

Perovskites Application in the Sensing of NPNC Biomarkers

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Perovskites are materials with a chemical formula of the form ABO_3 (oxide-based form) or ABX_3 (halogen-based form), ordered in a specific arrangement of atoms in a crystal lattice. It consists of a cubic unit cell in which an atom or ion is located at the center, surrounded by a larger octahedral cage of different atoms or ions. Non-protein nitrogenous compounds (NPNC) such as urea, uric acid, creatine, and creatinine are commonly used as biomarkers of human kidney health.

Keywords: non-enzymatic ; electrochemical ; perovskite-based sensor ; human health biomarkers

1. Sensing of Glucose

Glucose, as the major energy supply substance of organisms and an intermediate product of metabolism, plays a vital role in the physiology and biochemistry of living organisms. Glucose promotes the body's ability to remember, stimulates calcium absorption, and increases cellular communication ^[1]. However, the overexpression of serum glucose triggers adverse effects in the body, leading to chronic damage and dysfunction of various tissues, especially diabetic retinopathy, diabetic cataracts, and peripheral neuropathy. Hence, there is value in detecting and quantifying the glucose content in the human body. Traditional home blood glucose monitoring (HBGM) methods are invasive, involving blood analysis through a blood drop from one fingertip, the reaction of the glucose in the blood with the indicator band, and the final reading content on the glucometer screen. This type of biosensor is based on a thin layer of glucose oxidase (GOx) on an oxygen electrode. Thus, the readout is the amount of oxygen consumed by GOx during the enzymatic reaction with the substrate glucose ^[2]. In the test strip, a GOx enzyme reacts with glucose to create gluconic acid. The gluconic acid reacts with ferricyanide, which then combines to create ferrocyanide. The amount of ferrocyanide produced is related to the glucose concentration in the blood sample by an electronic current passing through the testing strip. The glucose concentration is indicated by the number displayed on the screen of the glucose testing meter. A compressive review of electrochemical glucose biosensors was reported by Wang ^[3]. According to this research, glucose biosensors can be categorized into three groups: (i) first-generation biosensors that require the use of a natural oxygen co-substrate, the generation and detection of hydrogen peroxide (H_2O_2), and the application of a relatively high potential on the electrode; (ii) second-generation biosensors that involve replacing the oxygen co-substrate with a synthetic electron acceptor capable of transporting electrons from the enzyme to the surface of the electrode; and (iii) third-generation biosensors that are based on the elimination of the electron-transfer mediator and the application of a relatively low operating potential.

Recently, the development of non-enzymatic glucose biosensors has been directed toward the obtention of non-invasive detection mechanisms, which avoids the hassle of taking a blood sample through determinations in other body biological fluids such as saliva, sweat, urine, and tears. The study of non-enzymatic sensors also has given rise to detection systems with reduced costs, avoiding the use of expensive and unstable enzymes. The use of enzymes presents the disadvantage of low stability, in addition to variations in sensory activity due to factors such as pH, the presence of inhibitory agents, and enzyme immobilization ^{[4][5][6]}. These non-invasive sensors are based on electrodes where the electrocatalytic oxidation of analytes is carried out on the surface. Therefore, the selection of the material for modifying the electrode surface, the electroactive area, morphology, porosity, and rugosity can significantly impact the final performance of the sensor ^[7]. $NiFe_2O_4$ (NFO) nanoparticles were used to immobilize GOx on a carbon paste electrode (CPE) coated with $LaNi_{0.5}Ti_{0.5}O_3$ (LNT) perovskite particles, finding that at a ratio of 20:1 LNT-NFO, the enzymes were effectively immobilized, obtaining a glucose sensor with a linear range of 0.5–10 mM and a detection limit of 0.04 mM ^[8]. In this sense, perovskites are materials able to retain a three-dimensional network microstructure while keeping large amounts of oxygen vacancies and accommodating different metallic elements within the lattice structure ^[9]. A recent review established that the mechanism for glucose electro-oxidation in perovskites is complex and similar to the mechanism of

H₂O₂ electro-oxidation [10]. This mechanism is known as the lattice-oxygen-mediated oxygen evolution reaction (LOM-OER), which involves the partial substitution of the cations of the A sites by divalent cations from group IIA of the periodic table, which can oxidize the cations of the B sites. The increase in these substitutions causes a reduction in the formation energy of oxygen vacancies, which transfer oxygen from the lattice to the adsorbed species, generating highly oxidative oxygen species known as superoxide ions.

The properties of the perovskites are mainly determined by the element, substitution, and occupation of the B sites [11]. In this sense, perovskites doped with noble metals (AB_{1-y}M_yO₃) may present improved stability and catalytic activity. To verify this, the SrPd_{1-y}M_yO₃ perovskites were studied, where M represents the noble metals tested (Ni²⁺, Cu²⁺, Au³⁺, and Pt²⁺) [12]. From these cations, Au³⁺ at low concentrations, i.e., 0 < y < 0.7, presented the best characteristics for glucose sensing since the anodic peak current increased, reaching a maximum value at y = 0.3. The dopant promoted the electronic properties of the perovskite and increased the charge transfer rate while stabilizing the orthorhombic structure. The glucose sensing in human serum showed a wide concentration range of 0.2–1000 µM, high sensitivity of 1.44 × 10⁴ µA/mM cm², low detection limit of 2.11 nM, and no interference from other molecules. Silver nanoparticles have been extensively studied as catalysts in various reactions due to their excellent electrical conductivity. Hence, by adding Ag NPs to a La-Ti perovskite (LaTiO₃-Ag_{0.2}), it has been possible to decrease the detection limit to 0.21 µM, increase the sensitivity to 784.14 µA/mM cm², and broaden the range of linear response of 2.5 µM to 4 mM [13].

Perovskites containing Ni at the B sites, known as nickelates (ANiO₃, where A = La, Nd, Eu, Gd, Dy, or Sm), have also attracted the attention of glucose sensing due to their specific conductivity, specific surface area, and electroactivity. The contribution of some of these rare earths in the A position of the perovskite, as in the case of Nd, is to provide a high specific conductivity to the electrode. For example, NdNiO₃ perovskite synthesized by the hydrothermal method and deposited on a carbon electrode showed excellent electrocatalytic behavior for the oxidation of glucose in human serum [14]. The electrochemical results showed a wide linear range from 0.5 µM to 4.6 mM, a sensitivity of 1105.1 µA/mM cm², and a detection limit of 0.3 µM. The non-enzymatic glucose oxidation mechanism in this type of perovskite involves the oxidation of nickel (Ni²⁺ → Ni³⁺ + e⁻) and the subsequent oxidation of glucose (Ni³⁺ + glucose → Ni²⁺ + glucolactone). LaNi_{0.5}Ti_{0.5}O₃ (LNT) deposited on a carbon paste electrode (CPE) showed a sensitivity to glucose of 1630.53 µA/mM cm², limit of detection of 0.07 µM, and linear range of 0.2 to 20 µM [15]. In electrodes modified with perovskites of La_{0.6}Sr_{0.4}FeO_{3-δ} and La_{0.6}Sr_{0.4}Co_{0.2}Fe_{0.8}O_{3-δ} (LSF and LSCF, respectively), the partial substitution of Fe by Co atoms increased the sensitivity towards the detection of H₂O₂ and glucose [16]. The partially occupied *d* orbitals allow these transition metals to assume different oxidation states through the loss or acquisition of electrons, which promote the formation of a surface layer of adsorbed OH species. Glucose detection results for the LSCF perovskite obtained at a potential of 0.5 V were a linear range of 0–200 µM, detection limit of 7 µM, and sensitivity of 285 µA/mM cm².

The formation of oxygen vacancies has also been identified as a key parameter to increase the glucose sensing capacity through surface area modification. He et al. studied the increase of oxygen vacancies by replacing trivalent cations such as La³⁺ with divalent cations such as Sr²⁺ or Ca²⁺. Additionally, the presence of these cations promotes a growth in surface area and structural stability. The doping of the SrPO₃ perovskite with Ca, in the concentration range of 0–0.7, allowed setting the formulation Sr_{1.7}Ca_{0.3}PdO₃ with optimal results in glucose sensing [17]. This performance was mainly attributed to the structural modification of the crystalline lattice, where the increase in the free volume allows a greater mobility of the surface lattice oxygen. The sensing results showed a linear range of 5 µM to 1.4 mM, a sensitivity of 306.9 µA/mM cm², and a detection limit of 0.0845 µM. Determining glucose in urine samples showed relative standard deviations lower than 4.17%. In the case of the perovskite La_{0.6}Sr_{0.4}CoO_{3-δ} (LSC), its behavior as a glucose sensor was enhanced by depositing on a reduced graphite oxide (RGO) electrode, presenting a detection range of 2–3350 µM, sensitivity of 330 µA/mM cm², and detection limit of 0.063 µM [18].

Surface area and grain size modification and the creation of porous structures represent an alternative strategy to improve the sensing performance of perovskite-based materials. For example, LaNiO₃ perovskites, prepared by electrospinning, showed a decrease in the diameter of the fibers after the removal of polyvinyl pyrrolidone by calcination, which worked as the binding agent. The reduction of the fibers decreased from 400 to 140 nm, giving rise to a rougher surface and increasing the specific surface area and the number of active sites for the electrochemical reaction. The Ni ions are the active sites with partially occupied *d* orbitals that produce two different oxidation states for the metal (Ni^{II}/Ni^{III}). The detection limit for glucose was 0.32 µM and a linear detection range of up to 1 mM. Electrospun and calcined La_{0.88}Sr_{0.12}MnO₃ perovskites deposited on a CPE presented a linear range of 0.05–100 µM, a sensitivity of 1111 µA/mM cm², and a detection limit of 32 nM [19]. The morphological characterization showed rough surface fibers with diameters of 200–300 nm and lengths of several micrometers. ABO₃-type perovskite structures were employed for modifying a carbon electrode with the electrospinning of porous La–Sr–Co–Ni–O (LSCNO) nanofibers [20]. According to these authors, when metallic elements such as Ni, Co, and Fe are placed in perovskite B sites, they promote a high activity in glucose sensing.

Then, after testing the perovskite's performance in glucose detection, they found an optimal composition of the form $\text{La}_{0.75}\text{Sr}_{0.25}\text{Co}_{0.5}\text{Ni}_{0.5}\text{O}_3$, showing a linear response in the range of 0.1–1.0 mM with a calibration sensitivity of $924 \pm 28 \mu\text{A}/\text{mM}$, a low detection limit of 0.083 mM, and $0.59 \pm 0.03 \text{ mM}$ of glucose in urine fluid sample.

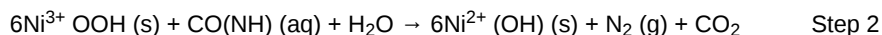
Because of its high electrocatalytic activity, perovskite-carbon nitride composite is an attractive potential candidate for interference-free sensing of glucose [21]. A perovskite-type cerium-aluminum oxide (CeAlO_3 , molar ratio Ce: Al of 1:1), in the form of polycrystalline powder, was ultrasonically mixed with carbon nitride (CN). The formed $\text{CeAlO}_3@\text{CN}$ composite was deposited on a glassy carbon electrode. Then, it was tested as the working electrode in a conventional electrochemical three-electrode cell configuration. The voltammetry detection of glucose showed a linear concentration range of 0.01–1034.5 μM and a detection limit of 0.86 nM. The amperometric determinations of glucose in human blood, saliva, and sweat were compared against those determined in a commercial glucometer, finding similar results with less than five percent error. A photoelectrochemical biosensor based on different semiconductor heterojunctions was constructed to reduce electron–hole pair recombination while retaining the oxidation potential of the composite material [22]. For this purpose, strontium titanate (SrTiO_3) perovskite was synthesized by hydrothermal method and employed as a working electrode in tandem with titanium oxide (TiO_2), polydopamine (PDA), and GOx. PDA was deposited on the final surface of the modified electrode and employed as the binder to increase the affinity between GOx and the perovskite. The $\text{TiO}_2\text{-SrTiO}_3\text{-PDA-GOx}$ system presented an extended linear range of 0–32 mM, a high sensitivity of $5.37 \mu\text{A}/\text{mM cm}^2$, a detection limit of 25.68 μM , and accurate detection of glucose in human serum in the range of 0–20 mM. The proposed mechanism describes hydrogen peroxide production from the surface reaction of glucose and GOx and its subsequent oxidation by photogenerated holes at the $\text{SrTiO}_3\text{-TiO}_2$ interface to generate a photocurrent. The SrPdO_3 perovskite coated with gold nanoparticles presented a synergistic effect with a higher current response for the electro-detection of glucose with a linear concentration range of 100 $\mu\text{M}/\text{L}$ to 6 mM/L, detection limit of 10 $\mu\text{M}/\text{L}$, and no interferences detection [17]. This system allowed the glucose oxidation mechanism explanation based on the oxidation potentials: (i) the dehydrogenation of the adsorbed glucose molecules (–0.55 V), (ii) oxidation of dehydrogenated glucose (+0.056 V) to gluconolactone, and (iii) conversion of gluconolactone to gluconate by reacting with hydroxyl ions in solution.

Other glucose sensing techniques not based on electrochemical measurements include colorimetry, fluorometry, and chemiluminescence. The hydrolysis reaction of glucose by GOx produces water, oxygen, and many protons, lowering the pH and harshening the environment of the sensing probe. Thus, cesium-lead-bromide perovskite quantum dots were coated with SiO_2 ($\text{CsPbBr}_3@\text{SiO}_2$ PQDs) to avoid environmental deterioration and the loss of optical properties such as light emission. This system showed excellent fluorescence properties with an absorption of UV radiation at a wavelength of 365 nm, an emission of green light at 525 nm, a notable selectivity to pH, and a detection limit of 18.5 μM [23]. The development of fluorescence measurements by eliminating multiple background interferences has led to a noticeable improvement in detection sensitivity and accuracy. This has been achieved by exciting a material at one wavelength and obtaining emission at two wavelengths. Metal halide perovskite nanocrystals present unique optical properties for this purpose. CsPbBr_3 hydride perovskite nanocrystals (PNCs) complexed with Cu NCs emit at two wavelengths: PNCs at 645 nm and NCs at 517 nm [24]. Upon the glucose sensing, the system presented a linear response range of 2.0–170.0 μM , a detection limit of 0.8 μM , and satisfactory results when tested with human serum. Electrochemiluminescence (ECL) sensors are based on the synergistic effect of electrochemical oxidation or reduction of a chemical compound that emits light. With $\text{LaTiO}_3\text{-Ag}_{0.1}$ deposited on a working electrode and luminol as a chemiluminescent agent, a promising glucose sensor was reported [25]. Properties as glucose sensors in human blood serum samples have a linear range of 0.01 μM –0.10 mM, a detection limit of 2.50 nM, and a sensitivity of $7.80 \times 10^2 \mu\text{A}/\text{mM cm}^2$. These properties are explained in terms of the increase of the active surface area of the working electrode caused by the perovskite composite oxide, while doping with Ag promotes the interfacial electron-transfer rate, and the luminol oxidation generates the ECL effect.

2. Sensing of Urea

According to the definition of the British encyclopedia [26], urea is a nitrogenous product of the metabolic breakdown of proteins in all mammal species, present in the urine, blood, bile, milk, and all mammal species perspiration. While the proteins break down, amino groups are removed from the amino acids, which could convert later to ammonia, which is toxic to the body and converted into urea by the liver. The urea then passes to the kidneys and is excreted in the urine. If this process is disturbed, ammonia levels begin to rise. High urea levels in blood and urine cause kidney failure, urinary tract obstruction, gastrointestinal bleeding, dehydration, burns, and shock, while lower levels of urea result in hepatic failure, nephritic syndrome, and cachexia [27][28][29]. The electrochemical oxidation of urea from perovskite-based sensors is catalyzed by cation B. In an alkaline medium, the adsorption of OH^- groups takes place on the electrode surface. Then, these groups react with the B metal to form the $\text{M}^{2+}(\text{OH})_2$ species. These molecules are oxidated to produce M^{3+}OOH ,

corresponding to the reactive species that carry out urea electrooxidation. $M^{3+}OOH$ reacts with $CO(NH_2)_2$ and water molecules to produce $M^{2+}(OH)_2$, N_2 and CO_2 . For example, Tran reported the synthesis of a nanocomposite based on CeO_2 -modified $LaNi_{0.6}Fe_{0.4}O_3$ perovskite and MWCNT [30]. Nickel cations were responsible for the catalytic activity, while the CeO_2 and multiwall carbon nanotubes imparted stability and increased the surface area, respectively. The authors report that the mechanism of electrochemical oxidation of urea is carried out in two steps, following the next reaction mechanism:



The urea detection level is of prime importance in clinical diagnosis and as a biomarker of the kidneys and liver functions. The most common methods for this important biomarker detection are UV-vis and infrared spectroscopy, ion and liquid chromatography-mass spectrometry, and fluorimetry [30][31][32][33]. Previously, the drawbacks of the enzymatic sensing of glucose, which are also applicable to urea, were mentioned. The typical amperometric urea biosensors are based upon the detection of ammonium ions resulting from the hydrolysis of urea in the presence of the enzyme urease viz. Due to the formation of ionic species by the reaction with the enzyme (NH_4^+ and HCO_3^-) in the presence of water, the electrochemical techniques become the suitable methodology for both the identification and quantification of urea. Thus, perovskite-based non-enzymatic detection systems for sensing analytes related to human kidney health were recently reported. Most of the studies have been focused on the improvement of (i) the surface area, (ii) imparting chemical stability, (iii) increasing the sensing performance, and (iv) reducing the cost of the fabrication of the sensor. For example, Tran et al. [30] used the perovskite of $LaNi_{0.6}Fe_{0.4}O_3$ in a composite with CeO_2 to improve the electro-oxidant activity towards urea. In addition, multi-wall carbon nanotubes (MWCNT) were included in the modified indium tin oxide (ITO) electrode, which improved the active electrochemical surface area. In the presence of urea, electrochemical oxidation is carried out by the reaction mechanism for the $Ni^{2+}(OH)_2/Ni^{3+}OOH$ pathway. Kumar et al. [34] reported the electrochemical detection of uric acid with a GP electrode modified with perovskite-type $XFeO_3$ (with $X = La, Ce, Pr, Sm, Gd, Tb$, and Dy). In every case, the perovskites were obtained in nanometric size with mainly cubic morphology in the 30 to 50 nm range. They found that the best charge transfer, using a standard $[Fe(CN)_6]^{4-}/[Fe(CN)_6]^{3-}$ redox system, occurred when $X = La$ with phosphate buffer saline (PBS) electrolyte at a pH of five. The modified electrode was tested with human serum and urine samples, finding high precision in its application in clinical diagnosis. Another study for the detection of uric acid employed a graphite paste electrode modified with a neodymium ortho-ferrite perovskite ($NdFeO_3$) to investigate the electrochemical behavior of the modified electrode. A standard $[Fe(CN)_6]^{4-}/[Fe(CN)_6]^{3-}$ redox system was used and compared against the unmodified electrode (blank). The modified electrode showed an oxidation current almost three times higher than the blank, evidencing its greater efficiency in charge transport. This was verified in the determination of uric acid at a pH of five, employing cyclic voltammetry. The sensor showed high stability after 30 days and high reproducibility [35]. Nanocubes of $ZnSnO_3$ perovskite, in its orthorhombic phase, were used for uric acid detection [36] by cyclic voltammetry and differential pulse voltammetry. Before analyte detection, a study to determine the optimal electrochemical surface-active area produced by the $ZnSnO_3$ charge was carried out. It was determined that with 6 μL of 0.5 wt% dispersion, an effective surface area of 0.1041 cm^2 was obtained, which favors the increment of the number of electrocatalytic active sites for redox reactions [36]. The detection of uric acid was attributed to the Sn^{2+}/Sn^{4+} oxidation states of tin, which favored the high electrochemical stability and sensor selectivity. Soundharraj et al. [37] reported urea sensing using a graphite electrode modified with zinc titanate ($ZnTiO_3$) nanoparticles prepared by the sol-gel method. The modified electrode showed a peak current increase in the presence of urea compared to graphite alone. This was related to the transfer of electrons from the amine groups to the $ZnTiO_3$ conduction band. The subsequent reduction in current with the increase in urea content was attributed to a delay in electrical conductivity due to an oxidation augment induced by the TiO_2 . Chakraborty et al. [38] employed a mixture of cerium copper oxide ($CeCuO_x$) and nickel oxide (NiO) nanoparticles, $CeCuO_x/NiO$, for the detection of Lipocalin 2 (LCN2). LCN2 higher than standard levels may indicate various human health conditions, including kidney damage. Before the assembly of the silicon-based electrodes, a heat treatment was performed on the $CeCuO_x$, which was corroborated by cyclic voltammetry, showing that annealing at 800 $^{\circ}C$ produced the best electrochemical behavior. Subsequently, when adding the NiO nanoparticles, an improvement in the electrochemical activity was observed, which was explained first as an electronic transfer reaction from $CeCuO_x$ to NiO , followed by a redox exchange reaction between Fe , $Fe^{3+/2+}$ in LCN2, and $Ni^{3+/2+}$ of the NiO nanoparticles. The sensor showed high stability for 14 days and high selectivity in the presence of common interferents in human serum. In addition, high sensing precision was found when compared with human serum samples spiked with LCN2.

Perovskite-type transition metal oxides have high catalytic activities due to high electronic conductivities. Manna et al. modified a glassy carbon electrode with the composite rhenium oxide (ReO_3)/reduced graphene oxide (rGO) for uric acid detection. The high electrochemical response of the composite was attributed to the plasmonic behavior at the edges of

the ReO_3 nanostructure, which favors electron transfer. It was found that the presence of interfering species, such as glucose and some metal ions, does not significantly alter the detection of uric acid. This study also indicated that the modified electrode has good repeatability, so it can be applied to real samples. Analyses of synthetic human serum spiked with uric acid showed an acceptable recovery, in the order of 10%.

It should be noted that other works dedicated to electrochemical non-enzymatic sensing with perovskites have reported the detection of some of the analytes of interest in this section. However, in these studies, these compounds were only used as interferents [39][40]; therefore, they were not considered in this review.

3. Sensing of Hydrogen Peroxide (H_2O_2)

Hydrogen peroxide (H_2O_2) is a reactive oxidant species (ROS) in all aerobic organisms [41][42]. It is a byproduct of aerobic metabolism and physiological processes such as immune activation [43], apoptosis [44], and cell growth [45]. Cells must remove this molecule due to oxidized biological targets, causing different diseases and pathologies [43] like cancer [46], cell damage [47], and inflammatory diseases [48].

H_2O_2 is not only a metabolic byproduct but also an oxidant reactive employed in food [49], pharmaceutical [50], cosmetics [51], and chemical industries [52]. Due to the relevance that H_2O_2 represents, its quantification and detection become necessary. In the last decades, hydrogen peroxide sensors have been developed, mainly based on colorimetric [53], fluorescent [54], and electrochemical [55] methodologies. This last kind of sensor has attracted much attention due to its relative facility for developing commercial sensor devices. The reported electrochemical hydrogen peroxide sensor could be also enzymatic [56] or non-enzymatic [57]. The non-enzymatic sensor generally requires metal compounds that make the redox reaction for hydrogen peroxide detection. Perovskite-based materials are bimetallic compounds that show excellent electrochemical and redox properties. Conventionally, these materials were employed to explore the redox properties of perovskites to develop electrochemical sensors. The most applied conventional formula of perovskites to sense hydrogen peroxide is also the ABO_3 form. The most employed elements are from the lanthanide group or alkaline earth metals, where La, Sr, and Ca are the most important. Cations in B positions are usually transition metals from the fourth group of the periodic table, in which Ni, Co, Fe, and Mn are more frequently reported [58]. These cations (B) are responsible for interacting with hydrogen peroxide through the redox reaction $\text{B}^{(\text{M})+}/\text{B}^{(\text{M}+1)+}$, producing molecular oxygen, water, and electrons as the responses of the electronic signal, which makes these substances electrochemically detectable and measurable. Due to lattice oxygen transfer, a redox mechanism occurs [18]. In contrast, the role of A cation is to stabilize the diverse B cation oxidation states in redox reactions. It has been reported that the general performance and redox behavior could be improved by partial substitution of A cations with the same element but with different oxidation states (A') [58]. The partial substitution of B with other cations with similar redox behaviors, B' , is a strategy to improve redox efficiency [59].

The development of materials as non-enzymatic H_2O_2 sensors has gone through several stages. The first studies focused on exploring different A cations and observing the electrocatalytic performance of these cations on H_2O_2 decomposition. For example, the enthalpy of the formation of various perovskite copper oxides (ACuO_3) with other A cations ($\text{A} = \text{La}, \text{Sr}, \text{Ba}, \text{Na}, \text{or K}$) and doped $\text{La}_{1-x}\text{CuO}_3$ perovskite (where $\text{X} = \text{Ca}, \text{Sr}, \text{or Ba}$) requires higher exothermic oxidation energies than lanthanum nickelates and cobalt [60]. The research on the effect of cation A and possible dopant agents was complemented by varying the ratio of each cation and observing their effects. In perovskite oxides of $\text{La}_{1-x}\text{Ca}_x\text{MnO}_3$ ($\text{X} = 0.0, 0.2, 0.4, \text{ and } 0.6$) using the citrate gel method, the concentration of calcium in perovskites affects the catalytic activity. This behavior is attributed to the non-stoichiometry δ -oxygen and the average valence states of Mn responsible for the catalytic activity in the decomposition of hydrogen peroxide in an alkaline solution [58].

Nevertheless, the perovskite doped with Sr showed the most sensing sensitivity of H_2O_2 [59]. The synthesis of $\text{La}_{1-x}\text{Sr}_x\text{MnO}_3$ perovskites by sol-gel methodology and calcinated between 550 and 750 °C for 5 h displayed considerable catalytic activity in an alkaline medium by reducing the analyte concentrations [61].

The morphology of perovskites also plays a fundamental role in sensor development since this strategy impacts the superficial area of the electrode and, therefore, the active sites available for detection and sensing of the interest analytes. In this sense, the synthesis methodology is the most significant variable in the resulting morphology. Perovskite nanostructures have been synthesized by hydrothermal, solvothermal, and electrospinning routes, evidencing significant changes in their morphology and physicochemical properties. Thus, the same CaTiO_3 system has been synthesized by several authors, evidencing a wide variety of morphologies ranging from butterfly-like dendrites [62], cubic particles [63], hollow micro-cubes [64], nanolamellate [65], and pyramids [37]. Also, a calcination procedure impacts the final morphology. For instance, electrospinning and sequential calcination of perovskite oxide LaNiO_3 nanofibers reduced the fiber's

diameter from 400 to 140 nm. The corresponding amperometry sensor of H_2O_2 and glucose developed with LaNiO_3 nanofibers showed a fast response and long-term stability [19]. Likewise, $\text{La}_{0.7}\text{Sr}_{0.3}\text{Mn}_{0.75}\text{Co}_{0.25}\text{O}_3$ nanofiber for sensing H_2O_2 in alkaline buffer, synthesized through electrospinning and calcination, was produced as fibers with a diameter in the 250 to 300 nm range [66]. Other morphologies reported are nano brushes of KNbO_3 synthesis by hydrothermal [67] and nano-structured perovskite oxide LaNiTiO [68].

Interest in the investigation to develop new perovskite structures, combining different cations (A and B), doped or pure, to improve some properties of the sensor, such as the limit of detection, the linear range of detection, the sensitivity, and the selectivity, increased after 2009. I.e., two B cations and their ratios have been studied to improve the sensing properties of LaCaBO perovskites. They described the behavior of carbon electrodes loaded with $\text{La}_{0.6}\text{Ca}_{0.4}\text{BO}_3$ (B = Cr, Mn, Fe, Co, and Ni) perovskites oxides prepared by the amorphous malate precursor method, which provides a specific surface area in the range of 8–30 m^2/g . These values are considerably larger than those obtained by the conventional acetate decomposition method [69]. The potentiometric response of these perovskites was tested in the presence of H_2O_2 , showing that material doped with Fe presented the best characteristics to develop a potentiometric sensor. Also, perovskite oxide $\text{La}_x\text{Sr}_{1-x}\text{CoYFe}_1\text{-YO}_{3-\delta}$ was synthesized by the citrate method, and a comparison of its sensing properties showed that cobalt added to the ferrite structure enhanced the sensitivity, the linear range, and the detection limit [16]. Also, SrFeO_3 perovskite doped with Ce showed that the presence of both cations improved the sensor performance [67]. A 3D macroporous SmCoO_3 perovskite hydrogen peroxide sensor presented the lowest detection limit of 0.004 μM and a linear detection in the 0.1 to 10,000 μM range, while the presence of glucose, ascorbic acid, dopamine, and uric acid did not show interference activity [68]. The properties of this sensor may be related to the hexagonally ordered microporous crystalline structure. Rare earth cuprate perovskites (Ln_2CuO_4 where Ln = Sm or La) were employed for sensing H_2O_2 and presented broad detection ranges [70]. The perovskite with Sm exhibited a detection range of 1.24–35,440 μM , while La showed a value of 0.50–23,350 μM but a lower LOD. Additionally, the highest sensitivity sensor toward H_2O_2 with a value of 1667.9 $\mu\text{A mM}^{-1} \text{cm}^{-2}$ was reported for a $\text{La}_{0.9}\text{Sr}_{0.1}\text{NiO}_3$ nanofiber perovskite; this structure provides excellent ion transport channels and contact sites for the H_2O_2 sensing [71].

Amperometric and potentiometric (cyclic voltammetry, CV) approaches are the most employed characterization techniques to evaluate the sensing capacity and electrocatalytic activity of perovskites. The way these electrochemical probes work is different. A potentiometric test measures the potential (voltage) between a working and reference electrode in a system with no significant current flow. The working electrode surface is modified so that changes in potential can be correlated with the changes in the analyte concentrations.

X-ray diffraction (XRD) is commonly employed to elucidate the perovskite crystalline structure. Ideally, perovskites of the form ABX_3 are arranged into a cubic cell, where the atoms are just touching each other, and the B–X distance is equal to $a/2$ and the A–X distance is $2a/2$, where a is the cube unit cell length and the relationship between radii of ions holds $RA + R_x = 2RB + R_x$ [72][73][74]. However, distorted structures with tetragonal, rhombohedral, or orthorhombic symmetry have been found, mainly arising from smaller A ions that induce a tilting of the BX_6 octahedra to optimize A–X bonding. ABO_3 perovskites, where cation A is typically larger than cation B, are known to exhibit cubic, hexagonal, or even polytypism consisting of mixed cubic and hexagonal stacking of AO_3 layers.

X-ray photoelectron spectroscopy (XPS) is also performed to probe the surface oxidation states and compositions, the binding energies, and the relative amounts of different metal and oxygen species on the surface available for the catalyst of the analyte. The characterization technique is also helpful in identifying the lattice oxygen species (O_2^-), highly oxidative oxygen species (O_2^{2-/O^-}), hydroxyl groups or the surface adsorbed oxygen (HO^-/O_2), and adsorbed molecular water (H_2O).

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