to ensure a steady oxygen supply to the enzyme.

GOx is a critical enzyme in biosensing applications, and recent reviews have highlighted its importance in both diagnostic and industrial settings [3]. Despite its extensive use, persistent challenges remain, such as optimizing enzyme stability, activity, and achieving sufficient oxygen delivery to the enzyme site. These challenges have led to a dual focus on enzyme-level optimization

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(e.g., through rational design and directed evolution) and device-level strategies (e.g., improving oxygen supply and electrode design) to enhance sensor performance.

A promising solution to overcome these challenges is the introduction of a gas phase directly at the biocatalytic interface. By incorporating a stable air reservoir, this design facilitates continuous oxygen supply to the enzyme, significantly enhancing the sensor's performance [4]. This strategy effectively alleviates the oxygen transport bottleneck commonly encountered in enzymatic electrochemical sensors.

In recent years, triphasic interfaces, which consist of gas, liquid, and solid phases, have emerged as a promising approach to alleviate oxygen limitations. These interfaces, with their ability to maintain an interfacial air reservoir, provide a steady supply of oxygen directly from the air, thus expanding the usable concentration window of oxidase biosensors [5]. Compared to traditional biphasic systems, triphasic configurations have been shown to significantly improve sensor sensitivity and linear range. This breakthrough has been demonstrated in various electrochemical sensors, particularly in bio-photoelectrochemical systems, where the combination of triphasic interfaces with superhydrophobic carbon textiles and semiconductor nanowires has led to improved charge collection and a wider glucose detection range.

Triphasic architectures are commonly realized through micro/nanostructured superhydrophobic coatings or porous scaffolds that retain interfacial air pockets or provide breathable gas channels [6]. These designs ensure an "always-on" air reservoir at the sensor surface, enabling continuous oxygen diffusion from the air and overcoming the limitations associated with liquid-phase oxygen transport. Among the materials used for triphasic interfaces, superhydrophobic carbon cloth has gained attention due to its combination of water repellency, flexibility, and thermal management. Carbon cloth not only enhances oxygen supply but also contributes to the mechanical and chemical durability of the biosensor.

Further research into bioelectrocatalytic systems has led to the development of flexible bioassays, such as those based on superhydrophobic PDMS micropillar arrays with carbon nanotube films. These systems demonstrate wide linear ranges, high selectivity, and excellent mechanical durability, making them promising for applications in wearable and portable biosensors. Additionally, these triphasic architectures complement other bioassay techniques, such as fluorescence-based assays, which measure GOx activity. The versatility of these biosensors is further demonstrated by studies exploring the optimization of GOx through directed evolution and high-throughput screening, which improves enzyme stability and activity for more demanding biosensing environments.

While current methods for enhancing oxygen delivery, such as external oxygenation, oxygen carriers, or microfluidic systems, introduce hardware complexity or slow sensor response, triphasic interfaces present a passive and device-level strategy that can sustain local oxygen levels without additional hardware. Furthermore, the integration of composition-matched controls is critical to isolate the specific impact of oxygen delivery from other factors, ensuring clearer insights into the technology's advantages.

This review aims to explore the potential of oxygen-enriched triphasic (gas-liquid-solid) interfaces for improving enzymatic electrochemical biosensors. Specifically, we focus on how superhydrophobic carbon cloth can be used to retain an interfacial air layer, enhancing sensor performance under ambient, low-potential conditions. By overcoming oxygen transport limitations, this technology holds promise for advancing oxidase-based biosensors.

This review further discusses the potential of triphasic architectures to: Improve sensitivity and linear range of electrochemical sensors; Influence sensor performance under varying oxygen

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availability conditions; Maintain specificity at low bias in the presence of common interferents; Correlate surface wettability and microstructure with electrochemical output.

By addressing these aspects, this review aims to establish oxygen-enriched triphasic architectures as a scalable, low-power solution for oxidase-based biosensing, offering comprehensive design guidelines for future sensor development across a wide range of bioelectrochemical platforms.

2. Technical review

2.1. Basic principles

In triphasic electrochemical biosensors, the coexistence of gas, liquid, and solid phases at the electrode surface creates a localized air reservoir that directly interfaces with the immobilized enzyme layer. This architecture enables molecular oxygen from the ambient atmosphere, not dissolved O_2 from the bulk electrolyte, to participate continuously in the enzymatic reaction. For glucose oxidase (GOx)-based systems, this sustained oxygen supply ensures that the catalytic cycle remains kinetically favorable even at high glucose concentrations, thereby mitigating substrate-induced oxygen depletion [7].

Critically, the enhanced oxygen availability increases the rate of glucose oxidation and concomitant hydrogen peroxide (H_2O_2) generation. Since H_2O_2 is commonly detected amperometrically at low applied potentials, its elevated local concentration translates directly into amplified electrochemical signals and improved sensitivity. Furthermore, the proximity of the gas phase reduces diffusion barriers for both reactants and products, facilitating faster mass transport and more efficient electron transfer at the electrode-enzyme interface [8]. These synergistic effects, continuous O_2 replenishment, boosted H_2O_2 yield, and accelerated interfacial kinetics, collectively underpin the superior analytical performance of triphasic sensors over conventional biphasic counterparts.

2.2. Material selection

The successful implementation of triphasic gas-liquid-solid interfaces relies heavily on the selection of appropriate materials that can sustain a stable interfacial air layer while supporting enzymatic activity and electrochemical transduction [9]. Among the most widely adopted platforms is superhydrophobic carbon cloth (CC), which serves simultaneously as a conductive scaffold, a gas-diffusion substrate, and a mechanically robust support. Its intrinsic porosity, combined with surface modifications that impart low surface energy (e.g., fluorination or deposition of hydrophobic nanoparticles), enables durable air pocket retention even under dynamic liquid contact.

Beyond carbon cloth, other material systems have been explored to construct functional triphasic architectures. These include: Porous carbon papers or felts, which offer high gas permeability and electrical conductivity; Micro/nanostructured polymer substrates (e.g., PDMS micropillar arrays coated with carbon nanotubes), engineered to trap air via capillary resistance; Hierarchically structured metal oxides or composites, where controlled roughness and chemical heterogeneity stabilize the Cassie-Baxter wetting state.

In all cases, the selected material must fulfill three core functions: (1) maintain a persistent gas phase at the electrode-electrolyte boundary, (2) provide sufficient surface area for enzyme immobilization, and (3) ensure efficient electron transport to the external circuit. The choice among these material platforms often depends on the target application, e.g., flexibility for wearables, thermal stability for implantable devices, or cost-effectiveness for disposable sensors.

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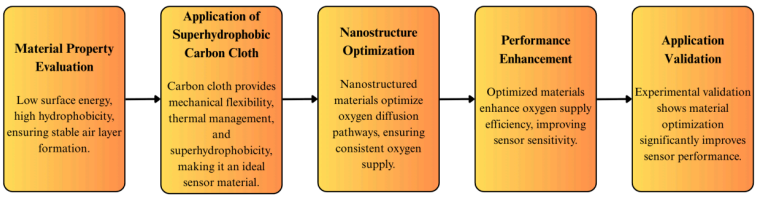

Figure 1. Material selection and optimization process

Figure 1 illustrates the process of selecting and optimizing materials for triphasic interfaces. It highlights the key steps involved in choosing materials like superhydrophobic carbon cloth and optimizing their properties to create a stable air reservoir and improve oxygen supply. These steps are essential for enhancing the sensor's performance and ensuring the stability of the triphasic interface.

Carbon cloth is particularly advantageous because, in addition to its superhydrophobic properties, it also combines mechanical flexibility and thermal management, making it an ideal candidate for flexible, durable, and even washable biosensors. The versatility of carbon cloth is further enhanced by its ability to support high surface areas, which contribute to the overall performance of the triphasic system [10].

In addition to superhydrophobic materials, nanostructured surfaces such as porous scaffolds or micro/nanostructured coatings play a critical role in enhancing the stability of the triphasic interface. These structures further support the formation of air pockets or provide breathable gas channels, improving the efficiency of oxygen diffusion across the interface [11]. By enhancing the oxygen diffusion pathway, these materials contribute to a more consistent and reliable oxygen supply, which in turn improves the biosensor's sensitivity and overall performance.

2.3. Application cases

Triphasic interfaces have been successfully applied in various electrochemical biosensors, particularly those based on GOx for glucose detection. The use of superhydrophobic carbon textiles as substrates has proven effective in extending the glucose detection window and enhancing sensor sensitivity, allowing for detection at higher glucose concentrations than traditional biosensors. The use of carbon paper or other porous materials for constructing triphasic electrodes has also demonstrated that the triphasic design is adaptable and reproducible across different substrates, reinforcing its potential for large-scale applications.

These interfaces have also been employed in bio-photoelectrochemical systems, where the combination of oxygen delivery from the air and efficient charge collection from semiconductor nanowires significantly enhances glucose detection sensitivity while lowering detection limits. The addition of light-responsive materials further boosts the performance of the triphasic system by providing an additional energy source, which is especially advantageous in applications requiring high sensitivity and low operating potentials.

Moreover, triphasic interfaces have found applications beyond glucose sensing. These interfaces are being explored for other bioelectrochemical assays, demonstrating their broad potential across various biosensing fields [12]. This includes applications in detecting other biomolecules, such as lactate or choline, as well as environmental pollutants. The versatility of triphasic interfaces, coupled with their capacity for enhanced oxygen delivery and improved charge transfer, positions them as a powerful solution for diverse biosensing applications.

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2.4. Future developments

The development of oxygen-enriched triphasic interfaces holds great promise for advancing biosensor technology, particularly in wearable devices, environmental monitoring, and biomedical diagnostics. In wearable biosensors, triphasic designs offer low power consumption and enhanced performance for real-time monitoring applications. By ensuring a continuous oxygen supply, triphasic biosensors can maintain stability and sensitivity even during prolonged use, making them well-suited for continuous health monitoring in wearable devices.

Environmental monitoring represents another key application area. Triphasic interfaces have the potential to significantly improve the sensitivity and accuracy of sensors used in monitoring air quality, including the detection of volatile organic compounds (VOCs) and other pollutants [13]. In industrial settings, where environmental conditions vary, the ability to maintain a steady oxygen supply at the sensing interface would enhance the sensor's reliability and performance, enabling more accurate monitoring of air quality.

Furthermore, the ability to integrate these interfaces into flexible and portable devices opens up new possibilities for point-of-care diagnostics. For applications requiring robust and scalable biosensors, such as disease detection in remote areas, triphasic biosensors could provide a practical solution due to their ability to operate efficiently without the need for complex hardware.

Despite the vast potential, several challenges remain in optimizing triphasic interfaces for broader applications. These challenges include improving the scalability of manufacturing processes and enhancing the stability of materials under varying environmental conditions. Additionally, further research is needed to refine the design of triphasic interfaces to maximize sensor sensitivity while minimizing interference from common electroactive species. Future studies will likely focus on exploring new material combinations, optimizing the fabrication techniques for large-scale production, and improving the durability and cost-effectiveness of triphasic biosensors.

3. Discussion

3.1. Technical challenges

Despite the significant potential of triphasic interfaces, several challenges remain in optimizing these systems for practical use. One major challenge is the stability of materials. While superhydrophobic materials like carbon cloth and PDMS micropillar arrays offer significant advantages in terms of air retention and flexibility, their long-term stability under various environmental conditions (such as exposure to moisture, temperature fluctuations, and mechanical deformation) must be carefully evaluated. In flexible or wearable biosensors, for instance, the material not only needs to maintain its superhydrophobic properties but also withstand repeated bending and stress without compromising its functionality. This is especially important for applications where devices are subjected to frequent mechanical deformation, as in wearable health-monitoring systems.

Another technical challenge lies in interface control. The formation and maintenance of a stable gas-liquid-solid interface depend heavily on the precise design and fabrication of the electrode surface. Variations in surface roughness, porosity, and wettability can lead to inconsistencies in the interface, which can affect sensor performance [14]. Ensuring uniform oxygen distribution throughout the electrode surface is also critical for achieving optimal sensor performance. The thickness of the interfacial air layer and its stability during operation are factors that must be

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carefully controlled to ensure the continuous supply of oxygen, thus preventing fluctuations in sensor output.

Finally, the scalability and manufacturability of triphasic electrode systems present another significant hurdle. While laboratory-scale experiments have shown great promise, translating these designs into cost-effective, large-scale manufacturing processes is still a challenge. Methods for fabricating uniform superhydrophobic surfaces and integrating them into flexible or large-area sensors must be developed to make triphasic systems viable for real-world applications. This requires innovations in fabrication techniques that can produce high-quality materials at a lower cost, enabling mass production of triphasic biosensors.

3.2. Design principles

Effective triphasic electrode design hinges not merely on material selection, but on the strategic integration of interfacial architecture and surface functionality to simultaneously satisfy competing requirements: stable gas-phase retention, efficient enzymatic catalysis, and rapid electrochemical transduction. Three core design principles guide this optimization.

First, wettability must be spatially and functionally engineered, not uniformly maximized. While a superhydrophobic background is essential for sustaining an air reservoir, excessive hydrophobicity impedes enzyme immobilization, as most oxidases (e.g., GOx) require a hydrated microenvironment to retain conformational stability and activity. Successful designs therefore adopt a dual-wettability strategy: a superhydrophobic matrix (e.g., fluorinated carbon cloth) provides the gas-diffusion backbone, while localized hydrophilic domains, introduced via plasma treatment, polymer grafting, or nanoparticle decoration, serve as anchoring sites for enzymes. This compartmentalization ensures that oxygen accesses the reaction zone directly from the gas phase, while the enzyme remains in a biocompatible aqueous pocket.

Second, pore architecture must balance gas permeability with catalytic surface area. The electrode's micro/nanostructure should feature hierarchical porosity: macropores (>1 μm) act as gas channels for bulk oxygen transport, while mesopores (2–50 nm) increase enzyme loading and shorten diffusion paths for glucose and H_2O_2. However, overly dense nanostructuring can collapse the air layer under hydrostatic pressure or electrolyte flow. Thus, optimal designs maintain a critical pore size distribution that stabilizes the Cassie state while maximizing accessible active sites.

Third, the triphasic interface must co-optimize mass transport and electron transfer. Since the electrochemical signal depends on H_2O_2 oxidation (or mediated electron shuttling), the conductive scaffold must extend into the immediate vicinity of the enzymatic reaction zone. Integrating high-aspect-ratio nanomaterials (e.g., CNTs, graphene flakes) within the hydrophilic enzyme zones reduces charge-transfer resistance without compromising air-layer integrity. Additionally, the electrode geometry should minimize diffusion distances between the gas-liquid-solid triple line and the current collector to prevent signal attenuation.

Collectively, these principles emphasize that high-performance triphasic biosensors emerge not from extreme material properties alone, but from intentional spatial orchestration of wettability, porosity, and conductivity at the microscale.

3.3. Interdisciplinary integration

The development of triphasic interfaces can be greatly enhanced through integration with other emerging technologies. One promising area is photoelectrochemical systems, where triphasic interfaces can be combined with light-responsive materials, such as TiO_2 nanowires, to enhance

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sensor performance under UV illumination [15]. This approach would allow for the simultaneous optimization of both oxygen delivery and electron transport, enabling more efficient energy harvesting and broader detection ranges. The synergy between these technologies could lead to biosensors with superior performance, particularly in applications requiring high sensitivity and low operating potentials.

Another significant area of integration is with flexible electronics and wearable biosensors. By combining triphasic systems with flexible substrates, such as PDMS or other soft materials, it is possible to create stretchable, bendable biosensors that maintain high performance even under mechanical deformation. This is especially relevant for wearable health-monitoring devices, which need to provide continuous, real-time glucose or other biomarker monitoring without losing sensitivity over time. The ability to incorporate triphasic interfaces into these devices opens up new possibilities for personalized healthcare and continuous monitoring systems.

Additionally, environmental monitoring can greatly benefit from the integration of triphasic biosensors. These sensors could be adapted for detecting various pollutants, such as volatile organic compounds (VOCs), by incorporating gas-phase sensing into the triphasic architecture. The high selectivity and sensitivity offered by triphasic interfaces make them particularly well-suited for outdoor or industrial settings, where environmental conditions are often unpredictable. This opens up avenues for more efficient and reliable monitoring of air quality and pollution levels.

3.4. Future work

The future of triphasic interfaces in biosensing holds tremendous potential for advancing sensor technology. As research in this area progresses, there are several key directions that could significantly enhance the performance and applicability of triphasic systems.

One crucial area of focus is the advancement of material design. While materials like superhydrophobic carbon cloth have shown promise, further research is needed to improve material versatility and functional properties. For instance, developing new composite materials that combine superhydrophobic properties with enhanced biocompatibility, mechanical strength, and environmental stability could open new avenues for triphasic biosensors in challenging environments, such as in extreme temperatures or high humidity. Additionally, novel nanomaterials could be explored to enhance the electron transport properties of triphasic systems, further boosting their sensor performance.

Another important avenue is the enhancement of integration with other emerging technologies. For example, microfluidics could be integrated with triphasic interfaces to create systems capable of handling complex fluid samples in real-time, enabling more advanced applications in diagnostics. Similarly, combining triphasic systems with bioelectronics and wearable technology could allow for continuous, non-invasive monitoring of biomarkers, further expanding the use of triphasic biosensors in healthcare and personalized medicine.

Furthermore, triphasic interfaces have the potential to benefit from the integration of artificial intelligence (AI) for data analysis and sensor optimization. By incorporating AI-driven algorithms, triphasic biosensors could automatically adapt to fluctuating environmental conditions, optimize sensing parameters, and provide real-time feedback to users. This could lead to more accurate and responsive biosensors, particularly in dynamic or complex environments, such as in wearable or point-of-care devices.

Finally, addressing the scalability and cost-effectiveness of triphasic electrode fabrication remains a crucial challenge. Scaling up manufacturing processes for triphasic systems will be necessary to move from laboratory prototypes to commercial products. Research into advanced fabrication

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techniques, such as 3D printing, roll-to-roll processing, or laser ablation, could enable the mass production of high-quality triphasic interfaces at lower costs, making these biosensors more accessible and widely applicable. Additionally, exploring methods to simplify the manufacturing process without compromising the performance or functionality of triphasic interfaces could drive their commercialization and broader adoption.

In conclusion, as these research challenges are addressed, triphasic interfaces are poised to significantly enhance the capabilities of biosensors. By improving material properties, integrating with advanced technologies, and optimizing manufacturing processes, triphasic biosensors could provide low-cost, high-performance solutions across a wide range of applications, from healthcare monitoring to environmental detection. Future developments will undoubtedly pave the way for more sophisticated, reliable, and scalable biosensors that offer improved sensitivity and adaptability, ultimately benefiting diverse fields of biomedical and environmental monitoring.

4. Conclusion

This review highlights the potential of triphasic gas-liquid-solid interfaces in improving the performance of biosensors, particularly in the context of oxygen-enriched enzymatic sensors. The introduction of a stable interfacial air layer, as demonstrated with superhydrophobic carbon cloth, significantly enhances the oxygen supply to the enzyme, thereby improving sensor sensitivity and extending the detection range. This triphasic configuration allows for more efficient oxygen delivery compared to conventional biphasic systems, addressing the critical limitation of oxygen transport that often constrains enzymatic reactions in biosensors.

The triphasic strategy offers a practical and scalable solution to overcoming oxygen limitation in electrochemical sensors. The ability to sustain oxygen availability at the sensing interface not only enhances enzymatic performance but also enables broader applications in various biosensing fields. Additionally, the use of superhydrophobic materials ensures that these sensors maintain robust performance even under low power conditions, without introducing additional complexity or hardware requirements. This makes triphasic interfaces a promising solution for low-cost, high-performance biosensors, especially in wearable and portable devices.

Future research directions in this field should focus on material innovation to improve the stability, flexibility, and durability of superhydrophobic substrates, particularly for applications in flexible and wearable biosensors. Advances in interface control, including better management of oxygen delivery and the optimization of the gas-liquid-solid boundary, will further enhance sensor performance and accuracy. Additionally, exploring multifunctional integration, where triphasic interfaces are combined with other technologies, such as photoelectrochemical systems, microfluidics, or AI-driven optimization, could open up new avenues for expanding the capabilities of triphasic biosensors.

Ultimately, as these challenges are addressed, the triphasic interface technology holds great promise for advancing biosensors, offering a scalable and efficient solution for a wide range of bioelectrochemical applications.

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