Low cost inkjet fabrication of glucose electrochemical sensors based on copper oxide

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by

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in partial fulfilment of the requirements for the degree of

Master of Science

in Materials Science and Engineering
at the Delft University of Technology

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Acknowledgements

Firstly, I would like to thank my supervisors Prof. Luca Magagnin, from Politecnico di Milano, and Prof. Maria Santofimia Navarro, from TU Delft, for giving me the opportunity to work on this thesis project. It has been an amazing experience for me and I feel sincerely grateful to them.

A big Thank you goes to my daily supervisor Roberto Bernasconi for his patience, support and useful insights. I have learnt so much working with him and I truly admire his knowledge and professionalism. I would also like to thank the group of people that constitutes the SEElab for their enthusiastic and determined approach to the research work. In particular, I thank Lorenzo Pedrazzetti and Alireza Molazemhosseini for their precious hints and help.

I thank my mum Mariella and my dad Sergio for their unconditional love and support, which I put on as an armor to face the past two years and to follow my dreams no matter what. Every single victory in my life is yours, I love you.

A huge Thank you to Ari, for guiding me through these years at university. I shared with you my happiest laughter and my saddest tears. Our lives recently took paths we had never imagined, but we overcame every obstacle together with strength and irony. We make a powerful team.

I would like to thank Simot and Kri for the crazy nights and warm hugs, for the days spent in the library and the cyclic arguments. Your sincere friendship is an essential part of my life. You are the family I chose.

I want thank all my friends in Milan for making these years unforgettable and my friends in Delft for making me feel like home in the Netherlands.

Since currently my life is split in two, one in and one out of the hospital, I would like to thank all the people that have been making that unusual second home less horrible than it can be: from nurses to doctors, secretaries and patients. Especially I thank Alexia for being much more than a doctor. No words can explain my gratitude for you.

I thank all the members of the B.LIVE foundation for giving young adults with serious diseases like me the possibility to live extraordinary experiences. You taught me that deep suffering represents a seed of joy that can flourish if managed in the right way.

Finally, I want to thank my friend Isa from the bottom of my heart. We have been through hell together, but your smile has always helped my to keep moving forward. You will always be a part of me.
Abstract

The availability of low cost, efficient and wearable glucose sensors is one of the prerequisites for managing diabetes, one of the most diffuse chronic disease worldwide. Efficient monitoring of glucose levels in diabetics patients is essential to control symptoms and prevent severe complications. Starting from this principle, wet metallization and low cost inkjet printing were employed in the present work to manufacture non-enzymatic electrochemical sensors. CuO nanoparticles were inkjet printed on platinum, which was electrodeposited on stainless steel. The active layer obtained in this way showed an acceptable linear range for glucose detection and a good sensitivity when used as sensor. The influence on performances of interfering species and curvature were investigated, demonstrating a negligible effect for the first and a decrease in linearity of the response and sensitivity for the latter. A second CuO based sensor was realized using a PET flexible substrate. Electroless deposition was employed for the formation of a conductive NiP layer on top of which the CuO nanoparticles were inkjet printed. Glucose sensing was demonstrated by amperometric measurement, but the manufacturing process still has to be optimized to achieve a linear response and acceptable values of sensitivity. Finally, the effectiveness of CuO nanoparticles towards glucose sensing was studied by printing CuO based inks on a Cu/graphene electrochemical sensor.
## Contents

List of Figures ix  
List of Tables xiii  

1 Background and State of the Art 3  
1.1 Inkjet Printing 3  
1.1.1 Printing Mechanism 4  
1.1.2 Fluid Property Requirements 6  
1.1.3 Ink Behaviour on Solid Non-permeable Substrates 8  
1.1.4 Inkjet Printed Sensing Devices 9  
1.1.5 Electrode Fabrication 14  
1.2 Wet Metallization 16  
1.2.1 Electrodeposition 16  
1.2.2 Electroless Deposition 20  
1.3 Glucose Sensors 27  
1.3.1 Glucose Sensors Generations 27  
1.3.2 Mechanism of Glucose Oxidation on the Electrode Surface 30  
1.3.3 CuO NEG sensors: Current and Emerging Technology 32  
1.3.4 Monitoring Glucose in Alternative Physiological Fluids 34  
1.4 Aim of the Work 37  

2 Experimental Methods 39  
2.1 Inkjet Printing 39  
2.2 Ink Preparation 40  
2.2.1 CuO Nanoparticles Synthesis 40  
2.2.2 Ink Formulation 40  
2.3 Electrodes Preparation 41  
2.3.1 Working Electrode on Stainless Steel Substrate 41  
2.3.2 Working Electrode on PET substrate 43  
2.4 Contact Angle Measurement 46  
2.4.1 Contact Angle 46  
2.4.2 Sessile Drop Technique 46  
2.5 Rheological Measurement 48  
2.5.1 Dynamic Shear Rheometer 48  
2.6 XRF Analysis 49  
2.6.1 Physical Basis 49  
2.6.2 Instrumentation 49  
2.7 SEM Analysis 51  
2.7.1 Physical Basis 51  
2.7.2 Instrumentation 53  
2.8 AFM Analysis 54  
2.8.1 Physical Basis 54  
2.8.2 Instrumentation 55  
2.9 TEM Analysis 56  
2.9.1 Physical Basis 56  
2.9.2 SAED 56  
2.10 Electrochemical Measurements 58  
2.10.1 Cyclic Voltammetry 58  
2.10.2 Chronoamperometry 59
# Results and Discussion

3.1 CuO Nanoparticles Characterization ................................................. 61
3.2 Ink Printability ................................................................................. 65
  3.2.1 Qualitative Ink Printability Evaluation ........................................ 65
  3.2.2 Quantitative Ink Printability Evaluation ...................................... 65
3.3 Sensor on Stainless Steel ................................................................. 70
  3.3.1 Sensor Fabrication and Characterization ...................................... 70
  3.3.2 Sensor Performances Evaluation ................................................ 78
3.4 Sensor on PET .................................................................................. 87
  3.4.1 Sensor Fabrication and Characterization ...................................... 87
  3.4.2 Sensor Performances Evaluation ................................................ 91

# Conclusions

4 Conclusions ......................................................................................... 93

## Graphene Based Sensor

A Graphene Based Sensor ................................................................. 95
  A.1 Sensor Fabrication ......................................................................... 95
    A.1.1 Functionalization with Gold NPs .............................................. 95
    A.1.2 Functionalization with Inkjet Printed CuO Layer ..................... 95
  A.2 Sensor Performances Evaluation ................................................ 97
    A.2.1 Chronoamperometry and Calibration ..................................... 97

Bibliography ......................................................................................... 99
List of Figures

1.1 The low cost HP 2225 printers eject droplets of ink in response to thermal excitation, that momentarily boils it [1]. .................................................. 3
1.2 Schematic illustration of a CIJ printhead. .................................................. 4
1.3 Printing mechanism of a thermal inkjet printhead. ................................. 5
1.4 Printing mechanism of a piezoelectric inkjet printer. ............................. 5
1.5 Satellites formation in an improper ink due to its high viscosity. ............. 6
1.6 This graph, whose coordinate system is defined by the Reynolds and the Weber number, illustrates the regime of fluid properties where inkjet printing is possible 7
1.7 Coffee drop effect mechanism ............................................................... 8
1.8 Scheme of the work principle for screen-printing technology [2] ................. 9
1.9 Polyaniline interdigitated electrodes (nanoPANI-IDAs) shown alone (a) and with a thermofoil heater (b). Exploded schematic diagram of the nanoPANI interdigitated array electrode showing the different layers of the sensor (c) [3]. ............... 12
1.10 Schematic illustration of strategy for selective cell patterning via modification of cell binding peptide (RGD peptide) on inkjet-printed PANI [4] .................. 13
1.11 SEM cross-sectional image of the 10-layer inkjet-printed $\text{TiO}_2$ electrode. [5]. .... 13
1.12 Printing, drying and sintering process of a silver nanoparticles based in on a Teonex Polyethylene Naphthalate PEN film .................................................. 14
1.13 Schematic representation of a reactive inkjet-printing process using a Au precursor ink and an ink incorporating a reducing agent to form the zerovalent metal directly on the substrate. .............................................................. 14
1.14 Schematic representation of the printed template (a), with four silver antennae in gray (left and right) surrounding a honeycomb structure (middle). Not drawn to scale. Inkjet printed honeycomb structure after photonic sintering and subsequent microwave flash sintering (b) [6]. ........................................ 15
1.15 Schematic drawing of the electrodeposition process .............................. 17
1.16 General mechanism of metal ion reduction ......................................... 18
1.17 pH dependence of galvanic displacement rate on amphoteric metals (solid line) and on nonamphoteric metals (broken line). ............................. 20
1.18 Polarization curves (Wagner–Traud diagram) for a generalized electroless deposition reaction. The dashed line indicates the curve for the complete electroless solution. The partial anodic and cathodic currents are represented by $i_a$ and $i_c$, respectively [7]. ................................................................. 22
1.19 Evans diagram of current–potential curves for a system with two different simultaneous electrochemical reactions [7]. ........................................ 23
1.20 Induction period for a copper electroless solution [8].............................. 23
1.21 Oxidation of D-glucose catalysed by glucose oxidase ............................... 27
1.22 Principles of electrochemical enzyme sensors according to their generation [9] ................................................................. 28
1.23 Number of papers on non-enzymatic glucose sensors [10] ...................... 29
1.24 An illustration of the concentric adsorption theory with adjacent adsorption sites proposed by Fletcher. $C_1$: hemiacetalic carbon atom. $R$: the other parts of the glucose molecule [10] .................................................. 30
1.25 A schematic illustration of the IHOAM model. $M^*$ is the reductive metal adsorption site, and $M[OH]_{ads}$ is the oxidative absorbed hydroxide radical. This figure illustrates how both oxidative and reductive processes are catalyzed at the metal electrode surface [10] ............... 31
A schematic diagram (not to scale) of the fabrication and application of CuO–MWCNTs glucose sensor. CuO nanoparticles are electrochemically deposited onto the MWCNTs array and the resulting nanocomposite generates electrochemical signals in the presence of glucose. [11].

1.28 Schematic of the microneedle glucose-sensing patch on the forearm [13].

1.29 A. Flexible glucose sensor. B. Glucose sensor integrated into a wearable wristband for non-invasive sensing in sweat [14].

2.1 PIXMA MX495 by Canon

2.2 Schematic representation of CuO NPs synthesis.

2.3 Schematic representation of the multilayer structure of the working electrode.

2.4 Electrolytic Pt Bath Setup

2.5 Schematic representation of the multilayer structure of the working electrode.

2.6 PET hydrolysis reaction and surface modification after immersion in NaOH.

2.7 Electroless NiP bath setup.

2.8 Contact angle formed by the liquid which spreads over the surface.

2.9 Graphical representation of the sessile drop technique.

2.10 Schematic representation of a dynamic shear rheometer.

2.11 FISHERSCOPE X-RAY XULM by Fisher.

2.12 Schematic representation of an XRF experiment.

2.13 EVO 50 EP SEM by Zeiss.

2.14 Scanning Electron Microscope signals scheme.

2.15 Schematic draw of an Atomic Force Microscope.

2.16 SOLVER PRO Atomic Force Microscope by NT-MDT.

2.17 Schematic draw of an Atomic Force Microscope.

2.18 Schematic draw of a Transmission Electron Microscope.

2.19 Three electrodes setup for electrochemical measurements.

2.20 (a) Triangular potential waveform employed in cyclic voltammetry. \( wE_{max} \) and \( E_{min} \) are the switching potentials; \( t_s \) is the switching time; \( E_i \) is the initial potential; (b) Current response of a cyclic voltammetry experiment with a triangular potential waveform.

2.21 Example of a chronoamperogram obtained by successive addition of 50 μmol \( l^{-1} \) DNPH in an electrochemical cell containing MeOH [16].

3.1 Picture of the dried CuO-NPs obtained with the one-step process described in the previous chapter.

3.2 TEM images of the copper oxide NPs dispersed in water/EG mixtures with increasing EG content.

3.3 SAED pattern of the copper oxide NPs.

3.4 NPs maximum diameter size distribution measured on the basis of TEM images shown in Fig.3.2.

3.5 comprehensive NPs maximum diameter size distribution of the three samples analyzed.

3.6 Dynamic viscosity as a function of the imposed shear rate for the 5% EG ink at three different temperatures: \( T=25°C, 30°C \) and \( 40°C \) respectively.

3.7 Dynamic viscosity as a function of the imposed shear rate for the 10% EG ink at three different temperatures: \( T=25°C, 30°C \) and \( 40°C \) respectively.

3.8 Dynamic viscosity as a function of the imposed shear rate for the 20% EG ink at three different temperatures: \( T=25°C, 30°C \) and \( 40°C \) respectively.

3.9 Dynamic viscosity at constant shear rate as a function of EG content, measured by varying the temperature.
3.10 Contact angle measurements showing the effect of EG on the wettability of the ink. .................................................. 68
3.11 Surface tension as a function of the EG content in the water/EG mixture. .... 69
3.12 Flexible CuO-NPs based working electrode on a stainless steel substrate. ... 70
3.13 SEM images of the CuO printed surface increasing the magnification. ........ 71
3.14 EDS spectra of four different areas underlined in Fig.3.13. ......................... 71
3.15 SEM images of the CuO printed surface increasing the magnification (the sample was previously sectioned). ......................... 72
3.16 SEM images of the electrode's section. ........................................ 73
3.17 Elemental mapping of Fe obtained with EDS analysis. ........................... 74
3.18 Elemental mapping of Cr obtained with EDS analysis. ........................... 74
3.19 Elemental mapping of Ni obtained with EDS analysis. ........................... 74
3.20 Elemental mapping of Pt obtained with EDS analysis. ........................... 75
3.21 Elemental mapping of Cu obtained with EDS analysis. ......................... 75
3.22 AFM image of 20μm x 20μm of the stainless steel surface. ................. 76
3.23 AFM image 20μm x 20μm of the platinum coating surface. ................. 76
3.24 AFM image 20μm x 20μm of the inkjet printed CuO layer surface. ......... 77
3.25 SEM and AFM images of the aggregates present on the surface of the CuO inkjet printed layer. ........................................ 77
3.26 Modified SS electrode. ............................................................. 78
3.27 Voltammetry experiment carried out cycling the potential between 0 and 0.8 V vs Ag/AgCl in aqueous 0.1 M NaOH solution at progressively higher glucose concentrations. ................................................. 79
3.28 Raw electrochemical response of the sensor as a consequence of progressive addition of 1mM glucose every 45s. ........................... 79
3.29 Calibration curves of two sensors made with the same fabrication steps. .......................... 80
3.30 Raw electrochemical response of the sensor as a consequence of progressive addition of 0.5mM glucose every 45s. ......................... 81
3.31 Calibration curve of the CuO based sensor obtained by progressively increasing glucose concentrations with steps of 0.5mM. ........................ 81
3.32 Minimum detection limit test results. ............................................ 82
3.33 Magnification of the minimum detection limit test results with superimposed mean current values for each step. ............................ 82
3.34 Testing templates employed to evaluate the effect of bending on the sensitivity of the sensor. ............................................. 83
3.35 SEM images of the SS electrode's section. ...................................... 84
3.36 Interference performances test results of the CuO printed sensor in presence of six different saccharides evaluated by amperometric measurements in 0.1M NaOH at 0.5V. Interfering species were added with a concentration of 0.01mM. Glucose was added with the concentration of 0.5mM. ................. 85
3.37 Interference performances test results of the CuO printed sensor in presence of six different saccharides evaluated by amperometric measurements in 0.1M NaOH at 0.5V. Interfering species were added with a concentration of 0.02mM. Glucose was added with the concentration of 0.5mM. ................. 85
3.38 NiP layer coating obtained by electroless deposition subsequently covered with an inkjet printed layer of CuO NPs. ............................. 87
3.39 SEM images of the CuO printed surface increasing the magnification (the sample was previously sectioned). ............................. 88
3.40 SEM images of the SS electrode's section. ...................................... 89
3.41 AFM image of 20μm x 20μm of CuO printed layer surface on top of PET. .... 89
3.42 Metallic Cu pattern obtained by multiple inkjet printing steps of a salt precursor and a reducing agent on top of a PET substrate. ..................... 90
3.43 Raw electrochemical response of the sensor as a consequence of progressive addition of 1mM glucose every 45s. ........................... 91
3.44 Calibration curve for the sensor on PET substrate. ............................. 92
A.1 Raman spectrum of CVD graphene on top of a copper substrate. 96
A.2 SEM image of the CuO NPs layer inkjet printed on top of the graphene based sensor. 96
A.3 Raw electrochemical response of the sensor as a consequence of progressive addition of 1mM glucose every 90s. 97
A.4 Calibration curve for the three graphene based sensors on metallic copper substrate. 98
List of Tables

1.1 Non-exhaustive list of electrochemical sensing application which exploit inkjet printing technology. .......................................................... 12
1.2 Non-exhaustive list of CuO based NEG sensors recently studied. ........ 33

2.1 Bath Formulation ........................................................................ 41
2.2 Working Conditions ................................................................ 42
2.3 Bath Formulation ........................................................................ 42
2.4 Working Conditions ................................................................ 43
2.5 Bath Formulation ........................................................................ 44
2.6 Working Conditions ................................................................ 44

3.1 Maximum diameter size distribution statistics. ......................... 64
3.2 Contact angle values. ................................................................. 68
3.3 Surface tension, density, dynamic viscosity, Ohnesorge number and its inverse for three inks formulated. .................................................. 68
3.4 Thicknesses of the layers that form the electrode. ................. 73
3.5 Performances of two identical inkjet printed CuO glucose sensors. . 80
3.6 Performances of the CuO based sensor obtained by progressively increasing glucose concentrations with steps of 0.5mM. ....................... 81
3.7 Performances of two the inkjet printed CuO glucose sensors in two different bending configurations. ............................................... 83
3.8 A comparison of the sensing performances of non-enzymatic nanostructured CuO glucose biosensor. ................................................ 86

A.1 Comparison of the performances of three different graphene based glucose sensors. ................................................................. 98
Introduction

Diabetes mellitus is an incurable chronic disease caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. Such a deficiency results in increased concentrations of glucose in the blood, which in turn damage many of the body’s systems, in particular the blood vessels and nerves. It affects almost 8.5% of the world population and ranks among the leading causes of death globally. Frequent monitoring of blood glucose levels is essential for managing the symptoms and to provide patients with a good quality of life, thus proper sensing devices are required to tackle this conspicuous health issue.

In the 1980s, a flourishing research activity in the field of biosensors led to the introduction of self-testing blood glucose meters, which are still widely used by diabetes patients. However, this intermittent traditional method of self-testing requires a small blood sample that is obtained through a finger prick, which might be painful and represent a non-negligible risk of infection compromising patient compliance.

Alternatively, continuous glucose monitoring (CGM) devices are less invasive and commonly consist of subcutaneously implanted amperometric biosensors that measure dynamically changing glucose levels in the skin interstitial fluid (ISF), and can provide alerts throughout the day and night.

Since current projections estimate an increase in the number of diabetics patients over the next few years, strong effort have been made to develop economically viable fabrication solutions for glucose monitoring devices able to keep up with the increasing demand. Inkjet printing, due to its flexibility and low-cost, represents an ideal candidate for the realization of customizable electrochemical non-enzymatic sensors. Specifically, this class of glucose sensing devices is characterized by a series of attractive features such as high sensitivity, long shelf life and simple architecture. Moreover, the absence of enzymes avoids problems of immobilization instability and reproducibility over time.

The choice of the material on which the glucose oxidation reaction exploited for sensing takes place is crucial for the realization of an efficient device. Typically, metals, both precious and non-precious, represent the material of choice, but recently the possibility to use metal oxides, graphene and other materials was demonstrated. Copper oxide constitutes an example of these alternative materials, being a p-type semiconductor with comparatively narrow band gap (1.2 eV), CuO is a good material for glucose sensing due to its high catalytic activity towards glucose oxidation and due to its low cost. Many reports are available on devices that employ CuO nanoparticles (NPs), nanospheres or other forms of this metal oxide.

In the present work, the realization of CuO NPs based sensors analogous to existing state-of-the-art devices, characterized by flexibility and considerably reduced low cost was developed. A single use approach is followed: the active part of the sensor is intended to be disposed after a certain period of use, while readout circuitry can be reused. Flexible substrates were applied in the perspective of a device that can be worn directly on patient’s skin, such as a bracelet. To keep the cost of the sensor as low as possible, wet metallization techniques and inkjet deposition from household printers were employed. Such low cost approach is also ideal to allow product customizability according to the specific needs of the patient. Final goal is to replicate the same performances of sensors fabricated with more elaborated techniques.
1

Background and State of the Art

1.1. Inkjet Printing

In the near future, a highly viable and innovative fusion of three technological areas (micro-electronics, chemistry and printing) is foreseen that will create markets with annual revenues estimated at more than €200 billion[17].

The combination of lightweight flexible substrates and inorganic nanostructured-materials with printing technologies leads to extremely cost-efficient electronic systems such as sensors and detectors, thin-film transistors, light-emitting devices, solar cells, contacts and conductive structures. Inkjet printing has become an important technology on account of its ability to precisely deposit picolitre volumes of solutions or suspensions in well-defined patterns. The need of a mask is removed, which leads to cost-savings, efficient use of materials and waste elimination. Furthermore, since inkjet printing is a non-contact deposition method, contamination is minimized. Despite all the advantages of this technology researchers have to deal with problems such as nozzle clogging, which easily occurs due to the agglomeration and precipitation of insoluble micro- or nanoparticles during the printing process. Therefore, the preparation of the inks should take into account specific values of dynamic viscosity and surface tension, which vary from printer to printer, in order to avoid clogging. Drying could also be a problem and the ability to form uniform printed films strictly depends on the type of substrate that is used.

The inkjet printing technology had been under research for many years and the technology appeared on the consumer market in the late 1980s. The first inkjet printer was introduced in 1948 by Siemens Elema in Sweden that worked upon the Lord Rayleigh principle in which a liquid stream breaks up into drops of uniform size and shape. In 1970s a massive development program was launched by IBM to adapt continuous inkjet technology for their printers and the first drop-on-demand inkjet method appeared. Many of the drop-on-demand inkjet systems were invented, developed and produced commercially in the 1970s.

Figure 1.1: The low cost HP 2225 printers eject droplets of ink in response to thermal excitation, that momentarily boils it [1].
and 1980s[18]. One of the most successful product during those years was the Hewlett-Packard’s ThinkJet family of portable thermal inkjet printers, one of which is showed in Fig. 1.1.

1.1.1. Printing Mechanism

Inkjet printing allows the user to recreate a two dimensions digital image, which is decoded by a transducer, by propelling droplets of ink onto a substrate, usually paper. In commercial printers the printheads are able to move along an horizontal axis, while the substrate is sliding below in the direction perpendicular to the axis; lab printers, though, could have different configuration in which the printheads and the substrate are both free to move along the x-y axes, which leads to a greater flexibility.

Depending on the method of ejecting droplets, the inkjet printing technologies are categorized into the continuous inkjet (CIJ) and Drop On Demand (DOD) inkjet systems. In a continuous type ink jet printer, conductive ink is forced through an orifice to form an ink filament (Fig. 1.2). The ink is vibrated at a constant frequency by piezo crystals vibrating to cause drops to regularly separate from the end of the filament.

A drop charging electrode located in the vicinity of the drop separation point is employed to selectively induce an electric charge on the conductive ink filament at the instant of drop separation. The separating drop captures the electric charge induced on the filament, and the charged drop is electrostatically deflected, for example to a drop catcher.

![Figure 1.2: Schematic illustration of a CIJ printhead.](image)

The achievable print resolution is relatively low due to the large diameter of the droplets (approximately 40µm). On the other hand it is an high-speed process, thus is widely used for graphical application such as industrial date coding, marking and labelling. Furthermore volatile solvent-based inks could be used, allowing rapid drying and proper adhesion on many substrates. Due to the toxicity of the volatile solvent CIJ is an environmentally unfriendly technology.

The DOD inkjet printer was first invented by Siemens in 1977. Droplets are ejected from the nozzles by a pressure pulse only when required. This pressure pulse can be generated exploiting different physical phenomena, thus DOD inkjet printers are commonly divided in two main types: thermal and piezoelectric.
1.1. Inkjet Printing

Thermal Inkjet Printing

Thermal inkjet printing technology consists in the presence of a resistive element in the ink chamber, whose heating leads to the formation of a droplet.

The quick increase of the resistive element temperature makes the ink evaporate and thus a bubble of air is created, which acts as pressure pulse to eject the droplet from the nozzle (Fig. 1.3). As soon as the droplet is ejected the chamber is subsequently filled back by ink. The printhead of thermal inkjet printer is not expensive and it has the ability to create small-size droplets. These printers, thought, have some limitation on the use of the ink because it has to be able to vaporize and to withstand high temperatures as well [18].

Piezoelectric Inkjet Printing

In the piezoelectric inkjet printers a piezoelectric material takes the place of the resistive element. When a voltage is applied, the piezoelectric material changes shape, generating a pressure pulse in the fluid, which forces a droplet of ink from the nozzle. The voltage applied, the pulse duration and the diameter of the nozzle control the size of the ink droplets (Fig. 1.4).

For these kind of printers there are far less restriction on the ink type, no need of a volatile component and the printheads have longer life. However, the hardware are costly due to the piezoelectric material, which is usually PZT (lead zirconium titanate) [18].
1.1.2. Fluid Property Requirements

Depending on the inkjet printer type, the inks should have appropriate properties to jet reliably and consistently. Viscosity, density, surface tension and, if a dispersion is used, particle size play a crucial role in the printability of the ink.

Viscosity of the inks has influence on droplet’s shape, drop spreading and final printed shape [18]. In particular, a too high viscosity leads to the formation of a long tail behind the head of the droplet that easily breaks into smaller droplets called satellites (Fig. 1.5), which produces an undesirable decrease in the print resolution.

![Figure 1.5: Satellites formation in an improper ink due to its high viscosity.](image)

Surface tension has to be controlled as well so that the droplets have a spheroidal shape while they are ejected from the nozzle, before impacting onto the substrate. If the surface tension of ink is too low, the ink will spread over the nozzle plate and cover the nozzle. On the other hand, if the ink’s surface tension is too high, the drive voltage requirement for piezoelectric printers will be insufficient to overcome the surface tension and eject a droplet. Thus solvents such as acetone and alcohol are often used to reduce surface tension [18].

Finally, since most of the inks used for inkjet printing are dispersion, the particle size represent one of the most critical property of the ink. Any suspended particle must be below a critical size to be ejected and avoid clogging of printhead nozzles. To prevent clogging of nozzles not only the particle size, but also the stability of the dispersion is important, which can be controlled by chemical and electrostatic treatments.

The aforementioned inks properties define a number of dimensionless groupings of physical constants, which are used to characterize the behaviour of liquid drops. The most useful constants are the Reynolds (Re), Weber (We) and Ohnesorge (Oh) numbers:

\[
Re = \frac{\nu \rho a}{\mu} \\
We = \frac{\nu^2 \rho a}{\gamma} \\
Oh = \frac{\sqrt{We}}{Re} = \frac{\mu}{\sqrt{\gamma \rho a}}
\]

where \(\rho\), \(\eta\) and \(\gamma\) are the density, dynamic viscosity and surface tension of the fluid respectively, \(\nu\) is the velocity and \(a\) is a characteristic length.

The earliest significant work attempting to understand the mechanism of drop generation was by Fromm [19]. According to his studies the Ohnesorge number is the appropriate physical constant to characterize the printability of an ink. In particular, the condition for stable drop generation that he proposed is the following: the inverse of the Ohnesorge number \(Z=1/Oh\) has to be greater than 2. This analysis was further refined by Reis and Derby
1.1. Inkjet Printing

Figure 1.6: This graph, whose coordinate system is defined by the Reynolds and the Weber number, illustrates the regime of fluid properties where inkjet printing is possible.

[20] who used numerical simulation of drop formation to propose the following range, $10 > Z > 1$ for a stable drop formation. At low values of $Z$, viscous dissipation prevents drop ejection, whereas at high values the primary drop is accompanied by a large number of satellite droplets. The printability of an ink can be verified by the use of the graph in Fig.1.6.
1.1.3. Ink Behaviour on Solid Non-permeable Substrates

Another essential parameter that has to be taken into account while dealing with inkjet printing is the ink behaviour on a substrate. For a proper understanding the impact mechanism has to be first investigated, which accounts to the first moments experienced by a droplet when it comes into contact with the substrate.

Van Dam and Le Clerc divided the process of impaction into three steps. In the first step, the droplet hits the substrate. In the second, the radius of the droplet-substrate interface expands to an order of magnitude greater than that of the in-flight droplet radius, with an accompanying swift fluid flow, which is radially outwards. In the third step, the fluid comes to rest after an initial rebound and a series of inertial oscillations that are dampened by viscous dissipation [21]. Van Dam and Le Clerc find out that the final radius of a droplet is larger for higher Weber numbers and that there is a clear influence of the surface energy of the substrate on the latter. Moreover, Nagel et al. found that splashing of a droplet occurs when a threshold pressure of the surrounding gas is reached and it scales with the molecular weight of the gas and the viscosity of the liquid [21].

Another phenomenon which has great influence on the final printed feature is the so-called “coffee drop” (Fig. 1.7), which describes the propensity for solute to deposit at the boundaries of a printed feature. The actual cause of coffee staining is the capillary flow induced by the differential evaporation rates across the drop: liquid evaporating from the edge is replenished by liquid from the interior. At the same time evaporation leads to a surface tension gradient inside the droplet which causes the so-called Marangoni effect. If the mass flow generated by the Marangoni effect is sufficiently strong the particles redistributes back to the centre of the droplet and coffee staining is reduced. Thus, for example, surfactants can be added to reduce the liquid’s surface tension gradient, disrupting the induced flow.

![Figure 1.7: Coffee drop effect mechanism.](image-url)
1.1.4. Inkjet Printed Sensing Devices

When evaluating the usefulness of inkjet printing technology in the context of the fabrication of (bio)chemical sensing devices, its peculiar deposition technology can be compared with other methods fulfilling an equivalent purpose. Of particular relevance are drop casting (manual pipetting), contact printing, pneumatic dispensing, screen printing, spin coating, and photolithography, among others. All methods transfer a controlled amount of liquid from a reservoir to user-selected spots on a substrate. They all allow flexible spatial control of the material deposition process. However, when it comes to achievable resolution, there are significant differences. Reproducible droplet formation in the picoliter volume order is nowadays routinely possible with almost any type of inkjet printing device, which make it superior to both drop casting and pneumatic dispensing in terms of resolution. In the case of contact printing, similar resolution to inkjet printing is achieved, but disadvantages include the risks of spot-to-spot cross-contamination and of damaging delicate substrates.

For the fabrication of printed electrodes or electrical contacts, screen printing is probably the most serious “competitor” of inkjet printing, since it enables very fast and low cost production with large numbers of replicates. From a material deposition point of view, screen printing allows for the fast creation of relatively thick films (20–100 μm). However, the achievable control over the layer thickness is low compared with inkjet printing. Furthermore, in terms of spatial control, a separate screen is required for every print pattern, which makes the technology useful for the reproduction of large numbers of identical layouts, but lacks the flexibility offered by inkjet printing when it comes to the free choice of shape and layout for device prototyping.

Spin coating is a fast and simple method for depositing thin, uniform layers of a material on comparably large areas. However, it does not offer the possibility of spatial control and, therefore, cannot compete with inkjet printing in terms of resolution and flexibility. Furthermore, it is not very economical in material use, since a significant amount of the coating liquid does not remain on the substrate, but is wasted.

The additive character of inkjet printing makes this method economically viable in terms of material use. In contrast, photolithography is based on a subtractive approach, resulting in the creation of waste. While photolithographic methods are superior to inkjet printing in terms of achievable resolution, their subtractive character calls for a larger number of processing steps and the selective removal of pre-deposited material requires a specific mask for every pattern, resulting in lower flexibility. The application of inkjet printing in the context of fabrication of chemical sensing devices is already widespread and it is continuously increasing with the prospects of simple laboratory scale prototyping and low cost mass fabrication.

Inkjet printing systems in the sensing devices industry are most commonly used as one fabrication tool among others to perform just a single specific material deposition task in the multi-step process leading to a complete device. Very often this deposition step concerns the formation of an electrically conducting trace (e.g., electrode, electric contact) or the active
sensing layer. In particular, the fabrication of metallic planar electrodes for electrochemical sensors, inkjet printing can already be regarded as a “routine” technology [22].

To give a clearer idea of inkjet printing potentiality in electrochemical sensing a non-exhaustive list of application is reported in table 1.1.

<table>
<thead>
<tr>
<th>Transduction mechanism</th>
<th>Sensing applications</th>
<th>Inkjet printed features</th>
<th>Printer Type</th>
<th>Substrate</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrochemical/potentiometric, amperometric</td>
<td>pH, glucose</td>
<td>Au and Ag electrodes (nanoparticles)</td>
<td>Dimatix DMP-2800</td>
<td>Piezo</td>
<td>Coated paper</td>
</tr>
<tr>
<td>Electrochemical/impedimetric</td>
<td>anti C-reactive protein (CRP) antibody</td>
<td>Au electrodes (nanoparticles)</td>
<td>Dimatix DMP-2800</td>
<td>Piezo</td>
<td>Coated paper</td>
</tr>
<tr>
<td>Electrochemical/amperometric</td>
<td>Interleukin-6 (cancer biomarker)</td>
<td>Au electrode array (nanoparticles)</td>
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<td>Piezo</td>
<td>Polyimide</td>
</tr>
<tr>
<td>Electrochemical/amperometric</td>
<td>O₂</td>
<td>Au electrode precursor (nanoparticles)</td>
<td>Epson R230</td>
<td>Piezo</td>
<td>Mixed cellulose ester</td>
</tr>
<tr>
<td>Electrochemical/amperometric</td>
<td>Aqueous NH₃</td>
<td>Polyaniline nanoparticle sensing layer</td>
<td>Dimatix DMP-2811</td>
<td>Piezo</td>
<td>Carbon electrodes on PET film</td>
</tr>
<tr>
<td>Electrochemical/amperometric</td>
<td>Epinephrine, norepinephrine, dopamine</td>
<td>Ammonium persulfate oxidant for vapor phase polymerization of polyaniline electrodes</td>
<td>Canon Pixma IP1300</td>
<td>Thermal</td>
<td>PET film</td>
</tr>
<tr>
<td>Electrochemical/amperometric</td>
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<td>PEDOT-PSSd film, Glucose oxidase or horseradish peroxidase enzyme</td>
<td>Olivetti Thermal</td>
<td>Thermal</td>
<td>indium tin oxide glass on cellulose acetate</td>
</tr>
<tr>
<td>Transduction mechanism</td>
<td>Sensing applications</td>
<td>Inkjet printed features</td>
<td>Printer Type</td>
<td>Substrate</td>
<td>Ref</td>
</tr>
<tr>
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</tr>
<tr>
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<td>Triglycerides</td>
<td>Au/PEDOT-PSS nanocomposite sensing layer</td>
<td>Dimatix</td>
<td>Carbon</td>
<td>[29]</td>
</tr>
<tr>
<td>amperometric</td>
<td></td>
<td></td>
<td>DMP-2800</td>
<td>electrodes</td>
<td></td>
</tr>
<tr>
<td>Electrochemical/</td>
<td>$H_2O_2$, NADH,</td>
<td>Graphene-PEDOT-PSS</td>
<td>Dimatix</td>
<td>Carbon</td>
<td>[30],</td>
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<tr>
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<td>Salbutamol</td>
<td>nanocomposite sensing</td>
<td></td>
<td>electrodes</td>
<td>[31]</td>
</tr>
<tr>
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<td>Chemical $O_2$ demand (COD)</td>
<td>$TiO_2$ photoanode</td>
<td>Epson</td>
<td>indium</td>
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<td>(nanoparticles)</td>
<td>R290</td>
<td>tin oxide</td>
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<tr>
<td>Electrochemical/</td>
<td>Ascorbic acid</td>
<td>Conducting polymer layer (polyaniline)</td>
<td>Dimatix</td>
<td>Screen-printed</td>
<td>[32]</td>
</tr>
<tr>
<td>chronoamperometric</td>
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<td></td>
<td>DMP-2800</td>
<td>carbon</td>
<td></td>
</tr>
<tr>
<td>Electrochemical/</td>
<td>Alcohol vapor</td>
<td>Conducting polymer – carbon nanotube composite electrodes</td>
<td>HP</td>
<td>Thermal PET</td>
<td>[33]</td>
</tr>
<tr>
<td>resistance</td>
<td></td>
<td></td>
<td>Deskjet 690C</td>
<td>sheets</td>
<td></td>
</tr>
<tr>
<td>Electrochemical/</td>
<td>Organic vapors</td>
<td>Reduced graphene oxide electrodes</td>
<td>HP 4250</td>
<td>Thermal PET</td>
<td>[34]</td>
</tr>
<tr>
<td>resistance</td>
<td></td>
<td></td>
<td></td>
<td>transparency</td>
<td></td>
</tr>
<tr>
<td>Electrochemical/</td>
<td>$CO_2$</td>
<td>$Pd$-polymer nanocomposite as seed-layer for electrode formation, PEDOT-PSS sensing layer</td>
<td>Dimatix</td>
<td>PET film</td>
<td>[35]</td>
</tr>
<tr>
<td>resistance</td>
<td></td>
<td></td>
<td>DMP-2800</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electrochemical/</td>
<td>Ethanol, methanol</td>
<td>PEDOT-PSS sensing layer</td>
<td>HP</td>
<td>Thermal Polyester</td>
<td>[36]</td>
</tr>
<tr>
<td>resistance</td>
<td>vapor</td>
<td></td>
<td>Deskjet 693C</td>
<td>film</td>
<td></td>
</tr>
</tbody>
</table>
Table 1.1: Non-exhaustive list of electrochemical sensing application which exploit inkjet printing technology.

<table>
<thead>
<tr>
<th>Transduction mechanism</th>
<th>Sensing applications</th>
<th>Inkjet printed features</th>
<th>Printer Type</th>
<th>Substrate</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrochemical/</td>
<td>( N\mathrm{H}_3 ) gas</td>
<td>Polyaniline nanoparticle sensing layer</td>
<td>Epson</td>
<td>C46/C48</td>
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<td>conductometric</td>
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<td></td>
<td>Piezo</td>
<td></td>
<td></td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>Silver</td>
<td>electrodes on PET film</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dimatix</td>
<td>DMP-2800</td>
<td>[38]</td>
</tr>
<tr>
<td></td>
<td>( \mathrm{CO}, \mathrm{H}_2, \mathrm{H}_2\mathrm{S}, \mathrm{NO} )</td>
<td>( \mathrm{WO}_3 ) nanoparticle sensing layer</td>
<td>Piezo</td>
<td>Si/SiO&lt;sub&gt;2&lt;/sub&gt; substrates with Ti/Pt electrodes</td>
<td></td>
</tr>
</tbody>
</table>

Inkjet Printing of Active Sensing Layers for Electrochemical Sensors

The coating of electrodes with an active sensing layer is an important application of inkjet printing. Frequently, that layer consists of a film of a conducting polymer with the purpose of fabricating an electrochemical gas sensor. In some cases, the polymer simultaneously acts as electrode and active sensing layer without the requirement of a separate underlying electrode substrate.

Crowley et al. [3] reported sensors for aqueous or gas phase ammonia detection obtained by a combination of screen printing of underlying silver or carbon electrodes and inkjet printing of dodecylbenzensulfonic acid (DBSA)-polyaniline (PANI) on a piezoelectric research-type or a piezoelectric desktop printer as shown in Fig. 1.9. The thickness of the deposited PANI chemiresistor films significantly influences the gas sensor properties such as the response time, stability and reproducibility. A great advantage of the inkjet printing approach is the flexible variation in the deposited total film thickness by simply varying the number of applied printing layers, which under the conditions used by the authors had a thickness of 170 nm.

![Figure 1.9](image-url)
A different approach to a PANI pattern printed from a thermal desktop inkjet printer has been reported for a biosensor targeting the detection of electrochemically active bio-molecules excreted from cells [4]. In this example, only an aqueous solution of an oxidant, ammonium persulfate, is inkjet patterned on the substrate. Doped PANI films are then created through vapor phase polymerization by exposing the patterned substrate to aniline monomer/HCl at 70 °C for 15 min (Fig. 1.10). With this method, dispersion stability of the printing ink is not an issue, since a simple aqueous solution is the only inkjet deposited material.

Yang et al. [5] have selected inkjet printing as a cost-effective mass production technology to produce robust and highly reproducible TiO₂ photoanodes, applied to the measurement of glucose, phenol, potassium hydrogen phthalate, glutaric acid, malonic acid, and chemical oxygen demand. A piezoelectric consumer inkjet printer was employed to deposit an aqueous colloidal TiO₂ particle (6 wt%) ink containing 1.8 wt% of carbowax as viscosity modifier onto indium tin oxide glass. The inkjet printed sensing layers were directly compared with those deposited by a conventional dip coating method. A more homogenous film formation is clearly confirmed for the inkjet printed electrode. Film thickness control was easily achieved by varying the number of printed layers (Fig. 1.11). The sensing results revealed that inkjet printed TiO₂ films have good fabrication reproducibility and that the relative standard deviations for independently fabricated electrodes are smaller compared with commonly used dip coated sensors.
1.1.5. Electrode Fabrication

Because of its simplicity and suitability for mass production, screen printing has become a major technology for the fabrication of planar electrodes, which form the basis for a variety of electrochemical sensors [22].

In particular, DOD inkjet printing technology has very attractive features for research and prototyping applications, where pattern flexibility is highly desired. Widely applied screen printed electrode materials are carbon, silver and gold. Several silver nanoparticle suspensions are commercially available and routinely used. These inks contain organic solvents and dispersing agents, which prevent aggregation of the particles.

After printing, the metal traces are sintered at high temperatures, usually above 200°C, in order to remove the solvent and the particles stabilizer, thus to gain sufficient conductivity. An oxygen sensor on flexible plastic substrate was made by Moya et al. [39] exploiting the described printing mechanism as shown in Fig. 1.12.

Another environmentally friendly approach for printing conductive paths on top of organic substrates is reactive inkjet printing. It involves the deposition of a metal precursor, which usually consists in a salt, in the first printing step, followed by the deposition of a reducing agent, such as sodium borohydride, in order to obtain the elemental metal forming the conductive path directly on the surface of the material 1.13. This process is solvent-free, requires no extended sintering and can be done by using a low-cost desktop printer since the risk of printer nozzle clogging is much less an issue due to the absence of suspended particles. A serious drawback, however, is the low conductivity [22]. Researchers are continuously attempting to find new methods to reduce the temperature and the time required for sintering of nanoparticles-based inks or to increase the conductivity of the traces obtained by printing the metal precursor.
Perelaer et al. [6] demonstrated a combination of photonic and microwave-induced sintering method for electrodes printed with a commercial silver nanopaste ink, which can be completed in 15 s and leads to 40% of bulk silver conductivity and thus compatible for a roll-to-roll fabrication process (Fig. 1.14).

Nie et al. [40] developed a valid approach to obtain highly conductive silver lines on PET substrate (50% of the conductivity of bulk silver) based on the thermal reduction of inkjet-printed silver citrate in the presence of 1,2-diaminopropane. This method has the advantage to be particle free.

![Inkjet printed honeycomb structures](image)

Figure 1.14: Schematic representation of the printed template (a), with four silver antennae in gray (left and right) surrounding a honeycomb structure (middle). Not drawn to scale. Inkjet printed honeycomb structure after photonic sintering and subsequent microwave flash sintering (b) [6].

While fabrication of metallic electrodes (mainly Ag and to a less degree also Au and Cu) by inkjet printing is becoming routine procedure, screen printing still remains the fabrication of choice for another important electrode material such as carbon. Printability of aqueous dispersions of carbon nanotube and reduced graphene oxide is reported [22], but it is still an emerging technology limited to research.
1.2. Wet Metallization

Wet metallization refers to any metal-coating technique that involves the use of a conductive solution containing ions of the metal required for the deposition. Metal coatings may be decorative, protective or functional and they can be applied onto both metallic and non-metallic substrates. Metallization currently is a widespread technology used in research as well as in industry. For the aim of this work electrodeposition and electroless deposition techniques are investigated.

1.2.1. Electrodeposition

Electrodeposition is a material production technology in which the metal ions contained in an electrolytic solution are reduced to atoms on a conducting substrate by applying an external voltage or current. The first electroplating attempt was made by Brugnatelli, who using the Volta pile, was able to plate gold on two large medals, but later development was reported only after 1830. The advent of the Daniell cell provided a more reliable current source needed for the formation of thin and continuous coatings [41]. Around the 1850s, practical electrolyte formulations for Zn, Ni and Sn plating among others were also developed, opening the way to engineering applications of coatings for both decorative and protective uses.

In the early days electrodeposition is routinely used and its applications strongly depend on the metal that is used. Au an Ag are commonly plated to embellish precious items such as art pieces or jewellery. Chromium combine a pleasant appearance with outstanding corrosion and abrasion resistance, while Zinc is used as a sacrificial protection by corroding preferentially. More specialized applications include Cu for electrical conductivity, alloys of Au with transitions metals to provide low contact resistance in printed circuit boards, Pt coatings for extreme environments such as high-temperature turbines, Pb and metal sulphides for soft bearings [41]. From the 1950s electrochemical processes progressed enough to be extended to electronic manufacturing, becoming a valid alternative, in combination with lithography, to vapour phase deposition methods. The advantage of electrodeposition was the capability to deposit only at conductive regions, therefore enabling for the first time purely additive growth and closer reproduction of the lithographic pattern resulting in fast production and low material waste.

The latest challenges of electroplating technology include the production of micro-sized parts by electroforming in three dimensions, competing with more complex additive manufacturing methods, and the integration of the electrical functionality with a variety of other functions, including biomedical ones in the fabrication of sensors and actuators.

Electrodeposition Process

The electrochemical growth of metallic films occurs in a container, or tank, with two electrodes immersed in the electrolyte, each being in contact to the same external electrical power source. The current that flows through the anode, which is positively charged, oxidises the metal atoms of the electrode, which then starts to dissolve. This reaction may be either dissolution of the anode or oxidation of an electrolyte species, in some case water. To maintain charge conservation, at the opposite electrode, the cathode, reduction of the metal ions contained in the electrolyte occurs leading to film growth as shown in Fig.1.15.

An unreactive salt is usually added in the solution to increase conductivity without reacting with the electrodes. Furthermore, a variety of neutral organic molecules adsorbing selectively at definite crystallographic facets are used to control growth morphology and crystallography, while surfactants are employed to control the surface energy at the substrate, thus affecting growth modes.
Stoichiometry

Electrodeposition, as any other electrochemical process, has to satisfy not only mass balance, but also the balance of charge. In 1833, Faraday postulated two laws that mathematically define mass and charge balance:

- The number of moles $n$ produced or consumed in an electrochemical reaction must be proportional to the charge flowing through the circuit, $Q$.

- The mass $W$ of different substances formed by the flow of the same charge is proportional to the charge to the molar mass $A$ divided by the oxidation state of the species, $z$.

$$W = \frac{AQ}{zF}$$  \hspace{1cm} (1.4)

Where $Q = it$ is the total charge flowing through the electrochemical cell during the process and the Faraday’s constant $F = 96.485 C/mol$ corresponds to the charge of one mole of electrons.

It is convenient to derive an equation in terms of film thickness $\delta$ generated by a current density applied for a given time:

$$\delta = \frac{AJt}{zF\rho}$$  \hspace{1cm} (1.5)

Where $\rho$ is the material density, $j$ is the current density and $t$ is the time of the process.

Thermodynamics

When a metal $Me$ is immersed in a solution containing the corresponding ion $Me^{z+}$, a dynamic equilibrium is achieved over time where the rate of metal dissolution from the electrode equals the rate of metal ion reduction:

$$Me^{z+} + ze^- \leftrightarrow Me$$  \hspace{1cm} (1.6)

A potential difference at the metal/electrolyte interface is induced by charge separation at the equilibrium condition. This potential difference can only be measured with respect to another electrochemical reaction that reaches equilibrium very quickly and it is stable over time, working as a reference electrode. One of such reactions is the hydrogen reduction/oxidation:

$$2H^+ + 2e^- \leftrightarrow H_2$$  \hspace{1cm} (1.7)

An arbitrary redox potential $E = (H^+/H_2) = 0$ under standard conditions is assigned to that reaction from which any other redox can be defined operationally by experimentally measuring the potential difference in a cell consisting of the hydrogen electrode and the electrode under study.
Under nonstandard states, the conditions of equilibrium change due to the activity of the ionic species, a quantity related to the ability of the system to perform work; activity approximates the concentration of the species in solution at concentration of the order of $10^{-3}$ mol and below. Under these conditions, the expression for the redox potential $E(\text{Me}^{2+}/\text{Me})$ becomes:

$$E = E^0 + \frac{RT}{zF} \log [\text{Me}^{2+}]$$

Where $E^0$ is the standard redox potential, $R = 8,314 \frac{\text{J}}{\text{mol} \cdot \text{K}}$ is the gas constant, $T$ the absolute temperature and $[\text{Me}^{2+}]$ the metal ion concentration in the electrolyte.

**Kinetics**

While thermodynamics provides a description of the behaviour at equilibrium and predicts whether a reaction is spontaneous, it does not provide any information about the reaction rates. This information is provided by kinetic equations.

When an external potential is applied to the circuit, the equilibrium condition is no more consistent and the electrode of interest may either be inert within a small range of applied potentials or current may start to flow immediately, leading to an electrochemical transformation. The driving force for this transformation is the so-called overpotential defined as:

$$\eta = V - E_{eq}$$

where $V$ is the applied voltage and $E_{eq}$ the equilibrium voltage.

The metal ions reduction to a metal on a substrate is a multiple steps process as is shown in Fig. Firstly, ions diffuse towards the electrode, then, as a consequence of a chemical reaction with the electrolyte, they form intermediate species which are subsequently adsorbed at the electrode surface, where they are reduced and finally incorporated in the growing film.

Each elementary step is characterized by its own reaction rate, the slowest of which is called the rate-determining step since it defines the velocity of the reduction process. In most cases the driving force for electron transfer is small and therefore this is the slowest step. It is possible to express the rate of electron transfer as a function of the overvoltage by using the Butler-Volmer equation [41]:

$$J = J_0 [e^{-a_f \eta} - e^{(1-a_f) \eta}]$$

(1.10)
1.2. Wet Metallization

Where $J$ is the current density, $J_0$ is the exchange current density, $\alpha$ is the transfer coefficient (a measure of the symmetry of the energy barrier for oxidation vs. reduction), $f = F \frac{z}{RT}$. This equation accounts for single electron transfer and it should be adjusted for the transfer of $z$ electrons:

$$J = J_0 [e^{-z\alpha f \eta} - e^{z(1-\alpha)f \eta}]$$  \hspace{1cm} (1.11)

Considering cathodic applied voltages, neglecting the second exponential terms and reframing the equation in the logarithmic form:

$$\eta = - \frac{RT}{z\alpha F} \log \frac{J}{J_0}$$  \hspace{1cm} (1.12)

Which represents the equation used by Tafel to describe experimental data in electrochemical kinetics.

Nickel Electrodeposition

Nickel plating, due to its excellent wear and corrosion resistance, is among the most common electrodeposition processes. Since Ni is more noble than low carbon steel it is widely used, in combination with a thin layer of Cr, to provide sacrificial protection to steel parts. Ni is also used as a structural material in electroforming, a near net shape electroplating process that uses a shaped mandrel to form the final part in a single piece. Electroplating is uniquely suitable to faithfully reproduce the shape of the mandrel due to the diffusional nature of the metal ion deposition process. Decorative bright Ni is obtained by the addition of a variety of proprietary additives and brighteners, mostly complex carbon and/or sulfur compounds.

The most popular electrolyte for Ni plating is the Watts bath, which contains Ni sulfate, chloride, and boric acid, as a buffering agent [41]. Electrodeposition of Ni on stainless steel, due to the passive layer which stands on top of its surface, could be challenging. Nickel Woods strike deposition represents a valid approach for stainless steel plating, which can be done in an extremely acid bath with high concentration of chloride or bromide [42].

Platinum Electrodeposition

Platinum has been widely used in ornaments and accessories because of its clear and subdued shine. It is also highly resistant to corrosion and high temperature and biocompatible as well. These extremely appealing properties of Pt make it suitable for high performance applications. However, platinum is one of the most noble metal, making it difficult to use in electrodeposition techniques. The commercially useful platinum plating baths mostly depend on chloroplatinic acid $H_2PtCl_6 \cdot 6H_2O$ or platinum diamino dinitrite $Pt(NH_3)_2(NO_2)_2$ [43].
1.2.2. Electroless Deposition

Electroless deposition refers to any plating process from aqueous solutions without an external current source. Even if this coating technique is predominantly related to metals and alloys, deposition of oxides, salts or polymers is also possible. For the aim of the work only electroless deposition of metals will be investigated.

Since electrodeposition of metals is always a cathodic reaction, the presence of specific reducing agents is necessary. The electroless processes are divided into two groups: galvanic displacement deposition and autocatalytic deposition.

Galvanic Displacement

This heterogeneous process involves a less noble metal immersed into an aqueous solution containing ions of a more noble metal. During galvanic displacement deposition the less noble metal is oxidized and dissolved while the ions of a more noble metal present in the solution are reduced leading to the deposition of the more noble metal [44].

- Anodic process:
  \[ M_1^0 \rightarrow M_1^{z_1^+} + z_1e^- \]  
  \[ (1.13) \]

- Cathodic process:
  \[ M_2^{z_2^+} + z_2e^- \rightarrow M_2^0 \]  
  \[ (1.14) \]

The electron produced due to oxidation of the metal \( M_1 \) are consumed for the reduction of the metallic ions of the metal \( M_2 \). Thus, combining cathodic and anodic processes a generic description of the galvanic displacement deposition can be obtained:

\[ M_2^{z_2^+} + \frac{z_2}{z_1}M_1^0 \rightarrow \frac{z_2}{z_1}M_1^{z_1^+} + M_2^0 \]  
\[ (1.15) \]

From a thermodynamic point of view, as long as there is a sufficient difference in the standard electrode potentials between the two metals, galvanic displacement occurs.

Figure 1.17: pH dependence of galvanic displacement rate on amphoteric metals (solid line) and on nonamphoteric metals (broken line).

The kinetics of the galvanic displacement reactions can be studied using the following generic reaction:

\[ \frac{dC}{dt} = -kC^n \]  
\[ (1.16) \]

where \( \frac{dC}{dt} \) is the rate of deposition, \( C \) is the concentration of ions of the more noble metal and \( n \) is the reaction number. \( k \) is the rate constant defined as:

\[ k = \frac{DA}{\delta V} \]  
\[ (1.17) \]
where \( D \) is the diffusion coefficient, \( A \) is the surface area on which the deposition takes place, \( \delta \) is the boundary layer thickness and \( V \) the volume of the electrolyte. These equations depend on numerous variables such as the electrolyte composition, additives, temperature, pH and nature of the substrate on which the deposition takes place. The pH dependence is generally given for simple solutions without complexing agents as shown in Fig. 1.17. While in the acidic solutions, generally, a decrease in pH leads to an increase in the rate of dissolution of the less noble metal, in solutions with alkaline pH a distinction between non-amphoteric metals and amphoteric should be done. For the latter an increase in pH leads to an increase of deposition of the more noble metal, while for non-amphoteric metals hydrolysis occurs for alkaline pH, which significantly reduces the rate of deposition. Hydrolysis products can be adsorbed at the less noble metal surface reducing the number of available active sites.

The presence of oxide films on the surface of the less noble metal generally leads to a delay in the initial stage of the galvanic deposition. Efficient removal of these films can be obtained by adding various complexing agents such as \( NH_3 \) or EDTA.

This relatively simple process does not require costly and complicated equipment, thus it is suitable for industrial scale applications such as purification in hydrometallurgical plants or removal of certain metals from waste solutions.

**Autocatalytic Deposition**

Autocatalytic deposition is the most commonly used coating process, which does not require an electrical current or potential. A wide variety of metals can be deposited by autocatalytic deposition, but Ni, Cu, Ag and Au represent the most important ones for practical applications.

The autocatalytic deposition proceeds only on the catalytically active surfaces and a typical bath requires: sources of metal ions, complexing and reducing agents. Sometimes, depending on a particular system, compounds classified as stabilizers and inhibitors are added in order to increase the stability of the plating solutions and to improve the properties of the deposit Djokić [44]. Sources of metal ions include any water soluble salt, whose choice is based on the solution stability, required properties of the deposited metal or alloy and the environment issues. Complexing agents, which increase the solution stability, are selected according to the metal ions, pH, temperature and other operating conditions. Finally, the reducing agents such as sodium hypophosphite (\( Na_2H_2PO_4 \)), sodium borohydride (\( NaBH_4 \)), hydrazine (\( N_2H_4 \)) and formaldehyde (\( H_2CO \)) should be soluble and reasonably stable in the plating solution.

Non-metallic surfaces must be properly activated before the metallization. The usual sensitization/activation steps are based on applications of \( SnCl_2 \) and \( PdCl_2 \) since palladium spots act as active sites for deposition.

The overall process of an autocatalytic deposition is described by the following equation:

\[
Me^{n+} + Red \rightarrow Me + Red^{n+}
\]  

(1.18)

In order that the overall electroless deposition reaction may proceed, the equilibrium [rest] potential of the reducing agent, must be more negative than that of the metal electrode, so that the reducing agent can function as an electron donor and as an electron acceptor. As stated in the mixed potential theory, the deposition results from the coupling of two partial electrochemical reactions: the cathodic reduction of metal ions and the anodic oxidation of the reducing agent. Both cathodic and anodic reactions occur simultaneously on the same activated surface to be plated; the electrons that appear in anodic oxidation of adsorbed reducing agent are transferred through the surface to the adsorbed metal ions, and the autocatalytic deposition of metal takes place. Since the formed metal layer has a catalytic effect on the reaction of anodic oxidation of the reducing agent, this process is said to be autocatalytic.

The kinetics of the process is estimated using the mixed potential theory, according to which the rate of a faradaic process is independent of other faradaic processes occurring simultaneously at the electrode and thus depends only on the electrode potential. Fig. 1.18
shows the Wagner-Traud diagram, which is a representation of the partial reactions polarization curves. The overall reaction of the electroless deposition can be described electrochemically in terms of three current–potential (i–E) curves:

- \( i_c = f(E) \) the current–potential curve for the reduction of \( Mz^+ \) ions;
- \( i_a = f(E) \) the current–potential curve for oxidation of the reducing agent;
- \( i_{total} = f(E) \) the current–potential curve for the overall electroless solution reaction.

The intersecting points for the polarization curves for anodic and cathodic partial reactions with the potential axis are their respective equilibrium potential values, denoted by \( E^{0}_{\text{Red}} \) and \( E^{0}_{Mz^+} \), respectively. The intersecting point of the polarization curve of the overall reaction occurs for \( i_a = i_c \), thus \( i_{total} = 0 \). The corresponding potential is called the mixed potential, \( E_{mp} \). Therefore, the rate of metal deposition equals the rate of reductant oxidation at \( E_{mp} \).

Figure 1.18: Polarization curves (Wagner–Traud diagram) for a generalized electroless deposition reaction. The dashed line indicates the curve for the complete electroless solution. The partial anodic and cathodic currents are represented by \( i_a \) and \( i_c \), respectively [7].

Alternatively, the current–potential curves for electroless metal deposition can be represented with the Evans diagram. In an Evans diagram the current is expressed in the abscissa in logarithmic scale, as shown in Fig.1.19. According to the Evans diagram of the mixed potential theory, the current–potential curves for individual processes, \( i_c = i_M = f(E) \) and \( i_a = i_{Red} = f(E) \), intersect. At the intersection, the ascissa is the deposition current density \( i_{dep} \), thus the rate at which electroless deposition occurs in terms of \( mA/cm^2 \), while the ordinate is the mixed potential \( E_{mp} \). Therefore, once the current density \( i \) is known from the current–potential curves diagram, the plating rate of metal can be easily calculated.

Steady-state electroless metal deposition at mixed potential \( E_{mp} \) is preceded by a non-steady-state period, called the induction period. The induction period is defined as the time necessary to reach the mixed potential \( E_{mp} \) at which steady-state metal deposition occurs. It is determined in a simple experiment in which a piece of metal is immersed in a solution for electroless deposition of a metal and the potential of the metal is recorded from the time of immersion until the steady-state mixed potential is established. A typical recorded curve for the electroless deposition of copper on copper substrate is shown in Fig.1.20.

Paunovic studied the induction period for the overall process, dividing it into dependence of the open-circuit potential (OCP) on the oxidation and reduction partial reactions, that is, into individual induction periods for each partial process. The OCP of the oxidating system
is reached instantaneously. From a comparison of these OCP curves, one can conclude that the rate of setting of the OCP of the reducing agent, is the rate-determining partial reaction in the setting of the steady-state mixed potential. The major factors that determine the time necessary to reach the rest potential of the reducing agent are the type and concentration of the ligand present [8].

There are two electrochemical methods for determination of the steady-state rate of an electrochemical reaction at the mixed potential. In the first method (the intercept method) the rate is determined as the current coordinate of the intersection of the high overpotential polarization curves for the partial cathodic and anodic processes, measured from the rest potential. In the second method (the low-over potential method) the rate is determined from the low-overpotential polarization data for partial cathodic and anodic processes, measured from the mixed potential. The rate of deposition may be calculated from these curves using the Le Roy equation:

$$i_{dep} = \frac{\sum_{j=1}^{n} i_j E_j}{\sum_{j=1}^{n} E_j^2}$$

$$E_j = 10^{\eta_j/b_a} - 10^{-\eta_j/b_c}$$

where $i_j$ and $h_j$ are current density and overpotential, respectively, at the $j$th point on the i–E curve; and $b_a$ and $b_c$ are the anodic and cathodic Tafel slopes, respectively. A comparison
of the results using this method and the rate of electroless copper deposition determined gravimetrically shows that the best results are obtained with the Le Roy equation applied to the polarization data in the anodic range [8].

Activation of Noncatalytic Surfaces

Noncatalytic surfaces (e.g., nonconductors, noncatalytic metals, noncatalytic semiconductors) have to be activated prior to electroless deposition. This activation is performed by generating catalytic nuclei on the surface of a noncatalytic material. Catalytic nuclei can be produced by photochemical or electrochemical activation process. The features of the latter will be investigated for a deeper understanding of the experimental methods used in this work.

In the electrochemical activation process, catalytic nuclei of a metal \( M \) on a noncatalytic surface \( S \) are generated in a oxidation-reduction reaction,

\[
M^{x+} + \text{Red} \rightarrow M + Ox
\]  

where \( M^{x+} \) is the metallic ion and \( M \) is the metal catalyst. The preferred catalyst is Pd, and thus the preferred nucleating agent \( M^{x+} \) is \( \text{Pd}^{2+} \) (from \( \text{PdCl}_2 \)). The preferred reducing agent \( \text{Red} \) in this case is \( \text{Sn}^{2+} \) ion (from \( \text{SnCl}_2 \)). In this example the overall reaction of activation, according to a simplified model, is

\[
\text{Pd}^{2+} + \text{Sn}^{2+} \rightarrow \text{Pd} + \text{Sn}^{4+}
\]  

\( \text{Sn}^{2+} \) can reduce \( \text{Pd}^{2+} \) ions since the standard oxidation–reduction potential of \( \text{Sn}^{4+}/\text{Sn}^{2+} \) is 0.15 V and that of \( \text{Pd}^{2+}/\text{Pd} \) is 0.987V. Therefore electrons flow from the more electronegative couple (here \( \text{Sn}^{4+}/\text{Sn}^{2+} \)) toward the less electronegative (more positive) couple (here \( \text{Pd}^{2+}/\text{Pd} \)). Electrochemical activation using \( \text{PdCl}_2 \) and \( \text{SnCl}_2 \) may be performed in either two steps or one step. Some nonconductors, such as the polymers polycarbonates and polystyrenes, must be subjected to a surface treatment prior to activation to ensure good adhesion of palladium nuclei. Surface treatment can include the use of chemical etchants for plastics or reactive gas plasma treatments [8].

• Two-Step Activation Process

In the first step, sensitization, \( \text{Sn}^{2+} \) ions are adsorbed on the nonconducting substrate \( S \) from the solution:

\[
S + \text{Sn}^{2+}_{\text{solution}} \rightarrow S \cdot \text{Sn}^{2+}_{\text{ads}}
\]  

where \( S \cdot \text{Sn}^{2+}_{\text{ads}} \) represents the adsorbed \( \text{Sn}^{2+} \) at the surface \( S \). These particles tend to agglomerate into dense clumps that are about 100 to 250 Å in size and the surface coverage is less than 25%. The sensitizer is applied to the substrate \( S \) by immersion of the substrate into the solution for 1 to 3 min. Alternatively, the surface of a nonconductor may be sprayed with sensitizer. Addition of aged stannic chloride (\( \text{SnCl}_4 \)) solution to the tin sensitizer solution results in an improved sensitizer. The improved sensitizer yields a greater number of active centers per unit surface area and a more uniform distribution. The density of adsorbed centers, using the conventional and improved sensitizers, is \( 10^{11} \) and \( 10^{12} \) particles per square centimeter, respectively. The diameter of adsorbed particles for both types of sensitizers is about 10 to 15Å.

The second step in the two-step process is nucleation. Nucleation is performed by immersion of a sensitized nonconductor into the nucleating solution for 0.5 to 2 min. The surface reaction between the stannous ions, \( \text{Sn}^{2+} \), adsorbed on the surface of the substrate ions, \( \text{Pd}^{2+} \), in the nucleator solution is

\[
S \cdot \text{Sn}^{2+}_{\text{ads}} + \text{Pd}^{2+}_{\text{solution}} \rightarrow S \cdot \text{Pd}_{\text{ads}} + \text{Sn}^{4+}_{\text{solution}}
\]  

The nucleation process produces small Pd catalytic sites dispersed on the surface of a substrate in an island network.
• **One-Step Activation Process**

In a one-step activation process, the sensitizing and nucleating solutions are combined into one solution. It is assumed that when this solution is made up, it contains various Sn–Pd chloride complexes. These complexes may subsequently transform into colloidal particles of metallic Pd or a metallic alloy (Sn/Pd) to form a colloidal dispersion. This dispersion is unstable and it may be stabilized by addition of an excess of Sn$^{4+}$ ions. In this case, Pd particles adsorbed on the nonconductor surface are surrounded by Sn$^{4+}$ ions. The latter must be removed by solubilizing before electroless plating so that the catalytic Pd on the surface will become exposed, freely available, to subsequent plating.

**Nickel-Phosphorous Electroless Deposition**

Electroless nickel coatings are widely employed in engineering application due to its unique characteristics, including excellent corrosion, wear and abrasion resistance, ductility, lubrication, soldering, and electrical properties. Moreover, these coatings are more uniform in thickness compared to the electroplated ones.

Nickel–phosphorus alloys, among electroless nickel coatings, represent 90% of the market share in industrial application. Their widespread use is due to the excellent corrosion protection in a variety of environments. On the other hand, phosphorus decrease the magnetic properties and the hardness of nickel. High hardness can be regained in such coatings through a heat treatment at 300–400 °C, which converts Ni–P amorphous alloy into crystalline Ni and a hard nickel phosphide phase. The degree of hardening depends on the content of phosphorus, heat treatment temperature, and time. However, an increase in the hardness of the deposit leads to a decrease in the corrosion resistance [45].

Ni-P baths can be classified on the basis of the pH at which the deposition occurs, thus it is a common practice to examine acidic and alkaline baths separately:

• **Acidic Baths**

Hot acid electroless nickel baths are used almost exclusively for the deposition of relatively thick coatings onto metals. Solutions which require high temperatures and an acid environment have several advantages over alkaline solutions. The coatings obtained in this fashion have superior physical properties and the bath solution is more stable during plating. Acidic baths can be sub-classified by their phosphorous content: 3–5% P coatings have excellent wear and corrosion resistance in concentrated caustic soda; 6–9% P coatings provide corrosion protection and abrasion resistance for most industrial applications and the production is economically favorable; 10–14% P coatings are very ductile and corrosion resistant, in particular against chlorides and simultaneous mechanical stress. A typical bath composition is 33 g/l nickel sulfate, 20 g/l sodium hypophosphite, 28 g/l lactic acid, 16 g/l sodium succinate, and 0.003 g/l lead (Pb$^{2+}$). Operating conditions are pH 5–6, temperature 85–95 °C, giving a deposition rate of 25 μm/h [45].

• **Alkaline Baths**

Hot alkaline nickel–phosphorus deposits are generally reduced by sodium hypophosphite and can work at both high and low temperatures by changing the bath composition. Therefore, an alkaline low temperature bath is a convenient way to deposit nickel on thermoplastic polymers such as PET, which may be damaged at high temperatures. A typical bath composition is 20 g/l nickel chloride, 24 g/l sodium hypophosphite, 45 g/l sodium citrate, and 30 g/l ammonium chloride. Operating conditions are pH 8–9, temperature 30–40 °C, giving a deposition rate of 8 μm/h. Since the main problem of these kind of baths is their instability, the effect of metal ion stabilizer has been extensively studied. Xiao et al. [46] studied the addition of Cd$^{2+}$ as a stabilizer. It increases the stability of the electroless nickel plating bath significantly, although it had negligible effect on the plating rate and P content of the Ni–P deposits within low concentration ranges. Ashassi-Sorkhabi et al.[47] studied the effects of added rare earth elements in the acidic hypophosphite based electroless nickel–phosphorus plating bath. An addition of optimum amounts of rare earth elements to the plating baths could increase the
deposition rate in comparison with the rare earth-free bath and refine the microstructure of the deposit and produce smooth and mirror-like coatings. Srinivasan and John formulated a new electroless bath using the nickel methane–sulfonate as the source of metal ions. The absence of sulfate ions increases the bath life of the electroless nickel bath solution. The small amount of nickel sulfonate is needed for replenishments due to higher solubility of the nickel sulfonate. The methane sulfonate anions increases in the concentration with age of the bath. The addition of calcium to the aged solution allows for the selective removal of orthophosphite thus increasing electroless nickel bath life [48].
1.3. Glucose Sensors

Diabetes mellitus is a chronic endocrine disease characterized by abnormal high levels of the sugar glucose in the blood. Hyperglycemia is the result of the pancreas’s failure to produce enough insulin (Type 1 diabetes) or a condition called insulin resistance in which cells fail to respond properly to insulin (Type 2 diabetes). Such disorder is a worldwide public health problem and represents one of the leading causes of death and disability in the world. According to the World Health Organization (WHO) and the International Diabetes Federation, its worldwide prevalence is projected to double over the next couple of decades, from 347 million people in 2005 to 700 million people in 2030. Notably, >80% of diabetic patients live in low- and middle-income countries. Even if there is still not a cure, the quality of life of diabetics has considerably increased with the development of devices that allow to tightly monitor glucose levels in blood, thus to better manage symptoms and avoid serious complications. The challenge of providing such reliable glycemic control remains the subject of a considerable amount of research.

Most of the traditional methods for measuring blood glucose levels employ electrochemical or colorimetric readout systems. Typical glucose level tests, including common handheld glucometers are done using a small blood sample that is generally obtained through a finger prick. The blood is subsequently introduced to a disposable test strip via capillary action. Although this testing enables accurate results, the finger prick method has had poor patient compliance in the past due to the fact that the patient’s finger must be pricked using a small pin or lancet to draw blood, which can be quite painful and it is associated with a non-negligible risk of infection, thus inconvenient for diabetics. In recent decades, various scientific groups have paid considerable attention to the development of more effective and continuous glucose monitoring techniques including invasive and non-invasive monitoring methods using both enzymatic and nonenzymatic sensing techniques. In attempts to improve patient compliance and comfort, recent advances to circumvent the finger prick method have even moved to examining the glucose in tears or the acetone in breath as markers for diabetes. These advances stand to make next-generation glucose sensors portable, easy to use, capable of instantaneous readout, lower cost, and pain free. Electrochemical glucose sensors play a leading role in this direction and they account for nearly 85% of the world market of biosensors.

1.3.1. Glucose Sensors Generations

The timeline of glucose sensor development can be divided into four primary generations. First generation sensors exploited the catalytic action of enzymes, such as glucose oxidase (GOx). The history of glucose enzyme electrodes starts with the first device developed in 1962 by Clark and Lyons from the Children Hospital in Cincinnati. In these devices the enzymes are immobilized on the surface of an electrode and act as catalyst for the reaction of glucose and oxygen. Glucose is then oxidized with the formation of gluconolactone and hydrogen peroxide as shown in Fig.1.21.

Figure 1.21: Oxidation of D-glucose catalysed by glucose oxidase.
In order to measure the initial concentration of glucose, the amount of hydrogen peroxide generated at the electrode by the reaction is detected. However, these sensors may not function effectively for oxygen deficient blood samples since the reaction requires free oxygen to act as the reaction mediator. Furthermore, first generation glucose sensors are highly sensitive to the interference caused by electroactive species in the blood, such as ascorbic acid, uric acid, and countless other drugs which could be present in the blood [50].

The second generation of glucose sensors relied on the use of a non-physiological, artificial mediator. In this way, oxygen can be replaced and the electron transport process is enhanced. The structure of these devices consist of an enzymatic catalyst immobilized on the surface of the artificial mediator. Various materials such as ferrocene derivatives and ferricyanide have been used as the artificial mediators. Even if second generation glucose sensors managed to overcome some limitation of the first generation device, their performance and sensitivity were still dependent on the changes in the pH of the medium and changes in the temperature and humidity of the electrode surface. A comparison between the structure of the four different generations of glucose sensors is sketched in Fig.1.22.

In the third generation of glucose sensor technology, the enzyme is immobilized directly on the electrode. The need for a reaction mediator is then eliminated making electron transfer process easier. Some nano- or micro-porous materials have been chosen as the substrate for immobilization of the GOx in order to maximize the surface area of the electrode, thus enhancing the electron transfer rate. Though third generation sensors were expected to show some improvement over their first and second generation counterparts, they still suffered from the limitations arising from the dependency of enzyme's activity on the temperature, humidity, interference. Moreover, some additional problems were encountered because the electron transfer process was inhibited by the thick enzyme layer [10].

Finally, the fourth generation of glucose sensor technology is substantially different from the others because it does not rely on the use of enzymes. There are disadvantages of using enzyme as a bio-catalyst for the measurement of glucose concentration. For instance, the unsatisfactory reproducibility of the enzyme-based sensors, the loss of the enzyme activity over time, and the instability of the immobilization of the enzyme limits industrial applications of this type of devices, particularly on continuous monitoring of glucose concentration in bioprocess. The scientific world has recently witnessed a tremendous interest of non-enzymatic glucose (NEG) sensors, as evidenced by the exponential increase in the number of publications over recent years (Fig.1.23).
Since this work is focused in NEG sensors, their current and emerging technologies as well as their properties and sensing mechanism will be investigated.
1.3.2. Mechanism of Glucose Oxidation on the Electrode Surface

While in traditional glucose sensors the enzyme is used as catalyst, in NEG sensors atoms at the surface of the electrode itself act as electrocatalysts. In this way the design of the device becomes much simpler and the rate of glucose oxidation and hence is detection is directly controlled by the chemical and physical properties of the electrode.

Although the mechanism of glucose oxidation at the electrode surface is still not fully understood, there are two main models which have been proposed to explain the above process. The first model, proposed by Pletcher [51], is known as the activated chemisorption model. According to this theory, the oxidation process is initiated through the adsorption of the glucose molecule on the electrode surface, which is then allowed to make a bond with the electrode surface, where the rate of oxidation of higher due to the presence of catalysts atoms. Meanwhile, the hydrogen atom attached to the hemiacetal carbon of the glucose molecule bonds with the electrode surface at a site adjacent to the bonded glucose (Fig. 1.24).

![Figure 1.24: An illustration of the concentric adsorption theory with adjacent adsorption sites proposed by Pletcher. C1: hemiacetal carbon atom. R: the other parts of the glucose molecule [10].](image_url)

Hydrogen extraction leads to a change in the oxidation state of glucose, which lowers the glucose-metal bond strength, thus resulting in desorption of the glucose molecule. Since this mechanism involves both adsorption (bond forming) and desorption (bond breaking) of glucose molecules, a bond of intermediate strength is desired for its optimization.

The second model is known as the ‘Incipient Hydrous Oxide Adatom Mediator’ (IHOAM) model and was proposed by Burke [52]. Burke originally formulated this model on the basis of the fact that metal atoms on the surface of the electrode are more reactive than bulk atoms due to their low lattice stabilization. These atoms undergo a pre-monolayer oxidation step, during which an incipient hydrous oxide layer is formed. The low coverage hydrous oxide, $M[OH]$, formed in the pre-monolayer oxidation process, is the effective oxidant (or mediator)
in electrocatalytic oxidation processes, whereas the low co-ordination surface metal atom, \( M^* \), is the effective reductant [53]. Fig. 1.25 shows a schematic illustration of the IHOAM model.
1.3.3. CuO NEG sensors: Current and Emerging Technology

During the last few years, several different metal oxides have been studied for applications in NEG sensors. Metal oxides possess excellent properties for electrochemical biosensors, namely: controllable size, functional biocompatibility, chemical stability, catalytic activity, enhanced electron-transfer kinetics and strong adsorption capabilities. In particular, copper oxide is a promising material for making NEG sensors. Copper oxide is a p-type semiconductor material with relatively narrow band gap (1.2eV) and it shows high electrochemical activity, is low cost, is non-toxic, and can be readily modified with other materials [10]. The oxidation mechanism of glucose on modified CuO-based electrodes in alkaline solution is believed to be assisted by the redox couple of Cu (II) and Cu (III) [54]. Specifically, the non-enzymatic electro-oxidation process of glucose on CuO surface in an alkaline medium proceeds as follows:

\[
CuO + OH^- \rightarrow CuOOH + e^-
\]  

\[
CuOOH + e^- + glucose \rightarrow CuO + OH^- + gluconic acid
\]  

Lots of work is going on in this field to develop new and novel techniques to fabricate CuO nanostructures. Gunasekaran group [11] developed a sensitive glucose sensor using vertically well-aligned MWCNTs array incorporated with CuO NPs nanocomposite in a simple and rapid two-step electrodeposition method (Fig. 1.26). The nanocomposite was synthesized by depositing Cu nanoparticles onto MWCNTs at constant potential and then they were oxidized into CuO by potential cycling.

Ahmad et al. [55] studied an inkjet-printed CuO NPs modified silver electrode for glucose detection. The CuO NPs were synthesized and developed into CuO NPs ink. Then, a thin layer of Ag (~100nm) was sputtered on the Si substrate followed by the direct printing of CuO NPs ink on Si/Ag substrates using drop-on-demand piezoelectric inkjet nozzle. The thickness of layer could be controlled by varying drop spacing (DS) method. The CV and amperometric measurements exhibited high specificity with good reproducibility and stability. This sensor showed useful linear concentration response for glucose which is up to ~18.45mM.

Espro et al. [56] reported a simple glucose sensor by growing CuO nanowires on Cu substrate “in situ” to avoid tedious hydrothermal synthesis. The growth of CuO was studied by SEM and electrochemical analysis was carried out by CV and amperometry. An interesting
approach for the fabrication of an ultrasensitive electrochemical glucose sensor based on a three different tailored-flower CuO micro/nanostructures (chrysanthemum-like, candock-like, and dandelion-like) grown on Cu foils was analysed [12]. The different nanostructures were obtained by varying the concentration of NaBH₄ in the solution. The dandelion-like CuO film electrode showed highest sensitivity which was attributed to many densely arranged subuliform nanobelts as building units possessing higher aspect ratio than that of nanoribbons and nanobelts, and thus better capability to shuttle electron between reaction and substrate for glucose measurement.

Some of the novel approaches explored for the fabrication of CuO based NEG sensors are listed in table 1.2.

<table>
<thead>
<tr>
<th>Electrode Matrix</th>
<th>Sensitivity $\mu A/mM \cdot cm^2$</th>
<th>Linear range mM</th>
<th>Limit of Detection $\mu M$</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>CuO nanoparticles onto MWCNTs</td>
<td>2190</td>
<td>up to 3</td>
<td>0,8</td>
<td>[11]</td>
</tr>
<tr>
<td>CuO-G-GCE</td>
<td>1360</td>
<td>0,002-4</td>
<td>0,7</td>
<td>[57]</td>
</tr>
<tr>
<td>CuO nanoflowers-Cu foil electrode</td>
<td>789,3</td>
<td>up to 3,1</td>
<td>/</td>
<td>[58]</td>
</tr>
<tr>
<td>CuO NPs</td>
<td>1430</td>
<td>0,04-6</td>
<td>5</td>
<td>[59]</td>
</tr>
<tr>
<td>CuO NPs modified Ag electrode</td>
<td>2762,5</td>
<td>0,05-18,45</td>
<td>$\approx$0,5</td>
<td>[55]</td>
</tr>
<tr>
<td>CuO nanowires on Cu</td>
<td>1800</td>
<td>up to 0,5</td>
<td>10</td>
<td>[56]</td>
</tr>
<tr>
<td>CuO nanoflowers</td>
<td></td>
<td></td>
<td></td>
<td>[58]</td>
</tr>
<tr>
<td>Chrysanthemum-like</td>
<td>3552</td>
<td>/</td>
<td>/</td>
<td></td>
</tr>
<tr>
<td>Candock-like</td>
<td>4078</td>
<td>/</td>
<td>/</td>
<td></td>
</tr>
<tr>
<td>Dandelion-like</td>
<td>5368</td>
<td>/</td>
<td>/</td>
<td></td>
</tr>
<tr>
<td>CuO microfibers/CuO nanoparticles/FTO</td>
<td>2321</td>
<td>up to 0,6</td>
<td>0,0022</td>
<td>[60]</td>
</tr>
<tr>
<td>CuO nanofibers/ITO</td>
<td>up to 1,3</td>
<td>0,04</td>
<td></td>
<td>[54]</td>
</tr>
<tr>
<td>Sandwich-structured</td>
<td>up to 3,2</td>
<td>1</td>
<td></td>
<td>[61]</td>
</tr>
<tr>
<td>CuO/GCE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1.2: Non-exhaustive list of CuO based NEG sensors recently studied.

A variety of different CuO-based electrodes configurations have been studied, changing the geometry of the CuO nanostructures or the multilayer composition of the electrode. While evaluating the possible use of these devices in diabetes patients it should be taken into account that glucose concentration in their blood varies from 2 to 40 mM [62], thus it is necessary to seek new approaches in order to widen the linear range of these sensors.
1.3.4. Monitoring Glucose in Alternative Physiological Fluids

Interstitial Fluid

Interstitial fluid is the extracellular fluid which surrounds tissue cells. Its similarity with blood in terms of composition and concentration of clinically important biomarkers makes it a valuable candidate for medical diagnostics. Specifically, interstitial fluid has been used for minimally invasive determination of inherited metabolic diseases, organ failure or drug efficacy. Consequently, methods for monitoring glucose via the skin have become very popular in recent years, where these approaches have been developed to counteract the challenges associated with patient compliance and invasive monitoring [62].

Microneedles and microneedle arrays can offer minimally invasive methods for electrochemical bio-sensing in Fig. 1.28. This concept was used in the development of a glucose-sensing patch by Jina et al. [13]. The device was designed in two compartments; the first containing the microneedle array and glucose biosensor with the second containing the electronics. Three screen-printed electrodes were used for quantifying glucose concentrations in the interstitial fluid, including a Pt-C working electrode covered with a layer of cross-linked bovine albumin serum and glucose oxidase. The sensing device was attached to the skin...
by an adhesive layer contouring the perimeter of the sensing pod. Detection was performed upon glucose diffusion into the microneedle array, wherein GOx could react to produce hydrogen peroxide. Tests have shown that this device can operate successfully for up to 72 h with only a 17 min lag time caused by the passive diffusion of analytes from blood into the interstitial fluid matrix [13]. Currently, the device must be recalibrated daily by the finger-prick approach.

Sweat

Sweat is one of the most accessible body fluids. Moreover, eccrine glands that excrete sweat can be found all over the body, which is extremely convenient in terms of sensing purposes. Sweat has been exploited for the detection of disease markers such as sodium, potassium, calcium, phosphate and glucose. It is also known that small-molecule drugs and their metabolites are present in sweat, thereby allowing the evaluation of drug efficacy. The reported glucose level in sweat for diabetics varies between 0.01 and 1mM for diabetics. The fluctuations in analyte concentrations result in a broad pH range of sweat, typically between pH 4.0–6.8 during exercise, which can impact on the effectiveness of chemical-sensing or biosensing techniques chosen for disease diagnosis or monitoring [62].

Gao et al. [14] recently reported a non-invasive and continuous wearable glucose-sweat sensing device (Fig. 1.29). Sensors integrated into this Bluetooth-enabled wristband detect skin temperature, sodium, potassium, lactate and glucose concentrations in sweat. Due to the complex nature of sweat, multiple sensors are required to provide a more comprehensive profile of sweat composition and enable data cross-comparisons. The sensors could be used for continuous operation for up to 2 h before the glucose and lactate sensing units were interchanged for fresh sensor arrays [14]. A minimum of 10 µL of sweat was required before any sweat analysis could be achieved. The sensors were placed close to the skin, to allow for immediate analysis of sweat as it emerged. This flexible wearable sensing system is a promising platform for tracking multiple physiological analytes during exercise.

Saliva

Saliva is a complex fluid containing many analytes that permeate from blood, thereby it has been investigated as an alternative fluid for non-invasive glucose sensing, with glucose levels ranging between 0.55 and 1.77mM for diabetics. Although a relationship between glucose levels in blood and saliva obviously exists, it is not well understood, and relating saliva concentration to therapeutic intervention is therefore more challenging. The main benefit being that saliva can be collected in a non-invasive fashion. A variety of new approaches have been reported for continuous and non-invasive glucose detection in saliva, using everyday dental platforms, including mouth guards and dentures, as well as novel devices, such as dental tattoos [62].

Zhang and co-workers [63] have fabricated a disposable microfluidic device for quantifying saliva-glucose concentrations by electrochemical methods. The intricate device design consisted of a working electrode functionalised with single-walled carbon nanotubes, to promote
electron transfer for the glucose oxidase reaction. Gold nanoparticles were incorporated to enhance this signal sensitivity, as well as to promote increased enzyme attachment [63].

Ocular Fluid

The fluid surrounding the eye and ocular tissue, also known as the aqueous humour, contains many analytes present in blood such as glucose, ascorbic acid, lactate, proteins, peptides, hormones, carbohydrates, electrolytes, lipids and chloride. As a result, this fluid has been investigated for non-invasive and continuous glucose monitoring. Recently, the Google[X] lab and Novartis have collaborated on the development of glucose sensing technologies in the aqueous humour. In 2014, they announced their goal to create a smart-contact lens (Fig.1.30), which they hoped would overcome glucose-monitoring obstacles associated with current methods, which are either invasive, in the case of implanted wearable devices or non-continuous, in the case of the finger-pricking approach [15]. This novel technology incorporates a GOx-based electrochemical enzymatic glucose sensor, in a microchip sandwiched between two layers of a soft contact lens. Using a contact lens as a sensing platform does hold many advantages, including real-time continuous and non-invasive glucose monitoring, as the lens would be in constant contact with the aqueous ocular fluid. Disposable contact lenses are typically replaced every 24 h, which is a reasonable period for reliable continuous biosensor operation. Blinking and tear secretion also allow for natural, fresh sample replenishment for accurate glucose concentration measurements throughout the day.

It is well known that critical side effects associated with diabetes are eye damage or blindness, due to glycation of proteins in the blood vessels of the eye. To account for such ocular deteriorations a detection function, potentially based on imaging, for glycation of vascularised tissues could later be incorporated into the lens [62].
1.4. Aim of the Work

As the number of diabetic patients is expected to increase in the future, extensive efforts to develop innovative glucose sensors have been made worldwide in order to properly manage this chronic disease, which might become an issue in terms of costs and administration. The challenge of the new research in glucose sensors is to develop wearable non-invasive devices that offer continuous monitoring of a user’s health and allowing them a better quality of life.

Therefore, the general aim of this work is the realization and characterization of a flexible CuO nanoparticles based non-enzymatic glucose sensors by low-cost inkjet fabrication. Specifically, two different devices are proposed: one platinum amperometric sensor on a stainless steel substrate and a Nickel Phosphorus (NiP) sensor on a polyethylene terephthalate (PET) substrate.

A commercial inkjet printer was employed to deposit the active CuO layer on top of the sensing device. This low-cost approach is the main innovative point that defines the potentiality of this work since it provides an excellent compromise in terms of fabrication cost, simplicity and design flexibility. In order to achieve the highest printability inks were prepared and characterized varying their formulation.

The choice of the materials for the design of the sensor was made on the assumption that the device has to be wearable allowing the highest comfort for the patient. In particular a PET substrate represents a good candidate for the purpose due to its biocompatibility, but patterning of metal thin films with a sufficient high conductivity on a polymeric material is still a challenging task, thus the process has to be further optimized. The influence of the mechanical stresses that arise as a consequence of bending was also investigated for the sensor on stainless steel.

Sensor’s performances were evaluated by electrochemical measurements in a three-electrode configuration, consisting of a working, a reference and a counter electrode. The range of linear current response obtained allows the sensor to be used in different physiological fluids. Selectivity towards glucose was also demonstrated by testing the samples in the presence of six saccharides as interfering species.

Considering the aforementioned properties, the devices proposed in this work are aimed to represent a cost-effective approach for the fabrication of novel non-enzymatic wearable glucose sensors.
2 Experimental Methods

2.1. Inkjet Printing

A commercially available PIXMA MX495 by Canon inkjet printer was employed for all the printing experiments. The ink was printed with a maximum resolution of 4800 x 1200 dpi (dots per inches) and a monochromatic printing speed of 9.9 ipm (images per minute). The printed device was placed in the middle of a paper sheet and stuck to it with common tape so that it could be easily removed after the printing step. As most desktop printers, PIXMA MX495 is a thermal inkjet printer and the inserted sheet is moved in a single direction by a set of rolls, while the printheads move back and forth in the perpendicular direction, depositing the ink following the digital input.

Commercial cartridges were opened, emptied from their original Canon ink, cleaned with abundant deionized water, filled with the CuO NPs ink and finally sealed to avoid leaks. PIXMA MX495 contains two different cartridges: one for black ink and one for colored inks, which is divided in three different compartments. Each printing step was performed by the creation of a .doc file, in which the selected area to be printed was filled with a color corresponding to a specific compartment of the cartridge. In this way, it was possible to print monochromatic areas and be sure that all the ink came from the same compartment of the cartridge.

Figure 2.1: PIXMA MX495 by Canon
2.2. Ink Preparation

2.2.1. CuO Nanoparticles Synthesis

A one-step precipitation approach was used to synthesize CuO nanoparticles [64]. A 200 ml of 0.2 M copper acetate solution \((\text{Cu}(\text{CH}_3\text{COO})_2)\) was mixed with 1 ml acetic acid \((\text{CH}_3\text{COOH})\) in a 400 ml flask. The solution was then heated up to 100 °C under vigorous stirring and 1 g of NaOH pellets was added in the boiling solution. After the addition of NaOH, the color of the solution turned from blue to black (pH=5-6). The sample was then cooled to room temperature. The precipitate solution was centrifuged at 4000 rpm for 1 min, washed with deionized water and ethanol in sequence and dried on a heater overnight.

![Schematic representation of CuO NPs synthesis.](image)

2.2.2. Ink Formulation

The ink used in this work was made of CuO spherical nanoparticles dispersed in a solution of deionized water and ethylene glycol (EG). In order to find the optimal printing conditions CuO NPs inks were formulated varying the concentration of CuO (0.5 % wt., 1 % wt. and 2 % wt.) and EG (5 % vol., 10 % vol. and 20 % vol.). Ink preparation was performed by first mixing water and EG in the desired volumetric concentration and then adding CuO. Weight percentage of CuO NPs is therefore referred to the final weight of water/EG + CuO mixture. The ink was then stirred for 24h and finally sonicated for 30 min with a *Falc Instruments* sonicator (50 kHz; 100 W). Printability of the inks was tested firstly on paper sheet and subsequently directly on the Pt layer of the electrode.
2.3. Electrodes Preparation

In this work two different types of working electrodes were made. Both of them were flexible, but they were assembled starting from two different substrate: firstly on a stainless steel foil, where Platinum deposition was easier, and then on a PET sheet, which is not conductive and thus can be patterned to obtain more easily the three electrodes configuration necessary for sensing purposes on the same sheet. These electrodes had an exposed area of about 1-1.5 cm² and were used for XRF and EDS analysis to evaluate their elemental composition and for SEM and AFM imaging in order to study their surface morphology and multilayer structure.

All the chemical necessary for this work are produced by Sigma-Aldrich and were used as provided.

2.3.1. Working Electrode on Stainless Steel Substrate

A 25 µm thick AISI 302 stainless steel (SS) sheet was acquired from Precision Brand and used as substrate for the fabrication of this electrode. The device had a multilayer structure as schematically showed in Fig. 2.3. The active area of the electrode was isolated by using Kapton polyimide tape. First of all a flash of Nickel was deposited on top of the stainless steel foil. Afterwards a thin layer of Platinum was electrodeposited. Finally, CuO NPs were inkjet printed on top of Platinum forming the active layer of the electrode.

Electrolytic Ni Bath

Plating on stainless steel can be quite challenging because of the passive oxide layer, which is present on the surface of the metal, that prevents strong adherence between the layers. Therefore, a strongly acidic bath was used, specifically a Wood’s strike bath, whose composition is summarized in table 2.1. The stainless steel foil was first immersed for 30s in 20% vol. HCl to facilitate the passive layer dissolution and then was put in the bath with the working parameters expressed in table 2.2. A nickel sheet was used as soluble anode.

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>NiCl₂</td>
<td>250 g/l</td>
</tr>
<tr>
<td>Concentrated HCl</td>
<td>250 ml/l</td>
</tr>
</tbody>
</table>

Table 2.1: Bath Formulation
### Table 2.2: Working Conditions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>Ambient</td>
</tr>
<tr>
<td>pH</td>
<td>Strongly Acid</td>
</tr>
<tr>
<td>Agitation</td>
<td>Vigorous</td>
</tr>
<tr>
<td>Deposition Time</td>
<td>1'</td>
</tr>
<tr>
<td>Current</td>
<td>400 mA/cm²</td>
</tr>
</tbody>
</table>

Electrolytic Pt Bath

A Platinum layer was needed as noble substrate on top of which CuO NPs can be inkjet printed. The bath formulation is listed in table 2.3. The setup comprehended a magnetic stirrer and a digital thermostat Fig.2.4. A precious metal oxides coated titanium anode was used and the working conditions are summarized in table 2.4. The pH was adjusted to the desired value by using a KOH solution.

![Electrolytic Pt Bath Setup](image)

Electrolytic Ni Bath

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration M</th>
</tr>
</thead>
<tbody>
<tr>
<td>$H_2PtCl_6 \cdot H_2O$</td>
<td>0,02</td>
</tr>
<tr>
<td>Triethylenetetramine</td>
<td>0,5</td>
</tr>
<tr>
<td>$NKO_3$</td>
<td>0,5</td>
</tr>
<tr>
<td>$HK_2O_4P$</td>
<td>0,2</td>
</tr>
</tbody>
</table>

![Electrolytic Pt Bath Setup](image)

Table 2.3: Bath Formulation
2.3. Electrodes Preparation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>60°C</td>
</tr>
<tr>
<td>pH</td>
<td>11.5</td>
</tr>
<tr>
<td>Agitation</td>
<td>Vigorous</td>
</tr>
<tr>
<td>Deposition Time</td>
<td>10'</td>
</tr>
<tr>
<td>Current</td>
<td>20 mA/cm²</td>
</tr>
</tbody>
</table>

Table 2.4: Working Conditions

CuO NPs Printed Layers
The active catalytic surface of the working electrode was finally deposited by inkjet printing the CuO-based ink on top of the Platinum layer. 25 printing steps guaranteed a sufficient amount of CuO nanoparticles and the desired thickness for electrochemical testing. After each printing step the samples were heated up to increase the rate of evaporation of the solvent and allow a better homogeneity of the film. The specimens then underwent annealing at a temperature of 150°C for 30 minutes.

2.3.2. Working Electrode on PET substrate

A PET sheet was used as substrate for the fabrication of this electrode. The device had a multilayer structure as schematically showed in Fig. 2.5. The design of the electrode is different from the one used for the electrode on stainless steel. Since PET is an insulator material, it does not conduct electricity and a conductive path that could connect the active area of the electrode to the testing system was needed. First of all the PET sheet underwent a surface pretreatment, whose aim was to increase the substrate wettability. Afterwards the PET surface was activated by growing Pd NPs as catalytic sites. A thin layer of Nickel-Phosphorous, which acted as conductive path, was then deposited by electroless deposition. Pt was evaporated on the NiP surface in order to obtain the noble substrate for CuO deposition. Finally, CuO NPs were inkjet printed on top of Nickel-Phosphorous forming the active layer of the electrode.

![Figure 2.5: Schematic representation of the multilayer structure of the working electrode.](image)

Surface Pretreatment
The PET surface was immersed for 30 min in a 3M NaOH solution to increase its wettability. When subjected to alkaline environment, the weak ester bonds in the polymer chain of PET break by mean of an hydrolysis reaction (the reverse reaction of esterification). As a result, hydrophilic groups (hydroxyl and carboxyl) are formed on top of the PET surface increasing its roughness and making it more hydrophilic (Fig. 2.6). Since this alkaline treatment destroys just a few nanometers of the PET surface, it has a little influence on the bulk properties of the material [65].

Surface Activation
In order to start electroless deposition the surface has to be activated by creating active catalytic sites on it. In this work, the pre-treated PET sheet underwent electrochemical activation using Pd as catalyst. A Neoganth 834 commercial Pd solution was employed, which
has a registered and unknown composition. It is declared that it needs 5 minutes of immersion to adsorb completely on the surface. The *Neoganth* has to be reduced to obtain metallic nanoparticles on the surface of the specimen. *NaBH₄* solution (20g/l) was used as reducing agent. The activation steps are:

- immersion of the PET sheet in the *Neoganth* solution for 5 minutes;
- reduction of the adsorbed *Neoganth* solution with the *NaBH₄* solution for about 1 minute;
- careful and deep wash in deionized water to remove any remaining of the reducing agent;
- the aforementioned steps were repeated three times to reach the desired nanoparticles surface density.

**Electroless NiP Bath**

NiP is electroless deposited as the conductive layer of the electrode. The bath composition is summarized in table 2.5 and the working conditions in table 2.6. The pH is adjusted with a *NH₃* solution in the formulation. The solution is assembled with a final volume of 200 ml and the setup comprehended a magnetic stirrer and a digital thermostat Fig.2.7. Vigorous stirring is required in order to avoid damage from hydrogen bubbles, but it might remove some of the Pd nanoparticles and leading to surface inhomogeneities if performed from the beginning of the reaction. Therefore the stirrer started to work 5-10 seconds after the immersion of the specimen.

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration g/l</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>NiSO₄</em></td>
<td>32</td>
</tr>
<tr>
<td>Sodium citrate</td>
<td>20</td>
</tr>
<tr>
<td><em>NH₄Cl</em></td>
<td>25</td>
</tr>
<tr>
<td>Sodium hypophosphite</td>
<td>28</td>
</tr>
</tbody>
</table>

Table 2.5: Bath Formulation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>45 °C</td>
</tr>
<tr>
<td>pH</td>
<td>9</td>
</tr>
<tr>
<td>Agitation</td>
<td>&gt;500rpm</td>
</tr>
<tr>
<td>Deposition Time</td>
<td>10'</td>
</tr>
</tbody>
</table>

Table 2.6: Working Conditions
2.3. Electrodes Preparation

Figure 2.7: Electroless NiP bath setup.

Platinum thin film
Platinum was evaporated on the NiP surface in order to obtain the noble metal layer necessary for sensing purposes. The thickness of such film was in the order of 80nm.

CuO NPs Printed Layers
As for the electrode on stainless steel, the active catalytic surface of the working electrode was finally deposited by inkjet printing the CuO-based ink on top of the Platinum layer. 60 printing steps guaranteed a sufficient amount of CuO nanoparticles and the desired thickness for electrochemical testing. After each printing step the samples were heated up to increase the rate of evaporation of the solvent and allow a better homogeneity of the film. The specimens then underwent annealing at a temperature of 60°C for 3 hours.
2.4. Contact Angle Measurement

Contact angle measurement was employed to evaluate the surface tension of the CuO-based ink for three different ethylene glycol concentrations: 5%, 10% and 20% respectively. Surface tension is a key parameter for determining the Ohnesorge number and thus the printability of an ink. Static contact angle measurement with the sessile drop method were performed on a pure silicon substrate, recorded and analysed by a KRÜSS DSA100 instrument with its relative software for Windows.

2.4.1. Contact Angle

Consider droplet of liquid on a flat, horizontal surface. The angle formed by the intersection of liquid–vapor and liquid–solid interface (geometrically integrated by drawing a tangent line from the contact point along with the liquid–vapor interface of the droplet) is called contact angle Fig.2.8. The shape of a liquid droplet is ideally determined by the liquid surface tension. Each molecule in the bulk is pulled equally in every direction by neighbouring liquid molecules, resulting in a zero net force. On the other hand, the surface molecules do not have neighboring molecules in all directions to balance the net force, but they are pulled inward creating an internal pressure. As result, the droplet contracts to maintain the lowest surface free energy assuming a spherical shape. The contact angle is an effective method to determine the physicochemical surface interactions of the three phases system formed by the drop, the substrate and surrounding air. The interface where the point at which liquid, solid, and vapor are coexisting is considered as “three phase contact line”. The magnitude of contact angle mainly depends on the steady of forces at the boundary line between solid, liquid, and vapor. Theoretically, contact angle value is expected to be the characteristic property of given surface/liquid in the particular environment. As first defined by Thomas Young, this is an energy balanced approach to the three equilibrium surface tensions and equation is derived as Young’s equation and given as:

\[ \gamma_{SG} - \gamma_{SL} = \gamma_{LG} \cos \theta \]  

(2.1)

where \( \theta \) represents the contact angle, \( \gamma_{LG}, \gamma_{SG}, \) and \( \gamma_{SL} \) symbolize the liquid–vapor, solid–vapor, and solid–liquid surface tensions, respectively. The Young’s equation was derived based on the hypothesis that, surface should be rigid, flat, nonreactive, inert, homogeneous, insoluble, smooth, and nonporous. A surface, which encounters all the assumption of the Young equation, is stated to as an ideal surface. Those parameters are usually not applicable in real surfaces. As a result, the contact angle value may vary from one point to another because of different surface tensions [66].

2.4.2. Sessile Drop Technique

One of the most extensively employed method for analyzing the contact angle is direct measurement of the tangent angle at three-phase equilibrium interfacial point using sessile drop method. An image of the adhering drop can be projected onto a screen and the outlines traced, then the angle is measured as shown in Fig.2.9. The accuracy of the method can be
improved by the use of comparatively high magnifications that allow the detailed investigation of the intersection of the solid surface and the drop profile. This method has the advantage of simplicity in operation and only small surface area of substrates and small amounts of liquid are required. On the other hand, small amounts of liquid leads to comparatively higher influence of impurities. The reproducibility and accuracy of contact angle measurement mainly depends on the assignment of the tangent line and consistency of the operator. It is essential to determine specific guidelines for operators to follow. Because, the drop might be unsymmetrical, it is advisable that contact angles be measured on both sides of the liquid drop profile and to use the averaged result. For a relatively large substrate, contact angles should be measured at multiple points to give an average value that is representative of the entire surface [66].
2.5. Rheological Measurement

Rheological measurements were done to determine the dynamic viscosity of the CuO-based ink for different ethylene glycol concentrations and temperatures: 5%, 10%, 20% and 25°C, 30°C, 40°C respectively. The results obtained were then used to calculate the Ohnesorge number and thus the printability of the inks. A Rheometrics DSR200 (Dynamic Stress Rheometer) was employed for all the measurements.

2.5.1. Dynamic Shear Rheometer

Dynamic shear rheometers have been in use for some time as a tool for research and development as well as for quality control in the manufacture of a wide range of materials. In the use of such rheometers, a small sample of a viscoelastic material is placed between two parallel circular plates spaced apart by a relatively small distance. One of the plates is oscillated to vary the angular position of the plate relative to the other plate with a torque provided by a precision electronic motor, under the control of a computer, and the angular displacement of the one plate relative to the other is measured precisely, usually by an optical encoder as shown in Fig. 2.10. The rheological properties of the material then are calculated by the computer, using the known torque and angular displacement. In order to maintain accuracy, the measurement of the torque and the angular displacement are maintained within an optimum range, within which range the quantities being measured are large enough to attain adequate resolution, yet are not so large as to be outside the range of accuracy of the measuring devices employed to determine both the torque and the angular displacement. Moreover, the measurement of torque and angular displacement must be carried out within the range where the relationship between stress and strain in the material being tested remains linear.
2.6. XRF Analysis

XRF was employed to quickly evaluate the rough elemental composition and the thickness of the different layers of the electrode structure. A FISHERSCOPE X-RAY XULM machine by Fisher (Fig. 2.11) was used to analyze the samples in this work.

![FISHERSCOPE X-RAY XULM by Fisher.](image)

2.6.1. Physical Basis

X-Ray Fluorescence (XRF) is a nondestructive method used for elemental analysis of materials. An X-ray source is used to irradiate the specimen and to cause the elements in the specimen to emit their characteristic X rays. A detector system is used to measure the positions of the fluorescent X-ray peaks for qualitative identification of the elements present, and to measure the intensities of the peaks for quantitative determination of the composition. All elements but low-Z elements—H, He, and Li—can be routinely analyzed by XRF. XRF can be used routinely for the simultaneous determination of elemental composition and thickness of thin films. The technique is nondestructive, rapid, precise, and potentially very accurate.

X-rays are highly energetic electromagnetic radiation of short wavelength. When an X-ray photon strikes an atom and knocks out an inner shell electron, if the incident photon has energy greater than the binding energy of the inner shell electron, a readjustment occurs in the atom by filling the inner shell vacancy with one of the outer electrons and simultaneously emitting an X-ray photon. The emitted photon (or fluorescent radiation) has the characteristic energy of the difference between the binding energies of the inner and the outer shells. The penetration depth of a high energy photon into a material is normally in the range.

XRF measurement were first reported in 1913 by H.G.J. Moseley, who found out that the square root of the frequency of the various X-ray lines exhibited a linear relationship with the atomic number of the element emitting the lines [67]. This fundamental “Moseley law” shows that each element has a characteristic X-ray spectrum and that the wavelengths vary in a regular fashion form one element to another.

2.6.2. Instrumentation

The instrumentation required to carry out XRF measurements normally comprises three major components: the primary X-ray source, the crystal spectrometer, and the detection system. A schematic X-ray experiment is shown in Fig2.12. Fluorescent X rays emitted from
the specimen are caused by high-energy incident X-rays generated by the X-ray tube. The fluorescent X rays from the specimen travel in a certain direction, pass through the primary collimator. The analyzing crystal, oriented to reflect from a set of crystal planes of known d-spacing, reflects one X-ray wavelength (\( \lambda \)) at a given angle (\( \theta \)) in accordance with Bragg's law:
\[
n\lambda = 2d \sin \theta
\]  
(2.2)

where \( n \) is a positive integer giving the order of reflection. By rotating the analyzing crystal at one-half the angular speed of the detector, the various wavelengths from the fluorescent X-rays are reflected one by one as the analyzing crystal makes the proper angle \( \theta \) for each wavelength. The intensity of at each wavelength is then recorded by the detector. This procedure is known also as the wavelength-dispersive method [67].

Figure 2.12: Schematic representation of an XRF experiment.
2.7. SEM Analysis

The morphology and the elemental composition of the multilayer electrodes structure was further studied with an *EVO 50 EP* SEM by *Zeiss* coupled with an *Inca* EDS (Energy Dispersive Spectroscopy) unit by *Oxford* (Fig. 2.13). The samples were put in liquid nitrogen and subsequently cut to isolate a section where all the layers were visible.

![EVO 50 EP SEM by Zeiss](image)

2.7.1. Physical Basis

In the SEM (Scanning Electron Microscope), a source of electrons is focused (in vacuum) into a fine probe that is rastered over the surface of the specimen. As the electrons penetrate the surface, a number of interactions occur that can result in the emission of electrons or photons from (or through) the surface. A reasonable fraction of the electrons emitted can be collected by appropriate detectors, and the output can be used to modulate the brightness of a cathode ray tube (CRT) whose x and y inputs are driven in synchronism with the x-y voltages rastering the electron beam. In this way an image is produced on the CRT; every point that the beam strikes on the sample is mapped directly onto a corresponding point on the screen.

The images in the SEM are produced by different sources shown in Fig. 2.14 and can be divided in three main types: secondary electron images, backscattered electron images, and elemental X-ray maps. Secondary and backscattered electrons are conventionally separated according to their energies. They are produced by different mechanisms.

When a high-energy primary electron interacts with an atom, it undergoes either inelastic scattering with atomic electrons or elastic scattering with the atomic nucleus. In an inelastic collision with an electron, part of the energy is transferred from one electron to the other. The emitted electron can exit the solid only if the energy transferred exceeds the work function of the material. When the energy of the emitted electron is less than about 50 eV, by convention it is referred to as a secondary electron (SE), or simply a secondary. Most of the emitted secondaries are produced within the first few nm of the surface. Secondaries which originate deeper in the specimen cannot be detected as they undergo additional inelastic collision that, by lowering their energy, trap them in the material.
Higher energy electrons are primary electrons that have been scattered without loss of kinetic energy by the nucleus of an atom, although these collisions may occur after the primary electron has already lost some of its energy to inelastic scattering.

Backscattered electrons (BSEs) are considered to be the electrons that exit the specimen with an energy greater than 50 eV, including Auger electrons. However most BSEs have energies comparable to the energy of the primary beam. The higher the atomic number of a material, the more likely it is that backscattering will occur. Therefore, as a beam passes from a low-Z to a high-Z area, the signal due to backscattering, and consequently the image brightness, will increase. As a consequence, elemental differences result in a built-in contrast [68].

The number of backscattered and secondary electrons emitted per incident electron are expressed by the backscattered electron yield $\eta$ and the secondary electron yield $\delta$:

$$\eta = \frac{i_{\text{BSE}}}{i_0} \quad (2.3)$$

$$\delta = \frac{i_{\text{SE}}}{i_0} \quad (2.4)$$

Both the secondary and backscattered electron yields increase with decreasing glancing angle of incidence because more scattering occurs closer to the surface. This is one of the major reasons why the SEM provides excellent topographical contrast, specifically in the SE mode. As the surface changes its slope, the number of secondary electrons produced changes as well, while for the BSEs this effect is not as prominent, since to fully realize it the BSE detector would have to be repositioned to measure forward scattering [68].

When the SEM is in primary electrons mode, the X-ray emission signal can be sorted by energy in an energy dispersive X-ray detector (EDS). The spectra obtained in this way are characteristic of the elements that produced them, thus images that show the spatial distribution of particular elements in the field of view can be made. Primary electrons can travel considerable distances into a solid before losing enough energy through collisions to be no longer able to generate X-ray emission. Therefore, the spatial resolution will rarely be better than 0.5 $\mu$m because the X-rays come from a large volume of the sample [68].
2.7.2. Instrumentation

Fig. 2.15 main features of the instrument are the electron column containing the electron source (the gun), the magnetic focusing lenses, the sample vacuum chamber and stage region (at the bottom of the column) and the electronics console containing the control panel, the electronic power supplies and the scanning modules. A solid state EDS X-ray detector is usually attached to the column and protrudes into the area immediately above the stage. The overall function of the electron gun is to produce a source of electrons emanating from as small a “spot” as possible. The lenses act to demagnify this spot and focus it onto a sample. The emission area might be a few μm in diameter and will be focused eventually into a spot as small as 1 or 2 nm on the specimen.

![Schematic of the electron optics constituting the SEM.](https://example.com/schematic.png)

The major electron sources are thermionic tungsten and LaB₆. In the former case, a tungsten filament is heated to temperatures in the range of 3000 °C, at which electrons are emitted by thermionic emission. LaB₆ has a lower work function than tungsten and thus can be operated at lower temperatures. However, LaB₆ filaments require a higher vacuum level than tungsten to achieve good stability and sufficient lifetime. Alternatively, field emission electron sources are used. They consist in very sharp tips subjected to a strong electric field that pushes electrons out of the material even at low temperatures.

Then the electron beam, generated by one of the aforementioned techniques, is defocused by a series of magnetic lenses as shown in Fig. 2.15, whose optical properties change depending on the magnitude of the current that flows through them. The top lenses are called condenser lenses and the focal length decreases as the current increases [68]. Therefore, increasing the current through the first lens leads to a reduction of the size of the image produced, but very high magnification images are inherently noisy, thus with poor resolution [68]. The final lens does the ultimate focusing of the beam onto the surface of the sample.
2.8. AFM Analysis

A SOLVER PRO Atomic Force Microscope by NT-MDT shown in Fig. 2.16 was employed at different stages of the manufacturing process to investigate the surface morphology and roughness of the different layers.

![SOLVER PRO Atomic Force Microscope by NT-MDT](image)

**Figure 2.16:** SOLVER PRO Atomic Force Microscope by NT-MDT.

2.8.1. Physical Basis

In AFM scanning, a cantilever with a sharp tip is used to scan over the surface of the sample. The cantilever probes the surface by sensing the force between surface and tip. The atoms respond to the developed Van der Waals force, which can be either short-range repulsive exchange interactions or longer-range attractive, depending on the type of contact. As the tip approaches the surface, the attractive Van der Waals force between the cantilever tip and sample deflects the cantilever toward the sample surface. When the cantilever tip is brought in contact with the sample surface, the repulsive van der Waals force develops, which pushes back the tip. These deflections, toward or away from the sample surface, are detected by a laser beam. The laser beam strikes the top of the cantilever and reflects back to a position-sensitive four-segment photodetector. The deflection in the cantilever is recorded by the photodetector. The segments of the photodetector are used to track the position of the laser spot on the detector and the angular deflections of the cantilever. While scanning, the AFM tip continuously moves back and forth along the surface features and the resulting deflections are recorded by the detector [69].

The tip and the cantilever are micro-machined components made of silicon or silicon nitride. The cantilever determines the force applied to the sample according to the deflection it undergoes. Its dimensions range from 100 to 200 μm in length, 10 to 40 μm in width, and 0.3 to 2 μm in thickness. The tip undergoes blunting due to continuous measurements, which leads to a poorer image quality. In contact mode, topography measurement of soft samples sometimes damages the tip or sample itself, owing to the higher contact forces involved in scanning.

The stiffness of the cantilever depends its dimension, shape, and material and it is a critic parameter to consider while making measurements with AFM. Soft materials require
2.8. AFM Analysis

A lower stiffness cantilever such as silicon nitride so that the cantilever is deflected in the measurement process without deforming the sample surface. The stiffness and resonant frequency are higher for shorter and thicker cantilevers. These types of cantilevers are used in non-contact mode for measurement on slightly harder and rough surfaces [69].

2.8.2. Instrumentation

The basic components of AFM are a laser diode, a photodetector which works as a scanner, a cantilever, and a sample stage capable of moving in x, y, and z directions (Fig. 2.17). In AFM, a sharp microfabricated tip attached to a cantilever is scanned across the sample. The cantilever is deflected due to the forces developed between the tip and the sample. The deflection is monitored using a laser and photodiode and is used to generate an image of the surface. The probe is placed on the end of a cantilever. The amount of force between the probe and sample depends on the spring constant (stiffness of the cantilever) and the distance between the probe and the sample surface. This force can be described using Hooke’s Law as \( F = kx \), where \( F \) is the force exerted on the cantilever during scanning, \( k \) is the spring constant or stiffness of the cantilever, and \( x \) is the cantilever deflection.

![Figure 2.17: Schematic draw of an Atomic Force Microscope.](image)
2.9. TEM Analysis

The characterization of the nanoparticles dispersed in the ink involved the use of a Transmission Electron Microscope (TEM). Specifically, a CM200 TEM by Philips was used in this work for measurements of the size and spatial distribution that are important parameters to take into account when studying the printability of a NPs based ink. Additionally, Selected Area Electron Diffraction (SAED), which is a crystallographic experimental techniques employed for the identification of crystal structures and crystal defects, was performed on the CuO NPs with the same machine.

2.9.1. Physical Basis

In TEM, an electron beam, generated from an electron gun, is focused by metal apertures and electromagnetic lens, which are present in the column of the machine, on a thin (less than 200 nm) sample placed onto the sample holder (or called TEM grid, consisting of metal frame and carbon-based film) equipped with a mechanical arm for controlling the position and holding it. The focusing mechanism is based on the wavelike character of electrons as they behave as negatively charged particles, then deflected by magnetic or electric fields. The signal in TEM is obtained from both undeflected and deflected electrons that penetrate the sample thickness and is delivered to a detector, usually a fluorescent screen, a film plate, or a video camera to convert the electron image information to a visible form. With a TEM high resolutions at the nanoscale can be obtained when the electrons in the beam have extremely short wavelengths. Such conditions are satisfied by applying several hundred kilovolts on the basis of the equation:

\[ \lambda \sim \frac{1.23}{\sqrt{V}} \]  

where \( \lambda \) is the wavelength and \( V \) is the acceleration voltage. Thanks to the high resolution of a TEM, even the fine structure of crystals can be determined by using the electrons with short wavelength. Additionally, to enhance the mean free path of electrons in the column of a TEM, the column in a TEM should maintain an extremely high vacuum since electrons cannot move in atmosphere. In Fig.2.18 a schematic draw of a TEM instrument, showing the location of a thin sample and the principal lenses within a TEM column, is illustrated [70][71].

2.9.2. SAED

The SAED technique uses a broad and parallel electron beam and a selected area aperture to define the region on the sample from where the diffraction pattern is obtained. The smallest area that can be selected by the aperture is (typically) approximately 500 nm in diameter. SAED patterns also yield either arrays of spots or ring patterns, which can be indexed for the structure analysis of the sample. Each spot of the pattern corresponds to a satisfied diffraction condition of the sample’s crystal structure. If the sample is tilted, the same crystal will stay under illumination, but different diffraction conditions will be activated, and different diffraction spots will appear or disappear. The accuracy and precision of \( d \)-spacings measurements in SAED patterns are critical for structure determinations, which require both careful calibration of the camera factors, such as camera length and camera constant and correction for elliptical distortion. This can be done by using a standard material, such as gold or platinum, sputtered and deposited onto the thin TEM sample, which can provide ring patterns of known \( d \)-spacings. Ideally, the size and thickness of the standard material is similar to that of the sample for the analysis [72].
Figure 2.18: Schematic draw of a Transmission Electron Microscope.
2.10. Electrochemical Measurements

The sensing performance of the CuO-based electrodes were investigated by mean of Cyclic Voltammetry (CV) and Chronoamperometry (CA) experiments. The working electrodes were tested in an electrolyte solution (0.1 NaOH) at ambient temperature. For all the experiments a Kapton adhesive sheet presenting a 5 mm x 5 mm window was applied to the sensor and a three electrodes setup, which is shown in Fig.2.19, was employed, whose components were:

- A CuONPs-based working electrode (WE);
- An Ag/AgCl KCl saturated reference electrode (RE);
- A platinum wire as counter electrode (CE);

The measurements were performed with an *EG&G Model 273 Potentiostat/Galvanostat* by *Princeton Applied Research*.

2.10.1. Cyclic Voltammetry

Cyclic voltammetry measurements were performed to evaluate the redox activity of the electrodes in the presence of glucose and the potential at which that activity was observed. The scanning voltages were analyzed at a scan rate of 50mV/s and they varied in the range of 0 and 0.8V.

**Basic Theory**

Cyclic voltammetry is one of the most commonly electrochemical measurement technique used and it consists in scanning the potential applied to a working electrode according to a triangular waveform and monitoring the resulting current flow Fig.2.20. The current response provides a powerful and direct insight into the energetics of redox reactions, the dynamics and reversibility of the electron transfer, as well as the rates of coupled chemical
reactions. Experimentally, the potential of a working electrode is cycled between two potential limits, driving the successive oxidation and reduction of an electroactive species that can be in solution or adsorbed at the electrode surface. By monitoring the current as a function of potential, current–potential voltammograms can be obtained that are unique to the system under study [73].

![Graph of electrochemical measurements](image)

Figure 2.20: (a) Triangular potential waveform employed in cyclic voltammetry. \( E_{\text{max}} \) and \( E_{\text{min}} \) are the switching potentials; \( t_s \) is the switching time; \( E_i \) is the initial potential; (b) Current response of a cyclic voltammetry experiment with a triangular potential waveform.

### 2.10.2. Chronoamperometry

Chronoamperometry measurements were performed to evaluate the sensing performance of the CuO based working electrodes. Specifically the sensitivity, the linear range and the detection limit were measured. In all the experiments, the concentration of glucose was gradually increased by steps of 0.5mM or 1mM or up to 7-8mM every 45 seconds. The samples were also tested in two bent configurations with different radius of curvature. The setup and the active area were the same as for CV measurements. Moreover, the selectivity of the electrodes was evaluated by addition of five different interfering species present in human blood (lactose, maltose, mannitol, sorbitol and fructose).

**Basic Theory**

Chronoamperometry is an electroanalytical technique that involves the application of a constant reducing or oxidizing potential to an indicator (working) electrode and the subsequent measurement of the resulting steady-state current. It is widely employed in the study of electrochemical sensors since the magnitude of the measured current is dependent on the concentration of the reduced or oxidized substance, which is usually the substance to detect.

This method offers the ability to distinguish selectively between a number of electroactive species in solutions by judicious selection of the applied potential and/or choice of electrode material. In cases where a nonspecific potential is applied, the resulting current may be contributed by several electroactive species. The careful choice of the composition of the supporting electrolyte may also be useful in improving the selectivity of amperometric methods. In cases where oxygen interferes, its removal by purging the solution with an inert gas such as nitrogen or argon may be necessary.

The electrochemical oxidation or reduction of an electroactive species on a suitable electrode material by the application of the desired potential results in either a steady-state anodic or cathodic current. According to the Cottrell equation, the resulting current can be
related to the concentration of the electroactive species as follows:

\[ I_f = \frac{nFA\sqrt{D}C_b}{\pi t} \]  

(2.6)

where \( I_f \) is the diffusion current (\( \mu A \)), \( t \) is the electrolysis time (s), \( n \) is the number of electrons involved in the electrode reaction, \( F \) is the Faraday constant, \( A \) is the electrode area (m\(^2\)), \( D \) is the diffusion coefficient of the electroactive species (m\(^2\) s\(^{-1}\)), and \( C_b \) is the bulk concentration of the electroactive species (mmol l\(^{-1}\)). The magnitude of the resulting current will thus be directly proportional to the concentration of the reduced or oxidized substance. This technique therefore provides a simple and reliable method for the quantification of various substances based on the relationship between the measured current and concentration of the analyte [74]. In Fig. 2.21 it is shown an example of the typical current-voltage response of a chronoamperometric experiment for sensor calibration.

When a metal is in contact with an electrolyte solution, a peculiar system forms at the surface, which is usually referred to as double layer. It consists in electrons, solvent molecules, electro-active species and ions in different arrangement and concentration as one proceeds from the metal to the solution. The metal solution interface behaves like an electrical capacitor in that it can store charge. In absence of any electrochemical reactions, the potential step produces a change in electron density of the double layer and a modification of ions distributions in solution. In response to the potential step, \( I_e \) decays exponentially following the expression:

\[ I_e = \frac{(E_f - E_i)}{R_s} e^{-\frac{t}{R_sC}} \]  

(2.7)

where \( R_s \) is the electrical resistance of the solution and \( C \) is the capacitance of the double layer and \( E_f - E_i \) the potential step applied to the sample. In the presence of glucose, when an appropriate step potential is applied at the working electrode, glucose oxidation occurs. The electrical current, response of the chemical reaction (\( I_f \)), increases compared to the bulk current, response of the potential step (\( I_e \)), proportionally to glucose concentration [74].

![Figure 2.21: Example of a chronoamperogram obtained by successive addition of 50 \( \mu \)mol l\(^{-1}\) DNPH in an electrochemical cell containing MeOH [16].](image-url)
3

Results and Discussion

In this chapter the results obtained will be discussed starting from the characterization of the ink and the evaluation of its printability, to the characterization of the electrodes and the evaluation of their electrochemical sensing performances. All the experimental analysis employed to get the results are described in the previous chapter.

3.1. CuO Nanoparticles Characterization

CuO nanoparticles Fig. 3.1 were the main component of the ink used in this work due to their catalytic activity in the presence of glucose. Therefore, a proper characterization, which included the evaluation of their morphology, size distribution and aggregation behavior, was needed.

Figure 3.1: Picture of the dried CuO-NPs obtained with the one-step process described in the previous chapter.

The samples used for the analysis consisted in a water/ethylene glycol (EG) mixture in which 1% wt. CuO nanoparticles were dispersed. Additives such as EG are necessary for stable drop formation for water based inks. Glycols are typically used as humectants for
their hygroscopic nature, which makes the solvent to evaporate more slowly. Moreover, these additives also act as rheology modifiers for the adjustment of surface tension and viscosity [75] in order to avoid premature nozzle clogging.

The effect of EG concentration on the nanoparticles distribution in the ink was studied by varying the composition of the mixture. Therefore, three different mixtures were studied, specifically with 5% vol., 10% vol. and 20% vol. EG. All the inks formulated were found to be stable for days, with no CuO NPs precipitation observed.

The size and morphology of the samples were analyzed at different magnifications by transmission electron microscopy (TEM) for each ethylene glycol concentration. The TEM images reveal that the product consists of almost equiaxial, thus spherical, particles with a regular morphology.

![Figure 3.2: TEM images of the copper oxide NPs dispersed in water/EG mixtures with increasing EG content.](image)

The TEM images obtained by evaporation of the aqueous component of the ink showed different aggregation behavior depending on the EG content. As visible from Fig.3.2, tendency to agglomerate is greatly amplified by a high EG content. Apparently, even the concentration of CuO nanoparticles increases with increasing EG content by observing the TEM images, but, since concentration of NPs was not varied during the preparation of the three samples (1%wt.), the different agglomeration states are solely due to different evaporation mechanisms of the inks. In particular, the Marangoni effect, which is described in Chapter 1, determines the aggregates morphology of an evaporated ink droplet. The Marangoni effect is strongly affected by the surface tension of the dispersion and by changing the content of EG, the surface tension of the droplet increases, thus leading to different morphologies of the aggregates [21].

The SAED patterns confirmed the purity of the CuO nanoparticles and evidenced the presence of only monoclinic CuO [76]. The various diffraction peaks present in the pattern come from different crystallographic planes, thus the nanoparticles were found to be polycrystalline. Specifically, diffraction rings for CuO (110), (002) and (111) planes are clearly visible in Fig.3.3.
3.1. CuO Nanoparticles Characterization

Figure 3.3: SAED pattern of the copper oxide NPs.

The size distribution of the particles was studied by manual length measurements on the TEM images, which were processed with the software ImageJ. The results for the three samples are displayed in Fig.3.4, which presented a nearly gaussian distribution.

Figure 3.4: NPs maximum diameter size distribution measured on the basis of TEM images shown in Fig.3.2.
Fig. 3.5 shows a comprehensive size distribution of the NPs for the three samples, whose mean diameter turned out to be in the range of 5 nm with an acceptable narrow size distribution (table 3.1). Therefore, the synthesized nanoparticles were homogeneous in terms of shape and size confirming the effectiveness of the one-step procedure employed for this work and described in the previous chapter.

![Graph showing size distribution](image)

**Figure 3.5:** comprehensive NPs maximum diameter size distribution of the three samples analyzed.

<table>
<thead>
<tr>
<th>Statistics</th>
<th>Value (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>5.25</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>1.65</td>
</tr>
</tbody>
</table>

**Table 3.1:** Maximum diameter size distribution statistics.
3.2. Ink Printability

Ink printability was evaluated by firstly varying the CuO-NPs concentration in the dispersion and secondly varying the EG content in the water/EG mixture. In both cases a first qualitative analysis was made by printing the inks on paper sheets, which are the standard substrates on which commercial office printers, like the one used in this work, operate. Afterwards, the printability was then visually observed on the SS/Ni/Pt substrates. Finally, in order to characterize printability in a more quantitative way, the most significant ink parameters (surface tension, density and dynamic viscosity) were determined and intercorrelated by calculating the Ohnesorge number, whose inverse number is widely used to evaluate printability of solutions.

3.2.1. Qualitative Ink Printability Evaluation

Three different CuO-NPs concentration were employed: namely 0.5 % wt., 1% wt. and 2 % wt. Generally, the inks were always printable for each concentration, but differences in quality and reproducibility were observed. In particular, the printing resulted uniform on paper and on Pt as well for 0.5% wt. and 1% wt, while for 2% wt. the printed surface turned out to be less homogeneous due to the presence of horizontal stripes. Such imperfections are typically observed when the ink flux in some nozzles is irregular. The flux is altered by progressive clogging of the nozzles due to agglomeration of the CuO-NPs on their internal surfaces. This phenomenon was observed for all EG concentration and it can be prevented by reducing the content of CuO-NPs in the formulation of the ink. Therefore, the %1 wt. ink was chosen as optimal for maximizing CuO concentration and at the same time reduce premature nozzle clogging.

The formulation of the ink was then optimized in terms of EG concentration. Three different mixture compositions were evaluated: namely 5% vol., 10% vol. and 20% vol. of EG. Generally, the inks were always printable for each concentration, but differences in quality and reproducibility were observed. In particular, the printing resulted uniform on paper and on Pt as well for 0.5% wt. and 1% wt, while for 2% wt. the printed surface turned out to be less homogeneous due to the presence of horizontal stripes. Such imperfections are typically observed when the ink flux in some nozzles is irregular. The flux is altered by progressive clogging of the nozzles due to agglomeration of the CuO-NPs on their internal surfaces. This phenomenon was observed for all EG concentration and it can be prevented by reducing the content of CuO-NPs in the formulation of the ink. Therefore, the %1 wt. ink was chosen as optimal for maximizing CuO concentration and at the same time reduce premature nozzle clogging.

The formulation of the ink was then optimized in terms of EG concentration. Three different mixture compositions were evaluated: namely 5% vol., 10% vol. and 20% vol. of EG. During preliminary tests on paper sheet, no major effects on the final quality were observed. Conversely, printing on Pt was found to be optimal at low EG concentrations. By increasing the EG content, the printed ink tended to coalescence in bigger droplets, thus compromising the wettability of the substrate and leaving behind areas with almost no ink on them.

3.2.2. Quantitative Ink Printability Evaluation

As mentioned before, the printability of the ink was then evaluated by calculating the Ohnesorge number, which is a dimensionless parameter that correlates the viscous forces to inertial and surface tension forces. The calculation was performed for three different inks with different EG content: 5% vol., 10% vol. and 20% vol. of EG in the mixture.

The physical properties of the inks required for the calculation were experimentally measured or obtained from scientific papers available in literature.

Dynamic Viscosity
The dynamic viscosity \( \mu \) was evaluated by rheological measurements with a shear rheometer at different temperatures. The results are exposed in the figures listed below.

The values of dynamic viscosity employed for the Ohnesorge number calculation were taken at a given shear rate equals to 400 \( s^{-1} \), because ink droplets ejection takes place at comparatively high speed, and summarized in Fig.3.9.

Since EG has a higher viscosity than water, an increase in the dynamic viscosity of the mixture with EG content was expected. Furthermore \( \mu \) decreased with temperature, which is a dependence typically observed in most fluids, and presented non-linear response to the applied shear rate. This latter effect may be due to the fact that the ink is a suspension of particles and not an homogeneous fluid.
Figure 3.6: Dynamic viscosity as a function of the imposed shear rate for the 5% EG ink at three different temperatures: T=25°C, 30°C and 40°C respectively.

Figure 3.7: Dynamic viscosity as a function of the imposed shear rate for the 10% EG ink at three different temperatures: T=25°C, 30°C and 40°C respectively.
3.2. Ink Printability

Figure 3.8: Dynamic viscosity as a function of the imposed shear rate for the 20% EG ink at three different temperatures: T=25°C, 30°C and 40°C respectively.

Figure 3.9: Dynamic viscosity at constant shear rate as a function of EG content, measured by varying the temperature.

Surface Tension

Surface tension values for the three different inks were obtained from the work of Mallinson et al. [77], who employed two chemical models to evaluate the surface tension and the viscosity of water/EG mixtures. Since the concentration of the CuO-NPs in the inks is only 1%wt., their effect on the surface tension was found to be negligible. Therefore, water/EG mixtures
can be used as references while measuring physical properties such as surface tension and density. The results significant for the aim of this work are displayed in Fig.3.11.

![Contact angle measurements showing the effect of EG on the wettability of the ink.](image)

The values found in literature were further demonstrated by contact angle measurements of the inks with the three different EG content as shown in Fig.3.10. The measurements were performed on a perfectly smooth gold surface, whose wettability is comparable to the one of Pt and the values obtained are listed in Table 3.2. An increase in the contact angle was observed for higher EG content, thus a reduction in the surface tension confirming the behavior observed qualitatively in Section 3.2.1. In order to maximize the wettability of the substrate, but at the same time having a sufficient amount of humectant in the ink, the optimal mixture formulation was then chosen to be 10% of EG.

<table>
<thead>
<tr>
<th>EG Content (%vol.)</th>
<th>Contact Angle</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>27°</td>
</tr>
<tr>
<td>10</td>
<td>32°</td>
</tr>
<tr>
<td>20</td>
<td>36°</td>
</tr>
</tbody>
</table>

Table 3.2: Contact angle values.

### Ohnesorge Number

The inverse of the Ohnesorge number $Z$ was then calculated since, as mentioned previously in Chapter 1, it is widely used to quantitatively evaluate the printability of solutions.

$$Z = \frac{1}{\text{Oh}} = \frac{\sqrt{\gamma \rho a}}{\mu}$$

where $a$ is the characteristic length, which in this case was the diameter of the nozzle set at 10 µm and $\gamma$, $\rho$ and $\mu$ surface tension, density and dynamic viscosity respectively. Table 3.3 recaps all the parameters for the different inks and for the two pure substances that constitute the base of the inks themselves.

<table>
<thead>
<tr>
<th>Fluid</th>
<th>Surface Tension ($mNm^{-1}$)</th>
<th>Density ($Kgm^{-3}$)</th>
<th>Dynamic Viscosity ($cPs$)</th>
<th>Z</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>$H_2O$</td>
<td>72.00</td>
<td>1000</td>
<td>0.89</td>
<td>30.15</td>
<td>[77]</td>
</tr>
<tr>
<td>EG 5% vol.</td>
<td>70.11</td>
<td>1004</td>
<td>2.13</td>
<td>12.48</td>
<td>[77]</td>
</tr>
<tr>
<td>EG 10% vol.</td>
<td>66.73</td>
<td>1015</td>
<td>2.45</td>
<td>10.62</td>
<td>[77]</td>
</tr>
<tr>
<td>EG 20% vol.</td>
<td>63.45</td>
<td>1026</td>
<td>2.75</td>
<td>9.28</td>
<td>[77]</td>
</tr>
<tr>
<td>EG</td>
<td>47.30</td>
<td>1110</td>
<td>16.9</td>
<td>1.36</td>
<td>[77]</td>
</tr>
</tbody>
</table>

Table 3.3: Surface tension, density, dynamic viscosity, Ohnesorge number and its inverse for three inks formulated.
The values obtained can be easily interpreted with the help of Fig.1.6. For low Z values, fluids are too viscous to allow stable drop formation. On the other hand, if Z is too high the phenomenon of satellite droplets becomes more and more significant compromising the resolution and thus the quality of the printed features. Therefore, fluids are defined printable for Z values that vary between 4 and 14. As expected, the use of water/EG mixtures brings ink printability in the suitable range since all the three inks formulated have acceptable Z values. Water and EG alone might be printed as well, but Z values too high or too low may lead either to unstable drop formation or to the presence of satellite drops.
3.3. Sensor on Stainless Steel

In this section the morphology and the elemental composition of the multilayer structure of the sensor on SS is analyzed employing SEM images and EDS spectra. Additionally, AFM measurements were performed during the different stages of the manufacturing in order investigate the morphology and the roughness of the surfaces of the different layers.

Finally, the sensing performances of the sensor are evaluated by using cyclic voltammetry and chronoamperometry analysis.

3.3.1. Sensor Fabrication and Characterization

A platinum layer was electrodeposited on a stainless steel substrate with the interposition of a Wood's Ni flash. CuO-NPs were then inkjet printed on top of the Pt surface because of their catalytic activity in the presence of glucose. A flexible, thus potentially wearable, electrode was obtained by using this fabrication process and it is shown in Fig.3.12.

Figure 3.12: Flexible CuO-NPs based working electrode on a stainless steel substrate.

From a mechanical stability point of view, the CuO NPs layer obtained from inkjet printing was found to be strongly adherent to Pt after the annealing process. CuO coatings easily resisted simple peel tests performed using Kapton polyimide tape and were found to be water stable.

SEM images of the CuO printed surface after annealing of two different electrodes at different magnification are listed below. Fig.3.14 shows the EDS spectra of four different areas of the sample underlined in Fig.3.13.
3.3. Sensor on Stainless Steel

Figure 3.13: SEM images of the CuO printed surface increasing the magnification.

Figure 3.14: EDS spectra of four different areas underlined in Fig. 3.13.
Fig. 3.13 shows the surface of a sample prepared with the ink containing the highest amount of EG (20%). As a consequence of ink agglomeration, an irregular film pattern was obtained, which can be avoided by optimizing the formulation of the ink, thus increasing its printability.

Formation of sub-micrometric aggregates on the surface of the coating was also visible from SEM analysis. These aggregates have a quasi-spherical shape and their mean maximum diameter, which was evaluated with the ImageJ software, is in the range of 1 μm. Such structures may be either present in the ink before jetting as a consequence of partial NPs agglomeration or they might have formed during the assembling of the coating. However, this defects turned out not to be detrimental for glucose sensing and did not affected the activity of copper oxide nanoparticles.

The EDS spectra displayed in Fig. 3.14 confirmed the expected elemental concentration of the sample. In particular, the Fe and Cr peaks derive from the stainless steel substrate, while the Cu and O peaks from the inkjet printed layer. The strike layer is detected as well as a Ni peak is present in all of the spectra. The Cl peak probably come from the strike, since Ni Wood’s baths have a high content of Cl, some of which might have been trapped during the electrodeposition of the film.

![Image of SEM analysis](image1.png)

Figure 3.15: SEM images of the CuO printed surface increasing the magnification (the sample was previously sectioned).

A second sample with the optimized ink composition was employed for SEM analysis 3.15. In this case the surface of the film is clearly homogeneous. Agglomerates with similar shape are still visible and have an average maximum diameter of 0.5 μm. Furthermore, small cracks are observed, which are probably due to mechanical stresses generated by sectioning the sample or by bending it multiple times. However, the functionality of the electrode was not affected by such defects of the coating.
3.3. Sensor on Stainless Steel

From the images obtained the surface morphology was found to be similar to micro-plotter printed CuO nanoparticles film [78]. The main difference between the latter and the films observed in this work was the presence of aggregates, which seem to be a typical defect of inkjet printed features though.

In order to better study the multilayer structure of the electrode, SEM analysis were performed after sectioning a sample in liquid nitrogen. The images obtained in this way are displayed below (Fig. 3.16).

Figure 3.16: SEM images of the electrode's section.

From these images the different layers are clearly visible and by using ImageJ was possible to measure the thicknesses of each, which are reported in table 3.4.

<table>
<thead>
<tr>
<th>Layer</th>
<th>Thickness (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ni flash</td>
<td>650</td>
</tr>
<tr>
<td>Electrolytic Pt</td>
<td>350</td>
</tr>
<tr>
<td>Inkjet printed CuO</td>
<td>170-220</td>
</tr>
</tbody>
</table>

Table 3.4: Thicknesses of the layers that form the electrode.

The composition of each layer was further investigated by EDS analysis, which made it easier to define each layer thickness and confirmed the expected elemental distribution. The results for the main elements present in the electrode are displayed in the following figures.
3. Results and Discussion

Figure 3.17: Elemental mapping of Fe obtained with EDS analysis.

Figure 3.18: Elemental mapping of Cr obtained with EDS analysis.

Figure 3.19: Elemental mapping of Ni obtained with EDS analysis.
3.3. Sensor on Stainless Steel

Figure 3.20: Elemental mapping of Pt obtained with EDS analysis.

Figure 3.21: Elemental mapping of Cu obtained with EDS analysis.
The CuO layer displayed in Fig. 3.16 was deposited by 25 repetitive printing steps. It is not clearly distinguishable from the Pt layer, but the EDS analysis confirmed the presence of CuO nanoparticles. The thickness of the CuO layer can be tuned by changing the number of printing steps. However, alignment problems may rise during multiple layers printing, since the paper handling system of the office printer employed is not designed for sequential printing. It was found that by carefully loading paper in the printer tray and by maintaining clean the drums that move the paper, alignment tolerances between 100 and 150 \( \mu \text{m} \) can be easily achieved.

It was possible to gain an acceptable uniformity of the CuO coating by the already cited optimization of the EG content. The chosen mixture allowed to obtain a reasonably low contact angle and good wettability. Therefore, droplets coagulation was avoided yielding good coating quality.

The morphology and the roughness of the aforementioned layers were further investigated by performing AFM analysis at different stages of the manufacturing. The results obtained are displayed below.

Figure 3.22: AFM image of 20\( \mu \text{m} \times 20\mu \text{m} \) of the stainless steel surface.

Figure 3.23: AFM image 20\( \mu \text{m} \times 20\mu \text{m} \) of the platinum coating surface.
Lamination signs on top of the stainless steel surface are clearly visible in Fig. 3.22 probably due to the action of the rolling mills during the manufacturing stage of the film. The average roughness ($R_a$) of the scanned area was measured on the basis of Fig. 3.22 and was found to be of 14.89 nm.

After depositing the strike Ni layer and the Pt coating, $R_a$ increased to 17.08 nm. Additionally, the stripes originally present in the SS layer almost disappeared, leaving a nodular structure, typical of an electrodeposited layer Fig. 3.23.

Finally, Fig. 3.24 depicts the AFM analysis of the Pt coating covered with the CuO printed layer. $R_a$ further increased to 38.74 nm. This increase is mostly ascribable to the presence of the already mentioned aggregates, whose height could be of hundreds of nanometers. Fig. 3.25 shows a comparison between a SEM image and an AFM one of the aggregates.

Before testing, the SS electrode was mounted on a PET sheet and covered with polyimide Kapton tape leaving a 5x5 mm window as active area (Fig. 3.26). These steps of the fabrication process were made in order to ease its use as a sensor.
3.3.2. Sensor Performances Evaluation

The anodic response registered in all experiments is a consequence of the already mentioned catalytic activity of CuO nanoparticles in the presence of glucose, which leads to glucose oxidation generating a current that was detected and used to calibrate an electrochemical sensor in this work.

The SS sample was also tested in three different setup configuration in order to study the effect of bending on sensitivity. Furthermore, the selectivity of the SS electrode was investigated by performing CA measurements in the presence of six different saccharides that can be found in human blood at lower concentrations compared to glucose.

Cyclic Voltammetry
The activity of CuO nanoparticles was first tested and confirmed by voltammetry measurements, whose results are shown in Fig.3.27. No peaks are visible when the electrolyte does not contain glucose, meaning that no redox reaction is undergoing. Conversely, as soon as the concentration of glucose in the NaOH solution is increased, anodic peaks start to appear in the voltage interval comprise between 0.5 V and 0.6 V, which is typical for glucose oxidation [79]. The reactions that takes place were already mentioned in Chapter 1 eq.1.25 and eq.1.26. The phenomenon gets more evident with high concentrations of glucose as it can be noted from the heights of the current peaks.
3.3. Sensor on Stainless Steel

Figure 3.27: Voltammetry experiment carried out cycling the potential between 0 and 0.8 V vs Ag/AgCl in aqueous 0.1 M NaOH solution at progressively higher glucose concentrations.

Chronoamperometry and Calibration

Once the voltage corresponding to glucose oxidation is known, chronoamperometry measurements can be performed. Therefore, a steady potential of 0.5 V was applied in order to optimize the current response, which was then recorded and plotted as a function of time as shown in Fig. 3.28. The high level of noise is mainly due to the stirring during the test, which was performed to favor uniform distribution of glucose in the electrolyte.

Figure 3.28: Raw electrochemical response of the sensor as a consequence of progressive addition of 1mM glucose every 45s.
The raw current response was then employed to build the calibration curve of the sensor by mediating currents values inside each step using the software *OriginPro 8*. The results are shown in Fig. 3.29. Since the low cost fabrication process and the use of a commercial inkjet printer might be expected to be an issue for the reproducibility of the work, two different sensors made with the same procedure were tested.

A linear response was observed for both sensors. By increasing glucose concentration the response progressively looses its linearity, which was acceptable up to 6 mM. The sensitivity of the two sensors was then evaluated by measuring the slopes of the two fittings. Results are summarized in table 3.7.

<table>
<thead>
<tr>
<th>Sensor</th>
<th>Linear Range (mM)</th>
<th>Correlation Coefficient</th>
<th>Sensitivity ($\mu AmM^{-1}cm^{-2}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1-6</td>
<td>0.9983</td>
<td>1588±25</td>
</tr>
<tr>
<td>2</td>
<td>1-6</td>
<td>0.9975</td>
<td>1660±31</td>
</tr>
</tbody>
</table>

Table 3.5: Performances of two identical inkjet printed CuO glucose sensors.

Sensitivities of the two sensors differ of only 4.3%, which is an acceptable level for a sensor printed using a commercial inkjet printer. In general, measured sensitivity is in agreement with state of the art sensors analogous to the one presented and considerably high in an acceptable linear range [78].

Chronoamperometry measurements were also performed by increasing glucose concentration in the 0,1M NaOH solution with steps of 0,5mM. The current response of the sensor in this setup is displayed in Fig. 3.30 and it showed a progressive increase in the signal noise after each glucose addition. For values of glucose concentration higher than 4mM the noise became dominant avoiding precise data analysis.

A calibration curve Fig. 3.31 was then built from the raw current response. Linearity was observed up to 5mM and the sensitivity of the sensor resulted comparable to the one obtained by adding 1mM of glucose each steps. The performances of the device are summarized in table 3.6.
3.3. Sensor on Stainless Steel

Figure 3.30: Raw electrochemical response of the sensor as a consequence of progressive addition of 0.5mM glucose every 45s.

Figure 3.31: Calibration curve of the CuO based sensor obtained by progressively increasing glucose concentrations with steps of 0.5mM.

<table>
<thead>
<tr>
<th>Linear Range (mM)</th>
<th>Correlation Coefficient</th>
<th>Sensitivity ((\mu A m M^{-1} cm^{-2}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-5</td>
<td>0.9964</td>
<td>1697±32</td>
</tr>
</tbody>
</table>

Table 3.6: Performances of the CuO based sensor obtained by progressively increasing glucose concentrations with steps of 0.5mM.
Limit of Detection

To evaluate the limit of detection (LOD), glucose concentration was increased by 0.5 µM steps until a signal was clearly detected. Each addition was made in the proximity of the working electrode and produced a sharp current peak Fig. ??, which immediately decayed due to stirring and homogenization of the electrolyte.

After stabilization, current values were mediated with OriginPro 8 for each glucose addition, as it was done for the previously mentioned calibration curve, and a discernible current step was observed also at 0.5 µM glucose concentration. These steps are clearly visible in the magnification of the raw current response shown in Fig. ??; This phenomenon implies that the sensor, similarly to analogous sensors described in the literature [78], is able to detect very low glucose amounts, down to 0.5 µM.
Electrochemical Measurements in Bent Configuration

The influence of mechanical bending was evaluated on the sensitivity of the CuO-NPs based electrode. In a bending process, the two sides of a flexible sheet vary linearly from tension to compression along its thickness. Tensile strains are present on the convex side, while the concave side experiences compressive strains. Since the CuO based sensor is expected to make contact with the human skin on the concave side, the tests were predisposed to induce compressive stresses on the sensing side of the electrode.

Influence of bending on the electrode was examined using a series of templates featuring fixed bending radii: 15 and 30 mm, shown in Fig. 3.34. These bending radii were selected with the idea that the glucose sensors will be worn as ring or bracelet.

![Testing templates employed to evaluate the effect of bending on the sensitivity of the sensor.](image)

Chronoamperometry measurements were then performed to obtain the calibration curves for the different radius of curvature. Performance parameters are listed in table 3.7.

<table>
<thead>
<tr>
<th>Radius of Curvature</th>
<th>Correlation Coefficient</th>
<th>Sensitivity ($\mu A m M^{-1} cm^{-2}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not flexed</td>
<td>0.9983</td>
<td>1588±25</td>
</tr>
<tr>
<td>30 mm</td>
<td>0.9968</td>
<td>1420±31</td>
</tr>
<tr>
<td>15 mm</td>
<td>0.9931</td>
<td>1379±43</td>
</tr>
</tbody>
</table>

Table 3.7: Performances of two the inkjet printed CuO glucose sensors in two different bending configurations.

The results showed that testing the samples in a bending configuration produced two major effect: loss of linearity and loss of sensitivity. Both phenomenon gets more significant by progressively increasing curvature as it can be clearly observed from the calibration curves displayed in Fig. 3.35. Quantitatively, the loss of linearity is confirmed by the decrease in the
correlation coefficient of the fittings, while the loss of sensitivity comes with a lower value of the calibration curve’s slope.

A possible explanation for the deterioration of the performances is the progressive damaging of the CuO layer according to a cracking mechanism. When the device is flexed, cracks may form on the surface, allowing contact of the electrolyte with the Pt layer and decreasing charge transfer. Sensitivity reduction as a consequence of bending has already been observed in CuO thin films based glucose sensors [80].

Selectivity

The selectivity of the printed electrode toward glucose in presence of interfering sugars was evaluated by amperometric measurements. Lactose, mannitol, maltose, sorbitol and fructose were chosen for the tests because they are commonly present in human blood and they are chemically/physically similar to glucose. Some interfering species typically present in human blood, like ascorbic acid or uric acid, were not considered due to their recognized low interfering power in alkaline electrolytes [78]. It has been demonstrated that concentration of interfering species in human blood is 30 to 50 times less than that of glucose [81]. In order to ensure the selectivity of the samples, much higher concentration of the interfering species was employed.

The current response of selectivity tests is shown in Fig. 3.36 and Fig. 3.37. No significant current increase is observed for any addition even by doubling the interfering species concentration. Therefore, the sensor was proved to be acceptably selective towards glucose and it might be efficiently employed for blood glycaemic index analysis.
Figure 3.36: Interference performance test results of the CuO printed sensor in presence of six different saccharides evaluated by amperometric measurements in 0.1M NaOH at 0.5V. Interfering species were added with a concentration of 0.01mM. Glucose was added with the concentration of 0.5mM.

Figure 3.37: Interference performance test results of the CuO printed sensor in presence of six different saccharides evaluated by amperometric measurements in 0.1M NaOH at 0.5V. Interfering species were added with a concentration of 0.02mM. Glucose was added with the concentration of 0.5mM.
Comparison of the Performances with other CuO-based Sensors

Table 3.8 compares the results obtained in this work to other reports. High-sensitivity detection of glucose in a wide linear range was achieved. The remarkable performances of this sensor can be attributed to the irregular surface morphology, which provided high specific surface area, and to the excellent adhesion of the CuO nanoparticles to thin platinum conductive film, which allowed high electron transfer rate.

<table>
<thead>
<tr>
<th>Electrode Matrix</th>
<th>Sensitivity $\mu A/m M \cdot cm^2$</th>
<th>Linear range $m M$</th>
<th>Limit of Detection $\mu M$</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>CuO NPs/Pt/Ni/SS inkjet printing</td>
<td>1660</td>
<td>1-6</td>
<td>0,5</td>
<td>this work</td>
</tr>
<tr>
<td>CuO NPs/Au/PET micro-plotter</td>
<td>2419,8</td>
<td>0,1-6,5</td>
<td>0,5</td>
<td>[78]</td>
</tr>
<tr>
<td>CuO nanoparticles onto MWCNTs</td>
<td>2190</td>
<td>up to 3</td>
<td>0,8</td>
<td>[11]</td>
</tr>
<tr>
<td>CuO-G-GCE</td>
<td>1360</td>
<td>0,002-4</td>
<td>0,7</td>
<td>[57]</td>
</tr>
<tr>
<td>CuO nanoflowers-Cu foil electrode</td>
<td>789,3</td>
<td>up to 3,1</td>
<td>/</td>
<td>[58]</td>
</tr>
<tr>
<td>CuO NPs</td>
<td>1430</td>
<td>0,04-6</td>
<td>5</td>
<td>[59]</td>
</tr>
<tr>
<td>CuO NPs modified Ag electrode</td>
<td>2762,5</td>
<td>0,05-18,45</td>
<td>≈0,5</td>
<td>[55]</td>
</tr>
<tr>
<td>CuO nanowires on Cu</td>
<td>1800</td>
<td>up to 0,5</td>
<td>10</td>
<td>[56]</td>
</tr>
<tr>
<td>CuO nanoflowers</td>
<td></td>
<td></td>
<td></td>
<td>[58]</td>
</tr>
<tr>
<td>Chrysanthemum-like</td>
<td>3552</td>
<td>/</td>
<td>/</td>
<td></td>
</tr>
<tr>
<td>Candock-like</td>
<td>4078</td>
<td>/</td>
<td>/</td>
<td></td>
</tr>
<tr>
<td>Dandelion-like</td>
<td>5368</td>
<td>/</td>
<td>/</td>
<td></td>
</tr>
<tr>
<td>CuO microfibers/CuO nanoparticles/FTO</td>
<td>2321</td>
<td>up to 0,6</td>
<td>0,0022</td>
<td>[60]</td>
</tr>
<tr>
<td>CuO nanofibers/ITO</td>
<td>up to 1,3</td>
<td>0,04</td>
<td>[54]</td>
<td></td>
</tr>
<tr>
<td>Sandwich-structured</td>
<td>up to 3,2</td>
<td>1</td>
<td>[61]</td>
<td></td>
</tr>
<tr>
<td>CuO/GCE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CuO nanospheres onto GCE</td>
<td>404,53</td>
<td>0-2,55</td>
<td>1</td>
<td>[82]</td>
</tr>
<tr>
<td>CuO NWs/Cu foil</td>
<td>490</td>
<td>0,0004-2</td>
<td>0,000049</td>
<td>[83]</td>
</tr>
<tr>
<td>CuO microspheres/SPE</td>
<td>26,59</td>
<td>2-9</td>
<td>20,6</td>
<td>[84]</td>
</tr>
<tr>
<td>Flower-shaped</td>
<td>709,52</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CuO/graphite</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CuO nanoseeds/Au/glass</td>
<td>1101</td>
<td>0,1-13,3</td>
<td>50</td>
<td>[86]</td>
</tr>
<tr>
<td>CuO SWCNT/ITO</td>
<td>1610</td>
<td>up to 1,8</td>
<td>0,00005</td>
<td>[87]</td>
</tr>
<tr>
<td>CuO-ZnO hybrid/Cu</td>
<td>408</td>
<td>0,1-1</td>
<td>18</td>
<td>[88]</td>
</tr>
<tr>
<td>nanoporous CuO/Cu</td>
<td>1066</td>
<td>0,1-2,04</td>
<td>2</td>
<td>[89]</td>
</tr>
<tr>
<td>CuO NFs/GCE</td>
<td>431,3</td>
<td>0,006-2,5</td>
<td>0,8</td>
<td>[90]</td>
</tr>
</tbody>
</table>

Table 3.8: A comparison of the sensing performances of non-enzymatic nanostructured CuO glucose biosensor.
3.4. Sensor on PET

In this section the morphology and the elemental composition of the multilayer structure of the sensor on PET substrate is analyzed employing SEM images. Additionally, AFM measurements were performed in order to better investigate the morphology and the roughness of the sensor surface.

Finally, the sensing performances of the sensor are evaluated by amperometric measurements and a calibration curve is built.

3.4.1. Sensor Fabrication and Characterization

The catalytic CuO-NPs were inkjet printed on top of a nickel-phosphorous coating obtained by electroless deposition. Metallic layers are not easily deposited on a PET surface, therefore proper surface treatment and activation are required. A flexible, thus potentially wearable, electrode was obtained by using this fabrication process and it is shown in Fig. 3.38.

![Figure 3.38: NiP layer coating obtained by electroless deposition subsequently covered with an inkjet printed layer of CuO NPs.](image)

The mechanical stability of this electrode was tested by simple peel test using Kapton polyimide. The CuO-NPs layer turned out to be adherent to the NiP surface after the annealing process, but not as strongly as it was on top of the electrodeposited platinum. Therefore, the electrode on PET have inferior mechanical stability compared to the one on stainless steel.
SEM images of the CuO printed surface after annealing of an electrode on PET are shown in Fig. 3.39. Three different magnifications were employed in order to better analyze specific features and defects present on the surface.

For this electrode as well, formation of micrometric aggregates on the surface of the coating was visible from SEM analysis. These aggregates have a quasi-spherical shape and their mean maximum diameter, which was evaluated with the ImageJ software, is in the range of 2 μm. From the images it can be observed that the concentration of these aggregates is higher compared to the electrode on stainless steel resulting in less uniformity. A poorer surface quality might compromise the sensitivity of the electrode. Furthermore, it is clearly visible that there are more small cracks breaking the surface uniformity compared to the SS electrode. They can be caused by the mechanical stresses generated after sectioning the sample or by multiple bending, but in any case the coating seems to have poorer mechanical properties and to be more brittle.

In order to better study the multilayer structure of the electrode, SEM analysis were performed after sectioning a sample in liquid nitrogen. The images obtained in this way are displayed below (Fig. 3.40).

From these images the different layers are clearly visible and by using ImageJ was possible to measure the thicknesses of each. In particular the NiP layer was found to be 600nm thick, while the CuO printed layer 750nm. The thickness of the latter was obtained by 60 to 70 printing steps, which explains why it is higher than the CuO layer thickness measured for the SS electrode. Fig. 3.41 shows an AFM image of the CuO printed layer on top of a PET surface which was used to quantitatively evaluate the roughness of the sample.
3.4. Sensor on PET

Figure 3.40: SEM images of the SS electrode’s section.

Figure 3.41: AFM image of 20\(\mu\)m x 20\(\mu\)m of CuO printed layer surface on top of PET.
Electrode Patterning

Since the process used in this work to deposit a conductive path on top of a PET substrate still has to be optimized, an alternative method was investigated. Specifically this method involved electrode patterning by means of a salt precursor and a reducing agent, which has already been mentioned in Section 1.1.5.

The color inkjet cartridge of the printed was emptied and properly cleaned. The cartridge is made of three different compartments: one of them was filled with a solution of copper sulfate 0.1 M and another one with sodium borohydride 20 g/l, which acted as reducing agent, and the third one was left empty. A conductive path made of metallic copper was then obtained by alternatively printing the same .doc file representing the desired pattern: once with the salt solution and subsequently with the reducing agent. The copper ions present in the salt undergo a redox reaction in presence of sodium borohydride which leads to the formation of pure Cu. By repeating this procedure multiple time it is possible to obtain a metallic feature on top of a PET substrate like the one showed in Fig. 3.42. The pattern turned out to be conductive by simply measuring a finite electrical resistance between different couples of points with an ohmmeter.

Coating uniformity and conductivity turned out to be too low to employ this pattern as a proper substrate for glucose sensing, but if further studied and optimized this method can be efficiently exploited for electrode fabrication thanks to its simplicity, low cost and flexibility (various shape can be easily obtained).

Figure 3.42: Metallic Cu pattern obtained by multiple inkjet printing steps of a salt precursor and a reducing agent on top of a PET substrate.
3.4.2. Sensor Performances Evaluation

A complete electrochemical characterization of the device was not possible since the fabrication process still has to be optimized. The major problem encountered was the low conductivity of the electroless deposited NiP layer, which prevented efficient electron transfer and consequently reduced the rate of glucose oxidation. However, potentiostatic tests showed that the electrode on PET can still detect glucose and a calibration curve can be built to use it as a sensor.

Chronoamperometry and Calibration

Chronoamperometry measurements were performed for the electrode on PET substrate in a 0.1 M NaOH solution. A steady potential of 0.5 V was imposed on the electrodes for the tests. The results demonstrated the ability of the sensor to detect glucose as it can be observed from its raw current response in Fig. 3.43.

Figure 3.43: Raw electrochemical response of the sensor as a consequence of progressive addition of 1mM glucose every 45s.

In this case as well, current values were mediated with OriginPro8 in order to build the calibration curve showed in Fig. 3.44. It can be immediately noted that glucose detection does not have a linear dependence for this sensor. However, the calibration curve can be fitted with an exponential function. The data obtain acceptably fit the exponential curve for glucose concentration up to 7mM. The fitting curve is drawn from the following function:

\[ y = y_0 + A \exp(R_0 x) \]  

(3.2)

where \( y_0 = 1.61 \cdot 10^{-3}, A = -1.58 \cdot 10^{-3} \) and \( R_0 = -0.3424 \) are the fitting parameters.

The loss of linearity can be attributed to the low conductivity of the NiP layer that could be improved by increasing its thickness or by replacing the film with a more conductive metallic pattern such as electroless deposited copper. Furthermore, since the thickness of the inkjet printed CuO layer is comparable to the one of other sensors found in literature [78], it did not cause the peculiar amperometric response of the sensor.
Figure 3.44: Calibration curve for the sensor on PET substrate.

The calibration curve clearly shows that linearity is lost, but that does not prevent the electrode to be used for rough sensing purposes.
In the present work, a flexible non-enzymatic electrochemical glucose sensor based on CuO nanoparticles as active material was presented. Such device is characterized by a multilayer structure composed of a conductive stainless steel substrate electroplated with Ni/Pt, on top of which a CuO NPs thin film was inkjet printed. The sensor was then mounted on a flexible PET supporting sheet and covered with polyimide Kapton tape to select the active area. Low cost techniques, including inkjet printing from office printers and wet metallization, were employed to accomplish the task.

Sensor manufacturing showed in general a good reproducibility. Concerning the measured sensing performances, a high sensitivity (around $1600 \text{AmM}^{-1}\text{cm}^{-2}$) and a wide linear range were achieved. Additionally, a low detection limit coupled with a good selectivity towards glucose, with respect to other saccharides that can be present in blood, allows the sensor to be used for frequent monitoring of diabetics patients through blood or other physiologic fluid analysis.

The sensors described, which was obtained by an ultra-low cost manufacturing route, may be an interesting candidate for applications requiring high customizability, wearability, low cost per unit and scalability.

Furthermore, the development of a sensor with similar features on a polymeric foil was also investigated. The choice of a non-metallic material is superior both in terms of biocompatibility and flexibility. Therefore, a PET sheet was employed as substrate. Metalization of the polymeric substrate by electroless deposition of NiP was necessary for the formation of a conductive path on top of which CuO NPs were inkjet printed. However, low values of conductivity were achieved, which lead to unsatisfactory sensing performances to use it for medical purposes.

A development of the present work might be focusing on the optimization of the metallic layer on PET. Conductivity can be enhanced by increasing the thickness of the film or by studying the electroless deposition of alternative and more conductive materials such as copper. Additionally, a non-conductive substrate allows electrical isolation of metallic patterns, thus reference and counter electrodes could be integrated on the same device.

Finally, the use of CuO-NPs based inks can be extended to a variety of other electrochemical sensors. Specifically, a first attempt was made in this project by inkjet printing the CuO film on a Cu/graphene sensor. Thanks to the catalytic activity of CuO nanoparticles, which can improve sensing performances towards glucose, the low-cost approach and the possibility to obtain elaborate patterns, inkjet printing of CuO-NPs based inks represents a good candidate for the development of innovative non-enzymatic glucose sensors. Extensive research in this field is necessary to tackle a disease that has the potentiality to become a worldwide health issue in the future.

A scientific paper was extracted from this master thesis project, which is currently in press, but not yet published [91].
The CuO NPs layer discussed in this work was inkjet printed on top of a graphene based glucose sensor in order to enhance its sensing performances. Such device was developed in a master thesis project at the SEElab, Politecnico di Milano [92]. The effect of graphene on such device towards glucose sensing was tested by chronoamperometric measurements in three different configurations: bare graphene, graphene functionalized with gold nanoparticles and with CuO NPs inkjet printed layer. A calibration curve was then obtained for each sensor to compare sensitivities.

A.1. Sensor Fabrication

Graphene was deposited on a copper substrate via atmospheric pressure chemical vapor deposition (CVD). The copper substrates were electrodeposited from a commercial bath and acted as metal catalyst in a CVD process employing methane and hydrogen. The presence of graphene on top of the copper substrate was demonstrated by Raman spectroscopy analysis. From the spectra in Fig. A.1 the three main peaks of graphene can unambiguously be identified [92].

A.1.1. Functionalization with Gold NPs

To optimize the functioning of the copper electrodes toward glucose oxidation, functionalization with gold nanoparticles was performed. Gold nanoparticles have been deposited on copper by galvanic displacement in a HAuCl₄ solution. The copper substrate covered with graphene was dipped in the solution either a single time for 40 seconds or 4 times for 10 seconds each time [92]. Deposition occurred on an area approximately of 5x5 mm isolated by Kapton tape.

A.1.2. Functionalization with Inkjet Printed CuO Layer

The CuO NPs based layer discussed in this work was deposited on graphene to enhance electron transfer during glucose oxidation. The CuO layer was inkjet printed on the sensor by 25 repetitive printing steps in order to achieve the desired amount of active material on top of the surface. SEM images Fig.A.2 showed the same morphologic features obtained for the sensors developed in this thesis project. Furthermore, the crystal grains of the metallic Cu substrate are visible.
Figure A.1: Raman spectrum of CVD graphene on top of a copper substrate.

Figure A.2: SEM image of the CuO NPs layer inkjet printed on top of the graphene based sensor.
A.2. Sensor Performances Evaluation

Preliminary chronoamperometry tests were performed to evaluate glucose detection. A complete electrochemical characterization of the sensor goes beyond the scope of this thesis project. The aim of these tests was to demonstrate the possibility to use the inkjet printed CuO layer on already functioning sensors in order to enhance their sensing performances. Therefore, the calibration curves of three different sensors were built not just to demonstrate the increase in sensitivity of the device with respect to the bare graphene, but also to compare the effect of the CuO layer with other functionalization techniques already employed in literature such as gold nanoparticles decoration.

A.2.1. Chronoamperometry and Calibration

Potentiostatic tests were performed on the three sensors at a constant voltage of 0.5V. Each step corresponds to an increase in glucose concentration of 1mM in a 0.1M NaOH solution, in which the samples were tested. The current response as a function of time (Fig. A.3) showed that higher currents were measured for CuO and Au decorated graphene with respect to bare graphene. Furthermore, it can be noticed that the noise in the signal of the CuO decorated sensor is lower compared to the Au decorated sensor. A similar current response for the two decorated sensors was then obtained, but the CuO layer allows lower signal noise.

![Figure A.3: Raw electrochemical response of the sensor as a consequence of progressive addition of 1mM glucose every 90s.](image)

The raw current responses were used to build the calibration curves of the three samples. Sensitivities and correlation coefficients are listed in table A.1. It turned out that the sensor functionalized with the CuO NPs layer had the highest sensitivity. Therefore, inkjet printing of active layers on top already functioning sensors can represent a valid alternative to the techniques, such deposition of gold nanoparticles by galvanic displacement, that nowadays are commonly used to enhance the performances of non enzymatic glucose sensors.
Figure A.4: Calibration curve for the three graphene based sensors on metallic copper substrate.

<table>
<thead>
<tr>
<th>Sensor</th>
<th>Correlation Coefficient</th>
<th>Sensitivity ($\mu A m M^{-1} cm^{-2}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bare Graphene</td>
<td>0.9965</td>
<td>803±18</td>
</tr>
<tr>
<td>Au on Graphene</td>
<td>0.9964</td>
<td>1556±35</td>
</tr>
<tr>
<td>CuO on Graphene</td>
<td>0.9954</td>
<td>1617±41</td>
</tr>
</tbody>
</table>

Table A.1: Comparison of the performances of three different graphene based glucose sensors.
Bibliography


