The Functionalization of Epitaxial Graphene on SiC with Nanoparticles towards Biosensing Capabilities

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Abstract
Graphene has been shown to be very powerful as a transducer in many biosensor applications due to its high sensitivity. This enables smaller surfaces and therefore less material consumption when producing sensors and consequently cheaper and more portable sensors compared to the commercially available sensors today. The electrical properties of graphene are very sensitive to gas exposure why presence of molecules or small changes in concentration could easily be detected when using graphene as a sensing layer. Graphene is sensitive towards many molecules and in order to detect and possibly identify gas molecules the surface needs to be functionalized. The intention of this project was to use nanoparticles (NPs) to further increase sensitivity and specificity towards selected molecules and also enable biofunctionalization of the NPs, and by that tune the electrical properties of the graphene. This study proposes the use of Fe$_3$O$_4$ and TiO$_2$ NPs to enable sensitive detection of volatile gases and possibly further functionalization of the NPs using biomolecules as a detecting agent in a liquid-phasebiosensor application. The interaction between graphene and NPs have been investigated using several surface characterization methods and electrical measurements for detection of gaseous molecules and also molecules in a liquid solution. The characterizing methods used are XPS, AFM with surface-potential mapping and Raman spectroscopy with reflectance mapping in order to investigate the NPs interaction with the graphene surface. Sensors where manufactured for gas-phase detection of CO, formaldehyde, benzene and NH$_3$ specifically and display differences in sensitivity and behavior of the Fe$_3$O$_4$ and TiO$_2$ NPs respectively. For liquid measurements the difference in behavior in two buffers was investigated using an in-house flow-cell setup. The surface characterizing measurements indicated that just a small difference could be found between the two NPs, however a significant change in sensor response could be detected as a function of coverage. The liquid and gas-phase measurements rendered information on differences in sensitivity between the NPs and between analytes where TiO$_2$ showed a higher level of sensitivity towards most of the gases investigated. Both Fe$_3$O$_4$ and TiO$_2$ NP coated graphene showed capability to detect formaldehyde and benzene down to 50 ppb and 5 ppb respectively. The sensitive gas detection could help protecting individuals being exposed to a hazardous level of volatile gases if concentrations increase rapidly or at a long term exposure with lower concentrations, improving safety and health where these gases are present.

Keyword
Abstract

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The electrical properties of graphene are very sensitive to gas exposure why presence of molecules or small changes in concentration could easily be detected when using graphene as a sensing layer. Graphene is sensitive towards many molecules and in order to detect and possibly identify gas molecules the surface needs to be functionalized. The intention of this project was to use nanoparticles (NPs) to further increase sensitivity and specificity towards selected molecules and also enable biofunctionalization of the NPs, and by that tune the electrical properties of the graphene. This study proposes the use of Fe$_3$O$_4$ and TiO$_2$ NPs to enable sensitive detection of volatile gases and possibly further functionalization of the NPs using biomolecules as a detecting agent in a liquid-phase biosensor application. The interaction between graphene and NPs have been investigated using several surface characterization methods and electrical measurements for detection of gaseous molecules and also molecules in a liquid solution. The characterizing methods used are XPS, AFM with surface-potential mapping and Raman spectroscopy with reflectance mapping in order to investigate the NPs interaction with the graphene surface. Sensors where manufactured for gas-phase detection of CO, formaldehyde, benzene and NH$_3$ specifically and display differences in sensitivity and behavior of the Fe$_3$O$_4$ and TiO$_2$ NPs respectively. For liquid measurements the difference in behavior in two buffers was investigated using an in-house flow-cell setup. The surface characterizing measurements indicated that just a small difference could be found between the two NPs, however a significant change in sensor response could be detected as a function of coverage. The liquid and gas-phase measurements rendered information on differences in sensitivity between the NPs and between analytes where TiO$_2$ showed a higher level of sensitivity towards most of the gases investigated. Both Fe$_3$O$_4$ and TiO$_2$ NP coated graphene showed capability to detect formaldehyde and benzene down to 50 ppb and 5 ppb respectively. The sensitive gas detection could help protecting individuals being exposed to a hazardous level of volatile gases if concentrations increase rapidly or at a long term exposure with lower concentrations, improving safety and health where these gases are present.
## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>2D</td>
<td>Two-dimensional</td>
</tr>
<tr>
<td>AFM</td>
<td>Atomic force microscopy</td>
</tr>
<tr>
<td>B.E.</td>
<td>Binding energy</td>
</tr>
<tr>
<td>CV</td>
<td>Cyclic voltammetry</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>EDL</td>
<td>Electrical double layer</td>
</tr>
<tr>
<td>EG-SiC</td>
<td>Epitaxial graphene on silicon carbide</td>
</tr>
<tr>
<td>EIS</td>
<td>Electrochemical impedance spectroscopy</td>
</tr>
<tr>
<td>FWHM</td>
<td>Full-width half-max</td>
</tr>
<tr>
<td>HC</td>
<td>Hollow cathode</td>
</tr>
<tr>
<td>ISFET</td>
<td>Ion sensitive field effect transistor</td>
</tr>
<tr>
<td>I-V</td>
<td>Current-voltage</td>
</tr>
<tr>
<td>NP</td>
<td>Nanoparticle</td>
</tr>
<tr>
<td>NP-EG-SiC</td>
<td>Nanoparticles on epitaxial graphene on silicon carbide</td>
</tr>
<tr>
<td>POM</td>
<td>Polyoxymethylene</td>
</tr>
<tr>
<td>RT</td>
<td>Room temperature</td>
</tr>
<tr>
<td>SAM</td>
<td>Self-assembled monolayer</td>
</tr>
<tr>
<td>SNP</td>
<td>Single nucleotide polymorphism</td>
</tr>
<tr>
<td>UHV</td>
<td>Ultra-high vacuum</td>
</tr>
<tr>
<td>WHO</td>
<td>World health organization</td>
</tr>
<tr>
<td>XPS</td>
<td>X-ray photoelectron spectroscopy</td>
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## Chemical Denotations

<table>
<thead>
<tr>
<th>Symbol</th>
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<tbody>
<tr>
<td>Ag</td>
<td>Silver</td>
</tr>
<tr>
<td>Au</td>
<td>Gold</td>
</tr>
<tr>
<td>Ar</td>
<td>Argon</td>
</tr>
<tr>
<td>CH₂O</td>
<td>Formaldehyde</td>
</tr>
<tr>
<td>CO</td>
<td>Carbon monoxide</td>
</tr>
<tr>
<td>COOH</td>
<td>Carboxylic group</td>
</tr>
<tr>
<td>Co₃O₄</td>
<td>Cobalt(II,III)oxide</td>
</tr>
<tr>
<td>CuO</td>
<td>Copper(II)oxide</td>
</tr>
<tr>
<td>Fe₂O₃</td>
<td>Iron(III)oxide</td>
</tr>
<tr>
<td>Fe₃O₄</td>
<td>Iron(II,III)oxide</td>
</tr>
<tr>
<td>H</td>
<td>Hydrogen</td>
</tr>
<tr>
<td>MnO₂</td>
<td>Manganese(IV)oxide</td>
</tr>
<tr>
<td>N₂</td>
<td>Nitrogen, gaseous</td>
</tr>
<tr>
<td>NH₂</td>
<td>Amine group</td>
</tr>
<tr>
<td>NH₃</td>
<td>Ammonia</td>
</tr>
<tr>
<td>O₂</td>
<td>Oxygen, gas</td>
</tr>
<tr>
<td>OH</td>
<td>Hydroxyl group</td>
</tr>
<tr>
<td>Pd</td>
<td>Palladium</td>
</tr>
<tr>
<td>P(O)(OH)₂</td>
<td>Phosphonic acid</td>
</tr>
<tr>
<td>Pt</td>
<td>Platina</td>
</tr>
<tr>
<td>Rh</td>
<td>Rhodium</td>
</tr>
<tr>
<td>Si</td>
<td>Silicon</td>
</tr>
<tr>
<td>SiC</td>
<td>Silicon carbide</td>
</tr>
<tr>
<td>TiO₂</td>
<td>Titanium(IV)oxide</td>
</tr>
<tr>
<td>W</td>
<td>Wolfram</td>
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Chapter 1

Introduction

Since the discovery of graphene in 2004 by Andre Geim and Konstantin “Kostya” Novoselov [1] many results have been achieved in investigating the intriguing properties of the material. Graphene possesses extraordinary thermal and electrical characteristics making it a new advanced material within several areas of research and applications in industries. Scientists have shown its advantages as a resistor, optical-, gas-, biosensor and many other applications. [2] Some of the uses in commerce and within research are actively pursued, while many more await in the future. [3] The study at hand investigates graphene sensitized by nanoparticles as a sensing layer for detection of gas-phase molecules and also pre-screening of liquid-phase measurements are done. Both strategies aid in building knowledge to apply when designing a biosensor system.

Graphene, as a word, is often used to describe several materials and constitutions of carbon lattices. Pristine graphene is a perfect, planar, one atom thick hexagonal lattice of carbon atoms, making it a 2-dimensional (2D) crystal structure. [4] In this thesis “graphene” refers to pristine graphene as a monolayer unless anything else is mentioned.

1.1 Background

Biosensors are an extremely useful tool in investigating the health of humans and detecting molecules from samples of the human body containing both exogenous organisms and endogenous material, or biomolecules. They are used in health care, detecting diseases caused by different pathogens and also in investigating DNA-constitutions that may lead to a number of defects in the human body. Sensitive biosensors offer a way to detect selected molecules with high specificity and thereby knowledge of how to treat a patient and to enable personalized healthcare. Regular lab-tests requires tests to be made or sent to a special facility where, often, advanced and expensive machines run them with a long time from sample taken to result and interpretation delivered. However, biosensors can be made to give a specific response when detecting a target molecule specifically and also immediately answer a question about presence of a substance. [5]

Graphene has shown intriguing possibilities in the field of biosensors due to its electrical properties and ability to react with biocompatible molecules and also changes in concentration and even conformation of the molecule used as a detecting agent. [6]

The high sensitivity of graphene-sensors enables smaller sized platforms and thereby increased portability of high performance biosensors. [5] Increased portability of biosensors means
improved distribution possibilities and miniaturization of samples when huge machines are made unnecessary. Also, the price of such a small analytical platform can be significantly reduced due to the smaller amount of materials needed.

Graphensic AB is a spin-off company from Linköping University research and is one of few manufacturers of high quality epitaxial graphene (EG) for multiple applications in the world. The company was founded in 2011 and has a patented method for a sublimation process, producing large area monolayer graphene on silicon carbide (SiC).

The number of areas in which monolayer EG can be applied are vast [2] and Graphensic AB are interested in expanding their market towards commercial applications within life-science and other areas. Thereby biosensors constitutes a huge opportunity and efforts are being made to investigate the possibilities of functionalization of Graphensic AB’s EG and a platform for biosensor purposes have been developed, described in section 2.2.1.

Graphensic AB has seen a need for an EG-based biosensor development on their platform to enable researchers and other potential customers to use the advantages of graphene in biosensor applications, without the need to have extensive knowledge about the material itself. Graphensic AB wants to investigate the impact a bio-functionalization of their EG will have and what future possibilities it possesses.

This project was done in collaboration between three research groups, molecular surface physics and nanoscience; applied sensor science; plasma and coatings physics, at IFM LiU and Graphensic AB. An open question was raised by Graphensic AB on how to obtain super sensitive graphene layers for (bio-) molecular detection in gas and liquid phases. The project required expertise from three research groups with knowledge in surface modifications, surface characterizations of hard and soft materials, nanoscience, plasma physics, gas-sensing and liquid-phase measurements.

This is a pilot study for future investigations on how to incorporate biomolecules for a biosensor application using graphene functionalized with nanoparticles as a starting-point for ultra-sensitive gas-phase and liquid-phase detections of different molecules.

A future prospect is in pharmacies where one today can buy tests for a number of deceases, allergies, infections and even genetic predispositions. Mostly these self-tests need to be sent by mail to a laboratory for analysis, while a few can be done completely in one’s home. [7] A biosensor on an EG-platform could in the future make all these tests faster, cheaper and more reliable. Graphene has shown superior properties to many other materials regarding its conductive characteristics at room-temperature together with its easily affected band-gap and high elasticity and strength. [5] These properties, alongside the inherently low noise in graphene devices, the linear and low density of states near the Dirac points, and the ultimate specific surface area, make graphene an extremely suitable material for use as an ultra-sensitive sensing transducer, more in section 2.1. [3]-[5]
Chapter 1. Introduction

1.2 Purpose
The purpose of this project is to investigate how EG-SiC can be modified, increasing its specificity, in order to be used as the active component in a biosensor device. The project further aims to investigate possibilities in decorating the EG with nanoparticles (NPs) before further functionalization of the NPs. The project should present a characterization of the modified surface together with an investigation of the effects on the characteristics of EG.

The study consists of characteristics of as-is EG-SiC, EG-SiC with NPs and EG-SiC with NPs used in a resistance measurement. Throughout the study, measurements will be conducted in order to follow the experiments and characterization of the modified surface. The aim is to investigate the modified EG-SiC as a sensing component including every step towards a functionalization, carefully examine the surfaces and with the smallest biomolecules as candidates to be detected in order to draw conclusions on EG-SiC-NPs abilities in a biosensor platform.

The results are intended to increase the basic knowledge of sensor surfaces such as graphene sensitized by nanoparticles within this field of research and to increase the understanding of graphene in applications such as biosensors for the future work of Graphensic AB especially in the field of producing a complete biosensor platform.

1.3 Method
Modifying the surface of EG-SiC with NPs requires knowledge of graphene’s chemical, physical and electrical properties and understanding the modifications effect on its ability to detect a specific change in the environment surrounding it. A literature study together with the knowledge of research groups at LiU and theoretical studies on graphene and measurement technologies will be the foundation when planning and performing experimental procedures and requirements. The requirements will be formed iteratively during the first period of the study, considering the seemingly limitless possibilities for functionalization. The difficulties and advantages of using NPs as a first step towards the functionalization of monolayer EG-SiC will be described in the theory section of this report.

Initially, as-is EG-SiC surfaces are studied in order to detect the influences of NP decoration and biofunctionalization thereof. These first experimental investigations will together with the theoretical studies give a hint to the robustness of the graphene and also provide basic knowledge to the difficulties in decorating Graphensic AB’s bio-sensing component, described in section 2.2.1.

Hollow cathode (HC) plasma sputtering of two different metal-oxides, titanium oxide (TiO$_2$) and iron oxide (Fe$_3$O$_4$), will provide information on EG’s interactions with NPs of these components.
and their electrochemical characteristics. The two alternatives of NPs on EG-SiC will be investigated using a number of methods for surface characterization, mentioned in the next section. The samples of EG-SiC with NPs will be used for a simple gas-detection as a first step in investigating the sensing performance. The gas-detection is to give a hint on the sensitivity, reactivity and recovery of the two NPs and thereby show which system is more promising for functionalization using biomolecules and what properties should be investigated further before applying a selected experimental setup.

In order to properly detect changes achieved on the surface of EG-SiC, with its effects on the properties of EG-SiC, and eventually NPs and NPs on EG-SiC with a functionalization, a number of characterization measurements are needed. The surface characterizing measurements will be performed multiple times in order to follow every step in the functionalization process, i.e. every time the EG has been used in a reaction, deposition or sensing process. As a start, measurements using methods that are easy to conduct and fast performing will be used in order to characterize the surfaces of the EG before conducting experiments using more time consuming equipment on samples which have displayed suitable characteristics for NP decoration and functionalization when using methods with a faster setup.

The methods with a faster setup that will give initial investigations are:

**AFM** – Render topographic images of the surface together with electrical measurements providing an image of differences in surface potential.

**Raman spectroscopy** – To investigate defects and “finger-print” changes due to the functionalization.

More time consuming methods using more advanced technology is:

**XPS** – To separate geographical differences with respect to compounds together with orientation of bound molecules.

Sensing experiments will be conducted using:

**Resistance measurements** – Detecting changes imposed by a functionalization and detection of target molecule.

AFM and Raman measurements will be used to gather information of the process towards functionalization and will be performed several times on each sample. XPS is intended for measurements on a few, more successful, samples and the sensing experiments, resistance, are to be performed using NP-decorated EG-SiC.
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Drawings and pictures have been made using:


Spectra and measurement results were rendered using:


**XPS** – KaleidaGraph for adjusting spectra and XPS-shift to fit theoretical peaks

**Raman** – SPC Graph 26b for looking at spectra and converting to other formats
Chapter 2

Theory

In order to consider a first step towards producing a functionalized surface of EG-SiC for the use as a biosensor, one needs to have knowledge of the physics, electronic properties and chemical characteristics of graphene. The theory presented intends to lay a foundation for the experiments to be performed as well as for discussing different techniques and results gained. First the properties of graphene will be presented followed by nanoparticles in combination with EG-SiC and lastly the techniques used for deposition of NPs, surface characterization and sensing. Readers of this project are assumed to have basic knowledge within physics, chemistry and biochemistry.

2.1 Graphene

As mentioned in the introduction, graphene is a monolayer of a hexagonal carbon lattice and is a superior material regarding its electronic, mechanical and chemical characteristics. [4] The discovery of graphene was made in 2004 by Kostya Novoselov and Andre Geim at the University of Manchester by using scotch tape to render as thin layers of graphite as possible. [1] A lot of research have been conducted on graphene within different fields, e.g. physics, mechanics, chemistry, biochemistry where some of its vast possibilities have started to unravel. [2]

Graphene exhibits special properties such as high surface area, high carrier mobility, zero bandgap and anomalous fractional quantum Hall effect at 25°C. The interesting properties in a biosensor application are the electrical, chemical and physical ones that are tempered with in a functionalization process. The changes made will also be of most importance for further functionalization processes. Graphene has shown high charge transfer with molecules in liquid, ambipolar electric field effect and gas adsorption, all suited for sensor applications. [2]

The resistivity of graphene decreases with increasing number of layers and the resistance decreases with increasing temperature. Conductivity in graphene have been shown to reach as high as $5.30 \pm 0.48 \cdot 10^3 \text{ W/mK}$ at room-temperature and has a high peak when moving from very low temperatures to higher ones, with a slope towards even higher temperatures. The carrier mobility may reach as high as $15 \text{ kcm}^2/\text{Vs}$, but decreases rapidly with more layers of graphene. The zero-bandgap characteristic of graphene makes its use as a measurement component difficult, but by altering the surface one can introduce a change in bandgap.
structures and thereby choose what conductive properties to have in a sensor application. [2] Resistivity and resistance are explained further in section 2.5.4.

A very important property of graphene is its extreme sensitivity to charge-transfer with organic molecules which has high potential in a biosensor application. Investigating doping and charge-transfer effects by halogen vapors have shown an increase in sensitivity with fewer layers, particularly higher sensitivity for mono- and bilayer graphene than any other constitution. Both electron-donating and -accepting molecules alter graphene’s resistivity and resistance, but in different ways. Aniline interacts with graphene to give higher resistance, while nitrophenyl gives a lower resistance. [2] The band structure of graphene renders great sensitivity towards chemical alterations. A small change in carrier concentration moves the Fermi level significantly, changing the conductivity characteristics of graphene where monolayer is more affected than bilayer. [8] The sensitivity is high enough to detect even single molecules and relates to the much reduced level of low frequency noise. The low frequency noise would derive from carrier mobility and carrier density instabilities, which graphene does not have. The high conductivity also contributes to reduction of other noises. [9]

The planar structure of the 2D crystal derives from the carbon atoms being sp² hybridized [2], which make bonds between the carbons very strong, 100 times stronger than steel. [3] A sp²-hybridization allows the molecular orbitals of carbon to be positioned in a planar arrangement [figure 1]. Carbon has four valence electrons that can be used to bond covalently with other elements, in graphene every carbon is bound to three other carbons. The one valence electron left, out of the four, is delocalized in π-orbitals. The π-orbitals are arranged perpendicular to the plane of the 2D crystal, making them free for interaction in-between carbons. This interaction is referred to as a delocalized π-binding system. The π-bonds make the graphene resistant to covalent modification, thus demanding harsh reactions in order to passivate the interacting π-electrons. [4]

![Figure 1](image)

**Figure 1** A) A small piece of a graphene sheet showing its hexagonal structure, B) Cut-out of two carbon rings with π-orbitals and red lines showing their interaction.

If the π-orbitals interaction is broken and an outside atom binds covalently to a carbon, the hybridization changes from sp² to sp³. [4] A carbon atom that is sp³-hybridized is no longer planar, but “stands up” in the form of a tetrahedron [figure 2]. This makes the graphene lose its electrical characteristics, at least close to the carbon that is sp³-hybridized, and this also affects other properties. Multilayer graphene can still maintain some graphene-like properties, but to a lesser extent, due to the layers beneath being intact. Since multilayer graphene does not have
the unique electronic properties of monolayer graphene, which makes it less useful for ultra-sensitive sensors.

**Figure 2** The difference between A) sp²-hybridized carbon and B) sp³-hybridized. The sp²-carbon is planar in its bindings, while the sp³-carbon is “standing up” as a tetrahedron and thus losing the possibility of a planar interaction with surrounding carbons in a graphene sheet.

### 2.1.1 Graphene on SiC

The process, as patented by Graphensic AB, for manufacturing monolayer graphene on SiC involves the sublimation of silicon (Si) from the substrate, thus creating a layer of graphene on top of the SiC [figure 3]. As Si is sublimated from the substrate a Si-poor C-rich layer forms, called buffer layer, upon which graphene is produced with further sublimation. [10] The graphene created on SiC is epitaxial, meaning it follows the structure of the substrate beneath it. This means that if there are defects in the substrate it will be reflected in the graphene structure. The process used by Graphensic AB has a high sublimation temperature under a flow of Ar-gas making the formation of graphene less prone to follow the defects of the substrate and thereby creating a more refined structure with fewer defects in the graphene layer. Their method allows for the graphene forming over a large area substrate of 2 inches in size, with the limitation being accessibility to larger surfaces of SiC. [11]

**Figure 3** A schematic picture of the sublimation process where Si leaves the substrate in different forms, creating a graphene layer when the carbon atoms (black) interact to form a lattice

The term “defects in graphene” includes a number of structural deviations from the hexagonal 2D crystal; bilayer, multilayer and holes to formations making the carbons arrange in a non-hexagonal manner and also substrate effects such as strain from step edges and chemical interactions between EG and the substrate. [12] All of the defects contribute in one way or another to change the electrical properties of graphene and also its reactivity to chemical alterations. It is a known phenomenon that reactions are prone to occur at defects in graphene and therefore gives rise to the problem of producing a uniform functionalization. If a uniform distribution of functional groups cannot be achieved, the quality of the functionalized graphene will only depend on the quality of the monolayer and one graphene lattice will not behave the same as another when attempting a functionalization process. [5]
Chapter 2. Theory

When Si is sublimated on top of the substrate a carbon layer, similar to graphene, first forms on top. This is called the buffer layer and it is on top of this layer that graphene is formed upon further sublimation. The buffer layer lacks graphene’s characteristic of delocalized π-electrons. One challenge remain now to produce perfect graphene on SiC is to reduce the presence of interactions between the buffer layer and the SiC substrate that remain after sublimation, [figure 4]. These interactions inhibit some of graphene’s electronic characteristics and reduce the carrier mobility of the graphene. Graphene in itself is a hydrophobic material and does not attract water from its environment, but rather repel water. The few binds between SiC and the buffer layer affects also the hydrophobic characteristics, making the graphene slightly hydrophilic and disturbances from water become possible. To circumvent these problems an intercalation of hydrogen can produce a more independent layer of graphene on SiC and give more free-standing properties of the monolayer. [13]

![Figure 4](image.png)

Figure 4 To the left showing buffer layer on SiC with intermediate interaction between the buffer layer and the substrate and to the right after intercalation with H, breaking most of the interactions between SiC and the buffer layer producing more free-standing graphene

2.2 Functionalization of graphene for biosensing purposes

A biosensor is an apparatus that detects molecules by having a biological component as the recognizing part of the sensor as a whole. The biological component is connected to a transducer, defined as the part of the sensor that converts recognition of physical or chemical nature into a signal. For chemical- and biosensors this signal is often electrical. The definition of a biosensor does not determine whether the molecule to be detected is a biomolecule or not, only the recognizing part as mentioned above. Graphene, in this sense, is used as the transducer of the sensor. [14]

Graphene has been used in many different setups for biosensing purposes, but the main applications are field-effect transistors (FETs), impedimetric sensors using EIS, electrochemical sensors and fluorescence detection. The FET-application detects a change in carrier density as the distribution of electric charges is altered by the binding of the target molecule and requires the change to take place so it affects the graphene. Impedimetric sensors detect very small changes in electrical properties, even as small as a single nucleotide polymorphism (SNP). Electrochemical sensors detect molecules in an electrolyte that actively seeks or donate electrons, also a very sensitive application. Graphene in fluorescence detection is used for its
Chapter 2. Theory

Quenching abilities and the setups can be varied in order to detect binding or dissociation of a certain molecule. [5]

The most sought after properties of graphene are its electrical and physical properties, as mentioned in section 2.1, but many investigations on its electrochemical and biochemical characteristics are being conducted and several applications have already been proven to work experimentally. Despite its popularity in modern science and biomedical research there is no effective method protocol derived for functionalization with biomolecules, but several different attempts have been and are being done. [2]

Some of the functionalizations of graphene are with glucose oxidase and horseradish peroxidase for glucose sensing, chitosan and Au-NP decorated graphene for oxygen and hydrogen peroxide detection, DNA sensors using reduced graphene oxide in an electrochemical sensing of the four nucleotides separately detecting even SNPs and antibody immobilization subsequently specifying detection of e.g. cancer biomarkers and other genetic products causing various diseases or disorders. [2]

The main ways of functionalizing graphene in order to increase its sensitivity and, mainly, specificity that have been employed can be divided into three approaches:

- Covalent binding [6]
- Non-covalent interactions [4]
- Nanoparticles of metals or metal oxides [4], [5]

Covalent binding of organic molecules to the surface of graphene give opportunities to further binding to the organic molecule using some biological agent, e.g. enzymes or other forms of protein, but the first use of covalent binding to graphene was done in order to make it soluble in organic solvents. The covalent attachment can be done through free radicals attacking the sp²-carbon’s available π-electrons and sharing them. As described this leads to sp³-hybridization of carbons and such a conformation is no longer viewed as graphene, they are considered defects. The radicals employed have mainly been in the form of heated diazonium salts, e.g. nitrophenyl, hydroxylated aryls and benzoyl peroxide. Another approach to covalently attach a molecule to graphene is by addition of dienophiles, e.g. azomethine ylide in a cycloaddition reaction, for increased solubility and subsequent reactions in a variety of applications. [4]

Non-covalent interactions use the delocalized π-bonding system of graphene through what is called π–π stacking or π–π interactions. The term is used to describe a rather complex set of interactions occurring between π-electrons in two compounds and is mostly constituted of ionic and Van der Waals forces. The nature of the interaction depends on whether the two compounds possess the same or different electronegativity- or electron doning/accepting characteristics, among many other things. Pyrene is a structure of four hexagonal rings that have delocalized π-electrons ready to interact with graphene and some derivatives have shown
good opportunities for functionalization with antibodies etc., namely PBSA (pyrene butanoic succinimidy l ester) and pyrenecarboxylic acid. [4]

Deposition of nanoparticles (NPs) and functionalization thereof using biomolecules are described in sections 2.3 – 2.4.

### 2.2.1 Graphensic AB’s biosensor platform

The biosensor platform developed at Graphensic AB consists of a chip holder and a sensor chip. The holder has a base, a top part with a cavity for liquids and a lid for sealing the top part and accessing it with electrodes. The base, top part and lid are made of polyoxymethylene (POM) and can hold 480 – 1280 µL of liquid, with/without the lid, and seals of a selected part of the chip with an O-ring. For interaction with the chip, the holder base fits a chip interface board that connects through a coaxial ribbon cable to a coaxial breakout board. [15]

The sensor chip is made with photolithographic etching using different masks for different layers. The graphene is exposed as channels between electrodes of gold connecting to the chip interface board. The channels have been developed with different widths, all 6 mm in length, of 857, 600 and 300 µm.

All put together makes an ion sensitive field effect transistor, ISFET, biosensor platform capable of measuring cyclic voltammetry (CV), current-voltage (I-V) and electrochemical impedance spectroscopy (EIS). [15] Some theory on an ISFET-setup is presented in section 2.5.4.2.

### 2.3 Nanoparticles on graphene

Some of the difficulties mentioned, sp³-hybridizing and the interacting π-electrons, with functionalizing graphene in section 2.1 are possible to avoid with deposition of nanoparticles (NPs) composed of different metals or metal oxides. Graphene is viewed upon as being an ideal material in depositing NPs with its mechanical durability, large surface area and beneficial conductivity and many different compounds have been used to manufacture NPs. [4] A number of constitutions using metals and metal oxides have shown altering interactions with graphene. Some interact in a more covalent way, while others are almost suspended on the surface interacting only loosely with graphene. [16]

Some metals, such as Pd, Au, Pt, Rh and Ag, are often used in different nanostructures presenting a variety of properties to the substrate they are assembled upon. It has been shown that Pd on graphene interact more with the surface compared to Ag and Au, and this is believed to be due to a more covalent attachment. To decorate graphene with Au is more difficult due to its very low affinity to monolayer graphene without defects (see 2.1.1). The affinity of Au to graphene is assisted by the sublayers in multilayer graphene and is almost insignificant when using a monolayer. [4]

Additionally, several metal oxides have been deposited on graphene as a substrate and used for a lot of different catalytic, conducting and chemical sensing applications. A few of the oxides...
studied are TiO$_2$, Fe$_3$O$_4$, Co$_3$O$_4$, CuO and MnO$_2$ that have been used for improving energy storage in batteries, photocatalytic water splitting, optoelectronics etc. [4]

Depositing NPs on graphene have been done in numerous ways and the choice on how to do so affects the graphene surface very differently. A method often mentioned is the reduction of metal salts using chemical, electrochemical, microwave and photo-induced techniques. These techniques often require the graphene sample to be dispersible in a solvent. [4] Deposition of metal oxides as NPs are performed by hydrolysis of metal oxide salts in a solvent followed by reduction or calcination of the sample and also by immersing the graphene in a solvent containing prepared NPs for self-assembly. [4] Another method employed for depositing NPs is thermal vaporization or electron-beam vaporization where the metal is vaporized in order for it to form small particles when “sprayed” upon the graphene surface. [16] One other method used for this purpose is magnetron sputtering, where plasma of different gases are used to eject atoms of a source which will constitute the deposited material (more on plasma sputtering in section 2.3.1). [17]

Depositing any kind of material on graphene alters the conductivity of the surface, which is called doping. This subject is tended to in section 2.5.4.1.

### 2.3.1 Plasma sputtering

Plasma sputtering refers to a material being sputtered by a plasma discharge, where ions in the plasma collide with the material and eject ions from the material into the plasma. There are some ways of achieving this and one is DC magnetron sputtering [17] and another is using a high power pulsed hollow cathode (HC) [18] to render NPs.

Using the HC method the growth of NPs in the plasma occurs in three different stages [figure 5] where the first, nucleation, is a collision between three metal particles, rendering a dimer of two of them. The dimers then grow by addition of single particles to form larger formations and when a certain size is reached they can form interactions in-between formations, coagulation. When coagulates grow in size, < 10 nm, they take on a negative charge distributed uniformly throughout the NP, accretion [figure 5]. The plasma is created by a discharge of electrons into the HC with a gas or a mix of gases, usually Ar and oxygen, making the gas molecules ionize and with pressure and temperature combinations achieve the special state called plasma. An advantage of using the HC method is the low temperature of the gas that can be applied. It has also been demonstrated that the size of the NPs can be controlled, 50-80 nm, by altering the power of the discharge, pressure and flow of gas. The degree of ionization is higher than other methods and the material density in the gas is also higher, due to the HC’s ability to eject it into a smaller area. When coagulates in the plasma take on a uniform charge they repel particles and thereby hinder further growth of the NPs. The size of the particles can also be determined by altering the characteristics of the electronic pulse through the HC, thereby controlling the degree of ionization. [18]
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The NPs are formed in the plasma, where they are in a vapor state, and increase in size the further the vapor gets from the HC. Vapor-clouds from different electrical pulses through the HC travel partly through one another and particles can interact in different stages of the NP-formation. As the particles get farther away from the HC, traveling with the gas flow, and increase in size the actual NPs take on a more solid form. A complete NP is believed to have interacted, in one way or another, with ten different vapor clouds from electronic pulses separated by time. [18]

![Figure 5](image.png)

*Figure 5 A schematic drawing of A) three metal particles colliding to form B) a dimer which C) attracts single particles to form clusters that D) coagulate and when a certain size repel particles, preventing further growth and has formed an NP moving towards its fluid state*

In this study, two different metal oxides will be sputtered on to graphene, TiO$_2$ and Fe$_3$O$_4$ NPs. Both metal oxides are produced in processes where Ar-gas is flowed through a HC and ionized to create plasma, while the HC is sputtered to release ions of metals. The difference between the methods are that the TiO$_2$ particles are produced using reactive sputtering, where oxygen is mixed with the Ar-gas in order to oxidize the Ti-HC ions, while the Fe$_3$O$_4$ NPs only uses Ar-gas and are later oxidized when exposed to air to create an Fe$_3$O$_4$-shell around an iron core.

2.4 Functionalization of NPs

Metal oxide NPs usually have hydroxyl (OH) groups exposed to their environment that, particularly when exposed to air or water, can be used to link biomolecules to the surface of the NPs. The anchoring group most suitable for binding to metal oxide NPs have proven to be phosphonic acids (P(O)(OH)$_2$) and carboxylic acids (COOH). Both P(O)(OH)$_2$ and COOH can adsorb to the surface of the metal oxide in different ways, forming a range of different interactions with the NP. The way of bonding is determined by several factors, such as pH, the tail of the anchoring group and the structure of the metal oxides. The interactions can be through hydrogen bonding, non-covalent interactions and covalent interactions with one of the oxygen’s or both to one metal oxide group or two separate ones. Also different derivatives of carboxylic acids have been applied in coupling to TiO$_2$, e.g. esters and amines. [19] Silicon containing groups, using Si as anchoring group, have been successfully grafted to the OH of TiO$_2$-NPs, forming an ester-like bond as Ti-O-Si-. This have been done in order to enhance sensitivity to gases and also the recovery time of the functionalized NPs. [20]

The attachment of carboxylate-terminated linkers on metal oxides has been performed using a few different techniques. Some exploit self-assembling of the molecules at room temperature, where the metal oxides on their substrate are immersed in a buffered solution containing the
linker molecule and left overnight with subsequent rinsing using pure buffer. In a form of self-assembling the pH of the solution used is critical and should be chosen between the particles iso-electric point (IEP) and the pKa of the linker’s carboxylate group, which will determine the charges of the two interacting specimens and the type of interaction occurring. [21] Others heat the immersed substrates and incubate only for a short time, sometimes using ultrasonication to assist in arranging the linker molecules. When a linker molecule is attached to the metal oxide particles, further functionalization of the unbound end is needed in order to sensitize the substrate towards some target molecule. The linker molecule is often ended with a carboxyl, amine, hydroxyl or ester group, where a sensitizing agent can be attached. [19] When choosing the ending of a linker, one must consider the function of the anchoring group. If the anchoring group is a carboxyl the tail group cannot be a carboxyl, since both anchoring group and tail will adsorb to the surface and few functional groups for further functionalization will be available, [figure 6].

![Figure 6](image)

To circumvent this problem, wanting the same group in both anchoring group and tail, one can apply a protection group on top of the carboxyl group. The protection group can then be removed after adsorption to the surface, giving a functional group that will not bind to the surface itself. [22]

In binding a sensitizing agent to the linker molecule, there are a range of techniques to choose from for every type of functional group. A few coupling are to use cyanogen bromide for OH-groups, carboimide for COOH, glutaraldehyde for NH₂ and acyl groups through treatment with hydrazides with nitrous acid. These coupling strategies are usually performed in order to immobilize an enzyme by covalent bonding to an NH₂-group in the enzyme. [22]

2.5 Measurement techniques

The measurements presented below are intended to, in combination, give a surface characterization of EG-SiC and NP-EG-SiC before and after sensing. The different methods give an insight into a vast range of properties of the samples and also a good indication on whether the experiments performed have been successful or not.
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The theory behind these methods is, in most cases, complex and needs to be understood to take full advantage of each technique when measuring. In this section the methods will be briefly described and it only contains basic descriptions of the physics, chemistry and electronic properties of the sample to measure upon that is used by the technology at hand. The descriptions are meant to give a basic understanding to the reader, before presenting results and later discussion.

2.5.1 AFM

Atomic force microscopy (AFM) is part of several other microscopy techniques called scanning probe microscopy, called so for using a proximal probe to scan a sample with down to nanometer scale and less. AFM in itself can be used in several different modes. These modes can be used with similar technical setup, but with a vast range of resolutions and parameters measured. [23] Herein AFM is described briefly and in a general manner.

The probe in AFM consists of a small tip on a cantilever attached to the regulatory unit. The angle of the cantilever is measured using a laser beam that reflects to a sensor mapping the results. [figure 7] In some setups the measured angle is rendered by the electric interaction between the sample and the tip, while in others it is the sheer difference in height of the sample moving the tip. Most of the measurements are a mix of the two. [24]

Tapping mode refers to when the cantilever is oscillating near its resonance frequency, closing in on the sample. At first the tip experiences attractive forces that changes to repulsive forces just before contact with the sample. The different forces change the frequency of the oscillation. This is registered by the sensor, rendering a topographical image in nanometer scale of the sample without physically interacting with it. The image of the surface, in tapping mode, is rendered by the tip tapping the surface in certain points. The operator may choose a specific area to investigate and the field of view is segmented into a number of lines, which the tip follows back and forth, and a number of points per line. The image is then presented line-by-line and point-by-point. The signal and its transformation can be viewed as sine-in-sine-out where the difference in amplitude renders the height information and the phase shift gives an image depending on differences in adhesion and viscoelasticity, among other things. [24]
Raman spectroscopy refers to methods measuring Raman scattering, inelastically scattered radiation of a different frequency than that of the incident light causing it. A brief description is that a photon in the incident light, often laser, is annihilated by a molecule in the sample, causing the formation of a new photon with a different energy. The energy of this new photon is lower than that of the incident photon, making the molecule in the sample gain energy and transit to a vibrational state. In the vibrational state another incident photon may interact with the molecule, causing the formation of yet another photon with higher energy than the incident one making the molecule lose energy. The scattered radiation of higher and lower frequency is measured respectively, depending on the method used. [25]

The spectrum from a certain molecule is like a fingerprint for that specific compound in that specific conformation. As such, Raman spectroscopy can be used to determine the conformation of a certain compound and also detect any changes made to it. Graphene has a characteristic spectrum with peaks indicating specific properties of the graphene layer. [26]

The D-peak [figure 8] can be related to defects in the graphene layer, see section 2.1.1, and should be absent in a perfect monolayer. The G'-peak’s, also called 2D-peak, shape is determined by the number of layers in the sample. It is most intense in monolayer graphene and exceeds the G-peak. The G-peak changes its appearance depending on the amount of layers and originates from different degenerative symmetry modes. These explanations to the different peaks are simplified and all of them depend on several properties of the sample. To properly describe them, i.e. full interpretation, one needs to have a great insight into quantum physics and quantum chemistry. Each peak can be related to the properties of the sample and comparisons between raw material and an altered sample can give important information on new properties introduced and original properties removed. [26]

Raman spectroscopy is a non-invasive method, it does not alter the sample measured upon, and cleaning of the sample is often unnecessary. The information obtained resembles that of an IR technique, but uses a different mode of the molecules. Raman spectroscopy and IR spectroscopy complement each other in that sense. [25]

A micro-Raman refers to a spectrometer using a smaller spot size than conventional ones, giving the possibility to investigate smaller areas. The basic principle uses a laser source that, via mirrors, affects the sample so that it produces scattered radiation. The radiation together with the incident light are split using a dichromatic mirror, guiding the Raman radiation to the detector. [27]
2.5.3 XPS

X-ray photoelectron spectroscopy uses the photoelectric effect, when electrons are excited by a photon with enough energy for the electron to be emitted from the atom. The photon needs to have more energy than the sum of binding energies and the work function in order for the electron to escape from the sample. The work function (\(\Phi\)) of an atom is described as a threshold i.e. the amount of extra energy, besides the energy needed to lift the electron from bound state (\(E_b\)) to the Fermi-level, required for one electron to leave the sample. The extra energy left will be measured as kinetic energy (\(E_k\)) \((h\nu = E_b + E_k + \Phi)\). The electrons are excited by x-rays and, when emitted, they are guided into an energy analyzer (two hemispheres with a potential between them). This makes the incoming electrons travel through half an orbit, but only electrons with the energy matching the pass energy will complete the half circle and reach the detector. The X-ray photon energy used to irradiate the sample is known and by measuring and counting electrons that are allowed to pass the energy analyzer, one can calculate their binding energy [figure 9]. This gives information about the binding energy of all the elements present in the sample and one can thus obtain the elemental composition of the sample, since every element has its unique electron structure. One can also distinguish elements in different environments (i.e. chemical shifts) such as different chemical environments in a chemical structure. The excitation and measurement needs to be performed under ultra-high vacuum (UHV). [18]

The energy of the x-rays is varied in order to excite different atoms at different binding energies. Usually one first measure a wide scan where one observe at a wide interval to identify all elements present. Then narrow scans are collected on each peak to identify the relative intensities, to gain chemical compositions and also to identify different chemical states of the same element. When a region of interest is found, the interval is selected and scanned several times with smaller energy steps to produce binding energy spectra with improved statistics and high energy resolution. [28]

X-rays in XPS are usually non-damaging, but may disturb or sometimes (after a long X-ray exposure time) also destroy some materials, for example certain self-assembled monolayers (SAMs). The fact that measurements must be performed under UHV conditions also restrict the choice of materials to be analyzed. There are materials that may disperse at such a low pressure. [18]

XPS is sometimes called electron spectroscopy for chemical analysis (ESCA) and is used to analyze chemical compositions with absolute binding energies, relative intensities and elements ratios by emitting core electrons of atoms. Shifts in binding energies indicate the chemical environments for the element. A specific setup of XPS, angle dependent, can determine the
geometry of compounds beside sits orientation relative to the substrate surface. A more electronegative environment usually shifts or broadens the elemental peak, in a XP spectrum, towards a higher binding energy. [28]

2.5.4 Resistance
The theory presented in this subchapter is meant to clarify quantities and give a background to what is measured, predominantly when performing gas-sensing experiments. The theory is also relevant when measuring cyclic voltammetry and for the next two subchapters.

It is important to distinguish between some nomenclatures when mentioning resistance. The most basic definition is that of resistance \( R \), defined as a material’s ability to oppose the flow of an electric current through it and is calculated as the potential over the current. A conductor’s resistance depends on its resistivity \( \rho \), length and cross-sectional area. Surface resistance \( R_s \) is the resistance between two contacts on one side of a substrate with known properties and placements. Surface resistivity \( \rho_s \) means the fraction with potential per the distance separating the electrodes as numerator and current per depth of the electrodes as denominator [figure 10]. \( R_s \) and \( \rho_s \) are quantified using ohm (Ω) and Ωm respectively. [29]

\[
R_s = \frac{U}{i} \\
\rho_s = \frac{U/\text{length}}{i/\text{depth}}
\]

When investigating a surface, regarding its resistance and resistivity, there are a lot of different factors influencing the electrical properties. If the surface is altered in any way or for example gas molecules adsorb to it the resistivity will change, since the carrier mobility and in some cases the carrier density will be altered. Attaching electrodes to the surface is an alteration in itself and the conductivity will depend on the electrode-surface interface. There are many environmental aspects affecting resistance and resistivity that one needs to be aware of when measuring and detecting any changes, for instance the temperature will influence the surface material depending on its constitution. Also the interfaces between electrodes and liquid or gas and between the surface and liquid or gas will affect many properties regarding the conductivity of the setup. [30]

There are three major effects influencing resistivity, not presented in detail here, of a surface. The size effect giving alterations depending on the thickness of the surface, the adsorption effect deriving from something adsorbing to the surface and the field effect brought about by changes in conductivity when exposing the surface material to an electric field. [30]
The measurement in itself can be performed using several different approaches, due to the connections between resistance, resistivity, potential and current [figure 10]. One can, for example, measure the potential and the current to calculate the resistance and resistivity, with knowledge of distances etc. As such, one can measure or have knowledge about two or more of the electrical parameters in order to gain knowledge about all of them. This does not mean that knowledge can be gained about the source of a change in e.g. resistance, but one can quantify it. To be able to say something about the source of changes in conductivity one has to control the environment around the setup for measurements very precisely and realize a lot about the different interfaces and effects that occur. Since the electrodes, wires, measurement equipment and the surface itself are conducting materials with, mostly, three-dimensional structures they all give rise to a super-positioned resistance. This also needs attention in order to fully understand the measurements conducted, but can be regarded as a background disturbance to be subtracted when measuring on the sample of interest.

When measuring resistance changes in a mixture of gases the response of the sensor is defined as the fraction of resistance in the mixture where the gas to be detected is \((R_g)\) and the resistance in the carrier gas \((R_0)\) \(\left(\frac{R_g + R_0}{R_0}\right)\). [17]

### 2.5.4.1 Doping

Without venturing deep into quantum physics and quantum mechanics, the concept of doping is here dabbled upon in a very simplified way.

When introducing elements to a conductive or semi-conductive material that change the number of electrons or holes present, the introduced element is doping the conductor. Electrons and holes are defined as charge carriers, where electrons represent negative charge carriers and holes positive charge carriers. A material that has a conduction aided predominantly by electrons is called an n-type conducting material and a material where holes dominate the aid of conduction is called a p-type conducting material. When a conductive surface is altered with a material that introduces more electrons to participate in conduction to the surface, the surface is n-type doped by the introduced material. As can be concluded, when holes are introduced the surface is p-type doped. [31]

Graphene in itself has been found to be a mixed-type conductor, with both electrons and holes aiding the conduction without one dominating over the other. Thereby the type of doping taking place when introducing e.g. nanoparticles or biomolecules to graphene is a very interesting aspect which is also connected to the change in band structure. [1] Graphene on SiC is n-type doped due to the interactions with the substrate mentioned in 2.1.1. [17]

### 2.5.4.2 ISFET

An ion sensitive field effect transistor (ISFET) is a device using an electrolyte in contact with an electrode. [19] It can be seen as a semiconductor-based biosensor with capability of being small in size and rendering responses fast. [32] The ISFET is constituted of two electrodes with a gate
insulator on top [figure 11]. The gate insulator is covered with an electrolyte, where a gate electrode is submerged. If one wants to only measure the resistance of the gate insulator, the current-voltage (I-V) is measured only between the two electrodes at the edges of the gate insulator. [19]

An ISFET application using biofunctionalized graphene as the gate insulator renders a biosensor that is sensitive to changes at the surface, inducing a change in resistance. [19] The measurements should be evaluated by comparing the responses in resistance with the complete sensitized graphene and thereafter with added target molecule. The comparison is necessary in order to distinguish the influence of the technical setups from addition of the target. [32] Ions in the electrolyte, buffer, are attracted to the gate insulator when a current flows through it. The insulator will be negatively charged, attracting positively charged ions forming a layer over the insulator. On top of the positively charged ion layer there will be attraction of negatively charged ions, forming an electrical double layer (EDL). The EDL can then be affected by changes in proximity to charged molecules or electronegative/-positive ones or direct interaction with molecules affecting the conductivity. [19]

The ISFET-device that Graphensic AB provides is based on a plastic, POM, base with a lid for liquid measurements. The base holds an interface board that connects the electrodes on a graphene chip with a coaxial ribbon cable that is split up to a breakout board with 20 connections. The starting point is to measure changes in resistance upon alternating pH in order to detect influences on conductivity and effect on the particles. More information on the ISFET-setup is available in [19].
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Chapter 3

Experimental

The individual procedures for all measurements and experiments are described below, in a simple manner in order to be easily understood. The overall procedure for a sample is pictured in [figure 12].

In [figure 12] one can see an overview of the process for a graphene sample in this project. Surface characterizations were performed as soon as the sample had taken part in an experiment in order to follow the impact on the graphene itself and NPs before and after sensing experiments. The measurements in-between the experiments are not the same for all samples, as they were divided into groups with similar topography and surface characteristics and treated the same within these groups. More time consuming measurements, e.g. XPS, were performed for samples selected to represent each group.

3.1 Materials and procedures

Below the separate procedures are described for each characterization measurement and for the use of graphene as a sensor-component. How the experiments were setup and what equipment and materials were used are described briefly.

3.1.1 Cleaning

Cleaning of the EG-SiC was done by ultrasonication in mQ-water, acetone and ethanol respectively and subsequently the samples were rinsed in mQ-water and dried under compressed air.

Cleaning of the samples was only performed before decorating with NPs, due to the NPs ability to dissociate from EG-SiC if attempting to clean them further.
3.1.2 Plasma sputtering of NPs
Both TiO$_2$ and Fe$_3$O$_4$ were sputtered using a HC-setup, see section 2.3.1, and a flow of Ar-gas under high vacuum pressure (~0.83 Torr), with a chamber base pressure of approximately $1\times10^{-6}$ Torr before flowing process gas.

3.1.2.1 TiO$_2$
First the HC and chamber were clean sputtered with a flow of only Ar-gas and a cleaning voltage of 295 V. The clean sputtering was altered between the source for NP-decoration and an alternative source perpendicular to the substrate holder. The clean sputtering was performed until the level of water in the chamber was low enough, approximately $1.3\times10^{-9}$ amu, in order to not interfere with the particles in their path to the substrate.

The substrate, EG-SiC, was exposed to the sputtered NP-flow for 2.5 min each with a DC-pulse in the HC of 1.5 kHz and a width of 80 ms. The bias applied on the substrates was 10 V and the flow of Ar-gas was 90 sccm. In order to oxidize the Ti and regulate the level of oxidization a flow of oxygen-gas was led into the chamber, 3 sccm.

An attempt to lower the coverage was done by a second sputtering, using the same parameters only shortening the exposure time to 15 s.

3.1.2.2 Fe$_3$O$_4$
When sputtering the Fe$_3$O$_4$-particles the bias over the substrates was kept at 3 V and a pulse of 1.2 kHz with a width of 80 ms was applied. The substrates were exposed to the plasma cloud for 1 min each and a flow of Ar-gas of 70 sccm entered the chamber. The current was approximately 6.44 A with a voltage of 370 V.

As mentioned the sputtering of an iron-HC renders iron particles that later oxidize, when exposed to air, to produce a Fe$_3$O$_4$-layer around an iron core and does not need addition of oxygen gas in the chamber.

A second sputtering of new samples was done where the time was reduced to 15 s in an attempt to reduce coverage.

3.1.3 AFM
The AFM topographical measurements were performed using a Quadrexed Enviroscope with a Nanoscope IVa controller. The samples were attached to a small metal plate using copper tape and the plate with the taped sample was put in a ceramic sample holder with metal pins for holding the plate and sample still. The chamber was closed by a glass door during the calibrations and measurements. At first an appropriate area of the sample was located and then the drive amplitude and amplitude set-point of the tip was altered together with gain parameters in order to give a clear image. During the calibrations the tip was working with a 1 Hz frequency and 128 lines with 128 samples per line. As the calibrations revealed a clearer
image, 256 lines with 256 samples per line were used to scan a 5 µm × 5 µm area. After taking
snapshots of the 5 µm² area a zoom-in was performed to an area of approximately 2.8 µm ×
2.8 µm. When a zoom-in had been performed another area of the sample was investigated,
starting with a 5 µm × 5 µm area.

Mapping of the surface potential was performed using an in-house produced plate for
grounding purposes, upon where the samples where attached on a metal plate by copper tape.
The data scale voltage was set to 0.1 V and scans were performed with a scan line in interleave
mode.

3.1.4 Raman spectroscopy
Raman spectroscopy was performed using a micro-Raman setup, described in [27]. First a
reflectance mapping of the samples was performed in order to select an area representative for
the whole sample, regarding topography etc., where Raman spectra were collected at an area
of 30 µm × 30 µm. From this area Raman scattering was measured at 230 points, rendering 230
spectra with Raman shifts from 879 – 3686 cm⁻¹, using 1.4 cm⁻¹ steps, a 50 µm slit and a laser
energy of 2330.7 meV. The background was subsequently subtracted from the spectra previous
to analysis, where the background was represented by measurement on a separate sample of
SiC.

3.1.5 XPS
The spectrometer used for XPS was a Microlab 310-F where the sample was put on a holder
and pinned with a copper cantilever to stay in place. The sample with holder was put in a
preparation chamber and the pressure lowered to approximately 10⁻⁸ Torr. When the correct
pressure was achieved, the sample was transferred to the analysis chamber. The energy of the
x-rays was kept at 1486.6 eV from an aluminum source and the surface was tilted 60°.

First a wide-scan was performed to investigate where the different peaks were located and
then fast narrow scans for the elements of interest, C1s, O1s, Si2p, Ti2p, Fe2p, N1s and S2p,
were used for setting up the parameters for the repetitive scan. The different intervals of
binding energies are shown in the results from XPS, section 4.3.

The spectra gained were fitted with theoretical peaks for the elements chemical bindings using
a full-width half-max (FWHM) value of 1.9 and a Lorentzian-Gaussian at 20 %, which have been
established to be the optimal settings for this type of samples and the equipment in use.
3.1.6 Gas-sensor and liquid measurements

Before measuring resistance, or rather I-V, over surfaces of EG-SiC-NP contacts on the surface itself needed to be constructed. This was done by thermal evaporation onto EG-SiC-NP masked with a shadow mask comprising 1 mm × 1 mm with a 2 mm pitch in vacuum. Starting with a pressure of $2 \times 10^{-6}$ Torr Ti was first vaporized from a wolfram (W) boat by increasing the current through the boat until the metal melted and then started to evaporate. The Ti was deposited to a thickness of about 52 Å using a z-ratio of 0.628. The process was then stopped and the current subsequently led through W-boats with gold that evaporated to a thickness of 530 Å, z-ratio of 0.381, on the Ti electrodes on the substrates. The final pressure after deposition was $\sim 3.5 \times 10^{-6}$ Torr, the electrodes were 1 × 1 mm in size and efforts were made to mask the center of the substrates using pieces of thin copper.

The samples with electrodes were subsequently mounted on a TO8 header with heater, temperature sensor (Pt100) and 16 pins for contacting. Gold wires were bonded to the connective pins and then connected to the electrodes on the samples using silver glue (Epotek E3081)

The gases to detect were mixed in a carrier gas consisting of 80 % N$_2$ and 20 % O$_2$, simulating air. The gases used for detection already had N$_2$ as a carrier gas, keeping the N$_2$/O$_2$ ratio constant when performing the gas sensing. The gases investigated were CO, formaldehyde, benzene and NH$_3$. This was done by applying 1 V over the samples and by changes in current, ranging from $\sim 0.08$ mA to $\sim 0.5$ mA, the resistance was calculated and plotted. The measurements were done at three different temperatures, i.e. room temperature (RT), 100 °C and 150 °C, where the sensor was stabilized in simulated air to begin with. After stabilization a sequence with specific gas mixes was executed in the order CO (500 ppm – 25 ppm), formaldehyde (1 ppm – 50 ppb), benzene (100 ppb – 5 ppb) and NH$_3$ (100 ppm – 5 ppm), where NH$_3$ needed to be removed by heating and a flow of N$_2$. Information on bottle-concentrations of the different gases and concentrations over the sensor can be found in Appendix D. An in-house gas-mixing system was used with a Keithley 2601 sourcemeter for applying voltage and measuring current. The software used to control the gas-mixing system was an in-house made program where percentages of gases were communicated in order to give specific concentrations of test-gases.

The sensors were tested for detection of NO$_2$ as well and showed large resistance changes for low concentrations. The changes in resistance were not identified as specific sensor detections and are therefore not presented as a result from the resistance measurements.

An initial test in liquid was performed where a potentiostat performed CV and measured resistance over a surface compared to an Ag/AgCl reference electrode (RE). In the initial test two different buffers were investigated in an in-house flow-cell where an external pump was used to expose the surface to 0.1 M PBS (pH 7.4) and 0.1 M HCl with 0.1 M NaCl (pH 1). Later on a series of concentrations of H$_2$O$_2$ in PBS were tested on the surfaces.
The liquid measurements were only performed on surfaces with Fe$_3$O$_4$-NPs because samples of a specific size were suitable in the flow-cell used and no more samples of that size were available.
Chapter 3. Experimental
Chapter 4

Results

The results presented are selected from a range of measurements to present adequate information for the conclusions drawn in this work. Some complementary results are presented in the appendix for each technique used. The sections are oriented by the three characterization techniques followed by the gas-sensing results and liquid measurements.

4.1 AFM

In [figure 13] The topography is clearly shown and so also surface potential of as-grown graphene, where the morphology does not correspond exactly with the surface potential. Monolayer graphene shows properties that render a higher potential and here presented with more yellow colors, while bilayer graphene has a lower potential that render more of the purple colors. The scale is inverted due to the surface potential being measured as $\Phi_S = \Phi_{\text{Tip}} - eV_{\text{CPD}}$ where $\Phi_S$ is the work-function of the sample surface and $\Phi_{\text{Tip}}$ the work-function of the tip.
Chapter 4. Results

4.1.1 TiO2-particles

The pieces of EG-SiC-NPs were investigated using tapping-mode AFM to give a topographical image of the surfaces and a hint on the degree with which the NPs are covering the different samples. [figure 14 A)] shows a topographical image of graphene decorated with TiO\textsubscript{2} NPs using a field of view of 5.0 µm x 5.0 µm. One can see the non-uniform coverage together with size differences of the particles and also some agglomeration (in the lower center of the image). The brighter the color, the higher the topography is and brown or black colors are ascribed to EG-SiC. The blue circle contains a NP that is approximately 79 nm in diameter, which was the predominantly occurring size. As can be seen there are some particles that are smaller and some that are bigger, sizes of single particles ranging from \sim 50 nm - \sim 85 nm. The even larger structures are agglomerates of two or more particles. From the parameters chosen when sputtering, the particles were expected to gain a size of approximately 60 nm – 70 nm. The NPs in [figure 14 A)] have a high coverage of the surface and couples as oligomers in some instances, but the surface of EG-SiC is still visible and there are small areas free from NPs. [figure 14 B)] displays a very different coverage of TiO\textsubscript{2} NPs (15 s deposition), but with a corresponding degree of agglomeration. The blue circle also contains a NP approximately 79 nm, with the same size distribution as in [figure 14 A)], in diameter and the three orange arrows shows a section of a step, rendered by the SiC and the graphene being epitaxial. As can be seen the particles does not follow the steps created by the substrate, nor do they avoid them.

The differences in surface potential for a sample decorated with TiO\textsubscript{2} (15 s deposition) can be seen in [figure 15], where the difference in potential over the surface is shown beside the topographical measurement performed simultaneously.

Figure 14 Two different samples with TiO\textsubscript{2}-NPs deposited for A) 2.5 min and B) 15 s
The potential over the surface ranges from -15.8 mV to 17.2 mV relative to the potential of the tip. As shown there are indications of the differences in monolayer and bilayer graphene when looking at the surface potential. The particles in the topographic image seem to be deposited both on mono and bilayer graphene, with more particles present on monolayer graphene.

Figure 15 Topographical image, A), with the phase-contrast, B), rendered simultaneously from a TiO$_2$-graphene sample

Figure 16 Height and potential mapping done simultaneously on graphene with TiO$_2$ nanoparticles
Chapter 4. Results

4.1.2 Fe$_3$O$_4$-particles

[Figure 17] A) and C) shows two different pieces of graphene decorated with Fe$_3$O$_4$-NPs with a field of view of 1 µm and 3 µm in width respectively due to the irregularities in topography when investigating areas in a greater field of view.

The blue circles contain particles that are approximately 60 nm in diameter in both A) and C), [figure 17]. As shown in the images, the iron oxide particles have formed clusters of both particles and agglomerates which lie on top of each other, rendering a very large height difference between layers of clusters. Both pieces were treated the same and display very similar degrees of agglomeration, coverage and topography. [figure 17 B]) shows the phase-contrast of A).

The potential in [figure 18 B)] has most of the particles at 50-60 mV relative to the potential of the tip and a higher potential of ~100 mV. The lowest potential measured was around 20 mV. [figure 17] and [figure 18] show samples where the sputtering was performed during 60 s.
The images in [figure 19] show topography and phase contrast for a sample sputtered for 15 s, giving a significantly lower coverage of Fe$_3$O$_4$-NPs. With the shorter time-span of 15 s the particles measured between 20–60 nm in diameter.

**Figure 18** A) height and B) surface potential for a sample with Fe$_3$O$_4$-particles on graphene, 60 s deposition time

**Figure 19** Height and phase contrast for a sample sputtered with Fe$_3$O$_4$ during a shorter time period, 15 s, over 3 µm × 3 µm
4.2 Raman spectroscopy

A reflectance mapping was done over an area of 30 µm x 30 µm with a 300 nm step, showing the variation of layers in the graphene [figure 20].

Yellow lines are occurring at two-layer graphene. The two-layer graphene has an area with the automatically calculated ratio of ~18 % of the total surface. The total area is in this case 30 µm x 30 µm. Monolayer graphene is shown in red and has accordingly a calculated ratio of ~82 % of the total surface.

The 30 µm² area was chosen after investigating the entire piece of EG-SiC and as being representative regarding its homogeneity in topography.

The reflectance mapping in [figure 20] shows a uniform piece of EG-SiC and all of the pieces investigated did not have the same appearance.

More reflectance mapping and Raman spectroscopy images of samples investigated in this study are shown in Appendix B.

The two spectra in [figure 21] are measured at separate points, one spectra derives from one point on the sample. The differences between mono- and bilayer graphene can be seen at the ratios between G- and 2D-peaks.

For monolayer graphene the 2D-peak becomes more prominent with respect to the G-peak and has a lower Raman shift. [26] The intensity of the G-peak for the monolayer is pointed out by the black dashed line to the left. The small irregular peaks to the left of the G-peak, encircled, are due to the buffer-layer and defects. [26]

In certain conformations of bilayer graphene there is an extra D-peak, higher in shift, that has not been explained properly yet. The G-peak’s shift does not depend on how many layers the sample has, but it shifts to the left with doping.

![Figure 20](reflectance_mapping.png)

**Figure 20** Reflectance mapping of graphene, showing 1-layer and 2-layers

![Figure 21](raman_spectra.png)

**Figure 21** Raman spectra from two different points on the same sample, showing mono- and bilayer graphene
by the substrate, a functionalization, the environment or strain and the width decreases compared to pristine graphene. The D’-peak also originates from defects while the 2D’-peak comes from a two-phonon interaction. [2]

4.2.1 TiO$_2$-particles

The two spectra in figure 22 shows the same sample before and after deposition of TiO$_2$-NPs with a high coverage. The differences are quite obvious, with the D-peak to the left, the shift of the G- and 2D-peak’s and an extra peak to the right of the 2D-peak. When another sample was investigated, having a shorter deposition time while sputtering, the graphene was still visible [figure 23]. The significant differences are the shifts of the G and 2D-peaks with the much lower intensity for the 2D-peak when having a high coverage. Note the slightly offset spectrum for monodispersed NPs indicating the G and 2D-peak should be even more similar to the as-grown graphene. In the D-peak area the shape of the spectrum for monodispersed NPs is almost the same as for graphene, with a slightly higher intensity. Compared to the D-peak of the high coverage NPs, the D-peak areas are almost insignificant in the other two spectra.
4.3 XPS

XPS-measurements were carried out on as-grown EG-SiC to gain a reference and detect changes in chemical constitutions upon altering the surface. After decorating the EG with NPs, the measurements were done again. [figure 24] shows a wide-scan of as-grown EG-SiC that was used in locating different binding energies (B.Es) to investigate further. The different intervals used for certain elements are presented in Appendix C where the denotations 1s and 2p refer to electrons occupying two separate energy levels or “shells”. To determine the different intervals to be examined, theoretical values for the elements were considered and then fast scans were performed to set the limits before scanning the intervals several times and render a mean.

The peak positions used to fit the raw intensities in order to identify chemical bindings for the elements investigated, were fitted with regards to [33]-[39] and also in fitting the peaks to give a sum as close to the measured intensity as possible. When fitting peak positions to an obtained spectrum, there is often broadenings or shifts that have several explanations. These shifts and can be correlated to chemical variance in the sample, solid state effects, contamination or impurities. [34]-[39] The broadenings are displayed by purple peaks in C1s and O1s spectra and in red in Si2p spectra.

The peak positions for the elements with specific chemical environment obtained correspond to precise values, but these values are as mentioned above fitted to [33]-[39] and to the raw intensities obtained. With each fitted peak, there is an exact value given.
4.3.1 TiO$_2$-particles

The C1s-spectra [figure 25] shows A) as-grown graphene and B) graphene with TiO$_2$-NPs. There are clear changes at the surface when decorating with particles at a high coverage. The SiC component of the C1s-peak found at 284.9 eV becomes somewhat less intense in relation to the carbon-peak at 283.8 eV. For the as-grown graphene [figure 25 A)] a pronounced shoulder at about 289 eV indicate oxidized species such as C-O, C=O and COOH and a shift in the shape of the curve takes place with less broadening, see [figure 25 B)], at the higher energy side. The Si2p-spectrum [figure 26] does not change much with decoration with particles, but a slight broadening is present in both cases on both sides of the theoretical values.

As-grown graphene showed small intensities of O1s, not shown here, that can be considered insignificant when comparing to the intensity with TiO$_2$. [figure 27]. The Ti2p-region had no signal present before deposition of particles. The result gave a split of $\Delta$eV = 5.6 eV and a background subtraction has been made, not affecting the relative intensities.
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For the results obtained the shifts in binding energy were correlated to theoretical values for Si2p in SiC-substrates. [38]

Figure 26 Spectra for Si2p in A) as-is graphene and B) graphene with TiO$_2$-particles

Figure 27 A) O1s-spectrum and B) Ti2p-spectrum for graphene with TiO$_2$-particles
4.3.2 Fe₃O₄-particles

![C1s spectra for as-is graphene A) and graphene with Fe₃O₄-particles B)](image)

C1s spectra for A) as-grown graphene and B) Fe₃O₄-coated graphene with a high coverage are shown, [figure 28]. The ratio between the carbon-peak and the SiC component of the C1s-peak changes, so that the SiC component is more visible for the Fe₃O₄ NP coated graphene. For the Si2p spectrum, [figure 29], there is no change in line shape visible after depositing the particles. As for the O1s-spectrum, [figure 30], a clear Fe₃O₄-peak is present, together with the two components with a higher binding energy. The split in the Fe2p-signal has a ΔeV of 13.6 eV together with small broadenings at the high binding energy side of each peak. This is in good agreement with earlier published XPS-data on Fe₃O₄.
Chapter 4. Results

Figure 29 Si2p for A) as-is graphene and B) with Fe$_3$O$_4$ particles

Figure 30 A) O1s and B) Fe2p for graphene with Fe$_3$O$_4$-particles
4.4 Gas sensing

4.4.1 TiO\textsubscript{2}-particles

Graphene samples sensitized with Fe\textsubscript{3}O\textsubscript{4} and TiO\textsubscript{2} NPs were investigated for gas detection. The resistance changes during exposure of A) CO, B) formaldehyde, C) benzene and D) NH\textsubscript{3} at three different temperatures, i.e. RT, 100°C and 150°C. The temperature giving the clearest response for all gases was 150 °C. Exposure to the gases to be detected rendered an increase in resistance over the surface, [figure 31], and the concentrations indicated in the figures are inserted just at the on-set time of exposure of the test gases. Examples from RT and 100 °C can be found in Appendix D.

For benzene the gas-mixer was able to give lower concentrations of gas, the lowest being 5 ppb, and so it was tested to find out detection limits.
Chapter 4. Results

[figure 32] shows resistance changes upon exposure to a set of low concentrations of benzene and 5 ppb is the lowest possible for the system available, still giving a clear response.

[Table 1] contains the calculated response and recovery times for the different gases to be detected. Three different values have been calculated relating to 30, 60 and 90 % of the response and recovery respectively. T90 refers to the time it takes for e.g. the resistance to reach 90 % of the full response and subsequently the time it takes for the resistance to reach 90 % of its recovered level.

<table>
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<th>Response time (s)</th>
<th>Recovery time (s)</th>
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<tr>
<td>TiO₂</td>
<td>CO</td>
<td>CH₂O</td>
</tr>
<tr>
<td>T30</td>
<td>138</td>
<td>133</td>
</tr>
<tr>
<td>T60</td>
<td>471</td>
<td>439</td>
</tr>
<tr>
<td>T90</td>
<td>1189</td>
<td>920</td>
</tr>
</tbody>
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Table 1 Calculated response- and recovery times for the different gases with the first exposure after stabilization in simulated air at 150 °C
4.4.2 Fe$_3$O$_4$-particles

When exposed to CO, formaldehyde, benzene and NH$_3$ the resistance decreased over the surface, [figure 33]. Clear responses to the test gases were observed at all of the tested operating temperatures, but there was a higher level of noise at RT. The concentrations indicated in the figures are inserted just at the on-set time of exposure of the test gases, where the resistance starts to drop.

[table 2] shows the calculated response times and recovery times for the different gases at 100 °C. The calculation was done as a mean value from the samples used and related to exposures where the time was 30 min for every step.
Chapter 4. Results

<table>
<thead>
<tr>
<th></th>
<th>Response time (s)</th>
<th>Recovery time (s)</th>
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</thead>
<tbody>
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<td>Fe$_3$O$_4$</td>
<td>CO</td>
<td>CH$_2$O</td>
</tr>
<tr>
<td>T30</td>
<td>138</td>
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<td>514</td>
</tr>
<tr>
<td>T90</td>
<td>1189</td>
<td>1217</td>
</tr>
</tbody>
</table>

Table 2 Calculated response- and recovery times for the four different analytes at 100 °C

As for the TiO$_2$-particles, lower concentrations of benzene were tested to see what could be detected. [figure 34] display the responses down to 5 ppb with clear changes in resistance for all concentrations, taking into account the heavy drift.

For all the gases tested the lowest concentration possible that the gas-mixing system in use could deliver correspond to 1 % in the total mixture.

Figure 34 Benzene response at lower concentrations and 150 °C
4.4.3 Sensitivities

In [figure 35] the sensitivities for varying concentrations of gases when exposed to the two NPs are shown. The sensor response has been calculated as the sum of the resistance in carrier gas and when exposed to the analyte divided by the resistance in the carrier gas. Worth noting is the concentration given in ppb for C), while for the others in ppm. The sensitivity calculated for NH₃, D), is further away from the true values than the others since the initial response never reached equilibrium and the following exposures to lower concentrations are affected by the level of saturation of the surfaces.

Figure 35 Sensitivities for the two different NPs towards different concentrations of A) CO, B) CH₂O, C) benzene and D) NH₃
4.5 Liquid measurements

4.5.1 Fe$_3$O$_4$-particles

The first liquid measurement was performed using 0.1 M PBS (pH 7.4) two times, 0.1 M HCl with 0.1 M NaCl (~pH 1) and then PBS again, respectively. As can be observed in [figure 36] the current over the surface descends for each measurement and particularly when measuring in HCl/NaCl and subsequently in PBS. A slight shift in the oxidation peak can also be observed, the potential reducing a little for each measurement.

A second measurement was performed for another sample with Fe$_3$O$_4$-NPs, treated exactly the same as the sample used in the first measurement. This time the CV displayed a very different behavior with two distinct peaks in HCl/NaCl-buffer. Subsequently in PBS the peaks disappeared. Worth noting is the significantly reduced current in [figure 37] compared to [figure 36]. For the second sample, a range of concentrations of H$_2$O$_2$ in PBS were also tested without any results indicating any kind of detection, results not shown here. The experiments where possible H$_2$O$_2$-detection was investigated, however, the sensing surfaces had already been used for a buffer-check and should only be considered as a screening and the information obtained on the behavior of the NP-decorated surfaces only as a pre-study.

![Figure 36 CV performed on EG-Fe$_3$O$_4$-NPs, low coverage, in the order 1) PBS 2) PBS 3) HCl/NaCl 4) PBS](image)

![Figure 37 CV performed on EG-Fe$_3$O$_4$-NPs, low coverage, in the order 1) HCl/NaCl and 2) PBS](image)
Chapter 5

Discussion and Conclusions

5.1 TiO$_2$-particles

The coverage shown in the AFM-results varies somewhat between samples, [figure 14], but they all have in common that the particles keep their spherical shape and have a flattened side towards the graphene as shown in phase-contrast images for low coverage NPs. The difference in coverage may have come from the graphene being damaged or the attempt to place a bias over the sample gave different results. Results from the second deposition, with shorter exposure time, indicate a lower coverage.

The Raman spectra in [figure 21] shows the same sample at two different points, one containing monolayer and the other bilayer graphene. What separate them are the shifts in the 2D-peak together with the ratio between the G- and the 2D-peaks. To the left of the G-peak there is a small cluster of peaks that are rendered from defects and the buffer layer, but the D-peak that corresponds to level of defects and structural deviances from monolayer graphene cannot be distinguished. After deposition of NPs, [figure 22], the D-peak is prominent, even to the G-peak, indicating on a much larger degree of defects in the graphene. Some intensity changes are to be expected when altering almost the entire surface with particles, but here the graphene has even been destroyed. When comparing to monodispersed particles [figure 23] one can also observe shifts of the G- and 2D-peaks and the much lower intensity of the 2D-peak, which indicates strain and also that the graphene is destroyed. The G-peak is blue-shifted which means the graphene is doped by holes. The results from Raman spectroscopy shows that it is very important what parameters are used when sputtering NPs on the surface. As concluded, the graphene is destroyed when a high coverage is gained and thereby its sensitivity as a transducer when constituting a sensor component is lost. When a low coverage of particles is achieved the graphene properties are intact and thus suitable for use as an ultra-sensitive transducer.

After deposition of particles the SiC-peak decreases in intensity relative to the carbon peak in XPS-results, [figure 25], which indicates that the substrate becomes somewhat shielded from photoemission. The SiC is still present, indicating that the particles do not cover the substrate completely, there are some areas available for photoemission. The high intensity purple peak is positioned at a binding energy related to C-OH groups, but also a broadening from the buffer layer where there are different forms of carbon’s. [38] The larger high-energy broadenings and
peaks can also be related to oxidized graphene where there are different forms of oxygen containing groups, i.e. carboxylic, hydroxyl, carbonyl and epoxide groups. [4]

The binding energy for Si2p [figure 26] does not change with NP-decoration which indicates that no chemical interactions occur between SiC and particles. This is an important support for the particles only interacting with graphene as a resistance measurement should only give results related to the graphene and the particles themselves. The broadenings on the slants indicate some alternative chemical interaction for Si, before and after NP-deposition, rendered from the buffer layer in combination with the substrate. [39]

A small indication of oxygen was present before decoration and can be related to either contamination by water or oxidation of the graphene, but probably a combination of the both. The ratio between intensities of O1s for the graphene before and after Np-deposition [figure 27] was very large, making the intensities from as-grown graphene negligible in the context.

In the Ti2p-region there was no detectable signal in as-is graphene, but a clear 2p-split after deposition. The $\Delta eV = 5.6$ eV corresponds well with literature and so does the smaller and broader characteristic of the $1/2$-electrons compared to the $3/2$-electrons. [33]

The particles should p-dope the graphene and give a response to CO, formaldehyde, benzene and NH$_3$ that result in a decrease in resistance. The inverted response [figure 31] in the results may be due to the particles p-doping the graphene extensively, moving the conduction band to the other side of the Fermi level where the surface can be regarded as a p-type conductor, contrary to as-grown EG-SiC which is n-type due to the interactions with the substrate. The gases giving the clearest response and detection at a low concentration were CH$_2$O and benzene, 50 ppb and 5 ppb respectively, whilst CO needed higher concentrations and did not render very clear responses. NH$_3$ sticks to the surface and gradually saturates it, making detection very difficult if there is not any NH$_3$ on the surface to start with since it never reaches any equilibrium in the time frame used for measurements in this study.

5.2 Fe$_3$O$_4$-particles

For a deposition time of 60 s the coverage is very high as shown in [figure 17] and there is evidence of multilayers in some areas. There is not a complete multilayer, but the layer beneath is clearly visible and there are small areas that have monolayer of particles. The phase-contrast, [figure 17 B)], shows that the particles have almost uniform adhesion and viscoelasticity characteristics, as they should when the image only show interactions between particles and not with graphene. The second deposition with lower exposure time rendered a significantly lower coverage, [figure 19], and phase contrast reveals NPs that are spherical.

Raman-spectroscopy results are unfortunately not included, since they were not performed within the time-frame of the study.
As shown in [figure 28] the C1s-spectrum does not change very much after deposition compared to before deposition, except for the ratios between the different components. The fact that the SiC-peak increases in relation to C-C should not occur and may be an indication of the measurements on as-is graphene being performed at an area containing very little monolayer. In these samples there were also indications of peaks and broadenings at the high energy side of the main peak that are ascribed to different forms of carbon interactions rendered by the buffer layer [38] and oxidized graphene. [4] The different forms of oxygen groups in oxidized graphene are presented in Appendix C. Similar to the TiO$_2$-particles, there was no indication of interactions with Si [figure 29].

For the Fe2p-region [figure 30] there was a weak signal prior to decoration that would not interfere with the strong signal after deposition. The metal oxide causes the baseline of the spectrum to ascend and broadenings can be seen after both of the split-peaks. The $\Delta$eV = 13.6 eV was the same as given in reference [33].

Here the response to gases [figure 33] render a decrease in resistance indicating that the surface can still be seen as a n-type conductor even though the particles should p-dope the graphene. These results point towards the graphene’s original conductivity, n-doped by the substrate, being conserved to a certain point when having a low coverage of NPs. The low coverage samples were very sensitive towards CH$_2$O and benzene, down to 50 ppb and 5 ppb respectively still giving a clear response. They were not as sensitive towards CO, being a smaller molecule with lower reactivity. Exposure to NH$_3$ rendered a large response and the surface seems to be more and more saturated by the gas sticking to it.

WHO has guidelines for exposure to a selected range of volatile gases, stating what concentrations are to be considered the maximum acceptable ones before being harmful and the time span it applies to. For CO it is 8.6 ppm at an 8 h exposure, formaldehyde 81 ppb for 30 min and for benzene there are none. The WHO is releasing new guidelines 2015 where the limits for formaldehyde will be lowered and benzene limits will be stated. The benzene limit is said to be set very low, around < 5 ppb. [40] This means that for the two NPs on graphene investigated the level of detection for formaldehyde has a high sensitivity, in relation to the exposure limit. For CO and benzene the detection needs to be lower than the ones presented in this study. However, the strong response to 5 ppb benzene indicates that the detection limit is in the lower ppb-range or possibly even in the high ppt-range.

The CV-measurements indicate that the Fe$_3$O$_4$-decorated surfaces are suitable for both gas-phase measurements as well as for liquid-phase measurements. They display interpretable forms of electrochemistry along the surface both when stop-flow was applied and in static conditions. When using HCl/NaCl-buffer, pH 1, the NPs seem to leave the surface either with the flow alone or by being chemically altered, dissolved in some way. The two different surfaces used did, however, display very different behavior regarding curve shapes and height in voltage. The samples had been treated in the same way in every aspect except for time stored before performing the measurements. They were stored in air-tight containers, but had been exposed
to air several times. Since the particles were spontaneously oxidized they might continue to oxidize when left for a longer time and form a more uniform oxide layer on the surface. The adhesion may also become different when further oxidizing the NPs. The attempt on H$_2$O$_2$-detection did not yield any results, but they were only performed on the second sample with very low voltage and when the potential had descended a lot already in PBS.

5.3 Summary

- The particles do seemingly not follow specific graphene conformations, monolayer or bilayer, but occur on both as well as on transition steps between mono- and bilayer areas. The same applies for steps in the SiC-substrate.
- When chemically interacting with carbons the NPs seemingly do not interact with SiC, but only with graphene.
- High coverage render entirely shielded or destroyed graphene for both NPs and also a much higher level of noise at gas-phase measurements.
- High coverage of TiO$_2$ seems to p-type dope the graphene to a larger extent than a high coverage of Fe$_3$O$_4$.
- Both NPs gave a very sensitive response towards formaldehyde and benzene, down to 50 ppb and 5 ppb respectively, where lower concentrations could not be tested due to experimental limitations.
- With increasing temperature, up to 150 °C, the level of noise reduced for both NPs, while an increase in coverage gave a higher level of noise, which is attributable to introduction of structural defects in the graphene.
- The TiO$_2$-NPs were more sensitive than Fe$_3$O$_4$ to CO and formaldehyde throughout all concentrations. For benzene the TiO$_2$ was less sensitive at 5 ppb than higher concentrations, but for NH$_3$ the reversed could be noted.
- Both NPs were sensitive towards NO$_2$, not presented in the results section.
- At a low pH, ~pH 1, the NPs dissociate or chemically dissolve and leave the surface.
- With time the Fe$_3$O$_4$-NPs seem to oxidize further, giving difficulties in measuring on them.
- The Fe$_3$O$_4$-NPs gave good indications on being suitable for both gas-phase and liquid-phase measurements.

5.4 Analysis and Process

In the beginning the samples at hand were surfaces with graphene on SiC that were investigated prior to any alterations at the same time as what particles to use was discussed. At first the idea of using Au-NPs was considered a good option in regards to the excessive studies done on Au, but after investigating the interactions with graphene the idea was discarded. The particles readily available for sputtering were the metal oxides used in the project and their interaction with graphene, from literature, seemed to be the ones suited for the purpose.
Chapter 5. Discussion and Conclusions

The surface characterization began with measurements on the surfaces before any alterations to see the condition of the graphene and in order to determine what properties might change with deposition of NPs. Raman spectroscopy and XPS were measured firstly and then the particles were sputtered onto the samples. When the particles had been deposited Raman and XPS were measured again together with AFM and surface potential mapping.

At this time the ambition was to perform a functionalization of the particles using a short alkane chain with COOH in one end and NH$_2$ in the other in order to use the technique described in 2.4 with pH as a regulator for the adsorption and then for further coupling to either an antibody or an enzyme. The number of samples and time remaining did however change the direction towards a gas-phase sensing together with an investigation on the effect of different pH on the particles in fluid that was considered more realistic, considering the available time and resources.

In order to perform sensing in gas-phase the surfaces with particles needed electrical contacts that were constituted by Au on an anchoring layer of Ti by thermal evaporation. In some cases the masking of the substrates was not fully covering and small spots of gold could be found on the surface. These small spots could be identified and used for possible shifts occurring regarding binding energies detected in XPS-measurements.

Unfortunately the etched graphene-chips, discussed in section 2.2.1, were not produced in time and therefore experiments using the biosensor platform from Graphensic AB for resistance measurements could not be performed. From the start the project was inclined towards using the biosensor platform for any liquid measurements. Giving the circumstances the planned liquid measurements were put on hold while investigating the possibilities for performing any within the timeframe.

Both iron oxide and titanium oxide were sputtered twice, on different substrates, with the ambition to lower coverage the second time. For iron oxide the coverage was very high after the first sputtering and the time was lowered from 60 s to 15 s, resulting in a lower coverage while maintaining size and conformation. The same was done for titanium oxide with new substrates, where the exposure time was also shortened to 15 s.

At this time it was decided to produce gas-sensing components using all the samples available and later use the leftovers for fluid measurements. Mounting of substrates onto sensor-headers was very time consuming as was the completion of an investigation for one sensor. The priority shifted towards gas-sensors. While fluid measurements were still considered important, it became a secondary priority.

5.4.1 Plasma sputtering of NPs
The AFM-images of the two different NPs [figure 14–19] clearly show differences in coverage and degree of agglomeration. The reason for this may be that Fe$_3$O$_4$ is actually sputtered as Fe-particles and later oxidize when exposed to air rendering a core-shell structure, while the TiO$_2$ is
5. Discussion and Conclusions

oxidized before encountering the substrate and does not oxidize further in air on its own, and the Fe-particles being magnetic. Pure metals should be affected more by the bias voltage applied over the substrate, because of their conducting characteristics compared to metal oxides. Another reason might be that the setup of the experiment was less successful, when sputtering TiO₂, in the contact between sample holder and substrate.

5.4.2 AFM
Measuring AFM in tapping mode gave important information on the coverage of NPs and their adsorption to the surface together with degree of agglomeration. The setup used required much handling of the substrates, in order to attach them to the sample holder using copper tape, and the significance of size differences in topography made the measurements more time consuming to deduct. For Fe₃O₄ the height differences confined measurements to smaller areas for field-of-view to render interpretable results.

Potential mapping of the samples did not render clear images of the surface potential. Here, again, the important information is the kind of interaction the NPs have with graphene and also the influence in relation to conductance.

5.4.3 Raman spectroscopy
Raman spectroscopy is a powerful tool to investigate characteristics of a surface and gives a lot of information. The interpretation of results, however, is rather complex and requires deep understanding of the interactions between photons, phonons, electrons and mechanical phenomena over the surface. Therefore the spectra obtained before and after an experiment will be compared and discussed from the changes that can be seen.

The Raman spectroscopy measurements were performed with a micro-Raman setup, giving a smaller spot of incident light on the surface. The results obtained gave vital information of defects introduced to the graphene samples when decorating them with NPs and also their condition regarding surface characteristics prior to altering them.

5.4.4 XPS
As mentioned there were indications of oxygen containing groups on the graphene from the beginning. This might give a hint as to what happens to this graphene when left for some time in air, in terms of oxidation or adsorption from not being stored under more inert conditions. Another suggestion can be the handling of it after production, when being packed and later handled in laboratory environments. Since XPS is performed under UHV-conditions the oxygen containing elements must be adsorbed or bound to the surface. Either way, the presence of oxygen gave very low intensities and was therefore ascribed to contamination. The wide-scan’s show more interfering signals at around 710 and 657 eV. These disturbances that should not be there are more difficult to explain, they could be due to contaminants on the sample or the sample holder, auger peaks from elements giving high intensities elsewhere or some part of it may be ascribed to the settings of the instrument used.
5.4.5 Gas sensing
The results from gas-measurements display some drift of the baseline in the graphs that either ascend or descend, depending on the sample used and also temperature, what mixes of gases the sample had been exposed to and exposure time. All samples had in common that both drift and noise were reduced when working at a higher temperature. In general, the gas-sensing was easily performed with an automated system mixing the gases and straightforward mounting of sensors in the flow cell and limitations came down to time required for stabilization, detection and recovery of sensors and also distortions in electrical equipment.

5.5 Gain to society
The study has suggested increased sensitivity and selectivity towards a number of gases and given a first surface characterization of the two NPs interaction with EG-SiC. The gas-detection proposes different level of doping with alternating coverage and also a difference between the two kinds of particles. The information obtained can be used as a starting point for further development of gas-sensors to be used in indoor environments for detection of toxic gases down to very low concentrations, with the ability to early detection of a hazardous environment if one stays in it for a longer period of time e.g. if working in it. Air quality monitoring and control is a fast growing field of research and innovation and efforts toward large scale sensor solutions for smart buildings with sensor controlled ventilation, along with air quality monitoring on city scale in new “smart cities” are currently being undertaken.

A better understanding of graphene’s interaction with different NPs give a foundation for developing even more sensitive gas-sensors as well as knowledge about their behavior in different environments and interactions with molecules. For a liquid application it is important to understand the characteristics of the NP-decorated EG in order to have an idea as to how they might behave when exposed to different chemical environments.

This project presents opportunities for environmental applications in e.g. air quality control and further provides a path towards biological recognitions that may be valuable in society for detecting dangerous gases or biological components that can help improve health for many people specifically or in general.

5.6 Ethical aspects
This project does not present any investigations that can be considered to raise ethical concerns, but the future use of the results might end up where ethical aspects are very important.

The fact that the project aims toward a further functionalization for biosensor applications give rise to the question of what purposes the final application is used in. If a sensor targets DNA-sequences there can be much information yielded depending on the sequence of choice. Should one be able to get information about one’s genomic predispositions for diseases and
Chapter 5. Discussion and Conclusions

who should obtain that information? Might it be used to determine relations between individuals? These DNA-information related questions have been stirring a lot of emotions and opinions for a long time and will not be discussed in this project, although they might be more relevant in the future uses.

Other aspects regard what a future biosensor application can be used to detect and what that information can say about an individual. Is there any part of that information that can be used by e.g. employers to determine whether a person is to be hired or by someone performing non-medical related investigations that might alter their view of a person socially and economically? The digging in these subjects is almost endless and should be considered when a decision is made as to what a biosensor is to detect.

This project as a whole has not been biased by any inputs or donations, but is an impartial investigation. The only thing altering the direction of the project has been time limitations and what samples were available.

5.7 Future prospects

This project has investigated the character of two different metal oxides on top of graphene on SiC as a first step towards a biosensor application. The results may be used as a base for further functionalization of the particles in order to sensitize them to target specific molecules.

If the process of depositing particles can be made reproducible enough to produce a specific result regarding patterning and coverage, the functionalization can be targeted to only attaching to them and not to graphene. A specific patterning of particles enables the conserving of graphene’s properties and thereby a new way to specifically functionalize a surface using biomolecules without being influenced by graphene thickness inhomogeneities of morphological differences on the graphene surface. The particles could also be deposited as nano-islands in order to increase their adhesion and as a way to define their distribution and uniformity.

The first investigation should be the reproducibility of depositing NPs on the graphene-channels of a chip and also characterize the interaction. Secondly the pH and buffer contents need to be investigated in order to find the optimum conditions for graphene with particles on this specific type of surface and later on for the sensitizing agents. The balance between what buffer molecules the NPs can handle, without aggregating or dissolving, and the desired properties for biological components may not be an easy task. One important aspect is the biosensor platform’s design which at this point is to work as an ISFET that requires a change in ion-concentration in the electrolyte or a change in charge distribution along the EDL.

The same can be said about any gaseous applications using the graphene with NPs, that if the process is made reproducible to a certain point it opens a number of possibilities. The detection of gases can be made very sensitive and when studying resistance changes one can separate
different gases by characterizing response curve features. The sensitivity of the sensors could allow for detection of very low concentrations of carcinogenic or toxic gases that can be harmful upon a long term exposure. This could be used in relevant environments such as laboratories or industries where chemicals are used that may produce gases by themselves or in a process. Another potentially bigger application would be in normal household environments and public buildings such as schools and hospitals. The WHO recently reported that about 99,000 premature deaths per year in Europe are attributed to household air pollution.
Acknowledgements

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[40] WHO guidelines for indoor air quality, “Chemical indoor air pollutants: selected pollutants”, 2010, [online]
Appendix A

AFM

Figure 38 A zoom-in on a piece of graphene with TiO$_2$-NPs, low coverage, with its phase-contrast mapping to

Figure 39 Two height images from different area of the same sample with high coverage TiO$_2$-NPs
Figure 40 Topography and surface potential for a high coverage sample with TiO$_2$-NPs

Figure 41 Monodispersed TiO$_2$ from the second sputtering with shorter exposure time and its potential
Appendix B

Raman

Figure 40 Reflectance mappings of pieces of EG-SIC containing ~50.8 % and ~77.2 % monolayer

Figure 41 Position, area, amplitude and FWHM for G- and 2D-peaks in a 4.2 µm² area
Appendix C

XPS

<table>
<thead>
<tr>
<th>Element</th>
<th>Lower limit (eV)</th>
<th>Higher limit (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Si2p</td>
<td>95</td>
<td>110</td>
</tr>
<tr>
<td>S2p</td>
<td>158</td>
<td>172</td>
</tr>
<tr>
<td>C1s</td>
<td>278</td>
<td>295</td>
</tr>
<tr>
<td>N1s</td>
<td>395</td>
<td>407</td>
</tr>
<tr>
<td>Ti2p</td>
<td>445</td>
<td>475</td>
</tr>
<tr>
<td>O1s</td>
<td>525</td>
<td>541</td>
</tr>
<tr>
<td>Fe2p</td>
<td>700</td>
<td>740</td>
</tr>
</tbody>
</table>

*Table 3* Intervals chosen for XPS-scans investigating presence of different elements

*Figure 42* The different forms of oxygen containing groups in oxidized graphene. A) carboxyl, B) epoxide, C) carbonyl and D) hydroxyl groups. The carbons showed are, or rather were, part of the graphene and are bound to other carbons
Appendix D

Resistance

<table>
<thead>
<tr>
<th>Analyte</th>
<th>[bottle]</th>
<th>Percentage</th>
<th>[sensor]</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO</td>
<td>2500 ppm</td>
<td>20</td>
<td>500 ppm</td>
</tr>
<tr>
<td>CH₂O</td>
<td>5 ppm</td>
<td>20</td>
<td>1 ppm</td>
</tr>
<tr>
<td>Benzene</td>
<td>500 ppb</td>
<td>50</td>
<td>250 ppb</td>
</tr>
<tr>
<td>NH₃</td>
<td>500 ppm</td>
<td>20</td>
<td>100 ppm</td>
</tr>
<tr>
<td>O₂</td>
<td>100 %</td>
<td>20</td>
<td>varying</td>
</tr>
<tr>
<td>N₂</td>
<td>100 %</td>
<td>varying</td>
<td>-</td>
</tr>
</tbody>
</table>

*Table 4* The different gaseous analytes and their concentrations. The concentration in bottle is diluted by N₂.

*Figure 43* A) benzene detection in RT and B) benzene detection in 100 °C for a sample decorated with low coverage TiO₂-NPs.

*Figure 44* Formaldehyde on Fe₃O₄-NPs at A) 100 °C and B) 150 °C.
Figure 45 Responses to CO at A) 100 °C and B) 150 °C over Fe₃O₄-NPs

Figure 46 Detection of NH₃ over TiO₂-particles at A) RT and B) 100 °C