COST is supported by the EU Framework Programme Horizon 2020

STSM Scientific Report

COST Action FP1405 ActInPak

Hugo Spieser

Electrochemical printed biosensors

Development of Smart and Safe packaging

LGP2 (Laboratory of Pulp and Paper Science and Graphic Arts), Grenoble, France
Home supervisor: Dr. Julien Bras

WCPC (Welsh Center for Printing and Coating), Swansea, Wales
Host supervision: Pr. David Gethin

Content

Introduction and context

This STSM is collaboration between the Laboratory of Pulp & Paper Science (LGP2, Grenoble, France) and the Welsh Centre for Printing and Coating (WCPC, Swansea, Wales). The LGP2 activity starts from wood science to converting biomass and functional printing. The laboratory is then considered as expert in: biorefinery, multiscale biobased materials, functionalization of surface & printing. On the other hand, the WCPC is today one of the World's leading centres for research and development of printing and coating processes such as screen, flexographic, lithographic, rotogravure, digital and pad printing. The WCPC focuses currently on three research themes: functional printing, 3D printing and Graphics/Packaging.

This STSM is relevant to my PhD project called “Smart and safe packaging”. Today, packaging is expected to fulfil more and more requirements such as being light, strong, recyclable, biodegradable, etc. More recently the potential for the integration of advanced functionalities such as sensing, active functions and security is being explored. In this framework, this STSM proposes to explore the development of electrochemical printed biosensors that could be adapted to packaging applications. The biosensing field met a booming enthusiasm during the 1980-1990 years and is still today a deeply studied sector. The most known and commercialized example is the blood glucose strip test sensing for diabetes monitoring. A biosensor is by definition a device composed of a sensitive biological component and a physicochemical detector. The interaction between the sensitive component and the target compound is sensed and measured by the physicochemical detector.

The STSM visit included training using two principles, electrochemistry and impedance and two sensors (electrochemical and electrical) were designed to achieve this. Work focused principally on the electrochemistry method and due to confidentiality purposes, the design of the electrical sensor and its characterization will not be displayed in this report. So the work carried out focused on the sensing of an antigen by an antibody fixed on a printed three-electrochemical sensor. The working electrode is made of carbon and antibody can be grafted on the surface. Interaction between this antibody-grafted carbon electrode and the targeted sensed antigen will lead to a change in the electrochemical behaviour of the electrode. However, one of the key steps of the preparation of the electrode is to properly graft the antibody. Indeed, the inert carbon electrode needs first to be functionalized with an active molecule that enables the grafting of the antibody. The organization of the STSM can be divided in two parts. Firstly a commercial screen printed carbon electrode was tested for antibody grafting and antigen detection. Secondly, a sensor was designed, screen-printed and tested for the same application.

Materials and Methods

Materials

Commercial screen printed electrodes (SPEs)

Commercial screen printed electrodes (SPEs) DRP 150 from DropSens (Spain) were used. The design is explained on Figure 1: the reference electrode (RE) is made of silver, the working electrode (WE) is made of carbon (4 mm diameter), the counter electrode (CE) is made of platinum and electrical contacts are made of silver. The dimensions of the device are the following: 3.4 x 1.0 x 0.05 cm (length x width x height). Cyclic voltammetry measurements were performed using an adapter for the SPEs linking to a potentiostat (see Figure 1).

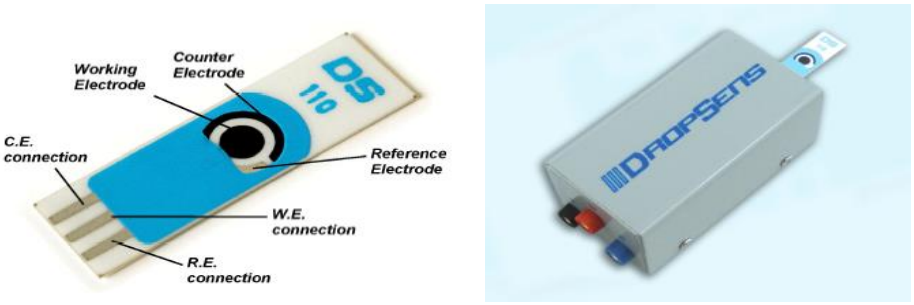

Figure 1: description of the SPEs DRP 150 from DropSens (left) and specific adapter for CV measurements (right).

Functionalizing agents and antibody/antigen system

For the purpose of training, rather than smart packaging application, an antibody/antigen system was tested targeting detection of the onset of Alzheimers and uses the antibody (AB) X and the antigen (AG) Y.

The chemical nature of the antigen/antibody couple (called here Y/X), and the functionalizing agent used to graft the antibody to the surface of the SPEs will not be displayed in this report as these are part of a highly confidential project that includes specific preparation protocols. The three different functionalisation agents are designated A, B and C. Similary the antigen preparation is confidential, however three concentrations were prepared.

Screen printing inks

For the screen printing, two different inks were used for the two different layers. The first layer was printed with a commercial Silver/Silver chloride ink, supplied by Gwent Electronic Materials (GEM) with code of (C2130809D5) and the second layer with a prototype carbon ink that has been prepared in the WCPC.

Methods

Preparation of the commercial SPEs and solutions

The surface of the SPEs was first looked at on an optical microscope to see if they are any damages or scratches that could possibly disturb the experiments. Electrochemical analyses are indeed highly sensitive and need damage-free surfaces. Dust was taken off using a nitrogen flow pistol.

The antibody solution was prepared by dissolving the powder in PBS (pH 7.5) and then prepared for grafting onto carbon using a confidential protocol that has been optimised for this purpose. A similar approach was set for the antigen, but at three concentrations ranging from 500 $ng.mL^{-1}$ to 1000 $ng.mL^{-1}$.

Screen printing

PET commercial sheets were used as substrates and the design sensor was printed on an ATMA AT-25PA screen printer. The design can be seen on Figure 2 with the two different layers, and the superposed result alongside. The two different layers can be printed with different inks and in this case, the first layer is composed of silver/silver chloride ink, and the second of carbon ink.

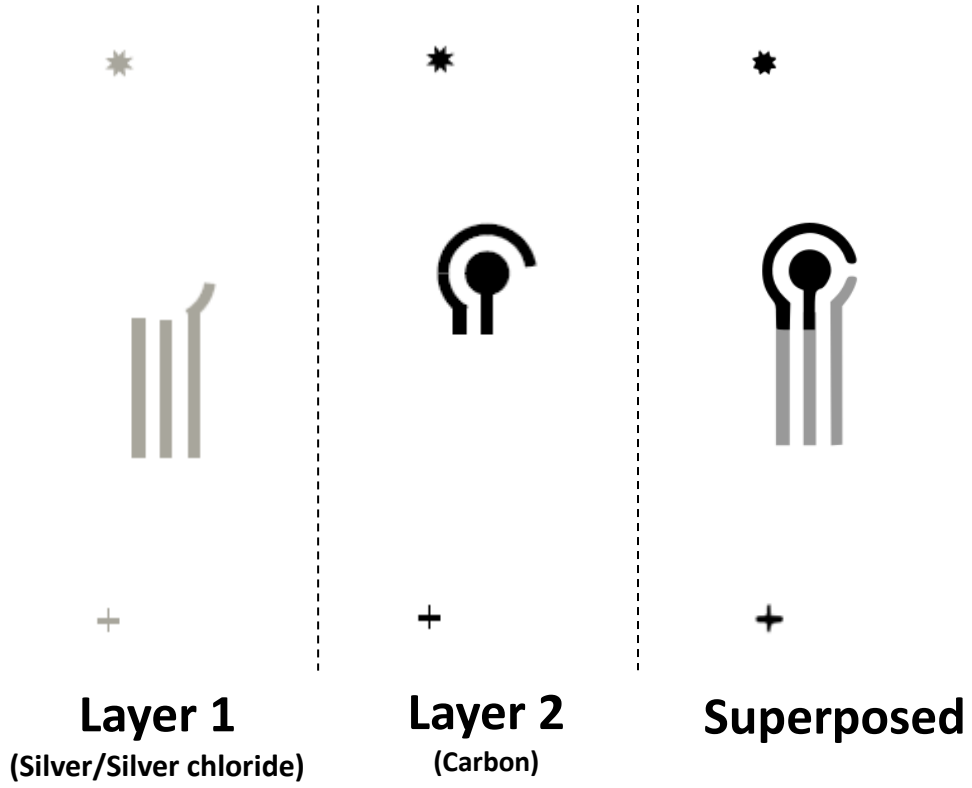

Figure 2: design of the electrochemical sensor.

When printing is complete the sensor design is as shown in Figure 3.

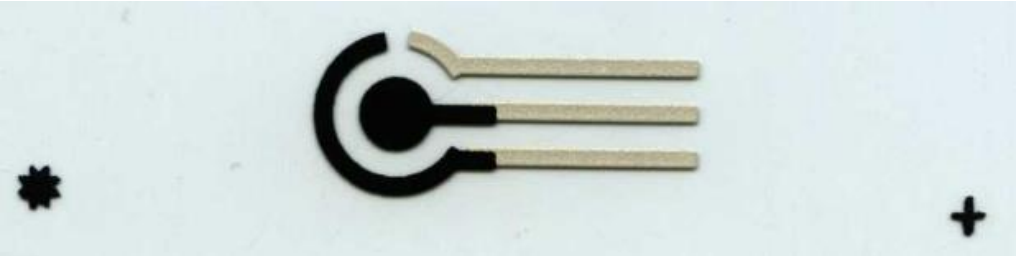

Figure 3: picture of the final printed electrochemical device with two-superposed layers.

Two different screen specifications were used for the printing: one specification for layer 1 and one specification for layer 2. The screens details are set out in Table 1. The theoretical ink deposit is given for a raw mesh and without taking in account of the Emulsion Over Mesh (EOM).

Table 1: Screens details for the screen printing process

	Screen 1	Screen 2
Mesh count (threads per cm)	61	110
Wire diameter (µm)	64	34
Emulsion over mesh (OEM) thickness (µm)	12	12
Screen angle (°)	22.5	22.5
Opening area (%)	37.16	39.19
Theoretical ink deposit ($cm^3.m^{-2}$)	47.57	25.65

The mesh and stencil were specified to deposit what is believed to be the right line thickness based on previous experience. Within the printing process, a drying step (130°C – 5 min) was conducted between each layer. Basically, during the screen printing process, the ink is applied through a screen-mesh and on the substrate. The general process is described on Figure 4.

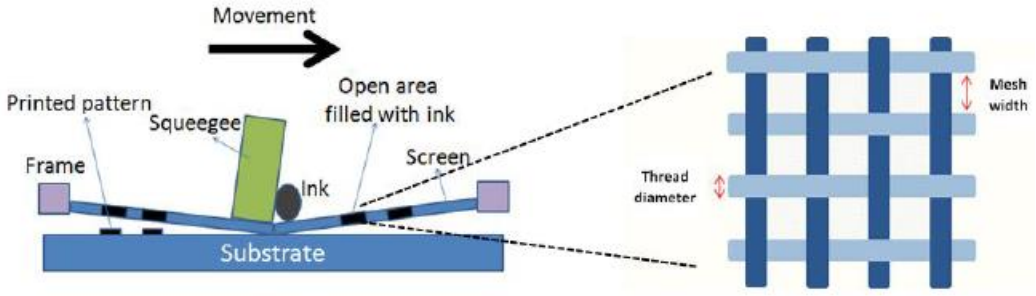

Figure 4: general description of the screen printing process.

Printing characterization

The as printed sensors morphology was characterized using a White light Interferometer Veeco Wyco NT9300. Seven different samples were tested and each measurement was made at least two times. Pictures were taken using an Alicona Infinite Focus at x10 magnification.

Cyclic voltammetry

The well-known redox couple potassium ferricyanide and potassium ferrocyanide ($K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$), is able to undergo both oxidation and reduction in a reversible redox reaction (equation below) resulting in both an anodic and cathodic peak.

$$Fe^{3+} \leftrightarrow Fe^{2+} + e^-$$

This is the solution that has been used in the experiment on the electrochemical sensor as an electrolyte. CV analysis was performed using an Autolab PGSTAT302N purchased from Metrohm.

SPE functionalization

The functionalization agents A, B and C were drop casted on the SPEs' working electrode (WE) using different volume (from 2 to 10 µL) and they were left to dry for 1 hour. After drying the SPEs were rinsed with distilled water for 10 s and dried with gentle nitrogen flow (a too harsh N_2 flow could damage the connection). Special care was taken not to wet the electrodes connection.

Antibody grafting

Antibody (AB) was deposited on the functionalized WE by the same method (drop-casting). 5 µL of AB solution was deposited on the surface of the WE and left for 10 min, but not to dry. The same washing/drying procedure was applied.

Antigen testing

Antigen solutions at three different concentrations were prepared: 500, 750 and 1000 $ng.mL^{-1}$. 10 µL of the desired solution was deposited on the functionalized/AB grafted WE and left to react for 10 min. It is important that the antigen solution does not dry completely on the surface. After that, the same washing/drying procedure was applied and characterizations done directly after the reaction.

Electrochemical analysis

Cyclic voltammetry (CV) was used to characterize the sensor. 10 µL of the electrolyte were deposited of the SPE (in order to cover the three electrodes WE, CE and RE) and the system was scanned from 0.6V to -0.6V with a scan rate of 50 $mV.s^{-1}$. Electrolyte solution is composed of $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (K3/K4) solution in Phosphate Buffer Solution (PBS) at pH 7.4. After measurements, the SPEs were rinsed with distilled water and dried with gentle N2 flow. This

procedure was applied for all the CV analyses at each step of the sensor preparation and the sensing process.

The goal of electrochemical analysis is to establish a link between the flow of electrons and the chemical changes taking place within a system. These chemical changes are often a reduction or an oxidation of a metal complex for inorganic chemistry. A potentiostat was used to vary the potential of an electrochemical system and the resulting collected current measured. The aspect of the oxidation and reduction peak are correlated to different parameters such as the formal potential of the studied couple, the number of electrons exchanged, the diffusion of the studied species, etc. Moreover, the aspect of those peaks is also correlated to the electrodes surface on which the reactions occur. The experimental electrochemical cell is composed of three electrodes: working electrode on which the electrochemical reactions occur, reference electrode against which the potential of the other electrodes are measured and the counter electrode which is used to complete the electrical circuit. These three electrodes are immersed in the electrolyte solution also containing the species of interest. The classic scheme of an electrochemical cell is presented on Figure 5 and can be compared with the SPEs electrode presented on Figure 1.

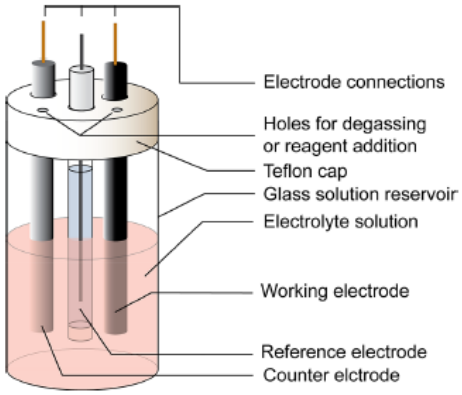

Figure 5: general scheme of a three-electrode electrochemical cell.

In this case we are looking at the behaviour of K3/K4 electrolyte on the SPEs. By varying the potential applied to the system, there is alternatively a reduction of Fe (III) (K3) into Fe (II) (K4) or an oxidation of Fe (II) (K4) to Fe (III) (K3). In order to gain familiarization with CV experiment, a basic CV curve obtained for the K3/K4 couple on an untreated commercial SPE was recorded and can be seen on Figure 6. This experiment was conducted following the parameters described in the Materials and Methods section.

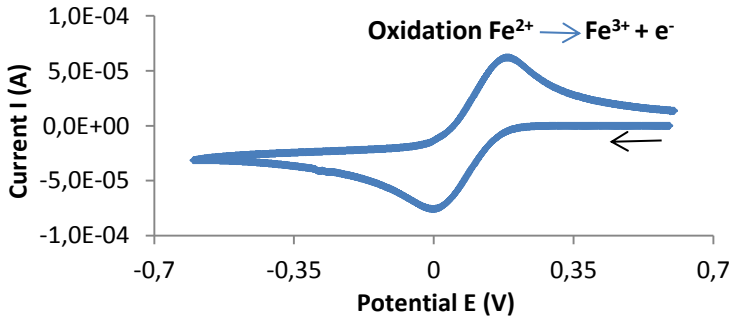

Figure 6: electrochemical analysis of untreated SPEs. Cyclic voltammetry was performed between -0.6 and 0.6 V at 50 $mV.s^{-1}$ in a Fe^{2+}/Fe^{3+} redox couple.

Different parameters can be sorted out from a CV curve: oxidation/reduction peak currents or voltages thus oxidation/reduction peak resistances, the amount of transferred charge during oxidation or reduction, difference between anodic and cathodic peak potentials, etc.

Results and discussion

Commercial SPEs

As described in the Materials and Methods section, three different steps are necessary to monitor all the process of this sensor: raw commercial SPEs functionalization, antibody (AB) grafting and antigen (AG) interaction responses (Figure 7).

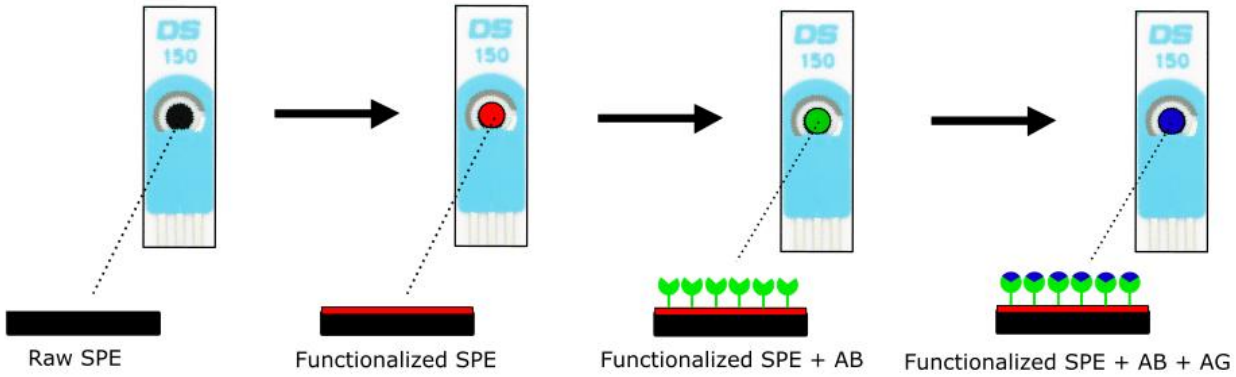

Figure 7: general scheme of the preparation of the sensor and the antigen sensing process.

At each step, CV curves were recorded using the reduction-oxidation system K3/K4, as the surface chemistry of the SPE dictates the aspect of the CV curves. If the surface chemistry of the SPEs changes, that means that there will be a change on the aspect of the CV curves. In this case, these changes could mean carbon functionalization, antibody grafting, or antigen/antibody interaction.

Functionalization was tested with different volumes deposited by drop-casting process. The deposited volume i.e. the thickness of coated layer is crucial for the sensing. Indeed, if the

deposited layer is too thick, there are no electron transports through the layer and toward the carbon WE and no signal can be then transported by the SPE to the potentiostat. If the layer is too thin, and there is some non-coated part on the surface of the WE, then the corresponding CV curve will be a superposition of raw WE and coated WE which hinders the interpretation.

The functionalization agents A, B, C (these are different solutions with different functional groups and contain amine and carboxylic groups, A is the solution without functional material, B contains amine group and C has carboxylic group) were drop-casted on SPEs using either 2 µL or 10 µl. It was found that 10 µL led to a too thick layer. Indeed, no oxidation/reduction of the electrolyte was measured for the 10 µL drop casted SPEs. This conclusion was made for all functionalization agents and the resulting graphs are not displayed here.

The functionalized SPEs' CV curves were compared to the blank reference and because of the modification of the electrochemical behaviour, functionalization was confirmed. (Figure 8)

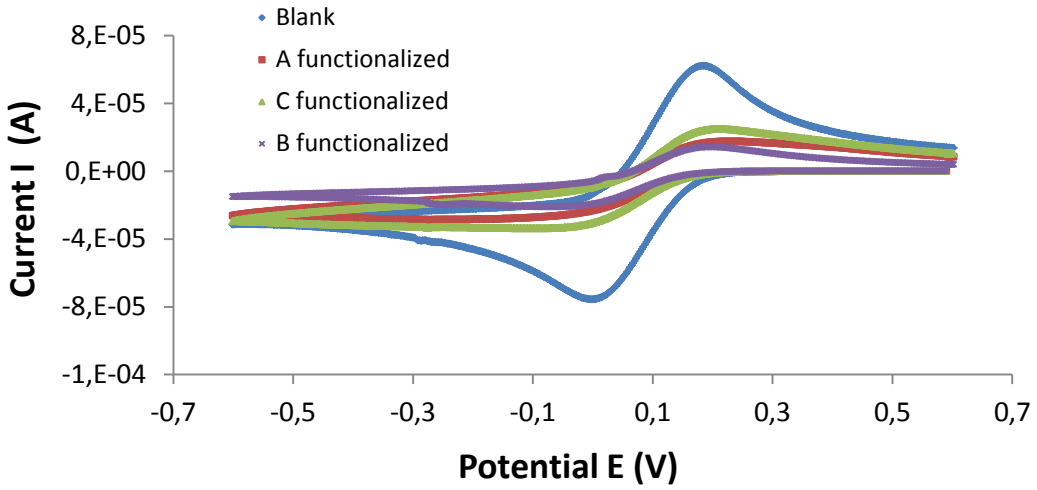

Figure 8: electrochemical analysis of A-, B- and C-functionalized SPEs compared to untreated SPE. Cyclic voltammetry was performed between -0.6 and 0.6 V at 50 mV.s^{-1} in a Fe^{2+}/Fe^{3+} redox couple.

The same method was used to investigate the antibody grafting and antigen response, on the A-, B- and C-functionalized SPEs. The results are displayed in figure 9, 10 and 11. For all samples, there are changes in the CV curves after antibody grafting indicating the successful attachment of AB. Also exposition to the antigen solutions modified the electrochemical behaviour, which proves the potential sensing capability.

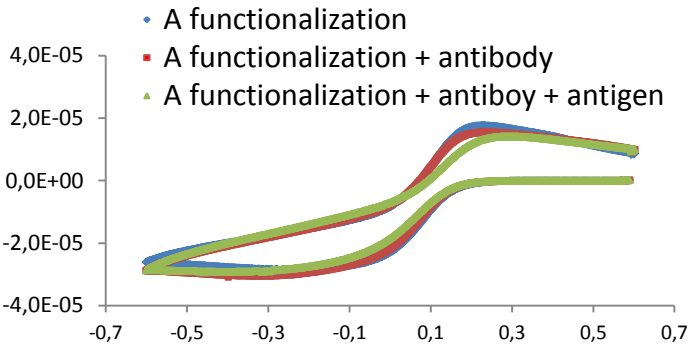

Figure 9: electrochemical analysis of A-functionalized SPEs with antibody grafting and antigen response. Cyclic voltammetry was performed between -0.6 and 0.6 V at 50 mV.s^{-1} in a Fe^{2+}/Fe^{3+} redox couple.

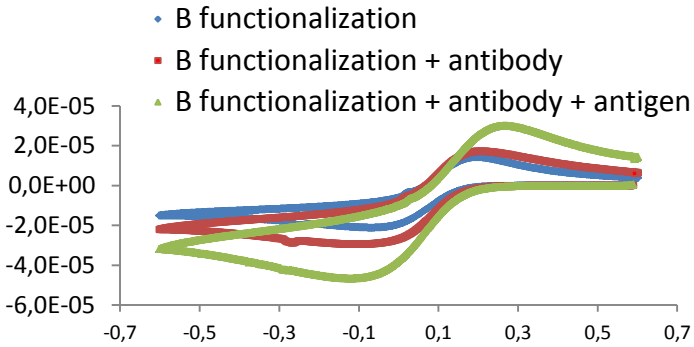

Figure 10: electrochemical analysis of B-functionalized SPEs with antibody grafting and antigen response. Cyclic voltammetry was performed between -0.6 and 0.6 V at 50 mV.s^{-1} in a Fe^{2+}/Fe^{3+} redox couple.

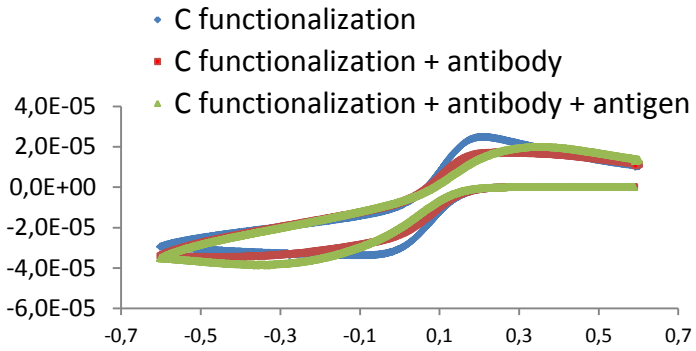

Figure 11: electrochemical analysis of C-functionalized SPEs with antibody grafting and antigen response. Cyclic voltammetry was performed between -0.6 and 0.6 V at 50 mV.s^{-1} in a Fe^{2+}/Fe^{3+} redox couple.

For quantitative analysis, only oxidation peaks will be investigated due to the symmetry of the graphs and reversibility of the redox reaction. The quantitative parameters relevant to compare between those steps are the amount of charge transferred during the oxidation (Q, coulomb), and the resistance of the system measured at the oxidation peak (R, ohm). In order to measure the quantity of charge transferred, the area below the oxidation peak was calculated using the trapezoidal rule (for positive current and potential, and using the scan rate 50 mV.s^{-1} as the step of the rule). The resistance was calculated by dividing the potential at the oxidation peak by the corresponding current. These data were calculated for each system and also for exposure of the antibody grafted SPEs to different concentration of antigen (500, 750 and 1000 ng.mL^{-1}). (Figure 12 and 13)

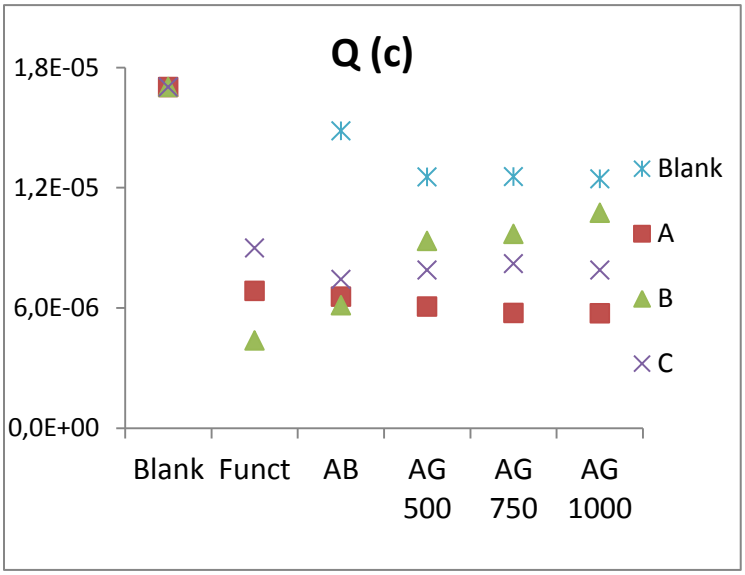

Figure 12: amount of charge transferred during the oxidation (Q) for the three (A, B and C) different functionalizations at the different steps of the sensing (Funct=functionalization step, AB=antibody grafting step, AG XXX=after exposure to antigen at XXX ng.mL^{-1} concentration)

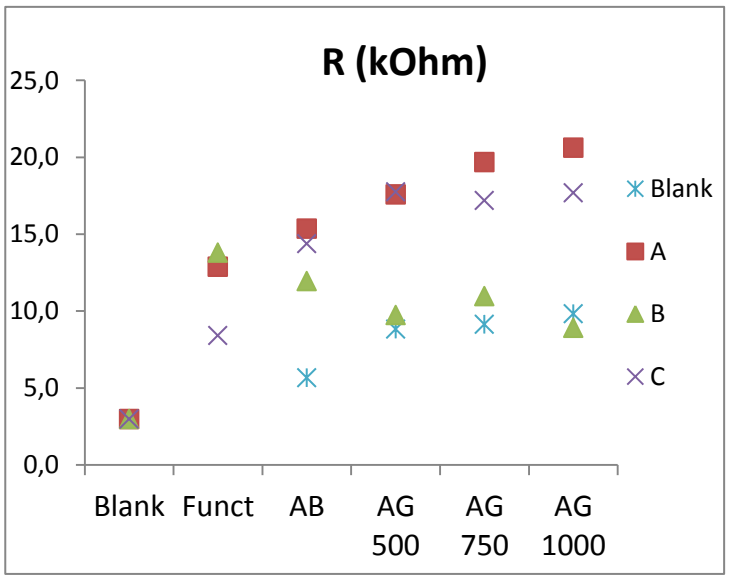

Figure 13: resistance of the system at the oxidation peak (R) for the three (A, B and C) different functionalizations at the different step of the sensing (Funct=functionalization step, AB=antibody grafting step, AG XXX=after exposure to antigen at XXX ng.mL^{-1} concentration)

Both graphs are showing that for the concentrations above 500 ng.mL^{-1}, all three samples are saturated and the sensing and detection limit has to be below 500 ng.mL^{-1}. These results are promising but remain preliminary tests and more characterizations tests are necessary to assess the performances of such sensors.

WCPC Printed SPEs

Printing morphology

As described in the Materials and Methods section, the design of the electrochemical electrodes printed in the WCPC are similar to the commercial SPEs. The major differences however are that the CE of commercial SPEs is made of platinum whereas it is made of carbon in the WCPC printed ones. Also the commercial SPEs substrate is made of ceramics whereas the WCPC ones were printed on flexible and transparent PET sheets. At the macroscale, the printing quality seems to be good enough (See Figure 3), however at the microscale there are small imperfections that could be eliminated through improved registration (the screen printing press did not have optical alignment capability).

In order to assess the quality of the SPEs developed in the WCPC, several parameters were measured using White Light Interferometry (see Materials and Methods section): the widths, thicknesses and roughness of the carbon and silver tracks, and the roughness of the carbon WE. The electrochemical sensor morphology was analysed using White Light Interferometry and Alicona imaging. The results are presented in the Table 2.

Table 2: morphology of the electrochemistry sensor assessed by white light interferometry

	Silver tracks	Carbon tracks	Carbon WE
Width (mm)	1.04 ± 0.01	1.07 ± 0.01	-
Thickness (µm)	17.57 ± 1.02	14.37 ± 0.62	-
Roughness (µm)	2.53 ± 0.13	12.91 ± 0.77	1.42 ± 0.29

The measured widths are consistent with the 1 mm designed width. Also the thicknesses and roughness are in line with what was expected. No interruptions of the tracks and no holes were seen and so the prints were concluded to be good for the sensing part. These conclusions were consistent with the pictures taken on the Alicona (Figure 14).

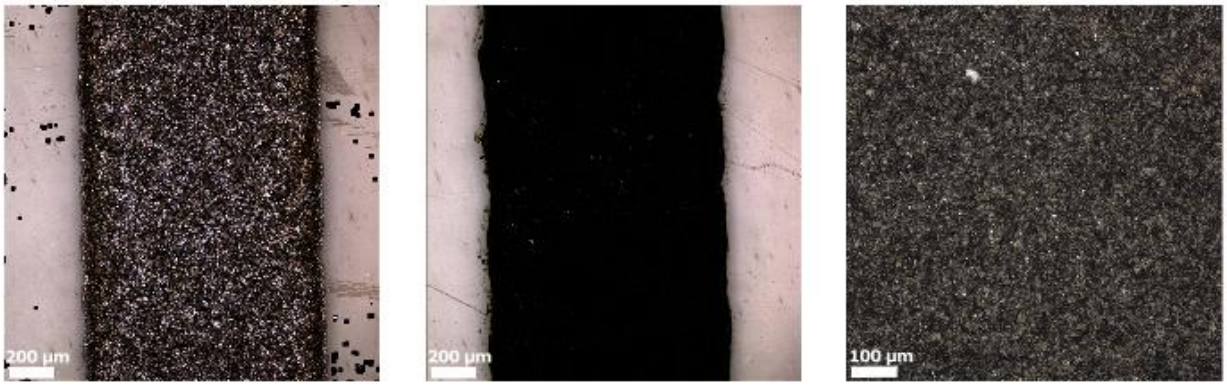

Figure 14: Alicona imaging for the silver track (left), the carbon track (middle) and the carbon WE (right) . All pictures were taken at x10 magnification.

The little black squares in the Alicona picture of the silver track (left) are missing part of the substrate. They are due to the reflection off the transparent PET during the acquisition of the pictures. However, during the white light interferometry measurements, it was noticed that the 3D topography of the tracks were different between the silver and the carbon tracks. An example of the 2D cross-section of the tracks is displayed on Figure 15 and was reconstituted from white light interferometry data.

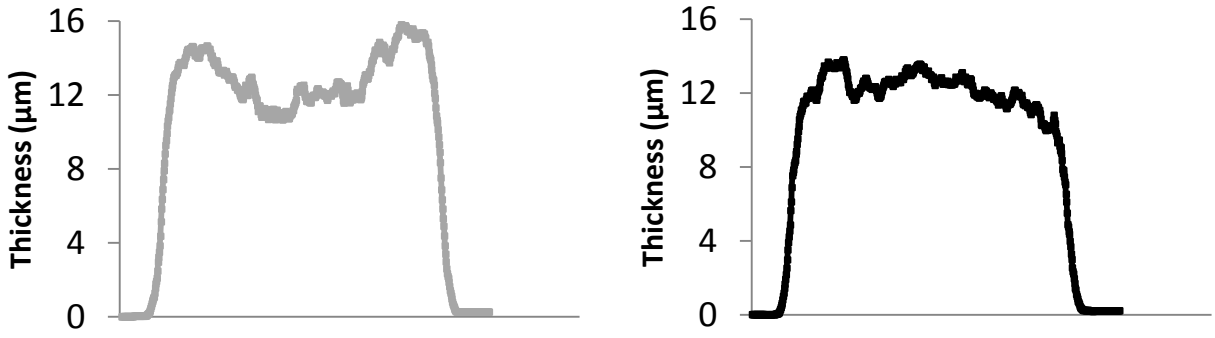

Figure 15: 2D cross-section topography of the silver track (left) and carbon track (right) reconstituted from white light interferometry data.

The left hand figure (silver track) shows the usual 'ears' at the sides – attributed to screen snap off and it is a characteristic feature. The prints have been established as good enough to be tested for electrochemical sensing.

Electrochemistry of the WCPC printed Sensor

A cyclic voltamogram of the WCPC printed sensor was recorded and compared with the commercial device as shown in Figure 16. There is very good agreement in the characteristic form.

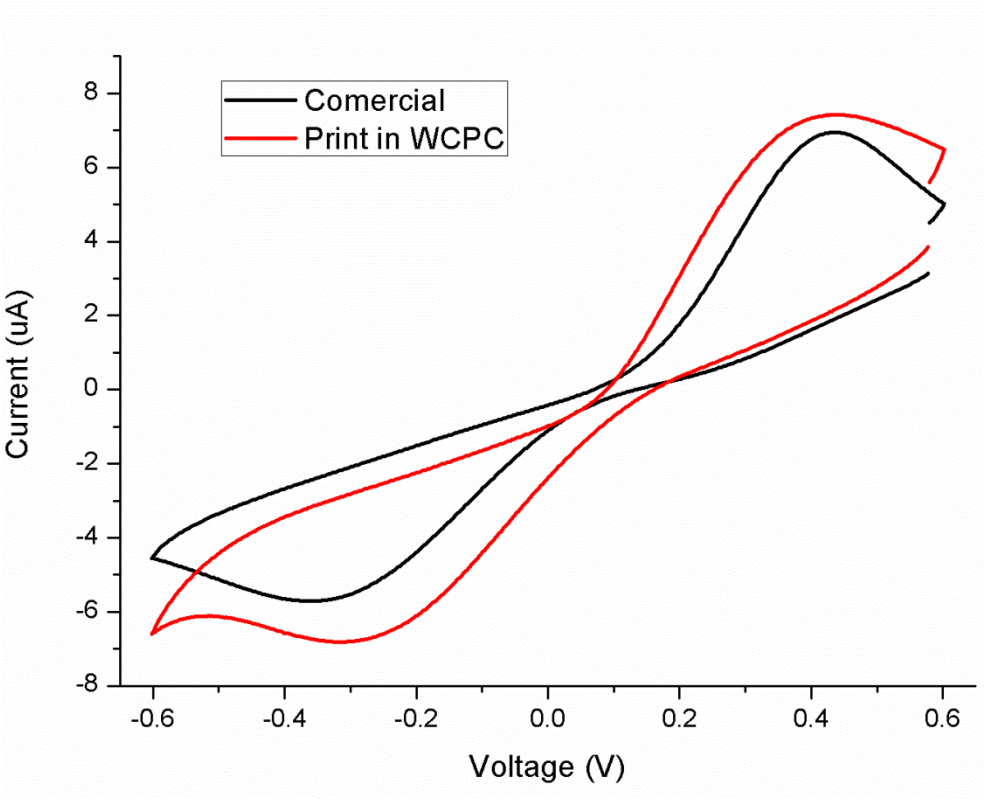

Figure 16: Comparison between electrochemical analysis of untreated SPEs commercial and printed in WCPC. Cyclic voltammetry was performed between -0.6 and 0.6 V at 50 mV.s^{-1} in a Fe^{2+}/Fe^{3+} redox couple.

Figure 16 shows the performance of the WCPC sensor design to be better than the commercial counterpart as the charge transfer is higher. Furthermore it supports the replacement of the platinum counter electrode with a lower cost carbon counterpart. However, due to time issue, it was not possible to proceed with the all process of antibody/antigen sensing, but it remains promising.

Conclusion and perspectives

This STSM was a great opportunity to initiate training and investigate the field of printed sensors. Due to the duration of the STSM and on-going project, the work was focused mainly on antibody/antigen sensor. Firstly commercial screen printed electrodes were used and prepared for the targeted application. Different types of functionalizing agent were used to enable the attachment of antibody onto the working electrode surface. Cyclic voltammetry was used to monitor the different steps of the preparation of the sensor, and all samples were found to be properly grafted with antibody due to a proper functionalization. Likewise, the responses to different concentration of antigen were investigated using the same characterization method yet detection limit was found to be lower than expected.

Secondly, an electrochemical sensor was printed using screen printing process on transparent and flexible PET substrate. The printing morphology was assessed using Alicona and white light interferometry and they were found to be good enough for the sensing part. However, cyclic voltamogram were recorded only for untreated WCPC printed electrochemical sensor due to a lack of time yet showed a higher charge transfer than the commercial ones.

Depending on the functionalization type and the desired interaction, the same principle can be applied to different sensors types and targeted molecules. For instance, those systems could be easily modified in order to work as a pH sensor, which is relevant in the smart packaging area as correlated to food freshness. I will specifically work on these smart packaging applications during the second half of my PhD. Indeed, as my PhD is a joint project between the LGP2 and the WCPC, I will be working full time in the WCPC at the beginning of 2019. This STSM is thus a beneficial and didactic experience. The future plan of action will be to focus on carbon based sensor in order to be able to physico-chemically functionalized the raw materials depending on the targeted sensing application (gas outbreak, physical failure, pH, etc.). Moreover a specific work will be conducted to adapt the raw materials and sensors characteristics in order to be able to process them by high speed reel to reel printing processes.

Acknowledgement

This STSM was a great opportunity to explore the field of electrochemical printed sensors and their characterization techniques. It was an interesting experience thanks to the different persons involved. I would like to thank everyone who helped me around in the laboratory and especially Pr. David Gethin for the hearty welcome and supervision, and Dr. Zari Tehrani for her patience and dedication.