Summary

Over the last decade nanotechnology have been substantially developed allowing the discovery of new downsized scale-based materials with different properties compared to their bulk counterparts. The evolution in the synthesis, preparation and application of new nano and microscale nanomaterials has opened new opportunities for the creation of new analytical devices with improved properties.

Carbon nanomaterials are widely used in electrochemistry due to their chemical inertness, relatively wide potential window, low background current, and suitability for different types of electroanalysis. Among carbon nanomaterials, carbon black has been recognized as an electrode material with comparable heterogeneous electron-transfer rate (HET) to other more *noble* carbon nanomaterials such as graphene or carbon nanotubes (CNTs), with the advantage of a much simpler manufacturing and lower costs.

Transition metal dichalcogenides (TMDs) are a family of compounds with MX_2 formula, where M is a transition metal element, typically from groups IV (Ti, Zr, Hf), V (V, Nb or Ta) and VI (Mo and W), and X is a chalcogen (S, Se or Te). These materials possess a layered structure. The atoms in of the MX_2 formula are strongly held together by covalent bonds, whereas each triatomic layer is only linked to its neighbors by weak Van der Waals interactions, forming layered materials. These properties allow a top-down approach for producing nanosheets of these compounds by using intercalation chemistry or by liquid phase exfoliation allowing individual sheets to be separated from each other forming 2D materials very easily. These features had led to an exponential growth in the research interest in the last five years. However, even though there is growing interest in these materials just few applications for sensing purposes has been developed. TMDs-based nanocomposites and hybrid nanoarchitectures are the most widespread. These strategies avoid restacking and expand the narrow potential window and low conductivity of TMDs, improving the general electrochemical performance. While TMDs have been widely explored as supercapacitors and catalyst for the hydrogen evolution reaction (HER), very little research has been carried out regarding their capabilities as sensing element, especially in the food analysis.

Detection of hydrogen peroxide is still of the paramount significance and it is conventionally carried out using platinum-based electrochemical sensors. Poor selectivity of the latter towards hydrogen peroxide reduction (due to reduction of oxygen at same potentials) requires detection by its oxidation at high anodic potential causing oxidation of easily oxidizable compounds worsen the selectivity. Another problem of platinum-based electrocatalysts is poisoning of their surface limiting, for example, analysis of sweat. Prussian blue-based electrodes solve these drawbacks. Prussian blue is selective to H_2O_2 reduction allowing its detection at low potential in the presence of oxygen. This almost completely avoids the problem of reductants. Prussian blue is insensitive to compounds poisoning platinum thus, for example, allowing analysis in cell culture media. For all these reasons, PB stands out as the best choice for measuring H_2O_2 in cell culture as marker of oxidative stress.

On the other hand, microfluidic systems and lab-on-a-chip (LOC) technologies offer excellent features to improve the analytical performance by reducing analysis time, decreasing extremely the consumption of sample and reagents, integrating multiplexed analysis, and provide the possibility of development of analysis integrated in cell culture. Electrochemical detection provides high sensitivity, allows miniaturization, and it is highly compatible with micro and nanotechnologies due to the simplicity of the instrumentation required. Nevertheless, the surface characteristics of nanomaterials can further improve the sensitivity as well as other important characteristics such as antifouling properties. Up to now the fabrication of these devices relied on clean-room-based fabrication method, which are not available for most laboratories. While clean room manufacturing may still provide powerful research-scale solutions, many clinical and biological applications have obviated some of the need for the ultrafine resolution of photolithographic techniques and hence are very suitable for being fabricated employing alternative manufacturing methods. Low-cost fabrication techniques (3D printing, laser cutting or xurography) and electrochemical detection create a powerful combination for the fabrication of ultra-low-cost disposable devices to perform (bio)-chemical assays.

Therefore, this exciting hybrid area of research is expected to make important contributions to diverse fields, leading to new capabilities that are currently beyond our reach and bringing major benefits to our quality of life.

In this doctoral thesis, different nano- and micromaterials such as carbon black (CB), transition metal dichalcogenides (TMDs) and Prussian Blue (PB), have been employed in the development of new (LOC-based) miniaturized strategies by exploiting their unique and improved electrochemical properties in two well-defined sensing topics:

1. Improved detection and determination of antioxidants in food samples.
2. Reliable detection of hydrogen peroxide as marker of oxidative stress (OS) in living cells.

To this end, this Doctoral Thesis has three defined objectives:

1. To synthetize and characterize novel TMDs-based nanomaterials.
2. To develop hybrid novel CB/TMDs-based electrochemical sensors for antioxidant determination in food samples.
3. To study oxidative stress in living cells and functional food protection on cell culture employing a PB-based electrochemical chip.

To achieve these objectives, the main milestones have been set as follow:

1. Study the exfoliation of group VI TMDs (MoS_2, WS_2, $MoSe_2$ and WSe_2) using different organic solvents and mix of water-surfactants.

2. Characterization of the exfoliated nanomaterials by scanning electron microscopy (SEM), UV-Vis spectroscopy and Raman spectroscopy.

3. Design, characterization, and evaluation of TMDs-based electrochemical transducers.

4. Evaluation of CB/TMDs hybrid transducers for PPs electrochemical determination in complex food environments.

5. Design, characterization, and development of PB-based electrochemical sensors able to detect H_2O_2 released from living cells as oxidative stress (OS) marker.

6. Design, characterization, and development of a PB-based electrochemical chip for real-time detection of H_2O_2 released from living cells as oxidative stress (OS) marker.

After a discussed background about the topics covered along this doctoral thesis in Chapter II, the results of this Doctoral Thesis have been divided in chapters III to IX. Finally, general conclusions and concluding remarks regarding this doctoral thesis are collected in chapter IX.

Chapter III deals with the synthesis, characterization, and applications of MoS_2, as representative TMDs, that in combination with CB have been investigated. While MoS_2 exhibited remarkable antifouling properties towards olive oil PPs but low sensitivity, CB present a high sensitivity with a poor fouling resistance. Interestingly, CB-MoS_2 nanohybrids combines the best properties of each individual nanomaterial with a higher sensitivity than CB and retained antifouling properties of MoS_2. The developed electroanalytical platform has been employed in the analysis of o-diphenolic content in olive oil and related products showing an impressive correlation without significant statically differences with a well-establish HPLC-UV method.

In Chapter IV, the developed CB-MoS_2 electrochemical sensor is applied to catechins determination in cocoa-samples. Again, the ability to merge CB ability to enhance the electrochemical response and the MoS_2 antifouling property was found. In fact, catechins can attach to carbon and CB modified electrodes by forming an electroactive product, which is totally hindered in the case of MoS_2 and CB-MoS_2 electrodes. Moreover, a fast extraction procedure to achieve fast and eco-friendly PPs evaluation is studied. The developed method results highly correlated with well-established methods for PPs content and antioxidant activity.

Chapter V is related to the development of a new electroanalytical platform based on other Group VI TMD, WS_2 and its combination with catechin-capped gold nanoparticles (WS_2/AuNP-CT) into a CB network. The nanomaterial-based synergistic effect of the sensor results in enhanced selectivity, sensitivity, and reproducibility in the simultaneous determination of three-hydroxycinnamic acid (hCN), caffeic, sinapic, and *p*-coumaric acids and their structural analogs in food samples. The assembly of WS_2/AuNP-CT into CB network exhibited a further conductivity enhancement without loss of antifouling performance. The nanomaterial-based synergistic effect of the sensor results in enhanced selectivity and sensitivity.

Chapter VI presents a comprehensive study comparing the electrochemical performance of Group VI TMDs (MoS_2, WS_2, $MoSe_2$ and WSe_2). Their electrochemical properties have been studied revealing a superior performance of the selenides versus the sulfides. This trend is also observed for catechol-containing flavonoids but also an enhanced antifouling propertied is observed for TMDs compared to carbon electrodes. More importantly, in this chapter an explanation to the mechanism involved in their antifouling properties is highlighted, giving a deeper response to findings found and discussed in the previous chapters.

In Chapter VII the combination of Carbon Black (CB) and electrodeposited Prussian Blue (PB) covered with a Nafion layer on Screen-Printed electrodes (CB/PB-SPE) was used for non-enzymatic H_2O_2 sensing in Neuroblastoma cell line (SH-SY5Y) challenged with 6-hidroxidopamine (6-OHDA) for modelling Parkinson's disease. CB was demonstrated to play a key role in the electrodeposition and further electrochemical performance of the developed device. The sensor showed detection limit in the nanomolar range and excellent selectivity in a complex environment such as the culture medium used, allowing the selective determination of very low amounts of H_2O_2 without interferences. In addition, in this work H_2O_2 was quantified not just detected upon the instantaneous release from cells challenged to a stressor as usually reported in literature.

In Chapter VIII an innovative electrochemical multiwell chip is designed to monitor the real-time release of H_2O_2 by HeLa cells. The chips are composed by a set 8 of electrochemical sensors and produced employing a benchtop microfabrication technology allowing their ultra-low-cost production. Electrodes were characterized, in terms of inherent electroactivity and stability. Electrochemical sensing of H_2O_2 was carried out at -100 mV vs Ag|AgCl, with a LOD of 0.1 µM and linear range between 1 and 1000 µM. These features allow the interference-free real-time detection of H_2O_2 in HeLa cell culture, which are directly cultured in the electrochemical chips enabling high-throughput analysis. As proof of the developed chips, cocoa extracts were employed to test their ability to decrease the H_2O_2 production from HeLa cells demonstrating a dose-dependent decrease.

The main and transversal conclusion of this Doctoral Thesis is the demonstration of the potential of miniaturized nanomaterial-based electrochemistry in two sensing relevant applications: the potential of TMD in the analysis of relevant PPs in food samples together with the ability of PB-based sensors to detect and quantify oxidative stress in different cell lines. Hence, the main conclusions derived from this Doctoral Thesis are:

1. The incorporation of TMD to other nanomaterials in electrochemical sensor technology has been demonstrated to be highly relevant, resulting in a synergistic approach that combines the unique physical and chemical properties of TMD with the intrinsic benefits of carbon nanomaterials.

   Even though the relatively low intrinsic conductivity and narrow electrochemical window of TMD, their hybridization with other nanomaterials has allowed improving their inherent properties. Two enhanced properties have been identified:

   - Apparent electrocatalysis towards catechol-containing PPs.

   - Impressive antifouling properties during the oxidation of the catechol-containing PPs compounds.

These findings have also been demonstrated for the other compounds of the group VI TMDs; $MoSe_2$ and WSe_2. The mechanism underlying their antifouling properties has also been proposed for the first time.

2. PB-based sensors have demonstrated its potential in the evaluation of oxidative stress in cell lines. The incorporation of PB-based sensors in LoC devices have permitted the culturing of cells and direct *in-situ* evaluation of their oxidative stress status and the effect of food functional PPs on it.

- PB-based electrochemical sensors have enabled a reliable detection of oxidative stress (OS) in living cells towards hydrogen peroxide monitorization in two different cell cultures SH-SY5Y and HeLa.

- The sensors have been PB-based electrochemical sensors integrated in a LoC device have enabled the detection of the produced H_2O_2 in the culturing of HeLa cells. The device was able to effectively detect a decrease in the H_2O_2 production response of HeLa cells treated with cocoa extracts in a dose-dependent-way.