Thermal and light irradiation effect on the electrocatalytic performance of Hemoglobin modified Co₃O₄-g-C₃N₄ nanomaterials for oxygen evolution reaction

Carlos Leal-Rodriguez,†a Daily Rodriguez-Padrón,†a Zeid A. Alothinman,*b Manuel Cano,*c Juan J. Giner-Casares,† Mario J. Muñoz-Batista,†d Sameh M. Osmanb and Rafael Luque*a,b

Oxygen evolution reaction (OER) plays a key role on the water splitting process and a high energy conversion efficiency is essential for the definitive advance of hydrogen-based technologies. Unfortunately, green and sustainable development of electrocatalysts for water oxidation is nowadays a real challenge. Herein, a successful mechanochemical method is proposed for the synthesis of a novel Hemoglobin (Hb) modified Co₃O₄/g-C₃N₄ composite nanomaterial. The controlled incorporation of Cobalt entities as well as the Hb functionalization, without affecting the g-C₃N₄ nanoarchitecture, was evaluated using different physicochemical techniques, such as X-ray diffraction, N₂-physical sorption, scanning electron microscopy, UV-visible and X-ray photoelectron spectroscopies. The beneficial effect of the resulting ternary bioconjugate together with the influence of the temperature and light irradiation were investigated by electrochemical analysis. Under 60 °C and light exposition, this electrocatalyst requires an overpotential of 370 mV to deliver the current density of 10 mA cm⁻², showing a Tafel slope of 66 mV dec⁻¹ and outstanding long-term stability for 600 OER cycles. This work paves the way for the controlled fabrication of multidimensional and multifunctional bio-electrocatalysts.

1. Introduction

The anthropogenic climate change together with the depletion of fossil fuels have motivated the rapid progress of hydrogen-based technologies, such as fuel cell and as carrier energy storage for harnessing renewable sources.¹⁻³ However, the future global application of these green and sustainable energy technologies requires the development of low-cost, efficient and durable electrocatalysts for electrochemical processes. For instance, the simplest method for solar to chemical energy conversion is water splitting, which involves the chemical breakdown of water into oxygen and hydrogen. These anodic and cathodic processes are known as oxygen (OER) and hydrogen (OER) evolution reactions, respectively. Although both need electrocatalysis, OER requires a higher theoretical overpotential of 1.23 V to provide a suitable current.⁴⁻⁷

Recently, transition-metal based electrocatalysts are emerging as an interesting alternative to the classical noble metals (e.g. Pt for HER and RuO₂/IrO₂ for OER).⁸⁻¹¹ Among all these chemical elements, Cobalt-based materials have been extensively investigated for water oxidation through different approaches, including oxides,¹²,¹³ hydroxides,¹⁴,¹⁵ chalcogenides,¹⁶ carbides,¹⁷ nitrides,¹⁸ silicates,¹⁹ phosphides,²⁰,²¹ alloys,²²,²³ etc., to name only a few. Another interesting strategy is focused on Cobalt-supported onto conducting carbonaceous composite materials, such as graphene,²⁴ carbon nanotubes,²⁵,²⁶ and its nitrogen-derivatives.²⁷,²⁸ The latter approximations provide not only an enhanced OER electrocatalytic performance but also a substantial reduction of the total Cobalt content.

Inspired in all these approaches and considering that graphite carbon nitride (g-C₃N₄) is a well-known metal-free electrocatalyst for HER and oxygen reduction reaction (ORR),²⁹⁻³² Wang et al.³³ and Zhu et al.³⁴ have recently reported two different Co₃O₄/g-C₃N₄ composite materials (i.e. Co₃O₄/g-C₃N₄ supported on N-doped graphene - 42.9%Co₃O₄@g-C₃N₄/NG- and Co₃O₄ supported on phosphorus-doped g-C₃N₄ -60%Co₃O₄/P-C₃N₄-, respectively) as efficient electrocatalysts for OER, demonstrating the enormous potential of this type of inorganic-organic hybrid combination. In this same direction, herein we propose a mechanochemical-assisted method to fabricate a novel multifunctional inorganic-organic biocomposite, which uses hemoglobin (Hb) as alternative material for reducing to 10 % the amount of Co₃O₄ into the Co₃O₄/g-C₃N₄ composite. Hb is a tetrameric redox protein formed by four polypeptide chains in which

![Scheme 1. Schematic representation of the proposed mechanochemical-assisted method. Molecules and NPs are not drawn to scale.](image)

Please do not adjust margins
each polypeptide domain encloses at least one iron active redox centre that provides both hierarchically structure and porous framework to the whole. This redox protein has been previously reported by Compton et al. as an outstanding electrocatalytic biomaterial for ORR. Our characterization results demonstrate the beneficial effect between three components (i.e. g-C3N4, Co and Hb acting as porous network, catalytic transition-metal and tertiary structure, respectively) and provide insights on the influence of the temperature and light irradiation on its electrocatalytic performance, paving the way for the rational design and easy fabrication of multidimensional and multifunctional electrocatalysts with desired performances for hydrogen-based technologies.

2. Experimental

2.1. Synthesis of materials

The graphitic carbon nitride material (g-C3N4) was obtained using a previously optimized protocol. The g-C3N4 (Fig. 1A) was obtained by calcination of melamine (Sigma-Aldrich) in a semi-closed crucible at 580 °C for 2 hours and using a heating rate of 5 °C min⁻¹. The obtained g-C3N4 was subsequently modified following a mechanochemical protocol to obtain ternary catalytic systems with minor amounts of CoO and hemoglobin (Hb). The preparation of Co3O4 spacers (pure reference and composites) was accomplished following a mechanochemical procedure at 350 rpm during 10 min. The binary samples (Co3O4/g-C3N4) was prepared employing the previously synthesized g-C3N4 and [Co(NO3)2·6H2O] as cobalt precursor. Four materials, containing different Co concentrations (1, 2.5, 5 and 10 wt. %), were prepared. The syntheses were performed in a Retsch PM100 ball mill, using a 125 mL reaction chamber under vacuum (10⁻⁶ Torr) and the measurements accomplished at room temperature using a conventional X-ray source with a Phoibos 150-MCD energy detector. XPS results were analyzed employing the XPS CASA software (version 2.3.15) and C 1s contribution utilized as energy calibration reference (284.6 eV). The UV-vis diffuse reflectance spectroscopy experiments were carried out using a LAMBDA 365 UV/Vis Spectrophotometer and the data processed employing the UV-Express software.

2.2. Characterization of materials

X-ray diffraction patterns were obtained using a Bruker D8 Advance Diffractometer with the LynxEye detector and acquired in a 2θ scan range from 10° to 70°. Phase identification was accomplished with Bruker Diffrac-plus Eva software. Brunauer–Emmett–Teller (BET) surface areas and porosity parameters were analyzed by N2 physisorption measurements (Micromeritics ASAP 2000 equipment). The morphology and elemental composition of the samples were investigated by Scanning Electron Microscopy (SEM)/X-ray Energy Dispersive Spectra (EDX) analysis acquired in the JEOL-SEM JSM-7800 LV scanning microscope. XPS experiments were performed on 4x4 mm² pellets in an ultrahigh vacuum multipurpose surface analysis instrument SpecTran. The samples were outgassed overnight in a pre-chamber under vacuum (10⁻⁶ Torr) and the measurements accomplished at room temperature using a conventional X-ray source with a Phoibos 150-MCD energy detector. XPS results were analyzed employing the XPS CASA software (version 2.3.15) and C 1s contribution utilized as energy calibration reference (284.6 eV). The UV-vis diffuse reflectance spectroscopy experiments were carried out using a LAMBDA 365 UV/Vis Spectrophotometer and the data processed employing the UV-Express software.

3. Results and discussion

Materials were prepared using a solvent-free (calcination/mechanochemistry) protocol (Scheme 1). Mechanochemical methods have broadly demonstrated their great applicability for nanomaterials design. A milling-assisted deposition of cobalt oxide nanoparticles (Co3O4 NPs) on the surface of g-C3N4 material was successfully achieved. Fig. 2 shows representative TEM images of the 10%Co/g-C3N4 composite sample and its pure constituents (i.e. Co3O4 NPs and g-C3N4). Further modification of the obtained materials with hemoglobin was also efficiently performed by a mechanochemical protocol. Operating conditions detailed described in experimental section allow obtaining a dominant Co3O4 phase as well as the stable deposition of the bio-entities hemoglobin, both with potential in electro catalytic applications. It is worth to highlight that solvent-free protocols and in particular mechanochemistry possesses an inherent sustainable character, avoiding the use of solvents and additional reagents in the synthesis of the materials. As well, the aforementioned methodology is highly

---

2 | J. Name., 2012, 00, 1-3

This journal is © The Royal Society of Chemistry 20xx
reproducible, versatile and simple, as well as allowing the preparation of a wide range of materials in short reaction times. Such advantages could motivate the scientific community to investigate the scalability and possible industrial applications of mechanochemistry.

The crystalline structures of the synthesized carbon nitride material and post-modified samples have been studied by X-ray diffraction analysis as shown in Fig. 3. Pure g-C₃N₄ as well as binary (XCo/g-C₃N₄) and ternary (Co/g-C₃N₄-Hb) composite samples showed as dominant contributions the two characteristic peaks of g-C₃N₄ that appear at 13.1° and 27.4°, associated with the crystallographic planes (100) and (002), respectively.⁴⁴-⁴⁶ These data suggest that the crystalline structure of the g-C₃N₄ material has not been modified after the mechanochemical treatment or after the incorporation of Co and Hb species. As well, it can be observed a small peak at 36.85°, associated with crystallographic plane (311), which was not found in the g-C₃N₄ sample. Such characteristic peak could be associated with Co₃O₄,⁴⁷ therefore confirming the successful formation of Co₃O₄ species and the effective deposition of cobalt counterpart on the g-C₃N₄ material.

![Fig. 3 XRD patterns of the prepared samples.](image)

The optical properties of the synthesized materials were studied by UV-vis spectroscopy, as shown in Fig. 4. The pure g-C₃N₄ presented the expected profile with absorption from UV to visible light and a bandgap value of 2.7 eV, which is in accordance with the expected profile for graphitic carbon nitride materials.⁵⁰,⁵¹ Although some variation in the absorption edge of the composite samples (Co/g-C₃N₄) can be observed, the profile bellow 450 nm is dominated by the major g-C₃N₄ component, leading to band gap values in a range of 2.7-2.9 eV. Incorporation of the Co entities on the surface of the g-C₃N₄ drove to an enhancement of the abortion above 450 nm. The broad contribution situated at ~580 nm (inset) has been previously detected and associated with direct transitions at 1.45 eV, corresponding to O²⁻ to Co³⁺ excitation charge transfer.⁵² The final modification with Hb described small differences in comparison to the composite support (10%CoOₓ/g-C₃N₄), with negligible variations of the bandgap and a decrease of the absorption band generated by the Co₃O₄ counterpart. Such finding motivated the use of these materials as photo-electro-catalysts using sunlight-type illumination source.

![Fig. 4 UV-vis spectra of the synthesized samples.](image)

The chemical properties of the samples were also studied by XPS measurements. The XPS regions of carbon 1s (C 1s), nitrogen 1s (N 1s), oxygen 1s (O 1s) and cobalt 2p (Co 2p) of 10% Co/g-C₃N₄ and 10% Co/g-C₃N₄-Hb samples were acquired, as shown in Fig. S1. It is important to note that very marked differences were detected in the C 1s region before and after the incorporation of Hb (Fig. S1A and S1E). In both samples, the C 1s XPS region showed three contributions corresponding to C–C, C–N and C–O, located around 284.6, 287.2 and 288.9 eV, respectively. The enhancement of the intensity of the C–C and C–O contributions in the 10% Co/g-C₃N₄-Hb sample can be exclusively attributed to the incorporation of the protein on the surface of the material.⁵³ In turn, in the sample 10% Co/g-C₃N₄-Hb, the peak located at 287.2 eV can be attributed to C–N bonds from structural moieties of g-C₃N₄,⁵⁴,⁵⁵ as well as from peptide bonds in the protein chains. The incorporation of Hb modified the

<table>
<thead>
<tr>
<th>Sample</th>
<th>Area BET (m²/g)</th>
<th>Pore volume (cm³/g)</th>
<th>Pore size (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>g-C₃N₄</td>
<td>25</td>
<td>0.10</td>
<td>20.1</td>
</tr>
<tr>
<td>1%Co/g-C₃N₄</td>
<td>25</td>
<td>0.10</td>
<td>19.8</td>
</tr>
<tr>
<td>2.5%Co/g-C₃N₄</td>
<td>24</td>
<td>0.09</td>
<td>19.3</td>
</tr>
<tr>
<td>5%Co/g-C₃N₄</td>
<td>23</td>
<td>0.09</td>
<td>18.9</td>
</tr>
<tr>
<td>10%Co/g-C₃N₄</td>
<td>19</td>
<td>0.08</td>
<td>18.5</td>
</tr>
<tr>
<td>10%Co/g-C₃N₄-Hb</td>
<td>19</td>
<td>0.08</td>
<td>18.5</td>
</tr>
</tbody>
</table>

The textural properties of the samples were determined by N₂ adsorption-desorption measurements and are shown in Table 1. The surface area of g-C₃N₄ material was found to be in good accordance with the reported values in the literature.³⁸,⁴⁸-⁵¹ In addition, it was observed a decreasing trend of this property with the increment of the cobalt concentration. Even if such variation was small, it could indicate that cobalt oxide particles were partially blocking the pores of the g-C₃N₄ structure. On the other hand, it was observed that the incorporation of Hb had a negligible influence on the surface of the materials. In any case, the small differences detected after functionalization with Co and Hb strongly suggested that the textural properties are given by the main component (g-C₃N₄). The same conclusion can be deduced from the pore volume and the pore size data.
The final XPS spectra of C 1s, by overlapping contributions, without considerable structural distortion of g-C₃N₄ sample. The deconvolution of N 1s XPS region, in both materials showed the presence of three bands around 398.7, 399.5 and 400.8 eV, which were attributed to C–N–C bonds, NH residues and π excitation in g-C₃N₄ based materials, respectively (Fig. S1B and S1F). In particular, for the protein modified material, the peak located at 398.7 eV can also be associated with nitrogen from Hb peptide bonds. On the other hand, the XPS region of O 1s exhibited two contributions in 530.9 and 532.0 eV, which can be assigned to O-Co/O and O-C bonds, respectively (Fig. S1C and S1G). The increased intensity of the O-C band (Fig. S1G) may be related to the incorporation of the protein on the surface of the 10% Co/g-C₃N₄ material. Fig. S1D and S1H showed the Co 2p XPS region. The relative low variations of the binding energies from Co²⁺ to Co³⁺ are difficult to precisely identify cobalt chemical environments in cobalt oxides materials. However, a dominant presence of Co³O₄ is suggested based on the peak shapes of the spectra and the relatively low intensity of satellite peaks, characteristic of Co³⁺ in CoO species.

The samples with the highest Co concentration were analysed by SEM-mapping. The obtained micrographs are shown in Fig. S2A-K. It could be observed the presence of carbon and nitrogen atoms, uniformly distributed. On the other hand, although some agglomerated areas are perceived in the oxygen and cobalt mapping images, it can be affirmed that cobalt oxide nanoparticles are homogeneously distributed over the surface of the g-C₃N₄ material. The 10%Co/g-C₃N₄-Hb sample was also analysed by SEM-mapping. The obtained results are shown in Fig. S2F-K. In this case, the analysis revealed the presence of sulphur in the sample, most likely from the polypeptide chains of hemoglobin, particularly in methionine and cysteine amino acids.

The electrocatalytic OER activity of the different synthesized composite materials and its constituent components were measured at room temperature and according to the detailed conditions in the experimental section. Fig. 5A shows their LSV plots, where a progressive increase of the OER activity of the different Co/g-C₃N₄ composites can be observed with the percentage of cobalt. Surprisingly, the 10%Co/g-C₃N₄ composite provides only a slight
lower OER activity than the 100%CoO₄ sample, indicating a favourable effect of the CoO₄ nanoparticles into the Co/g-C₃N₄ composite. In addition, the introduction of Hb into the 10%Co/g-C₃N₄ composite enhances significantly the resulting OER performance, which is even better than the one obtained for the 100%CoO₄ sample. This improvement can be attributed to the tertiary architecture of Hb with hierarchical structure and its iron active redox centre. Table 2 summarises some of the most important OER electrocatalytic parameters, which further confirm the observations above.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Onset potential (V)</th>
<th>Overpotential (mV at 10 mV·cm⁻²)</th>
<th>Maximum current density (mA·cm⁻²)</th>
<th>Tafel slope (mV·dec⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bare GC</td>
<td>1.70</td>
<td>-</td>
<td>1.0</td>
<td>-</td>
</tr>
<tr>
<td>g-C₃N₄</td>
<td>1.68</td>
<td>-</td>
<td>2.0</td>
<td>-</td>
</tr>
<tr>
<td>CoO₄</td>
<td>1.54</td>
<td>430</td>
<td>47.5</td>
<td>78.0</td>
</tr>
<tr>
<td>1%Co/g-C₃N₄</td>
<td>1.58</td>
<td>470</td>
<td>33.0</td>
<td>67.4</td>
</tr>
<tr>
<td>2.5%Co/g-C₃N₄</td>
<td>1.59</td>
<td>460</td>
<td>32.0</td>
<td>70.9</td>
</tr>
<tr>
<td>5%Co/g-C₃N₄</td>
<td>1.56</td>
<td>450</td>
<td>37.0</td>
<td>71.8</td>
</tr>
<tr>
<td>10%Co/g-C₃N₄-Hb</td>
<td>1.54</td>
<td>440</td>
<td>35.0</td>
<td>81.8</td>
</tr>
<tr>
<td>10%Co/g-C₃N₄-Hb</td>
<td>1.54</td>
<td>420</td>
<td>50.0</td>
<td>71.9</td>
</tr>
</tbody>
</table>

Next, in order to improve the OER electrocatalytic performance of this tricomponent composite (i.e. 10%Co/g-C₃N₄-Hb), LSV measurements were carried out under light irradiation and at 60 °C (Fig. 5C). The finding shows that the temperature increase combined with the visible light-mediated photoexcitation improves significantly the OER electrocatalytic response, providing both cathodic shift of the onset potential (from 1.54 to 1.51 V) and increase of the maximum current density (from 50 to 80 mA·cm⁻²), whilst the Tafel slopes maintained almost invariable as it can be observed in Fig. 5D. These results agree with the study reported by Zhang et al., who demonstrated an optimization of the HER activity of g-C₃N₄ through visible light irradiation. They also suggested that g-C₃N₄ was a stable semiconductor, whose band-gap covered both the water reduction and water oxidation potentials. On the other hand, the comparison against others Co-based electrocatalysts reported in the literature, our tricomponent composite material shows excellent properties as OER electrocatalyst, requiring an overpotential of 370 mV to deliver the current density of 10 mA·cm⁻², and with a Tafel slope of 66 mV·dec⁻¹. The latter values are quite similar that the ones reported recently for a 60%CoO₄/g-C₃N₄ modified GC electrode, despite of using a 50 % higher amount of CoO₄. Finally, a durability test was carried out in order to assess the deterioration of its electrocatalytic performance after 600 chronoamperometric OER cycles. Fig. 5E shows that the percentage of the initial current density practically did not change after all these cycles. Fig. 5F shows the LSV curves obtained before and after the long-term stability study with the tricomponent composite material, further confirming the outstanding electrocatalytic behaviour for water oxidation.

**Conclusions**

We have demonstrated the excellent features provided by mechanochemical method for the synthesis of a novel multidimensional and multifunctional biomanomaterial. The incorporation of small amounts of cobalt, as well as the functionalization with hemoglobin, was evaluated using different techniques such as XRD, N₂-physisorption, SEM, UV-vis and XPS. As it was revealed by XRD and N₂ adsorption-desorption, neither the incorporation of CoO₄ nor Hb altered the nanoarchitecture of the g-C₃N₄. Similarly, XPS analysis confirmed the maintenance of the native-like structure of this redox protein into the bionanocomposite. Finally, electrochemical analysis confirmed the outstanding electrocatalytic performance for OER of the tricomponent biomanomaterial under 60 °C and light irradiation. This work opens the route for the development of novel composite biomaterials for diverse technological applications.

**Conflicts of interest**

There are no conflicts to declare.

**Acknowledgements**

Rafael Luque gratefully acknowledges Spanish Ministry of Economy and Competitiveness (MINECO) for funding project CTQ2016-78289-P, co-financed with European Regional Development Funds (FEDER). Daily Rodriguez-Padrón also gratefully acknowledges MINECO for providing research contract under the same project. Support from the MINECO of Spain is acknowledged through the MANA (CTQ2017-83961-R) and JEANS (CTQ2017-92264-EXP) projects. J.J.G.-C. acknowledges the MINECO for a “Ramon y Cajal” contract (#RYC-2014-14956). M.C. thanks to the FEDER and the Andalusian Government (Consejería de Economía, Conocimiento, Empresas y Universidades, Junta de Andalucía) of Spain for the financial support through UCO-1263193 project. The authors also thank Prof. Dr. J. M. Rodríguez-Mellado of the Department of Physical Chemistry and Applied Thermodynamics at the UCO for kind access to the rotating disk equipment. This work was supported by the Distinguished Scientist Fellowship Program (DSFP) at King Saud University, Riyadh, Saudi Arabia.

**References**
