Electrochemistry of Amorphous Carbon Based Materials

Development of an electrochemical sensor for the detection of dopamine

Tommi Palomäki
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A doctoral dissertation completed for the degree of Doctor of Science (Technology) to be defended, with the permission of the Aalto University School of Electrical Engineering, at a public examination held at the lecture hall AS2 of the school on June 7th 2019 at 12:00.
Abstract

The prevalence of neurodegenerative disorders, such as Parkinson’s and Alzheimer’s diseases, will continue to increase as the population ages. This is expected to impose an increasing social and economic burden on societies.

Parkinson’s disease is characterized mainly by motor symptoms that are caused by the progressive degeneration of neurons that synthesize and release the neurotransmitter dopamine (DA). Current treatment methods, such as levodopa administration and deep brain stimulation, alleviate the symptoms but do not alter disease progression. Therefore, there is an evident need to understand the causes and progression of the disease and to develop new treatment methods. Since the abnormal neurotransmission of DA is linked to the onset of symptoms and progression of Parkinson’s disease, the real-time in vivo monitoring of DA levels could provide new information to understand the disease.

In this thesis, we have investigated the use of amorphous carbon (a-C) materials as electrochemical sensors for the sensitive and selective measurement of DA. There have been relatively few reports on the electrochemical properties of a-C, although it offers significant advantages, such as a wide potential window and a low background current, over commonly used electrode materials for sensor applications. We have studied the physical, chemical and electrochemical properties of different a-C materials using several bulk and surface characterization methods, computational simulations and electrochemical techniques with outer and inner sphere redox probes.

We have shown that deposition parameters can be varied to obtain a-C films with markedly different electrochemistry ranging from graphite-like, highly sp²-bonded carbon to diamond-like, highly sp³-bonded carbon. The sp²/sp³ bonding ratio will define the physical properties, in particular the electronic configuration that strongly correlates with the electrochemistry of a-C materials.

We have demonstrated the applicability of highly sp³-bonded tetrahedral amorphous carbon (ta-C) in electroanalytical applications by detecting physiologically relevant DA concentrations (40-85 nM) using cyclic voltammetry. The modification of ta-C with carbon nanotubes conferred the electrode the necessary selectivity to detect DA in the presence of its major interferents, ascorbic acid and uric acid, at physiological concentrations. Both the ta-C and the modified electrodes showed good biocompatibility, further emphasizing their potential as in vivo sensor materials.
Tekijä
Tommi Palomäki

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Amorfisten hiilimateriaalien sähkökemialliset ominaisuudet

Julkaisija
Sähköteknikan korkeakoulu

Yksikkö
Elektri- ja Automatointi

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Esseeväitöskirja

Tiivistelmä


Tulokset osoittavat, että amorfisen hiilen sähkökemiallisia ominaisuuksia voidaan muokata merkittävästi säätämällä depotielementtiarvoja pinnoituksen aikana. Ominaisuudet voivat vaihdella grafiittisen hiilien ja timantinkaltaisen hiilen välillä riippuen hiilimutosten sp²- ja sp³-sidoksen määrästä. Sidoksien sp²/sp³-suhte määrää amorfisen hiilen fysiikaaliset ominaisuudet ja erityisesti elektronikonfiguraation, joka korreloii voimakkaasti sähkökemiallisten ominaisuuksien kanssa.


Avainsanat
sähkökemia, amorfinen hiili, syklinen voltametri, sähkökemia, impedanssispektroskoopia, dopamiini, hermovaihtaja

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It is an honor to have Prof. Frank Marken as the opponent at the thesis defense. I would also like to acknowledge Prof. Jingdong Zhang and Prof. Jill Venton for the useful feedback during the pre-examination of this work.

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Finally, I would like to express my deepest appreciation to my wife Eunyoung for her love, trust and understanding, and to my sons Eliel and Mio for making us smile every day and reminding us of all the great things in life. This work is dedicated to you.

Otaniemi, May 12th 2019

Tommi Palomäki
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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AA</td>
<td>ascorbic acid</td>
</tr>
<tr>
<td>a-C</td>
<td>amorphous carbon</td>
</tr>
<tr>
<td>AFM</td>
<td>atomic force microscopy</td>
</tr>
<tr>
<td>APT</td>
<td>anodic pretreatment</td>
</tr>
<tr>
<td>BDD</td>
<td>boron doped diamond</td>
</tr>
<tr>
<td>CA</td>
<td>catechol</td>
</tr>
<tr>
<td>CFUBMS</td>
<td>closed-field unbalanced magnetron sputtering</td>
</tr>
<tr>
<td>CNF</td>
<td>carbon nanofiber</td>
</tr>
<tr>
<td>CNT</td>
<td>carbon nanotube</td>
</tr>
<tr>
<td>CP</td>
<td>carbon paste</td>
</tr>
<tr>
<td>CPE</td>
<td>constant phase element</td>
</tr>
<tr>
<td>CPT</td>
<td>cathodic pretreatment</td>
</tr>
<tr>
<td>CV</td>
<td>cyclic voltammetry</td>
</tr>
<tr>
<td>CVD</td>
<td>chemical vapour deposition</td>
</tr>
<tr>
<td>DA</td>
<td>dopamine</td>
</tr>
<tr>
<td>DAC</td>
<td>dopaminechrome</td>
</tr>
<tr>
<td>DAQ</td>
<td>dopamine quinone</td>
</tr>
<tr>
<td>DHBA</td>
<td>3,4-dihydroxybenzylamine</td>
</tr>
<tr>
<td>DLC</td>
<td>diamond-like carbon</td>
</tr>
<tr>
<td>DPV</td>
<td>differential pulse voltammetry</td>
</tr>
<tr>
<td>DOPAC</td>
<td>3,4-dihydroxyphenylacetic acid</td>
</tr>
<tr>
<td>DOS</td>
<td>density of states</td>
</tr>
<tr>
<td>ECF</td>
<td>extracellular fluid</td>
</tr>
<tr>
<td>EELS</td>
<td>electron energy loss spectroscopy</td>
</tr>
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</table>
EIS  electrochemical impedance spectroscopy
FcMeOH0/+  ferrocenemethanol redox couple
Fe2+/3+  iron redox couple
Fe(CN)64-/3-  ferrocyanide redox couple
fMRI  functional magnetic resonance imaging
FSACV  fast scan adsorption-controlled voltammetry
FSCV  fast scan cyclic voltammetry
GC  glassy carbon
GIXRD  grazing incidence X-ray diffraction
HIM  helium ion microscopy
HOPG  highly oriented pyrolytic graphite
HPLC  high-performance liquid chromatography
HVA  homovanillic acid (2-(4-hydroxy-3-methoxyphenol)acetic acid)
IrCl63-/2-  hexachloroiridate(IV) redox couple
ISR  inner sphere redox (probe)
LDAC  leucodopaminechrome
MC  methylcatechol
mNSC  mouse neural stem cell
MWCNT  multi-walled carbon nanotube
NA  nucleus accumbens
ND  nanodiamond
OSR  outer sphere redox (probe)
PET  positron emission tomography
PD  Parkinson’s disease
PVD  physical vapour deposition
Ru(NH3)62+/3+  hexaammineruthenium(III) redox couple
SCLC  space charge limited current
SEM  scanning electron microscopy
SPECT  single-photon emission computerized tomography
SSRM  spreading sheet resistance measurement
STS  scanning tunneling spectroscopy
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>SWV</td>
<td>square-wave voltammetry</td>
</tr>
<tr>
<td>ta-C</td>
<td>tetrahedral amorphous carbon</td>
</tr>
<tr>
<td>TEM</td>
<td>transmission electron microscopy</td>
</tr>
<tr>
<td>ToF-ERDA</td>
<td>time-of-flight elastic recoil detection analysis</td>
</tr>
<tr>
<td>UA</td>
<td>uric acid</td>
</tr>
<tr>
<td>XAS</td>
<td>X-ray absorption spectroscopy</td>
</tr>
<tr>
<td>XPS</td>
<td>X-ray photoelectron spectroscopy</td>
</tr>
<tr>
<td>XRR</td>
<td>X-ray reflectivity</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>$[^{123}]\text{IBZM}$</td>
<td>$[^{123}]$-labelled iodobenzamide</td>
</tr>
</tbody>
</table>
**List of Symbols**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A$</td>
<td>electrode area</td>
<td>cm$^2$</td>
</tr>
<tr>
<td>$C_{dl}$</td>
<td>double-layer capacitance</td>
<td>$\mu$F/cm$^2$</td>
</tr>
<tr>
<td>$D$</td>
<td>diffusion constant</td>
<td>cm$^2$/s</td>
</tr>
<tr>
<td>$E_p$</td>
<td>peak potential</td>
<td>V</td>
</tr>
<tr>
<td>$F$</td>
<td>Faraday constant</td>
<td>C</td>
</tr>
<tr>
<td>$i$</td>
<td>current</td>
<td>$\mu$A</td>
</tr>
<tr>
<td>$k^o$</td>
<td>standard reaction rate constant</td>
<td>cm/s</td>
</tr>
<tr>
<td>$m_T$</td>
<td>mass transfer coefficient</td>
<td>cm/s</td>
</tr>
<tr>
<td>$n$</td>
<td>number of electrons</td>
<td></td>
</tr>
<tr>
<td>$R$</td>
<td>gas constant</td>
<td>J mol$^{-1}$ K$^{-1}$</td>
</tr>
<tr>
<td>$R_{ct}$</td>
<td>charge transfer resistance</td>
<td>$\Omega$</td>
</tr>
<tr>
<td>$R_{transfer}$</td>
<td>charge transfer resistance</td>
<td>$\Omega$</td>
</tr>
<tr>
<td>$R_{transport}$</td>
<td>charge transport resistance</td>
<td>$\Omega$</td>
</tr>
<tr>
<td>$T$</td>
<td>temperature</td>
<td>K</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>transfer coefficient (dimensionless)</td>
<td></td>
</tr>
<tr>
<td>$\delta$</td>
<td>diffusion layer thickness</td>
<td>m</td>
</tr>
<tr>
<td>$\Lambda$</td>
<td>kinetic parameter (dimensionless)</td>
<td></td>
</tr>
<tr>
<td>$\upsilon$</td>
<td>scan rate</td>
<td>mV/s</td>
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<tr>
<td>$\psi$</td>
<td>kinetic parameter (dimensionless)</td>
<td></td>
</tr>
<tr>
<td>$\omega$</td>
<td>angular velocity (dimensionless)</td>
<td>rad/s</td>
</tr>
</tbody>
</table>
List of Publications

This doctoral dissertation consists of a summary and the following publications, which are referred to in the text by their roman numerals.


Author’s Contribution

**Publication 1:** Effect of power density on the electrochemical properties of undoped amorphous carbon (a-C) thin films

The first author defined the research plan and deposited the a-C films under the supervision of Prof. J. G. Han at the Center for Advanced Plasma Surface Technology at Sungkyunkwan University. M. A. Caro carried out the computational work and wrote the part in the manuscript about these results. N. Wester was responsible for Raman measurements, S. Sainio and L. Johansson for XPS measurements and analysis, J. Etula for XRR and GIXRD measurements. The first author conducted the CV, EIS, AFM and SSRM measurements and wrote the manuscript.

**Publication 2:** Characterization and Electrochemical Properties of Oxygenated Amorphous Carbon (a-C) Films

The first author defined the research plan and deposited the a-C:O films under the supervision of Prof. J. G. Han at the Center for Advanced Plasma Surface Technology at Sungkyunkwan University. The first author carried out the CV, EIS, AFM and SSRM measurements as well as interpreted the characterization results. N. Wester carried out Raman spectroscopy, L. Johansson XPS analysis, M. Laitinen and prof. T. Sajavaara ToF-ERDA measurements, K. Arstila took HIM images and H. Jiang TEM images. The first author wrote the manuscript.

**Publication 3:** Electron transport determines the electrochemical properties of tetrahedral amorphous carbon (ta-C) thin films

The first author outlined the research plan with N. Wester and carried out the CV, EIS and SSRM measurements. N. Wester deposited the ta-C films. M. A. Caro conducted the computational simulations and wrote the part of the manuscript about them. S. Sainio analyzed the ta-C films with XAS and V. Protopopova did the STS measurements. The first author wrote the manuscript.

**Publication 4:** Electrochemical reactions of catechol, methylcatechol and dopamine at tetrahedral amorphous carbon (ta-C) thin film electrodes

The first author outlined the research plan and analyzed the results together with Professors V. Climent, J. M. Feliu and T. Laurila. The author carried out the electrochemical experiments with the help of S. Chumillas at the University of Alicante. V. Protopopova and Prof. J. Koskinen provided the ta-C samples and M. Kauppila did the XPS measurements. The first author wrote the manuscript.
Publication 5: Unmodified and multi-walled carbon nanotube modified tetrahedral amorphous carbon (ta-C) films as in vivo sensor materials for sensitive and selective detection of dopamine

The first author outlined the research plan and carried out the electrochemical measurements. E. Peltola did the cell culture experiments and S. Sainio carried out XPS analysis. N. Wester provided the ta-C samples and carried out Raman spectroscopy. O. Pitkänen and Prof. K. Kordas deposited the MWCNT on ta-C. The first author analyzed the results and wrote the manuscript.

All the work was done under the guidance and supervision of Prof. Tomi Laurila.
List of Other Publications

The doctoral candidate has also contributed to the following publications, which are related to the topic of this dissertation but not part of it.


1. Introduction

1.1 Background

In its projected estimate for the period between 2005 and 2030, the World Health Organization (WHO) has estimated that the global burden of disease caused by neurodegenerative diseases, such as Parkinson’s disease (PD), Alzheimer’s disease and Huntington’s disease, will increase globally [1]. WHO estimates that PD currently affects 1% of the population over the age of 65 and its prevalence will increase as the population ages. Therefore, PD and other neurodegenerative disorders are expected to impose an increasing social and economic burden on societies. [1, 2]

PD is a chronic, slowly progressing neurodegenerative disorder characterized mainly by motor symptoms but as the disease progresses, non-motor symptoms such as psychiatric and cognitive impairment appear, contributing significantly to worsening quality of life and loss of autonomy. [1, 3] The cause of PD remains unknown but it is believed that both genetic and environmental factors lead to the progressive degeneration of dopaminergic neurons i.e. loss of neurons that synthesize and release dopamine (DA). [3-5] At present, there is no cure for PD and pharmacological treatment is symptomatic. In the early stages of the disease, treatments based on levodopa and DA agonists are usually successful in inhibiting the motor complications but long-term administration of levodopa causes fluctuations in motor functions. [1, 3] In patients with severe motor symptoms, deep brain stimulation (DBS) can be used as a form of treatment. DBS involves the implantation of a neurostimulator in the brain that sends continuous high-frequency electrical impulses through electrodes. DBS treatment significantly improves motor functions but there is no evidence that it alters disease progression. [6-8]

There is an evident need to understand the underlying causes and progression of PD, to develop therapeutic strategies to manage the disease, and to follow the efficacy of treatment methods to provide patient-centered care. Since the abnormal neurotransmission of DA is linked to PD, the real-time monitoring of DA levels in the brain would provide invaluable information on the disease. The main challenges associated with in vivo monitoring of DA are i) the high sensitivity required to detect nanomolar concentrations in the brain, ii) the high temporal resolution needed to observe fast DA transients that occur on sub-second time scale, and iii) sufficient chemical selectivity to distinguish between DA and other neurotransmitters present at much higher concentrations that interfere with its detection.

The main methods for detecting DA levels in vivo are microdialysis, imaging techniques and electrochemical methods such as fast scan cyclic voltammetry (FSCV) and amperometry. The advantage of electrochemical methods lies in the high temporal resolution, simple instrumentation and lower cost compared to the other methods [9].
The sensitivity achieved with FSCV and amperometry is in the physiologically relevant range and allows the in vivo detection of DA [9]. The main challenge with electrochemical methods is the chemical selectivity, which can be addressed by the choice of electrode material and the use of surface modification methods to block certain charged molecules or shift their oxidation potential. Furthermore, the electrode material needs to be biocompatible to avoid adverse effects on health and should be to some degree resistant to biofouling i.e. adhesion of proteins and other molecules that attenuate the current response of DA over time.

In this thesis, we have explored the use of amorphous carbon (a-C) and tetrahedral amorphous carbon (ta-C) for the electrochemical detection of DA. Carbon allotropes have been used extensively in electroanalytical applications and present a wide range of attractive physicochemical properties ranging from highly sp²-bonded graphitic materials such as graphene, carbon nanotubes (CNTs) and highly oriented pyrolytic graphite (HOPG), to highly sp³-bonded diamond-like carbon such as boron-doped diamond (BDD) and ta-C. [10] The advantage of carbon allotropes compared to other widely used electrode materials such as platinum and gold is their relatively wide potential window, electrocatalytic activity for a range of redox reactions, their low cost and ample availability [10]. The physical properties of amorphous carbon vary significantly depending on the fraction of sp² and sp³-bonded carbon atoms but their structure lacks long-range order compared to other carbon allotropes. [11, 12] Amorphous carbon materials have been relatively unstudied in electrochemical applications even if they present several attractive properties such as a wide potential window, low background current, chemical stability and good mechanical properties. [13-15] ta-C also has the advantage of being biocompatible [16] and resisting bacterial adhesion [17], which is important in in vivo sensor applications.

We foresee that in the future, a miniaturized carbon-based sensor could be implanted for prolonged DA monitoring in vivo and it may be possible to couple it with existing DBS neurostimulators to provide real-time feedback about physiological changes in PD patients.

1.2 Objectives and Overview of Publications

We have investigated a-C and ta-C as potential sensor materials for the electrochemical detection of DA. The two main objectives in this thesis were to

1. Deepen the knowledge on the electrochemical properties of a-C and ta-C, and
2. Fabricate a sensitive and selective amorphous carbon based sensor for the detection of DA

To understand the electrochemical properties of amorphous carbon materials, we deposited numerous a-C and ta-C thin films with varying deposition parameters and different surface modifications. We characterized in detail their physical, chemical and electrochemical properties using several characterization methods and redox molecules. The most promising materials were evaluated for their performance in the detection of DA in the presence of common interferents with CV. Table 1 summarizes the different electrode materials, characterization methods and redox molecules used in publications I-V.

In publication I, we investigated the effect of power density on the electrochemistry of a-C films prepared by closed-field unbalanced magnetron sputtering (CFUBMS). Recent reports suggest that nanocrystalline structures may be obtained with high-density plasma deposition
techniques such as CFUBMS, and that the nanocrystallinity may improve electron transfer kinetics. We characterized the films and computationally simulated a-C structures to understand their physicochemical properties that were then correlated with the electrochemical results. Although the films were not nanocrystalline, we observed a correlation between power density, bonding structure and oxidation potential of DA and AA.

In publication II, we deposited a series of a-C films with an increasing amount of incorporated oxygen to see its effect on the oxidation of DA and AA. Our hypothesis was that oxygen surface functional groups would strongly affect the redox reactions of these two inner sphere redox molecules but on the other hand might also affect the electron transport through the films. The films were thoroughly characterized to obtain information on the effect of oxygen on the structure of a-C and the physicochemical properties of the films. It was found that incorporated oxygen strongly affected the electrical properties of the films and the oxidation of both DA and AA.

Table 1. Summary of electrode materials, characterization methods and redox molecules used in the publications comprising this thesis.

<table>
<thead>
<tr>
<th>Publication</th>
<th>Electrode material</th>
<th>Characterization methods</th>
<th>Analytes/redox molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>a-C</td>
<td>GIXRD, XRR, Raman, AFM, SSRM, XPS, CV, EIS, computational simulations</td>
<td>Ru(NH₃)₆²⁺/³⁺, FcMeOH⁰/¹⁺, IrCl₆²⁻/³⁻, Fe²⁺/³⁺, DA, AA</td>
</tr>
<tr>
<td>II</td>
<td>a-C:O</td>
<td>TEM, HIM, AFM, SSRM, Raman, XPS, ToF-ERDA, EIS, CV</td>
<td>Ru(NH₃)₆²⁺/³⁺, Fe(CN)₆⁴⁻/³⁻, DA, AA</td>
</tr>
<tr>
<td>III</td>
<td>ta-C</td>
<td>Raman, TEM, SSRM, STS, XAS, CV, EIS, computational simulations</td>
<td>Ru(NH₃)₆²⁺/³⁺, FcMeOH⁰/¹⁺</td>
</tr>
<tr>
<td>IV</td>
<td>ta-C, GC</td>
<td>XPS, CV</td>
<td>DA, CA, MC</td>
</tr>
<tr>
<td>V</td>
<td>ta-C+</td>
<td>SEM, TEM, XPS, Raman, CV, computational simulations</td>
<td>DA, AA, UA</td>
</tr>
<tr>
<td></td>
<td>CNTs</td>
<td>biocompatibility (mNSC)</td>
<td></td>
</tr>
</tbody>
</table>

In publication III, we studied the effect of film thickness in the range of 7 to 100 nm on the electrochemical properties of undoped ta-C. We used CV and EIS with outer sphere redox probes to demonstrate the strong correlation between film thickness and electron transfer rate. Computational methods were also used to rationalize the results. The electrochemical properties of undoped ta-C films have been studied very little in literature and the role of film thickness has generally not been considered.

In publication IV, the electrochemical behaviour of DA was studied at ta-C electrodes and compared to that of catechol and methylcatechol. GC was also used, because it has been studied extensively as an electrode materials and represents a well-known benchmark material for comparison with ta-C. The scope of the publication was to understand the electrochemical reactions of DA on ta-C and observe to what extent it is passivated.

In publication V, ta-C was investigated as a potential in vivo sensor material to detect DA in the presence of ascorbic acid and uric acid, which are the main interferents in the brain. The
performance of ta-C was compared to ta-C modified with CNTs, since there are many reports on the superior properties of carbon nanotubes in electroanalytical applications. The biocompatibility of ta-C and CNT-modified ta-C was assessed with cell culture of mouse neural stem cells (mNSCs). Both the bare and modified ta-C electrodes could detect DA in the nanomolar range needed for \textit{in vivo} sensors, but major differences were observed in the selectivity of the materials.
2. Characterization Methods

This chapter introduces the characterization methods that were used in publications I-V and summarized in Table 1. The focus is on cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS), as they were the main electrochemical methods used in all the publications. We briefly introduce their theory and discuss their use in the characterization of amorphous carbon thin films. We also talk about the outer and inner sphere redox probes that were used in the electrochemical characterization and their interaction with the electrode surface. Finally, we briefly introduce the wide range of physical and chemical characterization methods that were used to investigate the electrode materials. A detailed characterization of the chemical and physical properties of the carbon materials is necessary in order to make correlations with their electrochemical properties.

2.1 Cyclic Voltammetry

Cyclic voltammetry is a fast, versatile and relatively simple method for investigating electrochemical reactions and electrode materials. In publication IV, we used CV to investigate the electrochemical behaviour of DA at ta-C electrodes and we were able to observe part of its oxidation pathway. In publications I, II and III, we used CV to study the electrochemical properties of amorphous carbon films with the use of several redox probes. CV can also be used to test the performance of potential sensor materials by determining their sensitivity and selectivity toward specific analytes such as in publication V, where we used this method to study the potential of ta-C+CNT electrodes as in vivo DA sensors. The fast temporal resolution this method offers makes it the most frequently used technique for the investigation of rapid neurotransmission events, although other methods such as DPV offer higher selectivity [9].

In the CV experiment, the potential of the working electrode is scanned linearly between an initial potential and a switching potential. The direction of the linear scan is reversed at the switching potential and this potential range is then scanned for several cycles. The scan rate \( \nu \) indicates the rate at which the potential is changed. The potential limits are chosen so that the oxidation and reduction of the desired electroactive species lie within this potential window. The potential window is also limited by the oxygen and hydrogen evolution reactions in aqueous solutions. [18]

The forward or anodic scan causes the electroactive species to oxidize, while the reverse or cathodic scan causes it to reduce back to the initial state. Both reactions are observed as current peaks in the cyclic voltammograms, since electrochemical reactions involve the transfer of electrons. An important parameter is the peak potential separation, \( \Delta E_p \), between the oxidation and reduction peak potentials. It is a measure of the reversibility of the electrochemical reactions. For a reversible reaction, \( \Delta E_p \) will be equal to \( 59/n \) mV at room temperature, where \( n \) is the number of transferred electrons according to the Nernst equation [18, 19].
The ratio of peak currents $i_{\text{pa}} / i_{\text{pc}}$ is a measure of the stability of the chemical species involved in the redox reaction. A value of 1 indicates a reversible reaction, where all the oxidized species are reduced, whereas any deviation from this value indicates complications in the oxidation process or the existence of coupled homogeneous chemical reactions as will be seen in the case of DA in publication IV.

**Figure 1.** A typical cyclic voltammogram for a reversible reaction showing the parameters of interest.

The current response at the electrode results from faradaic and non-faradaic processes. The faradaic processes involve electron transfer across the electrode-solution interface and arise from the redox reactions of the analyte in solution. The faradaic current obeys Faraday’s law i.e. it is proportional to the amount of electrolyzed solution species. Non-faradaic processes are caused by changes in the electrical double layer, adsorption/desorption processes, rearrangement of dipole moments etc. and do not involve electron transfer over the electrode-solution interface.

The electrical double layer acts as a capacitor and when the potential is scanned in the CV experiments, it will cause a charging current $i = AC_{\text{dl}} \text{v}$ to always flow, which is proportional to the electrode area ($A$), double layer capacitance ($C_{\text{dl}}$) and scan rate. Because of this, the faradaic current must always be measured from the baseline of the charging current but it may be quite difficult especially for the reduction peak as seen in Figure 1. Therefore, the measurement of peak currents in CV is imprecise and it is not an ideal method for quantitative evaluation of properties derived from peak heights such as the concentration of analytes. It is better suited for qualitative analysis of electrochemical systems. [18]

**Butler-Volmer kinetics**

The Butler-Volmer kinetic model is central in the study of electrode kinetics and relates the electrode current to the electrode potential:

$$i = i_c - i_a = nF A k^0 \left[ c_{\text{ox}} \exp \left( \frac{-\alpha F (E - E^\theta)}{RT} \right) - c_{\text{red}} \exp \left( \frac{1 - \alpha) F (E - E^\theta)}{RT} \right) \right]$$
where \( n \) is the number of electrons involved in the reaction, \( F \) is the Faraday constant (9.64853 \( \times 10^4 \) C), \( k^0 \) is the standard reaction rate constant, \( c \) is the concentration of the oxidized and reduced forms of the redox species, \( \alpha \) is the transfer coefficient, \( E \) is the potential of the electrode, \( E^0_f \) is the formal potential of the redox species, \( R \) is the gas constant (8.31447 J mol\(^{-1}\) K\(^{-1}\)) and \( T \) is the temperature. The subscripts \( a \) and \( c \) denote anodic and cathodic, respectively. We can see from the equation that the currents depend strongly on the applied voltage and the total current is equal to the sum of the cathodic and anodic currents. The limiting cases of the Butler-Volmer expression give the Nernst equation at equilibrium and the Tafel equations at high overpotentials. At equilibrium, the net current is zero, because the anodic and cathodic currents are of equal magnitude. The electrode will adopt a potential based on the bulk concentrations of the oxidized and reduced species that obey the Nernst Equation:

\[
E = E^0_f + \frac{RT}{F} \ln \left( \frac{c_{\text{ox}}}{c_{\text{red}}} \right)
\]

**Reversibility of the electrochemical reaction**

The reversibility of the electrochemical reaction is determined by the relative speeds of the electron transfer kinetics (expressed by \( k^0 \)) and mass transport (expressed by the mass transfer coefficient \( m_T \)). Both quantities have the same units (cm/s) and can thus be directly compared with each other. If the electron transfer kinetics are so fast that the net rate of reaction is totally controlled by the rate at which the electroactive species is transported to the electrode surface, the electrochemical reaction is reversible and \( k^0 \gg m_T \). On the other hand, if the net rate of reaction is totally controlled by the electron transfer kinetics, the reaction is irreversible and \( k^0 \ll m_T \). [19]

Matsuda and Ayabe [20] proposed the parameter \( \Lambda \) for assessing the reversibility of an electrochemical reaction:

\[
\Lambda = \frac{k^0}{m_T} = \frac{k^0}{\left( \frac{FDv}{RT} \right)^{1/2}}
\]

where \( D \) is the diffusion constant of the oxidizing species. They suggested the following limits for the reversible, quasi-reversible and irreversible cases (where \( T = 298 \) K, \( n = 1 \) and \( \alpha \sim 0.5 \) have been used to obtain the values relating to \( \Lambda \)):

- **Reversible:** \( \Lambda \geq 15 \)  \( k^0 \geq 0.3 (nv)^{1/2} \) cm s\(^{-1} \)
- **Quasi-reversible:** \( 15 > \Lambda > 10^{-3} \)  \( 0.3 \times v^{1/2} > k^0 > 2 \times 10^{-5} (nv)^{1/2} \) cm s\(^{-1} \)
- **Irreversible:** \( \Lambda \leq 10^{-3} \)  \( k^0 \leq 2 \times 10^{-5} (nv)^{1/2} \) cm s\(^{-1} \)

The conditions show that the observed reversibility depends in each case on the scan rate and that any reaction can be made irreversible by increasing the scan rate sufficiently. This is because at sufficiently fast scan rates the diffusion layer becomes thin enough that mass transport becomes faster than the electron transfer kinetics [19, 20]
Determining $k^0$ – the Nicholson method

The standard reaction rate constant is a measure of the kinetic facility of a redox couple. A system with a large $k^0$ will achieve equilibrium faster than a system with a small $k^0$. The value of $k^0$ is specific for a certain electrochemical system including the electrode material at which the reaction occurs. Thus, it is an important electrochemical parameter for directly comparing different electrode materials.

Nicholson [21] proposed a method for deriving the value of $k^0$ in quasi-reversible systems by observing the variation of $\Delta E_p$ with scan rate. They introduced the kinetic parameter $\psi$ that is related to the equation proposed by Matsuda and Ayabe by

$$\psi = \Delta E_p^{1/2} = \frac{k^0}{\left(\frac{\pi F D u}{R T}\right)^{1/2}}$$

The value of $\psi$ can be determined by tabulated values of $\Delta E_p$ with the assumption that $T = 298$ K and $\alpha = 0.5$. The uncompensated resistance must be sufficiently small that the resulting voltage drop is negligible compared to $\Delta E_p$.

2.2 Electrochemical Impedance Spectroscopy

Electrochemical impedance spectroscopy (EIS) has been used extensively in corrosion [22], battery and fuel cell [23] as well as sensor research [24]. Several important parameters of an electrochemical system can be obtained with this technique such as the charge transfer resistance and double-layer capacitance in sensor application, or polarization resistance and pore resistance in corrosion analysis. EIS can also be used to investigate the effect of surface modifications in composite electrodes that are often used to improve electron transfer and sensitivity towards specific analytes. Physical properties of the electrode such as surface roughness and porosity can also be estimated. In addition, EIS is increasingly finding use in the field of biosensors to study for example antibody-antigen binding and as an actual method for signal transduction [25, 26]. In publications I-III we used EIS to investigate several types of a-C and ta-C films to observe the effect of power density, oxygenation and film thickness on the electrochemical properties of these materials.

In EIS experiments, the impedance of the electrochemical system is measured as a function of frequency with a potentiostat. The electrochemical cell is kept at a constant potential on top of which a sinusoidal input voltage of small amplitude, typically 10-20 mV, is applied. The current output is then recorded over a chosen frequency range. The data is generally presented graphically as a Nyquist plot, where the imaginary component of the impedance is plotted against the real component, or in a Bode plot, where impedance and phase angle are plotted against frequency.

Nyquist plots are used to derive important electrochemical parameters such the solution resistance ($R_s$), charge transfer resistance ($R_{ct}$) and double-layer capacitance ($C_{dl}$) by fitting the experimental data with an equivalent circuit model. The most common equivalent circuit used to model the interface between an electrode and an electrolyte solution is the Randles circuit model shown in Figure 2. It is composed of a solution resistance in series with a double-layer capacitance, charge transfer resistance and a Warburg element connected in parallel. This circuit can be used to satisfactorily model simple electrochemical systems comprised for example
of an outer sphere redox molecule at an unmodified metal electrode. Modifying electrodes with other materials or adsorption phenomena at the electrode surface generally lead to more complicated equivalent circuit models where additional RC-circuits need to be introduced.

![Figure 2](image)

**Figure 2.** A) Nyquist plot obtained from EIS measurements of a 30 nm thick ta-C film. B) Equivalent Randles circuit model used to fit the Nyquist plot in A. The resistors in the dashed area are part of a modified Randles circuit model we proposed in publication III to explain charge transport in ta-C (see text for details).

The solution resistance is obtained as the distance between the y-axis and the intersect of the half-circle with the x-axis in the high-frequency region of the Nyquist plot as shown in Figure 2A. It describes the impedance that arises from the solution.

The charge transfer resistance is equal to the distance between the intersects of the half-circle and the x-axis. \( R_{ct} \) is a measure of the rate of charge transfer and therefore defines the kinetically controlled region of the Nyquist plot. When the current is small and the effect of mass transfer can be omitted, the reaction is governed solely by electron transfer kinetics and \( R_{ct} \) can be expressed as

\[
R_{ct} = \frac{RT}{nFi_0}
\]

where the variables have the same meaning as before and \( i_0 \) is the exchange current density given by \( i_0 = FK^oc \), where \( c \) is the concentration of the electroactive species. By inserting the latter equation for \( i_0 \) above, we see that \( R_{ct} \) is related to the reaction rate constant \( (k^o) \) and we obtain the following equation

\[
k^o = \frac{RT}{n^2F^2AR_{ct}(c_{O}^b)^{\alpha}(c_{R}^b)^{1-\alpha}}
\]

where \( A \) is the geometrical area of the electrode, \( \alpha \) is the transfer coefficient (usually \( \alpha = 0.5 \)). The subscripts \( O \) and \( R \) denote the oxidized and reduced species, respectively, and the superscript \( b \) denotes the bulk concentration.

The double-layer capacitance arises from the charge distribution at the interface between the electrode surface and the electrolyte. Solvent molecules adsorbed on the electrode act like a dielectric in a conventional capacitor. \( C_{dl} \) can be estimated by the maximum of the imaginary
part \(Z''_{\text{max}}\) of the semi-circle in the Nyquist plot (Figure 2A). Generally, the capacitance in the Randles circuit is replaced by a constant phase element (CPE) because \(C_d\) does not behave ideally like a capacitor in real experiments. The CPE accounts for inhomogeneities on the electrode surface, which is not ideally smooth and presents areas that differ in electrochemical activity such as passivated areas and active sites at grain boundaries for example. The inhomogeneities lead to a distribution of \(k_o\) values (and hence \(R_{ct}\) values) and time constants that need to be fitted with a CPE.

The parameter \(a\) indicates the deviation of the CPE’s characteristics from an ideal capacitor \((a = 1\) for an ideal capacitor). The \(C_{dl}\) values were calculated with the Gamry Echem Analyst software using the following equation proposed by Hsu and Mansfeld [27]:

\[
C_{dl} = A_{dl}(\omega)^{a-1}
\]

where \(A_{dl}\) is a parameter of the CPE (in addition to \(a\)) and \(\omega\) is the angular frequency defined as \(\omega = 2\pi f\). The frequency \(f\) corresponds to the maximum of the imaginary part \((-Z_{\text{imag}})\) in the semicircle of the Nyquist plot.

The Warburg element accounts for the impedance due to the diffusion of the solution species and is dependent on frequency. At high frequencies, the Warburg impedance is small because the reactants do not have time to diffuse far from the electrode surface. Thus, the contribution from the Warburg impedance is seen only at low frequencies when the reactants have time to diffuse farther. On the Nyquist plot, a diagonal line with a slope of 45° characterizes the Warburg impedance and defines the diffusion-controlled region (Figure 2A). Inhomogeneities on the electrode surface lead to a lowering in the slope of the diagonal line because the concentration of reactants is not equal at a constant distance from the surface. In reality, the concentration follows the surface roughness and shape of the electrode introducing unideal behaviour. The Warburg element is only valid in the case of semi-infinite diffusion and in other cases, different diffusion elements must be used in the equivalent circuit model. If a homogeneous chemical reaction follows the electrochemical reaction, a Gerischer impedance is typically used instead.

In publication III, we proposed a modified Randles circuit model, where \(R_{ct}\) is composed of the series connection of charge transfer resistance and charge transport resistance. \(R_{\text{transfer}}\) represents the charge transfer from the redox species in solution to the surface of the electrode, whereas \(R_{\text{transport}}\) represents the charge transport through the ta-C film. This modification is shown in the dashed box in Figure 2B.

### 2.3 Inner and Outer Sphere Redox Probes

The redox probes used in the electrochemical characterization of carbon electrodes are generally classified as outer or inner sphere based on their interaction with the electrode surface. Inner sphere redox (ISR) probes can be further classified based on the type of surface interaction as described for example by Chen & McCreery [10, 28].

Outer sphere redox (OSR) probes are mostly insensitive to the surface chemistry of the electrode material and their oxidation proceeds without adsorption or rearrangements of the inner coordination shell. The electron transfer depends solely on the electronic interaction with the electrode and thus, OSR probes can offer information on the electronic properties of the electrode material. Known OSR probes include at least hexaammineruthenium(III)
(Ru(NH₃)₆²⁺/³⁺), hexachloroiridate(IV) (IrCl₆³⁻/²⁻) and ferrocenemethanol (FcMeOH⁰/¹⁺). [10, 28] However, there is recent evidence that the latter adsorbs on HOPG, calling into question its outer sphere character [29].

As opposed to OSR probes, inner sphere redox (ISR) probes are heavily dependent on surface chemistry as their oxidation requires specific interactions with the surface and the formation of a common ligand. [18] Chen & McCreery have observed that the oxidation of ISR probes may depend on adsorption, surface oxygen content or specific oxygen functional groups such as carbonyl or hydroxyl groups. For example, carbonyl groups can catalyze the oxidation of Fe²⁺/³⁺ at carbon electrodes [10]. We have also observed that the oxidations of DA and AA are affected by surface oxygen content in publication II.¹ Ferrocyanide (Fe(CN)₆⁴⁻/³⁻) has long been considered an OSR probe and has been used extensively to probe the electrochemical properties of carbon electrodes. However, recent reports have shown that its oxidation is sensitive to the nature and concentration of cations in the electrolyte and its electron transfer involves changes in its solvation shell. [30-32] Thus, it may be safer to consider Fe(CN)₆⁴⁻/³⁻ as an ISR probe.

2.4 Other Characterization Methods

Several characterization methods were used in publications I-V to study the physical and chemical properties of the deposited a-c and ta-C films. Detailed characterization is needed to find correlations between physical, chemical and electrochemical properties. The characterization methods are summarized in Table 1 and this section briefly describes them.

**Scanning electron microscopy**

Scanning electron microscopy (SEM) was used to image the surface morphology of the electrode materials. In SEM, a focused beam of electrons is raster scanned on the surface of the sample in a vacuum chamber. The images are produced either by secondary electrons or by back-scattered electrons. Secondary electrons are ejected from the surface region due to inelastic collisions with the incident beam electrons, whereas back-scattered electrons results from the beam electrons that are reflected because of elastic scattering. Back-scattered electrons give a better contrast between areas with different composition, because heavier elements scatter more strongly and appear brighter on the SEM images than lighter elements. The samples for SEM must be conductive to avoid electrostatic charge from building up. In our case, the a-C and ta-C films were sufficiently conductive that no surface coatings were needed to avoid this problem. [33]

**Helium ion microscopy**

Helium ion microscopy (HIM) was also used to image the surface of the electrode materials but as opposed to SEM, this method uses a focused beam of helium ions instead of electrons. HIM improves the resolution and depth of field of the image, because of the shorter De Broglie wavelength of the helium ions and the use of ultra-high brightness gas field ionization sources. It has been estimated that the resolution is five times better than that of field-emission SEMs.

¹ McCreery classifies DA and AA as oxide insensitive but DA requires adsorption to oxidize. See discussion in section 4.3 on this topic.
HIM finds use especially in the imaging of insulating samples and uncoated biological samples. [34, 35]

Transmission electron microscopy

In transmission electron microscopy (TEM), a focused beam of electrons is transmitted through a very thin sample (generally < 100 nm) and the transmitted electrons carry the information from which the TEM image is formed. High-resolution TEM (HRTEM) allows the imaging of atomic structures. Some electrons in the beam are scattered at angles corresponding to the crystal structure of the sample and form a diffraction pattern. Analysis of the diffraction pattern gives information on the crystal structure, crystal defects etc. In publication II, we used HRTEM to observe the microstructure of the oxygenated a-C films and the presence of nano-crystalline areas. In publications III and IV, we used HRTEM to investigate the interfacial region in cross-sectional samples of the ta-C and ta-C+CNT films, respectively, deposited on a titanium adhesion layer on top of a silicon substrate. Cross-sectional samples were prepared by focused ion beam (FIB).

X-ray photoelectron spectroscopy

X-ray photoelectron spectroscopy (XPS) is a non-destructive method for analyzing the surface chemistry of a sample in vacuum. The sample is irradiated with X-rays while the kinetic energy and number of electrons that escape the surface region are measured. The probing depth is generally about 10 nm. Each element (except hydrogen) produces a characteristic XPS spectra, from which it can be identified. The number of electrons permits quantitative analysis of elemental composition but the characteristic peaks must be normalized. Small shifts in binding energy due to local bonding environment gives information on the chemical and electronic state of the surface region. For example, the bonding configuration of carbon and of carbon to oxygen is often investigated in this manner, although the fitting of XPS high-resolution spectra is not unambiguous. We have used XPS in all the publications to study the surface chemical composition, in particular the relative amount and nature of surface oxygen functional groups and carbon bonding. In publication IV, we used XPS to obtain evidence of polydopamine formation following the electrochemical oxidation of DA on ta-C electrodes.

X-ray absorption spectroscopy

X-ray absorption spectroscopy (XAS) is a similar technique to XPS but enables a much more detailed characterization of the sample under study. In XAS, a synchrotron radiation source is used to irradiate the sample at specific energies for each material. The transitions from core electronic states to excited electronic states and to continuum are measured and each transition can be observed in the resulting spectra. This gives information on the local bonding states and electronic structure of the sample in much more detail than XPS that only measures transitions to continuum. [36] In publication III, we used total electron yield and Auger electron yield spectra, which give information from different depths in ta-C films, to show the existence of a sp$^{2}$-rich surface layer.

Time-of-flight elastic recoil detection analysis
Time-of-flight elastic recoil detection analysis (ToF-ERDA) was used to obtain elemental depth profiles of oxygenated a-C films in publication II. Whereas XPS and XAS only give information from the surface region, ERDA can measure bulk concentrations of a thin film. This is achieved by accelerating a beam of ions towards a sample and measuring the recoiled atoms due to elastic interactions with the incident beam ions. ERDA is a destructive technique with the advantage of being able to measure hydrogen content as opposed to XAS and XPS. [37]

**Grazing incidence X-ray diffraction**

In grazing incidence X-ray diffraction (GIXRD), a beam of X-rays is directed at a sample at small incident angles and the crystalline structure of the sample causes the incident X-rays to diffract, giving a diffraction pattern. GIXRD is used to characterize the atomic structure of materials and was used in publication I to look for nanocrystalline areas in a-C films deposited by closed-field unbalanced magnetron sputtering.

**X-ray reflectivity**

X-ray reflectivity (XRR) was used to measure the thickness, mass density and root mean square roughness ($R_{\text{rms}}$) of the a-C films in publication I. This technique uses a beam of X-rays directed at the sample surface and measures the intensity of the reflected X-rays in the specular direction. The XRR data is analyzed by fitting a simulated curve to the experimental data.

**Raman spectroscopy**

We used visible Raman spectroscopy to study the bonding configuration of a-C and ta-C films in publications I, II, III and to estimate qualitatively the quantity of defects in carbon nanotubes in publication V. In this method, a monochromatic laser beam interacts with the molecular vibrations of the sample and causes the laser energy to shift. The shift in energy is then used to obtain information on the vibrational and rotational modes in a material.

**Scanning probe microscopy**

Several scanning probe modes were used in publications I, II and III to investigate the surface topography and electrical properties of a-C and ta-C films. In all the operating modes, a probe is raster scanned on the sample surface. The probe can be scanned in either a constant interaction mode, in which a feedback loop is used to keep the interaction (such as current or cantilever deflection) constant by changing probe height, or in constant height mode, in which the height is kept constant and the interaction is recorded. Conductive tips are used for electrical measurements and the probe diameter at the tip determines the resolution for each scanning probe mode. Atomic force microscopy (AFM) was used to measure surface roughness, scanning spreading resistance microscopy (SSRM) was used to measure the average current flowing through the thin films and scanning tunneling microscopy (STM) was used to investigate the density of states (DOS) near the Fermi level and mobility band gaps.
3. Physical and Electrochemical Properties of Amorphous Carbon

Carbon materials are widely used in electroanalytical applications due to their wide potential window, low background current and the large extent to which their structure and surface chemistry can be modified. Thus, conventional electrode materials such as graphite, carbon paste (CP) and glassy carbon (GC) have been extensively studied in literature. [10] More recently, several new carbon materials have emerged including carbon nanotubes (CNTs), carbon nanofibers (CNFs), graphene and boron-doped diamond (BDD) [10, 38-40]. A large part of the research has focused on these novel electrode materials due to their unique properties. Nevertheless, amorphous carbon, which can be extensively modified to present diamond-like or graphite-like electrochemical properties, has been much less investigated than BDD although it possesses similar electrochemical properties.

In this thesis, we have characterized and studied the electrochemical properties of amorphous carbon electrodes with an emphasis on finding correlations between the physical, chemical and electrochemical properties. In this chapter, we introduce amorphous carbon materials starting from the main deposition methods to their physical and electrical properties before finally looking in more detail into their electrochemistry. The results from publications I, II and III are presented in the following sections.

3.1 Deposition Methods

The physical, chemical and electrochemical properties of amorphous carbon films depend on the ratio of sp²/sp³-bonded carbon as well as the presence of incorporated impurities such as hydrogen, nitrogen and possible metal impurities from the deposition chamber. The bonding structure can be controlled by adjusting the deposition parameters such as working pressure, temperature and kinetic energy of the ions. Impurities can be incorporated by introducing reactive gases in the deposition chamber during film growth. [11, 12, 41] The different deposition methods and adjustable parameters lead to a wide variety of different amorphous carbon materials as can be seen in Figure 3. The most commonly used physical (PVD) and chemical vapour deposition (CVD) methods for carbon synthesis have been summarized in Table 2. Typical values are reported, which can be improved with the use of specialized equipment such as high-density plasma sources (for example electron cyclotron wave resonance sources in CVD processes), irradiation of the film surface (ion plating) or the use of mass-selective components to control the deposited ions (called mass-selected ion beam deposition). The a-C films used in publication I and II were deposited by closed-field unbalance magnetron sputtering (CFUBMS) and the ta-C films in publications III, IV and V by filtered cathodic vacuum arc (FCVA). These two deposition methods will be discussed in more detail below.
Filtered cathodic vacuum arc

In FCVA deposition, a vacuum arc discharge is initiated between a planar graphite cathode and an anode by touching the cathode with a striker electrode. This produces a high ion density plasma of energetic carbon ions (up to $10^{13}$ cm$^{-3}$). The macroparticles that are ejected when the arc discharge hits the graphite cathode are filtered by passing the plasma in a 90° or S-bend magnetic filter duct. The electrons and positively charged ions follow the filter whereas macroparticles and neutral particles are filtered producing a nearly fully ionized plasma. The plasma beam is condensed onto a substrate to produce the carbon film. The arc discharge is generally pulsed or it is guided by a magnetic field to move the arc spot around the graphite cathode to avoid cathode erosion and improve stability. [12, 41]

The carbon ions in the plasma have a kinetic energy of 10-30 eV. By applying a high DC or RF self-bias voltage to the substrate, the incident ions are accelerated with the additional energy given by the potential difference between the plasma and substrate. [41] The high kinetic energy of the carbon ions results in a high fraction of sp$^3$ bonded carbon. ta-C films with an sp$^3$ fraction up to 85-90% have been reported by using this deposition method [43, 44]. The neutral plasma allows the deposition onto insulating substrates but an adhesion interlayer or alloying is often required with thick ta-C films because of high internal stress in ta-C films. [12, 41] We have used Ti interlayers to improve adhesion, which has the additional advantage of improving the electrical contact at the Si/Ti/ta-C interface in electrodes as was observed in publication III. FCVA produces carbon films with very low smoothness and high hardness but some defects are introduced by the ejected particles from the graphite target, even with filtering. [12]

Closed-field unbalanced magnetron sputtering

In magnetron sputtering, energetic ions generated in a glow discharge plasma, typically Ar$^+$ ions, are driven to a negatively charged target (cathode). The collision of the ions causes the target material to be removed i.e. sputtered, and the ejected particles can deposit on the substrate material placed in front of the target to form a thin film.
Table 2. Description of the most common deposition methods for synthesis of a-C and ta-C films. Typical values are reported but can be significantly improved with specialized equipment. For the kinetic energy, rough estimation of the mean kinetic energy of plasma without substrate biasing is presented in parentheses.

<table>
<thead>
<tr>
<th>Deposition method</th>
<th>Process (PVD/CVD, description)</th>
<th>Kinetic energy (eV)</th>
<th>Deposition rate (nm/s)</th>
<th>Film properties</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct ion beam</td>
<td>PVD, ionization of hydrocarbon gas such as methane and acceleration of ions to substrate</td>
<td>50-750</td>
<td>0.1-1</td>
<td>- typically 15-40 at.% hydrogen</td>
<td>[41, 45]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- a-C, ta-C (sp³ fraction up to 80%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- possibility for precursor gases</td>
<td></td>
</tr>
<tr>
<td>Filtered cathodic arc</td>
<td>PVD, carbon ions produced by vacuum arc discharge between graphite cathode and grounded anode are filtered (to eliminate particles and neutral species) and accelerated toward a substrate</td>
<td>100-2500 (10-30 eV + biasing)</td>
<td>0.1-1</td>
<td>- hydrogen-free films</td>
<td>[41, 43, 44]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- ta-C (sp³ fraction up to 80-90%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- possibility for precursor gases</td>
<td></td>
</tr>
<tr>
<td>Pulsed-laser ablation</td>
<td>PVD, a high power laser pulse is directed at a graphite or polycarbon target producing a plasma plume that is accelerated onto a substrate</td>
<td>1-1000 (3-600 eV + biasing)</td>
<td>0.1-1</td>
<td>- hydrogen-free films</td>
<td>[46, 47]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- a-C, ta-C (sp³ fraction up to 95%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- possibility for precursor gases</td>
<td></td>
</tr>
<tr>
<td>Magnetron sputtering</td>
<td>PVD, sputtering of graphite target with Ar ion plasma</td>
<td>1-1000 (1-20 eV + biasing)</td>
<td>0.1-10</td>
<td>- low hydrogen content (&lt;10 at.%)</td>
<td>[46, 48-50]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- a-C and ta-C films</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- possibility for precursor gases</td>
<td></td>
</tr>
<tr>
<td>Plasma-enhanced</td>
<td>CVD, RF plasma dissociation of a hydrocarbon precursor (acetylene, methane) and adsorption on substrate and chemical bonding to other atoms</td>
<td>1-30</td>
<td>0.1-10</td>
<td>- hydrogenated films (up to 50 at. %)</td>
<td>[12, 41, 51-53]</td>
</tr>
<tr>
<td>chemical vapour deposition</td>
<td></td>
<td></td>
<td></td>
<td>- a-C:H (sp³ fraction up to ~50%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- precursor gas needed</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- film H/C ratio depends on precursor gas</td>
<td></td>
</tr>
</tbody>
</table>
Closed-field unbalanced magnetron sputtering was used to deposited a-C films in publications I and II. This method overcomes some of the limitations of conventional sputtering such as low deposition rate, low ionization degree of the plasma and high substrate temperature. [54, 55] The low ionization degree results in a low total ion flux towards the growing film. As a consequence, a high bias voltage is required to increase the energy of the deposited atoms but this also leads to problems such as subplantation of Ar+ ions leading to lattice defects, high residual stress and a deterioration of the quality of the substrate/film interface and poor film adhesion. [54, 56] The main difference between magnetron sputtering and conventional sputtering is the use of magnetrons to strongly confine the plasma to the target region. Electrons trapped by the magnetic field undergo more collisions with gas molecules producing more ions that are driven to the target. This in turn leads to higher sputtering rates and thus to higher deposition rates. The higher ionization efficiency allows the discharge to be maintained at a lower deposition pressure. [56]

In an unbalanced magnetron configuration, the magnets are positioned so that some of the field lines are directed towards the substrate. The electrons are no longer confined to the target region, but follow the magnetic field lines to reach the substrate. As a result, high ion currents reach the substrate, without the need to externally bias it, producing higher quality films. [55, 56]

In a system with several magnetrons with opposing magnets of opposed polarity, the configuration is called closed-field. Only few electrons are lost to chamber walls, because the magnetic field lines between magnetrons form a closed trap maintaining a dense plasma in the substrate region. This leads to a high total ion flux and bombardment of the growing film. [56]

CFUBMS can be used to deposit pure metals, ceramics, alloy films with multiple targets and more than one material, reactivity sputtered coatings by controlling the partial pressure of a reactive gas, or graded films that have different properties at the substrate/film interface and film surface. [55] One example of graded film is the deposition of a multilayer diamond-like carbon (DLC) film with improved adhesion to the substrate [57]. Some of the most common deposition parameters that are altered to produce markedly different amorphous carbon films are the target power density [58], substrate bias voltage [59], gas pressure [60], AC or DC pulse frequency [61] and use of reactive gases [62].

### 3.2 Structure, Growth Mechanism and Surface Chemistry

#### Structure

The structure of a-C consists of sp, sp2 and sp3-bonded carbon atoms in amorphous network without any long-range order. Caro et al. showed in a computational study that the sp2 sites in a-C tend to cluster and their arrangement depends on the sp2/sp3 fraction. As the density in the a-C structures increased, the sp2 sites tended to cluster in olefinic chains, and as it decreased, there was an increase in aromatic ring formation although olefinic chains were still predominant. [63]

In the case of ta-C, the bulk is mostly sp3 bonded, but the surface is composed of a low-density, sp2-rich layer. Davis et al. showed by cross-sectional electron energy loss spectroscopy (EELS) that ta-C films deposited with 320 eV ion energy had a 1.3 ± 0.3 nm thick surface layer compared to 0.4 ± 0.2 nm with 35 eV. The bulk sp3 fraction was 80-90 %. According to the authors, the thickness of the sp2 surface layers depends on ion energy but not on deposition
temperature. [64] The presence of a 5 nm thick sp\textsuperscript{2} layer mixed with oxygen and silicon at the substrate interface was also confirmed. [64, 65] We could also verify the existence of this surface sp\textsuperscript{2} layer by XAS measurements carried on 7 and 15 nm thick ta-C films in publication III and reference [66]. This confirms that the sp\textsuperscript{2}-rich surface layer is similar regardless of film thickness and sp\textsuperscript{3} fraction.

Figure 4. Simulated atomic structure of A) a-C, where green, light blue and dark blue atoms represent sp, sp\textsuperscript{2} and sp\textsuperscript{3} bonded C atoms and red is oxygen, and B) ta-C, where purple, red, orange and yellow atoms represent sp, sp\textsuperscript{2} and sp\textsuperscript{3} bonded C atoms. B) Reprinted with permission from [67]. Copyright (2018) American Physical Society.

**Growth mechanisms**

The consensus in literature is that the growth of highly sp\textsuperscript{3}-bonded a-C (or ta-C) occurs by subplantation of the incident ions. In this model, highly energetic incident ions can penetrate into the growing film either directly or by a knock-on collision. The penetration of the ions below the surface of the film increases the local density causing the local bonding to form sp\textsuperscript{3} bonded carbon. [12, 68, 69] Robertson has estimated the penetration threshold to be of the order of 30 eV [69] and maximum sp\textsuperscript{3} content is achieved at ion energies of around 100 eV [69, 70]. Below the penetration threshold, the incident ions do not have enough energy to penetrate the film and will either adhere to the surface forming sp\textsuperscript{2} or simply bounce off. [42, 69] However, if the ion energy is too high, the excess energy is dissipated in the form of heat. This causes relaxation of the density increment and results in the formation of sp\textsuperscript{2} carbon as density decreases. [12, 69]

Very recently, Caro et al. proposed the peening model as a new mechanism that explains the growth of highly sp\textsuperscript{3}-bonded amorphous carbon (Figure 4B). [67] Their methodology relied on machine learning based molecular dynamics simulations. It is the first computational model that can reproduce a-C structures with the highest sp\textsuperscript{3} fraction (85-90%) obtained experimentally. In the peening model, the incident ions displace carbon atoms at the impact site where the density decreases. The incident ions and knock-on atoms are deposited as sp\textsuperscript{2} carbon.
However, further away from the impact site, the pressure waves created by the impacting ions result in a rearrangement of the bonds to sp³.

**Surface chemistry**

Carbon surfaces can form a wide variety of surface functional groups and react readily with ambient air to form oxides. Without rigorous pretreatments, oxygen functional groups will therefore be present on carbon surfaces, which has considerable implications on their electrochemistry (electron transfer kinetics, adsorption, electrostatic effects among others). [10]

The surface functionalization and reactivity of ta-C have been recently investigated using machine learning based molecular dynamics and Monte Carlo simulations of ta-C. [71] The incorporation of hydrogen lead to an increase in sp³-bonded carbon atoms in the surface region (topmost 3 Å) as the surface sp² carbon became saturated by hydrogen. In the most hydrogenated ta-C:H structures, the most abundant hydrogen groups were sp³ C-H with 53 ± 8 % and doubly hydrogenated CH₂ with 9 ± 5 %. In contrast, methyl groups (-CH₃) were not found on the surface. [71] Three main surface oxygen functional groups were observed when the surface of ta-C was oxygenated. The most abundant group was ketone, followed closely by epoxy groups and a small amount of ether groups at a temperature of 300 K. [71] The abundance of ketone groups is qualitatively consistent with experimentally results by XAS. [66]

Caro et al. used machine learning combined with density functional theory to calculate the adsorption energies of different surface functional groups to atomic motifs (carbon sp, sp² and sp³ sites) to give an insight on the surface reactivity of a-C. [72] Carbon sp sites were found predominantly only in the surface region of a-C, while sp³ sites were found mostly in the bulk. Carbon sp² sites were found in both the surface and bulk. It was shown, by calculating the adsorption energies of several functional groups such as hydrogen (-H), ketone (=O), epoxide (-O-), hydroxyl (-OH) and carboxylic acid (-COOH) groups on each atomic motif on the surface of a-C, that sp sites were more reactive than sp² and sp³ sites. Carbon sp³ sites showed extremely poor adsorption due to the chemically inert nature of diamond. Interestingly, atomic motifs with the same coordination (i.e. same amount of neighbour atoms) showed different adsorption energies for the same surface functional group depending on small variation in bond length and angle. The authors also noted that the local density of states (LDOS) around the Fermi level correlated strongly with adsorption energies with a higher LDOS corresponding to more negative adsorption energies and vice versa. [72]

In publication II, we studied the effect of increasing oxygen content on the electrochemical properties of a-C and observed that it strongly affects the oxidation of inner sphere redox molecules such as DA and AA (see section 3.5 for details).

### 3.3 Electrical Properties

Carbon can form several different kinds of crystalline and disordered structures depending on its bonding configuration. All the structures have distinctive physical characteristics that range from the extremely hard, chemically inert and electrically resistive diamond to the softer, electrically conductive graphite. These distinctive characteristics stem from the fact that carbon atoms can adopt three hybridizations, sp, sp² and sp³. In the sp³ configuration, the carbon atom’s four valence electrons form sp³ orbitals that make strong σ bonds to adjacent atoms in a tetrahedral geometry such as in diamond. In the sp² configuration, for example in graphite,
three valence electrons form sp² orbitals that form σ bonds in a plane. The fourth valence electron forms a weaker π orbital that bonds to an adjacent atom perpendicular to the σ plane. In the sp¹ configuration, two valence electrons form σ orbitals and the remaining two electrons π orbitals. All the orbitals lie on a different axis perpendicular to each other.

The σ and π bonds form filled σ and π states in the valence band and empty σ* and π* states in the conduction band. The σ and σ* states are separated by a wider gap than the π and π* states that lie around the Fermi level. Thus, the electronic properties are governed by the sp² fraction in amorphous carbon. In amorphous materials, the electronic states in the middle of the valence and conduction bands are called extended states. The presence of sp² bonded carbon in the sp³ matrix introduces localized states in the gap region (as opposed to delocalized electrons in aromatic sp² structures). The band tails that extend from the band edges into the band gap can have even in small amounts a significant contribution to the overall electronic properties. Since the gap contains localized states, it is called a pseudogap. [11, 12, 73]

The conduction mechanism of a-C has been studied with a model used for non-crystalline semiconductors introduced by Mott and Davis. [74] Based on this model, it has been suggested that the conduction mechanism at room temperature is either variable-range hopping [11], hopping within a bandtail distribution of localized π states [75], or hopping between neighbouring sp² islands embedded in a sp³ matrix when the sp²/sp³ ratio is below the percolation limit [76]. Above room temperature, Dasgupta et al. suggest that the conduction mechanism is always variable-range hopping at band tails [76]. Whether this hopping mechanism holds for the a-C films prepared by CFUBMS, which have a significantly higher sp² fraction, is not clear. Baker et al. proposed that the conduction mechanism of GC, which is an amorphous, sp²-bonded material, is composed of a variable range hopping component and a metallic component [77]. This conduction model could be closer to that of the a-C films used in this work.

The electronic properties of ta-C depend strongly on the film thickness because it affects the fraction of sp² and sp³ bonded carbon. [78, 79] We observed in publication III, that the fraction of sp² increases with decreasing ta-C film thickness from 100 to 7 nm. This correlated with a decrease in mobility gap and an increase in the current flow through the films. The highest current was observed for the 7 nm thick film, which also corresponded to the highest charge carrier mobility among the studied films. The computational studies were also consistent with the decrease in mobility gap with decreasing sp³ fraction. The use of adhesion layers such as Ti can have an impact on the electronic properties in the case of very thin ta-C films, which was observed by scanning tunneling spectroscopy (STS) [78].

We also studied the conduction mechanism in ta-C and found that in the range of 7 to 30 nm, it obeys a space charge limited current (SCLC) model with monoenergetic shallow traps in the band gap. The SCLC model is used for dielectric thin films and has been shown to provide good fits for several types of diamond, a-C, a-C:H, ta-C and ta-C:N films [80, 81]. The conduction mechanism in ta-C films with a thickness below 7 nm did not follow the same model, most likely because the ta-C film was not uniform and the underlying Ti layer was oxidized. Films thicker than 30 nm were omitted from the work. [79]

### 3.4 Electrochemical Properties of a-C

The electrochemical properties of a-C depend strongly on the sp²/sp³ ratio, since the bonding structure will determine the physical properties of the material. As was discussed earlier in
section 3.3, the sp² fraction determines the electrical properties of a-C and thus a high sp² fraction will lead to graphite-like electrochemical properties whereas a high sp³ fraction will lead to diamond-like properties.

We have collected a large amount of electrochemical data on a-C films from literature and the results are summarized in Table 5. From the data, it can be concluded that 1) the potential windows are generally large, up to around 3.5 V, 2) the double layer capacitances are low, 3) most reports deal with nitrogen-doped a-C films, and 4) all in all, there are very few reports on the electrochemistry of a-C and especially undoped films. The low conductivity is usually improved by nitrogen incorporation that leads to an increase in sp² fraction and higher electrochemical reactivity [82, 83].

In publication I, we investigated the undoped a-C films deposited by CFUBMS. We varied the power density during deposition to see its effect on the physical and electrochemical properties. The a-C films were then characterized with several OSR and ISR probes (listed in Table 1) using CV and EIS. The potential windows (Figure 5A) ranged from 2.77 to 2.93 V (without a clear trend as a function of power density) and were larger than the potential window of GC (1.97 V) but lower than that of ta-C (3.52 V). The background currents of the a-C films were also between those of GC and ta-C as can be seen from the CVs.

The electrochemical parameters obtained from EIS measurements are shown in Table 3. As can be seen, the $C_{dl}$ values were between 0.86 and 1.02 μF cm⁻² for all the a-C films and close to what has been cited in literature. Again, these values are in between those of GC (33 μF cm⁻²) [84] and ta-C (0.18 μF cm⁻² from publication III). The $R_{ct}$ values decreased as a function of power density, which correlated with the increase in current through the films observed by SSRM. The apparent reaction rate constant ($k_{0,app}$) slightly increased with power density and showed a clear difference between 10-15 and 20-25 W/cm² films.

The CV measurements showed that electron transfer kinetics was reversible for the positively charged Ru(NH₃)₆²⁺/³⁺ and FcMeOH⁰⁺/¹⁺ probes, whereas the negatively charged IrCl₆²⁻/³⁻ showed quasi-reversible behavior with diminishing peak-to-peak separation from 94 ± 6 mV to 72 ± 1 mV as power density increased. Since all the a-C films contained an almost equal amount of negatively charged oxygen functional groups, electrostatic repulsion as the sole cause of the non-reversible behavior was ruled out. However, it is possible that the negatively
charged oxygen groups change the reaction kinetics of IrCl$_6^{2-/3-}$ from adiabatic to non-adiabatic, because electron transfer must happen over a longer distance due to the repulsive charge. In that case, DOS would affect the electron transfer and based on the simulations the highest DOS is observed at the 25 W/cm$^2$ film that also had the lowest Δ$E_p$.

Table 3. The Nyquist plots in Figure 5B were fitted with a Randles equivalent circuit model to obtain the parameters. Potential windows were determined with CV and are the same as in Figure 5A.

<table>
<thead>
<tr>
<th>Power density (W/cm$^2$)</th>
<th>EIS parameters ± STD (N ≥ 3)</th>
<th>Potential Window (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R$_s$ (Ω)</td>
<td>A</td>
</tr>
<tr>
<td>10</td>
<td>30 ± 17</td>
<td>0.87 ± 0.13</td>
</tr>
<tr>
<td>15</td>
<td>16 ± 4</td>
<td>0.95 ± 0.03</td>
</tr>
<tr>
<td>20</td>
<td>16 ± 0</td>
<td>0.89 ± 0.02</td>
</tr>
<tr>
<td>25</td>
<td>18 ± 4</td>
<td>0.86 ± 0.11</td>
</tr>
</tbody>
</table>

We used Fe$^{2+/3+}$, whose oxidation is catalyzed by the presence of carbonyl groups [10, 85] to further assess possible differences in surface oxygen functional groups. However, there was no difference in the Δ$E_p$ values of the a-C films, which were within the standard deviation. Therefore, there were no observable differences in surface oxygen functional groups, which was consistent with the XPS results showing an equal amount of COO and COOO groups on all the films.

Table 4. Results from CV measurements. Note that for AA, the oxidation potential is reported instead of peak potential separation.

<table>
<thead>
<tr>
<th>Power density (W/cm$^2$)</th>
<th>Δ$E_p$ (mV) (N ≥ 2) at 100 mV/s</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ru(NH$_3$)$_6^{2+/3+}$</td>
</tr>
<tr>
<td>10</td>
<td>62 ± 1</td>
</tr>
<tr>
<td>15</td>
<td>61 ± 2</td>
</tr>
<tr>
<td>20</td>
<td>61 ± 1</td>
</tr>
<tr>
<td>25</td>
<td>60 ± 2</td>
</tr>
</tbody>
</table>

The oxidation of dopamine (Figure 6A) was quasi-reversible with a decrease in Δ$E_p$ from 250 ± 18 to 157 ± 19 mV with increasing power density. Oxidation of DA requires the molecule to first adsorb on the surface and thus it is heavily sensitive to the surface chemistry of the electrode material. Nevertheless, the decrease in Δ$E_p$ and oxidation potential as a function of power density cannot be explained by changes in surface chemistry (detectable at least by XPS), since the a-C films were almost identical chemically. Differences in surface roughness and feature size were also very small and their effect on DA adsorption can thus be considered minimal. In this case, the differences in structural and electrical properties, evidenced by the increase in I$_D$/I$_G$ and the variation in DOS, could affect the oxidation of DA. It has been hypothesized that π-π interactions play a significant role in the electron transfer of DA and thus a higher DOS or clustering of the sp$^2$ phase could facilitate the electron transfer process.
The oxidation potential of ascorbic acid shifts from +0.52 V to +0.38 V (vs Ag/AgCl) with increasing power density (Figure 6B). Like DA, AA is also a surface sensitive redox probe and its electrochemical behavior on the a-C films cannot be explained by differences in surface chemistry in this case. Therefore, the changes in structural and electrical properties most likely explain the behavior of AA at the a-C films.

![Figure 6](image-url)

**Figure 6.** Cyclic voltammograms of A) 1 mM Da in PBS and B) 1 mM AA in PBS at a-C electrodes. Scan rate 100 mV/s.

### 3.5 Electrochemical Properties of Oxygenated a-C

In publication II, we deposited oxygenated a-C films by CFUBMS. This was achieved by introducing oxygen at varying flow rates in the chamber during deposition. The aim was to functionalize the surface with varying amounts of oxygen functional groups to see their effect on the oxidation of DA and AA, because surface modification has been shown to strongly affect the kinetics of redox reaction at carbon electrodes [10].

Benchikh et al. showed that a cathodic pretreatment (CPT) (6 cycles between +0.3 and -1.5 V vs SCE in 0.5 M H₂SO₄ at 50 mV/s) improved the heterogeneous electron transfer rate constant of Fe(CN)₆⁴⁻/₃⁻, Ru(NH₃)₆²⁺/³⁺ and IrCl₆³⁻/²⁻ whereas an anodic pretreatment (APT) deteriorated them [86]. Unfortunately, a lack of characterization of the pretreatments’ effect prevents the rationalization of the observed changes in reaction kinetics. Benlahsen et al. showed with scanning electrochemical microscopy that the surface reactivity of a-C:N was not uniform and showed areas with high and low apparent standard rate constants. After cathodic pretreatment, the surface reactivity became uniform on the whole sample surface. The authors attributed the lowly reactive sites to sp³ C-N sites and highly reactive sites to sp² C=N sites based on XPS. Medeiros et al. showed that the oxidation peak potentials of DA can be decreased and that of AA increased by anodic pretreatment (3 mA cm⁻² during 60 s in 0.1 M KOH), allowing for the simultaneous detection of both analytes by square-wave voltammetry (SWV) at a-C:N electrodes [87]. In a subsequent article, Medeiros et al. determined by XPS that APT leads to the increase in oxygen at the surface with a relative increase in C-OH and O-C=O and relative decrease in C-O and C=O groups as compared to an as-grown film. CPT had the opposite effect. The anodically pretreated a-C:N electrodes (with the same pretreatment as described above)
Table 5. Electrochemical properties of various a-C films retrieved from literature.

<table>
<thead>
<tr>
<th>Material (deposition method)</th>
<th>Substrate + film thickness</th>
<th>sp³ (%)</th>
<th>( I_{D}/I_{G} )</th>
<th>Doping (at. %)</th>
<th>H content (%)</th>
<th>Resistivity (Ω cm)</th>
<th>Potential window (V)</th>
<th>( \Delta E_{p} ) (mV)</th>
<th>( \Delta E_{p} ) (mV)</th>
<th>( C_{dl} ) (μF/cm²)</th>
<th>( R_{ct} ) (Ω)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>a-C:H:N (ID ECR)</td>
<td>Si/Ti/SS + 60 nm</td>
<td>-</td>
<td>5-24</td>
<td>32-20</td>
<td>( 10^3-10^4 )</td>
<td>3.7</td>
<td>(0.5 M LiClO₄)</td>
<td>-</td>
<td>380-995</td>
<td>430-995</td>
<td>454-496</td>
<td>[88]</td>
</tr>
<tr>
<td>a-C:N (RF MS)</td>
<td>Si + 200 nm</td>
<td>-</td>
<td>13</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3.44-4.48</td>
<td>-</td>
<td>[89]</td>
</tr>
<tr>
<td>a-C:N (RF MS)</td>
<td>Ti + 100 nm</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>1.5x10⁶</td>
<td>3.6</td>
<td>(0.5 M LiClO₄)</td>
<td>-</td>
<td>70</td>
<td>3.7</td>
<td>450-490</td>
<td>[82]</td>
</tr>
<tr>
<td>a-C:H:N (RF MS)</td>
<td>Ti + 100 nm</td>
<td>-</td>
<td>-</td>
<td>32</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>360</td>
<td>-</td>
<td>[83]</td>
</tr>
<tr>
<td>a-C:N (RF MS)</td>
<td>Si + 200 nm</td>
<td>-</td>
<td>13</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.67</td>
<td>154</td>
<td>[83]</td>
</tr>
<tr>
<td>a-C:N (RF MS)</td>
<td>Si + 200 nm</td>
<td>-</td>
<td>1.98-4.72</td>
<td>-</td>
<td>400-1.5</td>
<td>3.6-3.2</td>
<td>(0.5 M LiClO₄)</td>
<td>-</td>
<td>70-240</td>
<td>-</td>
<td>-</td>
<td>[90]</td>
</tr>
<tr>
<td>a-C:N (RF MS)</td>
<td>Ti + 100 nm</td>
<td>-</td>
<td>6-26</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.8-5.9</td>
<td>25-322</td>
<td>[91]</td>
</tr>
<tr>
<td>a-C:N:H (PECVD)</td>
<td>Si + 1500 nm</td>
<td>28.1</td>
<td>0.99</td>
<td>8.2</td>
<td>0.70</td>
<td>2.97</td>
<td>(0.1 M H₂SO₄)</td>
<td>124</td>
<td>250</td>
<td>7.2-17.3</td>
<td>-</td>
<td>[92]</td>
</tr>
<tr>
<td>a-C (PLD)</td>
<td>Si₃N₄ + 50 nm</td>
<td>0.49</td>
<td>0</td>
<td>158</td>
<td>-</td>
<td>2.67</td>
<td>(1 mM in 0.1 M Na₂SO₄, 10 mV/s)</td>
<td>490</td>
<td>-</td>
<td>400</td>
<td>-</td>
<td>[93]</td>
</tr>
<tr>
<td>a-C:B (PLD)</td>
<td>Si₃N₄ + 50 nm</td>
<td>0.96</td>
<td>8</td>
<td>4.6</td>
<td>-</td>
<td>2.67</td>
<td>(1 mM in 0.1 M NaCl, 10 mV/s)</td>
<td>400</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>[94]</td>
</tr>
<tr>
<td>a-C:N (DC MS)</td>
<td>SS + 200 nm</td>
<td>2.5</td>
<td>16</td>
<td>0.14</td>
<td>-</td>
<td>3.0</td>
<td>(0.5 M LiClO₄)</td>
<td>-</td>
<td>-</td>
<td>46</td>
<td>1.7</td>
<td>[86]</td>
</tr>
<tr>
<td>a-C:N</td>
<td>SS + 400 nm</td>
<td>-</td>
<td>11</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>300</td>
<td>[94]</td>
</tr>
</tbody>
</table>

ID ECR = integrated distributed electron cyclotron resonance, MS = magnetron sputtering, PLD = pulsed laser deposition, RF = radio frequency, SS = stainless steel

\(^a\) Amount of nitrogen was varied in ta-C films. Value ranges in Table refer to samples with lowest and highest N content, respectively. \(^b\) Values for 0.21 V (vs SCE), which is close to the formal potential of the ferro-ferricyanide redox pair. The values vary slightly with angular velocity. \(^c\) Estimated from CV. \(^d\) Nitrogen partial pressure was varied from 0 to 0.15 Pa during deposition and the value range refers to samples with lowest and highest partial pressure, respectively. \(^e\) Partial pressure was varied between 0.01 and 0.08 Pa. \( C_{dl} \) and \( R_{ct} \) values vary depending on electrochemical pretreatment. \(^f\) Values for as grown 200 nm thick a-C:N sample. EIS in Ru(NH₃)₆²⁺/³⁺. \(^g\) Estimated from CV for the as grown film.
reduced the peak-to-peak separation of Fe(CN)$_6^{3+/4-}$ compared to as-grown films and had a DA detection limit of 66 nM in the presence of AA using SWV. Cathodically pretreated a-C:N improved peak-to-peak separation of Fe(CN)$_6^{3+/4-}$ (consistent with Benchikh et al. [86]) but could not resolve between the oxidation peaks of DA and AA. [94]

![Figure 7. A) Potential windows and B) Nyquist plots. Inset shows a magnification of the high-frequency region.](image)

The oxygenated a-C films in publication II had potential windows of about 3.20 V, except for the most highly oxygenated film (O14) that had a slightly narrower potential window of 2.91 V as shown in Figure 7A. As the oxygen content increased, the background current also increased and clear oxidation and reduction waves became more apparent at about +0.6 V and -0.5 V (vs Ag/AgCl), respectively. Since the $C_{dl}$ values obtained from fitting the EIS results (Table 6) were almost the same for all the films (about 1 $\mu$F/cm$^2$), the increase in background current is attributed to the redox reactions of the surface oxygen functional groups that increase with oxygen content.

### Table 6. Parameters obtained by fitting the Nyquist plots with the Randles circuit model.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$a$</th>
<th>$R_s$ (Ω)</th>
<th>$R_{ct}$ (Ω)</th>
<th>$C_{dl}$ ($\mu$F/cm$^2$)</th>
<th>$k^0$ (cm/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ref</td>
<td>0.95 ± 0.0</td>
<td>24 ± 8.4</td>
<td>44 ± 21</td>
<td>0.98 ± 0.1</td>
<td>0.047 ± 0.0</td>
</tr>
<tr>
<td>O2</td>
<td>0.99 ± 0.0</td>
<td>15 ± 0.6</td>
<td>55 ± 4.3</td>
<td>1.1 ± 0.0</td>
<td>0.028 ± 0.0</td>
</tr>
<tr>
<td>O6</td>
<td>0.99 ± 0.0</td>
<td>16 ± 0.6</td>
<td>50 ± 14</td>
<td>1.1 ± 0.0</td>
<td>0.033 ± 0.0</td>
</tr>
<tr>
<td>O10</td>
<td>0.97 ± 0.0</td>
<td>16 ± 0.3</td>
<td>7540 ± 4500</td>
<td>1.1 ± 0.1</td>
<td>(0.28 ± 0.15) x 10$^{-3}$</td>
</tr>
<tr>
<td>O14</td>
<td>0.97 ± 0.0</td>
<td>45 ± 45</td>
<td>7470 ± 2620</td>
<td>1.1 ± 0.0</td>
<td>(0.22 ± 0.10) x 10$^{-3}$</td>
</tr>
</tbody>
</table>

The $R_{ct}$ values showed a clear division between the reference and lightly oxygenated films with a relatively low $R_{ct}$ of 44-55 Ω compared to the highly oxygenated films with very high $R_{ct}$ of 7.5 x 10$^3$ Ω. This is clearly seen in the magnitude of the semicircles in the Nyquist plots in Figure 7B. This is consistent with the significant decrease in current through the films measured by SSRM and indicates that the resistivity of the films drastically increases when the oxygen content reaches a certain level. ToF-ERDA showed that this corresponds to a limit of 11 at. % in the bulk film, beyond which the extra oxygen is not incorporated in the films although oxygen inflow was increased during deposition. The increasing hydrogen content in the bulk may also
affect adversely the electrical properties of the a-C films. The substantial change in the electrical properties of the a-C films prevented to some extent the detailed investigation of the reaction kinetics of ISR probes at highly oxygenated films, because the effect of the electrical properties and varying surface chemistry could not be differentiated. XPS showed that the surface oxygen content increased steadily with oxygen inflow and a cap for maximum oxygen content was not observed as in the bulk. The O/C ratio increased from 0.09 for the reference sample to 0.19 for sample O14. ToF-ERDA indicated that the amount of oxygen was practically uniform throughout the a-C films excluding the hypothesis of a non-conductive oxide film at the surface of the films.

Table 7. Potential windows and $\Delta E_p$ data for 1 mM Ru(NH$_3$)$_6^{2+/3+}$ in 1 M KCl, 1 mM Fe(CN)$_6^{4-/3-}$ in 1 M KCl, 100 μM dopamine in PBS and $E_{pa}$ for 1 mM ascorbic acid in PBS. Scan rate 100 mV/s.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Potential window (V)</th>
<th>$\Delta E_p$ (mV) ± STD (N = 3)</th>
<th>$E_{pa}$ (mV) ± STD (N = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ru(NH$_3$)$_6^{2+/3+}$</td>
<td>Fe(CN)$_6^{4-/3-}$</td>
</tr>
<tr>
<td>Ref</td>
<td>3.17</td>
<td>59.8 ± 0.0</td>
<td>139 ± 12</td>
</tr>
<tr>
<td>O2</td>
<td>3.21</td>
<td>62.8 ± 0.8</td>
<td>306 ± 51</td>
</tr>
<tr>
<td>O6</td>
<td>3.11</td>
<td>61.5 ± 1.2</td>
<td>235 ± 14</td>
</tr>
<tr>
<td>O10</td>
<td>3.18</td>
<td>170 ± 20</td>
<td>646 ± 38</td>
</tr>
<tr>
<td>O14</td>
<td>2.91</td>
<td>397 ± 47</td>
<td>599 ± 84</td>
</tr>
</tbody>
</table>

As expected from the changes in $R_{ct}$, the electron transfer was reversible for Ru(NH$_3$)$_6^{2+/3+}$ at the lightly oxygenated films and changed to quasireversible for the highly oxygenated films. Since Ru(NH$_3$)$_6^{2+/3+}$ is an OSR probe, the increase in $\Delta E_p$ is most likely related to the slower electron transport through the films (as opposed to electron transfer from the solution species to the electrode) as the oxygen content increases.

The CVs of DA in Figure 8A show that the $\Delta E_p$ values increased steadily from 124 mV (ref) to 303 mV (O14). Dopamine is considered a surface sensitive redox probe that it is not significantly affected by surface oxygen functionalities [10, 95] and whose oxidation is catalyzed by the presence of hydrogen bonding sites to facilitate proton-assisted electron transfer [96]. However, our results indicate that the increase in surface oxygen functional groups slows down the electron transfer of DA at the lightly oxygenated a-C films (where the contribution from deteriorating electrical properties is small based on $R_{ct}$ values). Electrostatic repulsion can be neglected due to the protonated amine group of DA at neutral pH giving it a positive charge.

Figure 8. A) CV of 100 μM DA in PBS and B) 1 mM AA in PBS. Scan rate 100 mV/s.
The oxidation potential of AA increases with increasing oxygen content as shown in Figure 8B. AA is also classified as a surface sensitive redox probe, but its sensitivity to oxygen functional groups has been debated. Chen and McCreery have shown that AA is insensitive to surface oxides on freshly prepared GC although longer exposure to air shifts its oxidation potential [28]. On the other hand, Notsu et al. reported that oxygen plasma treatment and electrochemical oxidation of BDD greatly increased the oxidation potential of AA [97]. The authors suggested that the underlying cause is the increased amount of repulsive dipole-dipole moments between the oxidized BDD surface (and in particular carbonyl groups) and AA. In our study, the amount of carboxyl and aldehyde/ketone groups (and their relative amount among C-O bonds) increased with oxygen content in the a-C films, which could explain the increasing oxidation potential. However, contribution of these specific groups is not unequivocal, since all the oxygen functional groups increased on the surface.

### 3.6 Electrochemical Properties of ta-C

The electrochemical properties of ta-C resemble those of diamond films due to the very high fraction of sp³ carbon that confers their chemical inertness, electrical resistivity and hardness. This leads to large potential windows, low background currents and low double layer capacitance values that characterize these materials. The low background current improves the signal-to-noise ratio and allows for smaller currents to be detected. The advantage of ta-C over diamond is the low deposition temperature (room temperature vs 500–800 °C) that allows the use of various substrate materials and makes ta-C deposition compatible with integrated circuit processing.

While the high sp³ fraction leads in many way to desirable electrochemical properties, it also makes ta-C and diamond films poor conductors and they are generally doped by nitrogen and boron, respectively, to improve their electrical properties. Nitrogen doping increases the sp² fraction in ta-C [98, 99], which lowers the band gap and increases conductivity of the film. Indeed, most of the literature on ta-C refers to the N-doped films, because of their attractive electrochemical properties. Table 9 gives an extensive overview on the electrochemical characterization data of various ta-C and DLC films gathered from literature. From the data, several conclusions can be drawn. The water windows are large, ranging from 2.6 to 3.8 V (depending on sp³ fraction, current thresholds and electrolyte), and the double-layer capacitances are generally within 20 to 40 μF cm⁻². The peak-to-peak separation for Ru(NH₃)₆²⁺/³⁺ indicates that electron transfer is reversible for this redox probe (with a few exceptions). Jarosova et al. compared the heterogeneous electron transfer rate of several redox probes (methyl viologen, Ru(NH₃)₆²⁺/³⁺, Fe(CN)₆⁴⁻/³⁻, ferrocene carboxylic acid (FCA) and IrCl₆³⁻/²⁻) at ta-C:N, GC and BDD electrodes and found that ta-C:N shows reversible electron transfer for all the studied probes. The rates of heterogeneous electron transfer were comparable to or better than those of GC. The capacitive background current (41 μF cm⁻²) was lower than that of GC (71 μF cm⁻²) and higher than that of BDD (10 μF cm⁻²). [32] An alternative way to improve the electrochemical properties of ta-C, and which has been almost entirely disregarded in literature, is to decrease the film thickness. As was discussed in section 3.4, the electrical properties of ta-C depend significantly on film thickness.

In publication III, we investigated the effect of ta-C film thickness on the electrochemical properties of ta-C by using CV and EIS. The electron transfer kinetics of ta-C were assessed with FeMeOH⁰/⁰⁺ and Ru(NH₃)₆²⁺/³⁺, since the reaction kinetics of these OSR probes can be
related to the varying electrical properties of the ta-C films (as opposed to changes in surface chemistry). The cyclic voltammograms for 1 mM Ru(NH₃)₆²⁺/³⁺ are shown in Figure 9A and the corresponding ΔEₚ data in Table 8. It can be seen that the electron transfer was reversible at 7 and 15 nm ta-C films and shifted to quasi-reversible from 30 nm. Similar behaviour was observed for FcMeOH⁰/⁺ with a shift to quasi-reversible behaviour at 30 nm ta-C films.

The Nyquist plots obtained from the EIS measurements (Figure 9B) were fitted with the modified Randles circuit model shown in Figure 2B and the obtained parameters are summarized in Table 10. The Cdl values were low, decreasing from 8.06 to 0.18 μF cm⁻² as the thickness increased from 7 to 100 nm, as expected based on the inert character of the films. The values are similar with BDD electrodes (10 μF cm⁻²) [32] and most undoped ta-C films. Values reported for ta-C:N (20-40 μF cm⁻²) are a bit higher due to the doping. The Rct values increased from 4.50 to 720 Ω with increasing thickness, which is consistent with the observed increase in ΔEₚ for FcMeOH⁰/⁺ and Ru(NH₃)₆²⁺/³⁺ in the CV measurements. The increasing Rct explain the very low apparent heterogeneous rate constants (k₀_app) as the film thickness increases. In the low frequency range, the straight line with a slope of circa 45° indicates that the response was controlled by diffusion.

**Table 8. ΔEₚ values of the ta-C thin films in 1 mM Ru(NH₃)₆²⁺/³⁺ in 1 M KCl.**

<table>
<thead>
<tr>
<th>Scan rate (mV/s)</th>
<th>7 nm</th>
<th>15 nm</th>
<th>30 nm</th>
<th>50 nm</th>
<th>100 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>55.3 ± 1.0</td>
<td>58.9 ± 0.8</td>
<td>62.9 ± 1.4</td>
<td>64.9 ± 1.4</td>
<td>69.9 ± 0.8</td>
</tr>
<tr>
<td>50</td>
<td>57.5 ± 1.7</td>
<td>56.8 ± 0.8</td>
<td>67.2 ± 3.3</td>
<td>72.6 ± 0.9</td>
<td>84.5 ± 1.2</td>
</tr>
<tr>
<td>100</td>
<td>57.1 ± 2.9</td>
<td>57.8 ± 0.0</td>
<td>71.1 ± 2.5</td>
<td>79.5 ± 0.5</td>
<td>96.1 ± 0.5</td>
</tr>
<tr>
<td>200</td>
<td>56.7 ± 1.5</td>
<td>59.3 ± 1.2</td>
<td>77.7 ± 2.4</td>
<td>88.7 ± 1.6</td>
<td>111.3 ± 2.9</td>
</tr>
<tr>
<td>400</td>
<td>59.2 ± 0.5</td>
<td>61.2 ± 1.2</td>
<td>88.9 ± 2.1</td>
<td>101.2 ± 2.2</td>
<td>132.0 ± 2.9</td>
</tr>
</tbody>
</table>

In our modified Randles circuit model, Rct is composed of the series connection of charge transfer resistance and charge transport resistance. Rtransfer represents the charge transfer from the
<table>
<thead>
<tr>
<th>Material (deposition method)</th>
<th>Substrate &amp; film thickness</th>
<th>sp³ (%)</th>
<th>I_D/I_G</th>
<th>Doping (at. %)</th>
<th>H content (%)</th>
<th>Resistivity (Ω cm)</th>
<th>Potential window (V)</th>
<th>ΔEp (mV) Ru(NH₃)₆²⁺/³⁺</th>
<th>ΔEp (mV) Fe(CN)₆⁴⁻/³⁻</th>
<th>Cₛ (µF/cm²)</th>
<th>Rₛ (Ω)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLC 125 W (RF MS)</td>
<td>Si or Pt + 30 nm</td>
<td>-</td>
<td>-</td>
<td>1.69</td>
<td>0.18</td>
<td>-2.7 (0.1 M PBS)</td>
<td>-440</td>
<td>-</td>
<td>-</td>
<td>38.5</td>
<td>-</td>
<td>[100]</td>
</tr>
<tr>
<td>DLC 500 W (RF MS)</td>
<td>Si or Pt + 30-350 nm</td>
<td>-</td>
<td>-</td>
<td>0.94</td>
<td>0.036</td>
<td>-2.7</td>
<td>82</td>
<td>-</td>
<td>-</td>
<td>37.1</td>
<td>-</td>
<td>[100]</td>
</tr>
<tr>
<td>ta-C:N (FCVA)</td>
<td>Si + 40 nm</td>
<td>70-80</td>
<td>-</td>
<td>10</td>
<td>-</td>
<td>3.8 (1 M HClO₄)</td>
<td>-60⁰</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>[101]</td>
</tr>
<tr>
<td>ta-C:N (FCVA)</td>
<td>Ta + 25 nm</td>
<td>-</td>
<td>5</td>
<td>10</td>
<td>-</td>
<td>-2.55 (1 M HCl)</td>
<td>285⁰</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>[102]</td>
</tr>
<tr>
<td>DLC+Pt (reactive MS)</td>
<td>Si + 70 nm</td>
<td>-</td>
<td>0.5-15</td>
<td>-</td>
<td>-</td>
<td>10⁻⁷ - 10⁻⁸ (EIS)</td>
<td>-</td>
<td>-</td>
<td>&gt;360</td>
<td>0.02-4</td>
<td>-</td>
<td>[103]</td>
</tr>
<tr>
<td>DLC 900 W (DC MS)</td>
<td>Si + 196 nm</td>
<td>-</td>
<td>0.85c</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.571</td>
<td>301</td>
<td>[104]</td>
</tr>
<tr>
<td>ta:C:N⁹ (FCVA)</td>
<td>Si + 40 nm</td>
<td>-</td>
<td>-</td>
<td>3.3 x 10⁻⁴</td>
<td>-</td>
<td>1100-400 (10 mM in 0.01 M PBS, 0.1 V/s)</td>
<td>-</td>
<td>1.5-0.2 MΩ</td>
<td>-</td>
<td>-</td>
<td>[105]</td>
<td></td>
</tr>
<tr>
<td>ta-C:N⁶ (PLA)</td>
<td>Si + 200 nm</td>
<td>56-42</td>
<td>-</td>
<td>0.64-1.12</td>
<td>0.13% N/C</td>
<td>613-1.1</td>
<td>3.13-2.78 (0.5 M H₂SO₄)</td>
<td>58-59</td>
<td>119-84</td>
<td>20-30</td>
<td>-</td>
<td>[98]</td>
</tr>
<tr>
<td>ta-C:N⁷ (LA PVD)</td>
<td>Si + 200 nm</td>
<td>50</td>
<td>0.79</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>59-65</td>
<td>95</td>
<td>41</td>
<td>-</td>
<td>[32]</td>
</tr>
<tr>
<td>ta-C:N (PLA)</td>
<td>Si + 200 nm</td>
<td>60</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>≥3 (0.1 M H₂SO₄)</td>
<td>115⁰</td>
<td>179</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>[106]</td>
</tr>
<tr>
<td>ta-C:N (PLA)</td>
<td>Si + 200 nm</td>
<td>50</td>
<td>0.72</td>
<td>-</td>
<td>-</td>
<td>3.1 (0.5 M H₂SO₄)</td>
<td>59</td>
<td>170</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>[107]</td>
</tr>
</tbody>
</table>

FCVA = filtered cathodic vacuum arc, HTZ = hydrochlorothiazide, LA = laser-arc, MS = magnetron sputtering, RF = radio frequency, PLA = pulsed laser-arc, PB = phosphate buffer, PROP = propranolol

¹ Estimated value from CV. ² Amount of nitrogen was varied in ta-C films. Value ranges in Table refer to samples with lowest and highest N content. ³ Estimated from graph.
redox species in solution to the electrode, whereas $R_{\text{transport}}$ represents the charge transport through the ta-C film. The charge transfer is estimated to be equal for all ta-C films, because the surface is composed of a sp$^2$-rich layer regardless of film thickness as was observed by XAS and computational studies [63]. However, the varying thickness strongly affects the electrical properties of ta-C (section 3.4) and thus we believe that the changes in $R_{ct}$ observed between the ta-C films are due to the changes in charge transport through the films, not charge transfer. The experimental and computational results showing an increase of the mobility gap and decrease in current through the films as the thickness increases support this finding. Naturally, the fitting only gives the total value, $R_{ct}$, but if $R_{\text{transfer}}$ is equal for all the films, we can estimate the magnitude of $R_{\text{transport}}$.

Table 10. Results obtained from fitting the Nyquist plots with the modified Randles circuit model.

<table>
<thead>
<tr>
<th>Thickness (nm)</th>
<th>a</th>
<th>$R_s$ (Ω)</th>
<th>$R_{ct}$ (Ω)</th>
<th>$C_{dl}$ (μF/cm$^2$)</th>
<th>$k_{0\text{app}}$ (cm/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>0.68</td>
<td>13.4</td>
<td>4.50</td>
<td>8.06</td>
<td>0.34</td>
</tr>
<tr>
<td>15</td>
<td>0.58</td>
<td>15.7</td>
<td>6.50</td>
<td>6.52</td>
<td>0.23</td>
</tr>
<tr>
<td>30</td>
<td>0.86</td>
<td>16.4</td>
<td>116.6</td>
<td>0.54</td>
<td>0.013</td>
</tr>
<tr>
<td>50</td>
<td>0.88</td>
<td>16.9</td>
<td>252.3</td>
<td>0.34</td>
<td>0.0060</td>
</tr>
<tr>
<td>100</td>
<td>0.83</td>
<td>11.0</td>
<td>719.8</td>
<td>0.18</td>
<td>0.0021</td>
</tr>
</tbody>
</table>

We also investigated the effect of a Ti adhesion layer between Si and ta-C on the electrochemical properties of the films. We observed that the $R_{ct}$ values increase with increasing thickness whether Ti is used or not, but its presence lowers the resistance value. This effect was most significant at the 7 nm film, where the inclusion of Ti gave an $R_{ct}$ value of 7.3 against 55.5 without Ti. $C_{dl}$ values were slightly higher with the Ti layer. This result support our view that the charge transport through the electrode limits the overall reaction kinetics.

Comparing the $R_{ct}$ values obtained in publication III and literature, there is as much as a 6-order of magnitude difference. The discrepancy can most likely be attributed to the difference in film thickness and use of a Ti interlayer and conductive Si substrate. Most articles listed in Table 9 use a thick ta-C layer (tens to hundreds of nm) and no metal interlayer. Zeng and Cachet et al. observed that a Ti interlayer improves the electrical contact between ta-C and a-C:H:N films, respectively [108]. Cachet et al. showed that this can be seen as an improvement in the apparent reactivity of the a-C:H:N film as evidenced by a decrease in peak-to-peak separation from 595 to 445 mV in 10 mM Fe(CN)$_6^{3-/4-}$ [108]. The use of a conductive silicon substrate in our study may also improve the electrical properties.
4. Dopamine and Its Electrochemical Detection

Dopamine is one of the main neurotransmitters in the mammalian brain. It affects cognitive, behavioral and motor functions in the human body and its abnormal transmission is associated with several neurological disorders such as PD, schizophrenia, attention deficit hyperactivity disorder, Huntington’s disease, obsessive compulsive disorder and Tourette’s syndrome. [109, 110] DA is involved in neuronal plasticity, learning, memory, brain reward system and attention span, although the precise mechanisms by which it mediates these effects is largely unknown owing to the complexity of DA systems. [9, 109, 111] Since DA is crucially implicated in so many functions in the brain, its accurate detection and real-time monitoring are of great interest in several research fields.

This chapter focuses on the electrochemical detection of dopamine and its oxidation mechanism. Ascorbic acid, uric acid, DOPAC and homovanillic acid (HVA), which are the main interferents in the electrochemical detection of DA, are also discussed from the same aspects. Results from publications IV and V are presented.

4.1 Challenges of In Vivo Dopamine Detection

Dopamine (3,4-dihydroxyphenethylamine) belongs to the family of catecholamines with its derivatives epinephrine and norepinephrine. The catecholamines are electroactive compounds that can be detected directly by electrochemical methods as they undergo redox reactions at the surface of an electrode. The main interferents in the electrochemical detection of dopamine are other electroactive substances found in the same brain areas and oxidizing at the same potential. These include the dopamine metabolites DOPAC and HVA, and the antioxidants ascorbic acid and uric acid. [112-115] The interference from other neurotransmitters such as norepinephrine can be minimized by selecting dopaminergic regions with low norepinephrine content [116]. However, the sensor material still needs to be highly selective to detect dopamine in the presence of the main interferents, since they are found at much higher concentrations (see Table 14).

The concentration of DA in the extracellular fluid depends on the rates of release, uptake and mass transport. Dopaminergic neurons release DA in asynchronous, low-frequency tonic firing or periodically in synchronous high-frequency phasic firing. Tonic firing determines the basal concentration of DA while phasic firing results in high-concentration transients. [113, 117] The basal levels of DA in the extracellular fluid (ECF) of the brain are in the low nanomolar range (Table 12). Thus, highly sensitive sensors are needed to detect physiologically relevant concentrations of DA. Furthermore, Wightman et al. have reported that when electrical stimulus is
initiated, a DA concentration change is detected within 100 milliseconds and concentrations return to the original baseline within a few seconds after termination [113]. This requires the sensor to have high temporal resolution, because the observed changes in concentration associated with release and uptake of DA occur in sub-second timescale. The sensor material should also be resistant to biofouling to some degree, since proteins and other molecules adsorb on its surface and attenuate the current response over time. These challenges place heavy constraints on the measurement technique and material selection that both play key roles in the performance of the electrochemical sensor.

4.2 Methods for Measuring Dopamine Levels

Microdialysis

Microdialysis is the most commonly used method for monitoring extracellular DA, because of its high chemical selectivity [116, 118]. A microdialysis probe is inserted in the area of interest inside the brain and small molecules can diffuse through the probe membrane. The dialysate solution, containing the analytes, can then be analyzed with any external analysis method giving microdialysis its high chemical resolution. [119] Generally, high-performance liquid chromatography (HPLC) is used to identify and quantify the analytes. This allows microdialysis to accurately measure absolute levels of species in the ECF with the advantage to measure also electrochemically inactive species. [119] The disadvantages are the limited time resolution, typically \( \geq 1 \) min, and low spatial resolution compared to electrochemical techniques. The spatial resolution is determined by the probe size, which is typically 200-300 \( \mu \)m in diameter. [9, 120] The low temporal resolution allows for evaluating the effect of pharmacological agents on neurotransmitter levels but not to evaluate the role of neurotransmitters on behavioural events, because synaptic signalling occurs in subsecond time scale. [9, 118] Additionally, microdialysis is an invasive technique that causes tissue damage in the region under study. It has been debated whether the trauma caused by the probe may alter the extracellular concentration of DA and other neurotransmitters near the implantation site. [119, 120]

By coupling microdialysis to a liquid chromatograph with electrochemical detection, Wightman et al. estimated the basal concentration of dopamine in the ECF of the caudate nucleus to be around 30 nM. [113] Parsons & Justice have measured the basal concentration of DA to be about 4 nM in the nucleus accumbens (NA) of rats [121], whereas a basal concentration of 10-15 nM was measured in the striatum of rats by Smith et al. [122]. Church et al. reported an extracellular DA concentration of 20-30 nM in rat striatum [123], while Yim et al. reported a basal concentration of around 10 nM in rat NA [124]. All these results were obtained by microdialysis and are summarized in Table 12.

Imaging techniques

The imaging techniques used in the measurement of dopamine are positron emission tomography (PET), single-photon emission computerized tomography (SPECT) and functional magnetic resonance imaging (fMRI). Imaging techniques are non-invasive and can provide neuronal activity maps with good resolution, but they measure neurotransmitters indirectly.
PET detects gamma rays emitted by positron-emitting radioactive nuclides attached to biomolecules of interest. PET has been used to measure synaptic concentration changes of dopamine in the living brain. [125] The principle is based on the competition between endogeneous DA and radiolabeled ligands for binding to dopamine D1 or D2 receptors. Changes in synaptic concentration of dopamine causes changes in receptor occupancy that can be seen as changes in the binding potential of the radioactive ligand. [126, 127] One of the advantages of PET using endogeneous competition is that it can estimate intrasynaptic D2 receptor occupancy and intrasynaptic DA levels. A disadvantage of PET is the lack of understanding of the multiple factors that affect ligand binding and competition with endogeneous DA and lack of temporal resolution that could help correlate DA levels with behavior. [126]

Functional magnetic imaging (fMRI) maps brain activity by measuring changes in blood oxygen levels associated with brain activity. When neuronal activity increases in a specific area of the brain, there is an increased amount of cerebral blood flow to that area to deliver nutrients, concomitant with a change in ratio of oxygenated to deoxygenated hemoglobin. The brain activity map is based on blood-oxygenation-level dependent contrast that results from the difference in magnetic properties of oxygenated and deoxygenated hemoglobin. [128] Therefore, this technique does not provide a direct or quantitative measurement of DA but can be used to observe the activation of dopaminergic areas following for example behavioral stimuli [129] or drug administration [130]. Compared to PET, this technique offers a better spatial and temporal resolution.

Ross et al. estimated the synaptic concentration of DA to be 40-60 nM in mouse striatum using the specific binding of the radiolabelled D2 receptor agonist [3H]NPA at baseline and following dopamine depletion with DA depletors [131, 132]. Scintillation spectroscopy was used to measure the binding of [3H]NPA. The results was in good agreement with SPECT imaging of the D2 radiotracer [123I]IBZM before and after acute DA depletion in the human brain giving an estimate of around 45 nM for the synaptic concentration. [125] Laruelle et al. emphasized that since as much as 40% of D2 receptors are situated outside the synapse, values obtained by in vivo competition of endogeneous DA and radiolabelled ligands likely underes-timate true synaptic concentration. [125] Dewey et al. estimated the concentration of synaptic DA to be 8-12 nM in the striatum of baboons with radiolabelled D2 receptor antagonist [11C]raclopride using PET [133].

**Electrochemical methods**

Electrochemical methods are based on the oxidation-reduction (redox) reactions of electroactive molecules at an electrode. The redox reactions are chemical reactions that involve the transfer of electrons between the molecule of interest and the electrode surface. The current formed by the transfer of electrons is proportional to the amount of reactants and it is measured as a function of potential or time. There are several electrochemical methods that all have their strengths and weaknesses. In general, electrochemical methods have good temporal resolution and their instrumentation is simple and inexpensive. In the case of in vivo DA measurements, the challenges are related to the chemical selectivity, fouling of the electrode surface and the invasiveness of the method. The lack of selectivity is due to the close oxidation potential of DA and other biomolecules such as DOPAC, HVA, UA and AA. Fouling is caused by the accumulation of reaction products or other biological compounds such as proteins that accumulate on the electrode surface and attenuate the current response over time. Issues related to
selectivity and fouling can be addressed by the appropriate choice of electrode material and measuring method. The kinetics of the redox reactions and extent of fouling depend on the physical and chemical properties of the electrode material [134], which was observed for example between GC and ta-C in publication IV.

The most common electrochemical methods used in the in vivo detection of DA are fast scan cyclic voltammetry (FSCV) and amperometry. In amperometry, a constant potential sufficient to oxidize the analyte of interest is applied to the electrode. All the electroactive molecules that can oxidize at that potential will produce a current response. The electrode detects changes in analyte concentrations with high temporal resolution, but it is unable to differentiate between the current contribution of the different oxidizing molecules. Therefore, amperometry lacks the selectivity to detect dopamine when interferents are present. FSCV provides more selectivity than amperometry because the potential is linearly scanned at a very fast rate, which shifts the oxidation potential of DOPAC and AA compared to DA, because of their slower electron transfer kinetics [115]. But since the method relies on background current subtraction to extract the DA current response, it can only measure changes in DA concentration (not basal levels) and it is prone to changes in pH that shift the background current. [116] Both amperometry and FSCV have been shown to have a high temporal resolution that allows the correlation between transient DA release from neurons and behavioral patterns [9, 135-137]. In vivo measurements of dopamine are commonly carried out with carbon fiber electrodes with a diameter below 10 μm, which makes the spatial resolution very high compared to non-electrochemical techniques. [9]

Table 11. Comparison of methods to detect and measure DA in vivo.

<table>
<thead>
<tr>
<th>Detection method</th>
<th>Temporal resolution</th>
<th>Spatial resolution</th>
<th>Equipment cost ($)</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microdialysis</td>
<td>≥ 1 min</td>
<td>&lt; 1 mm</td>
<td>100 k</td>
<td>Chemical resolution</td>
<td>Invasive</td>
<td>[118, 119]</td>
</tr>
<tr>
<td>PET</td>
<td>1-2 min</td>
<td>4-6 mm</td>
<td>8 M</td>
<td>Non-invasive</td>
<td>Indirect measurement, ligand interaction with endogeneous analyte not known well</td>
<td>[128, 138-140]</td>
</tr>
<tr>
<td>fMRI</td>
<td>4-6 s</td>
<td>2-3 mm</td>
<td>2 M</td>
<td>Non-invasive, high-resolution anatomic scans at the same time</td>
<td>Indirect measurement, no quantitative data</td>
<td>[128, 138, 140]</td>
</tr>
<tr>
<td>FSCV</td>
<td>&lt; 1 s</td>
<td>&lt; 1 mm</td>
<td>20 k</td>
<td>Simple instrumentation</td>
<td>Invasive, limited selectivity, only electrochemically active molecules</td>
<td>[9]</td>
</tr>
<tr>
<td>Amperometry</td>
<td>&lt; 1 s</td>
<td>&lt; 1 mm</td>
<td>20 k</td>
<td>Simple instrumentation</td>
<td>Invasive, no selectivity, only electrochemically active molecules</td>
<td>[9]</td>
</tr>
</tbody>
</table>

* With signal processing subsecond time resolution has been reported [128, 138]

Venton et al., using amperometry at carbon fiber electrodes implanted in the CP of anesthetized rats and finite-difference diffusion model to fit the experimental data, estimated that the concentrations of DA released in the ECF are 120±20 nM following electrical stimulus. With the obtained parameters for diffusion, uptake and release, they estimated basal dopamine levels at 30 nM with simulations in the striatal tissue of mouse. According to the results, tonic
firing results in spatially uniform DA concentration that are temporally static. Phasic firing, in contrast, results in large temporal and spatial fluctuations. [141] Gonon et al. have used carbon fiber electrodes to electrochemically measure dopamine levels in the striatum of anesthetized rats. [114] Selectivity between AA and DA was achieved by electrochemical treatment in PBS, while the response from DOPAC was eliminated by administrating pargyline to the rats to inhibit its synthesis. The measurement was done using a combination of differential pulse voltammetry (DPV) and normal pulse voltammetry (NPV) to achieve higher sensitivity and selectivity while minimizing surface fouling by keeping the electrode at rest potential between the pulses. They reported an estimated DA level of 15-25 nM. Kawagoe et. al have estimated using FSCV at Naftion-coated carbon nanofibers that the extracellular concentration of DA is 6 nM but the synaptic concentration can reach 200 nM during transients. In the study, they stimulated the striatum of anesthetized rats with frequencies close to the physiological firing rate, measured the DA concentration and then extrapolated the data to the unstimulated state. [142]

Transient DA concentrations arising from natural stimuli (as opposed to evoked electrical stimuli through an implanted electrode) have been measured with FSCV. Robinson et al. have shown that sub-second transients (200-900 ms) of 200 to 500 nM DA occurred in the nucleus accumbens (NA) core of male rats upon exposure to receptive female rats. Measurements were made with 5 μm carbon fiber electrodes using FSCV. [135] In another study, the same group showed that transients of 240 nM occurred in the caudate putamen (CP), nucleus accumbens (NA) and olfactory tubercle (OT) during conspecific periods (when an isolated rat could interact with another rat for 30 s). Measurements were done with a carbon fiber electrode using FSCV. The range of concentration spanned from 80 to 1350 nM in 125 transients identified by FSCV in 17 rats tested. [117] Yoshimi et al. were able to measure release of DA (<100 nM) in the monkey striatum using amperometry at BDD electrodes (deposited on tungsten needles) following reward-predicting cues [136].

Transients evoked by electrical stimulus can have higher concentration than natural transients. For example Ford et al. measured a release of 950 nM of dopamine evoked by a single stimulus (600 ms) in the striatum of a mouse brain slice with a peak time of 130 ms after stimulus. However, the same stimulus resulted in a release of 50 nM in the ventral tegmental area (brain slice) with a 180 ms peak time. [143] The release of DA depends on the stimulation parameters. The concentration of DA is a function of pulse frequency and duration. [142]

Recent advances have allowed to directly measure basal levels of DA using electrochemical techniques. Wightman’s group used a convolution procedure for prediction and removal of non-faradaic contributions to the background current in FSCV [144]. These experiments resulted in an estimate of basal dopamine levels of 41 ± 13 nM in the NA of rats. Certain surface redox active species such as quinone groups and other oxygen functional groups formed during potential cycling introduce some error in the background subtraction and remain a challenge. To overcome this, specific holding potentials can be used to avoid oxidation of surface oxygen groups, the carbon electrode can be selected to minimize capacitive charging currents and the measurement parameters such as scan rate and modulation of waveform application frequencies can be varied to increase sensitivity [144].

Heien’s group showed that fast-scan controlled-adsorption voltammetry (FSCAV) can be used to directly measure basal concentrations of DA in vivo [145]. This technique applies first a triangular waveform (−0.4 V to 1.3 V at 1200 V/s) for 2 seconds, then holds the electrode at a negative potential (-0.4 V) for 10 seconds to allow DA to adsorb to the electrode surface until equilibrium is reached, and lastly re-applies the triangular waveform to measure the adsorbed dopamine concentration. Recently, they have also used FSCAV to measure DA levels in the monkey ventral tegmental area and the monkey nucleus accumbens [145].
DA. The background current is removed using convolution theory, as in the basal measurements by Wightman’s group, which provides more accurate results than a simple background subtraction. Atcherley et al. obtained a basal DA concentration of $90 \pm 9$ nM in mouse nucleus accumbens with FSCAV [145].

### 4.3 Oxidation Mechanism of Dopamine

Dopamine oxidizes in a $2e^- \cdot 2H^+$ transfer process to form dopamine quinone (DAQ) as shown in Figure 10. The order of electron and proton transfer is pH-dependent and has been proposed to follow the scheme of squares. [146-148] This model has been applied to interpret the electrochemical properties of quinone/hydroquinone redox systems in general. This model is based on the assumptions that protonations are at equilibrium and that the electron transfer step is rate-determining. According to the model, the reaction pathway depends heavily on the acid dissociation constants ($pK_a$) values of the quinone species and the pH of the electrochemical environment that will affect the starting point of the oxidation process. At neutral pH, Laviron proposed a $H^+ \cdot e^- \cdot H^+ \cdot e^-$ transfer sequence for hydroquinone at Pt electrode [146] and Deakin proposed the same sequence for methylcatechol, 3,4-dihydroxybenzylamine (DHBA) and 3,4-dihydroxyphenylacetic acid (DOPAC) at carbon paste electrode [147, 148].

![Figure 10. Oxidation pathway of a neutral dopamine molecule.](image)

After the first oxidation step, DAQ undergoes an intramolecular cyclization step by 1,4-Michael addition with its amine side chain to form leucodopaminechrome (LDAC), which can further oxidize to dopaminechrome (DAC) as shown in Figure 10. Thus, the overall reaction pathway can be defined as following an ECE mechanism that has been proposed for DA and other catecholamines at carbon, Pt and Au electrodes [149-151]. An ECC mechanism, in which LDAC is reduced by DAQ to form DAC has also been suggested [149, 152].
Table 12. In vivo measurements of basal and transient levels of DA using different detection methods.

<table>
<thead>
<tr>
<th>Method</th>
<th>Estimated DA (nM)</th>
<th>Brain area (animal)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>Transient (stimulus)</td>
<td></td>
</tr>
<tr>
<td>Microdialysis</td>
<td>30</td>
<td>caudate nucleus (rat)</td>
<td>[113]</td>
</tr>
<tr>
<td>Microdialysis</td>
<td>4</td>
<td>nucleus accumbens (rat)</td>
<td>[121]</td>
</tr>
<tr>
<td>Microdialysis</td>
<td>10-15</td>
<td>striatum (rat)</td>
<td>[122]</td>
</tr>
<tr>
<td>Microdialysis</td>
<td>20-30</td>
<td>striatum (rat)</td>
<td>[123]</td>
</tr>
<tr>
<td>Microdialysis</td>
<td>10</td>
<td>nucleus accumbens (rat)</td>
<td>[124]</td>
</tr>
<tr>
<td>Scintillation spectroscopy</td>
<td>40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>striatum (mouse)</td>
<td>[132]</td>
</tr>
<tr>
<td>Imaging (SPECT)</td>
<td>60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>striatum (mouse)</td>
<td>[131]</td>
</tr>
<tr>
<td>Imaging (PET)</td>
<td>45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>brain (human)</td>
<td>[125]</td>
</tr>
<tr>
<td>Electrochemical (DNPV, carbon fiber)</td>
<td>8-12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>striatum (baboon)</td>
<td>[133]</td>
</tr>
<tr>
<td>Electrochemical (FSCV, carbon fiber)</td>
<td>15-25</td>
<td>striatum (rat)</td>
<td>[114]</td>
</tr>
<tr>
<td>Electrochemical (FSCAV)</td>
<td>41</td>
<td>Nucleus accumbens (rat)</td>
<td>[144]</td>
</tr>
<tr>
<td>Electrochemical (FSCAV)</td>
<td>90</td>
<td>Nucleus accumbens (mouse)</td>
<td>[145]</td>
</tr>
<tr>
<td>Electrochemical (FSCV, carbon fiber+Nafion)</td>
<td>6 200&lt;sup&gt;a&lt;/sup&gt; (electrical)</td>
<td>striatum (rat)</td>
<td>[142]</td>
</tr>
<tr>
<td>Electrochemical (amperometry, carbon fiber)</td>
<td>30 120 (electrical)</td>
<td>caudate putamen (rat)</td>
<td>[141]</td>
</tr>
<tr>
<td>Electrochemical (FSCV, carbon fiber)</td>
<td>- 200-500 (sexual behavior)</td>
<td>nucleus accumbens (rat)</td>
<td>[135]</td>
</tr>
<tr>
<td>Electrochemical (FSCV, carbon fiber)</td>
<td>- 240 (conspecific interaction)</td>
<td>striatum (rat)</td>
<td>[117]</td>
</tr>
<tr>
<td>Electrochemical (amperometry, BDD)</td>
<td>- &lt;100 (reward response)</td>
<td>striatum (monkey)</td>
<td>[136]</td>
</tr>
</tbody>
</table>

DNPV = differential normal pulse voltammetry, FSCAV = fast-scan controlled-adsorption voltammetry
<sup>a</sup> synaptic concentration

It is well established that DAC may undergo further reactions to form a melanin-like polymer film known as polydopamine. Self-polymerization of DA to form polydopamine films has been shown to occur at the surface of a wide range of materials in alkaline solutions. [153] The formation of this film also severely passivates the electrode surface resulting in a loss of DA signal in vitro as seen for example in publication IV for ta-C and GC.

It should be noted that the adsorption of DA on the surface of the electrode is necessary for the oxidation to occur. Duvall et al. showed that the oxidation of DA is completely inhibited by a monolayer of triphenylmethylphenyl groups adsorbed on GC, while it had minor effects on the oxidation of an outer sphere redox probe such as Ru(NH₃)₆²⁺/³⁺ [95, 96].

In publication IV, we investigated the electrochemical behaviour of DA and two structurally related compounds, catechol and 4-methylcatechol, at ta-C electrodes in different pH solu-
tions. We observed that the oxidation of DA is quasireversible in neutral phosphate buffer solution (PBS, pH 7.2) as can be seen in Figure 11 A and B. The main redox peaks corresponding to the DA/DAQ pair are accompanied by the appearance of the secondary redox peaks at lower potential. This is in agreement with reaction pathway of DA described above. The second redox peaks are assigned to the LDAC/DAC pair. The low $I_{red}/I_{ox}$ ratio also indicates that a coupled homogeneous reaction occurs. Comparing the CVs at 50 mV/s and 500 mV/s, the magnitude of the DAQ reduction wave is more significant at higher scan rate. This is because the scan rate is comparable to the reaction rate of the intracyclization step and only little DAQ is consumed before it is reduced back to DA.

Table 13. Electrochemical data for CA, MC and DA at ta-C and GC electrodes. Data taken from CV scans at 50 mV/s in PBS (pH 7.2). Slopes are from measurements at ta-C only.

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Slope $\log(I_{ox})$ vs $\log(\nu)$</th>
<th>ta-C $E_{ox}$ vs pH</th>
<th>$\Delta E_p$</th>
<th>$I_{red}/I_{ox}$</th>
<th>GC $\Delta E_p$</th>
<th>$I_{red}/I_{ox}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA</td>
<td>0.50</td>
<td>-0.060</td>
<td>416</td>
<td>0.78</td>
<td>92</td>
<td>0.96</td>
</tr>
<tr>
<td>MC</td>
<td>0.48</td>
<td>-0.057</td>
<td>336</td>
<td>0.80</td>
<td>78</td>
<td>0.98</td>
</tr>
<tr>
<td>DA</td>
<td>0.47</td>
<td>-0.060</td>
<td>369</td>
<td>0.11</td>
<td>62</td>
<td>0.65</td>
</tr>
</tbody>
</table>

At low pH, the redox peaks of LDAC/DAC are not observed as can be seen from Figure 11C. This is because the amine side chain of dopamine is deprotonated, which prevents the intracyclization reaction from happening [149, 150]. This also prevents or considerably slows down the formation of reaction products that block the ta-C surface, since the peak currents do not decrease significantly. Nevertheless, the $I_{red}/I_{ox}$ ratio was around 0.60 for scan rates ranging from 10 to 500 mV/s, indicating that some coupled chemical reaction occurs. It is likely a hydroxylation reaction caused by nucleophilic attack of H$_2$O that has been observed for CA and MC [154, 155] and proposed also for DA [152].

It can also be seen in the 10 consecutive CVs in Figure 11A and B, that the peak currents strongly decrease while the peak potential separation increases. This implicates severe surface fouling that passivates the electrodes. This was observed in particular for slow scan rates, where a large proportion of DAQ undergoes the intracyclization reaction leading to the formation of polydopamine. We confirmed by XPS an increase in nitrogen content from 1 to over 6 at.% for ta-C samples that had been cycled in 1 mM DA compared to unused samples. The nitrogen was attributed to amine side chain of DA oxidation products that polymerized and adhered on ta-C, since the solution does not contain this element. We also observed that CA and MC strongly passivate the ta-C surface in neutral and alkaline solutions and attributed this to formation of a polymer layer as in the case of DA. It is known that the o-quinone formed by catechol oxidation can undergo dimerization and subsequent polymerization reactions to form a polycatechol film similar to polydopamine [156] and it has been observed at several electrodes including GC [157], Au [158] and BDD [159]. It is expected that MC also polymerizes in the same way forming a passivating polymer film [154, 160].

Some of the electrochemical trends observed for the studied molecules is summarized in Table 13. The plots of oxidation peak potential $E_{ox}$ vs pH gave slopes that are very close to -59 mV/pH for all the studied molecules, which is consistent with a $2e^- \cdot 2H^+$ transfer process at neutral pH. The slopes of $\log(I_{ox})$ vs $\log(\nu)$ gave values that are very close 0.50, indicating a diffusion-controlled process whereas an adsorption-controlled process would theoretically give a value of 1.
The electron transfer kinetics were faster at GC than at ta-C electrodes as can be observed from the $\Delta E_p$ (Table 13) values and this was the case for CA, MC and DA at all pH values studied in publication IV. In addition, looking at the CVs in Figure 11 and the ratio of $I_{red}/I_{ox}$, GC was less susceptible to fouling than ta-C.

Figure 11. CVs of 1 mM DA in PBS (pH 7.2) at ta-C electrodes using a scan rate of A) 50mV/s and B) 500 mV/s compared to C) GC electrode in the same solution (50 mV/s). D) 1 mM DA in H2SO4 (pH 0.65) at ta-C electrode (50 mV/s).

### 4.4 Ascorbic Acid

#### Functions in the body

Ascorbic acid (also known as vitamin C) is an important antioxidant in the human brain. At physiological pH, it exists as a hydrophilic anion, often referred to as ascorbate [161, 162]. In addition to its neuroprotective role as an antioxidant, AA partakes in many other biochemical functions as well. It is used as a cofactor in enzymatic reactions such as the synthesis of catecholamine neurotransmitters [161, 163, 164] and in the production of collagen [161, 162]. AA has also been linked to neural maturation (differentiation of stem cells to neurons) and neurotransmission of dopamine and glutamate. Since oxidative stress is strongly related to the development of neurodegenerative diseases such as Parkinson’s, Alzheimer’s and Huntington’s, its role as a potential therapeutic agent has also been investigated [162].

In all its bodily functions, ascorbic acid acts as a one-electron donor forming the ascorbate radical (or semidehydroascorbic acid) [162]. As an antioxidant, it is able to reduce reactive
oxygen and nitrogen species including hydroxyl, alkoxy, peroxyl, thyl and oxosulphuric radicals generated during cellular metabolism to less harmful species. Following the first electron transfer, the ascorbate radical can further oxidizes to dehydroascorbic acid (DHA). Both oxidation states can be reduced back to the original form by reducing agents such as nicotinamide dinucleotide (NADH) or glutathione (GSH). Semidehydroascorbic acid is quite stable, whereas DHA decomposes to diketogulonic acid by hydrolysis and then to oxalic and L-threonic acids. Ascorbic acid can also regenerate other antioxidants such as tocopherol and uric acid to their reduced forms. [161-164] The interaction between AA and UA has implications in the detection of DA, when all three are measured simultaneously in a ternary solution as will be seen in publication V.

**In vivo concentration**

The concentration of AA in the brain varies depending on brain region. Post mortem measurements in humans have shown that the highest levels of AA are found in the nucleus accumbens and hippocampus and lowest in substantia nigra. [161] It has been estimated that the concentration inside neurons is about 10 mM and the overall concentration of brain tissue 1-3 mM [161, 165]. The concentration outside of the neurons in the ECF and CSF is lower. Measurements using CV with carbon fiber microelectrodes in anaesthetized rats have given an ascorbate concentration of 500 μM in CSF and 200-400 μM in ECF (260-300 μM in cortex, 220 μM in striatum and 380 μM in corpus callosum). [166] Miele and Fillenz measured the ECF concentration of ascorbate in the rat striatum and obtained a value of 416 ±66 μM using a combination of microdialysis and amperometry with a carbon paste electrode [167]. Lönnrot et al. obtained a concentration of 197 ± 33 μM in human CSF extracted by lumbar puncture and analyzed with HPLC [168]. This value was close to the value obtained by Bowman et al. from human patients with mild to moderate Alzheimer’s disease (129 μM) [169] and Reiber et al. from CSF in healthy patients (158 μM) [170]. Mefford et al. carried out a comprehensive study of ascorbate levels in the human brain with liquid chromatography coupled with electrochemical detection. The group found out that depending on patient and brain area, the concentrations vary between 50 to 276 μg/g of wet tissue, corresponding to about 300 to 1600 μM. No correlation between catecholamine-rich and AA-rich regions was found but on gross tissue basis, the concentration of AA is 2-3 orders of magnitude greater than that of catecholamines everywhere in the brain. [171]

**Electrochemical oxidation of AA**

Two pathways have been proposed for the electrochemical oxidation of ascorbic acid at neutral pH, namely the direct oxidation pathway observed at most electrode materials and the dissociative adsorption pathway observed solely at Pt electrodes.

In the direct oxidation pathway (Figure 12), AA is deprotonated to an ascorbate monoanion at neutral pH (pKa1 = 4.1). Ascorbate is then oxidized in a reversible 2e⁻ 1H⁺ transfer process to dehydroascorbic acid (DHA) followed by an irreversible chemical reaction to 2,3-diketogulonic acid (DKG). The first step in the oxidation process is a 1e⁻ 1H⁺ oxidation to form a radical anion that then undergoes in a second step a 1e⁻ oxidation to form DHA. [172-178] Finally, DHA undergoes an irreversible chemical reaction by hydrolysis that gives DKG. [176, 179] It is to be
noted that DHA is generally found in solution in its bicyclic form after an intermediate hydrolysis step, which is omitted from Figure 12 giving only the final product, DKG [174, 178]. Early literature did not address the order of the electron and proton transfers in the first oxidation step of ascorbate, but more recently it has been proposed that the electron transfer happens first, giving an overall ECE mechanism before the irreversible chemical step to DKG [147, 180]. Ruiz et al. suggested that the electron transfer steps are reversible and the rate-determining step is the irreversible chemical reaction following charge transfer [174], whereas Prieto et al. suggested that the second proton transfer step is rate determining [180]. Perone & Kretlow observed the reduction wave of DHA at scan rates over 100 V/s at hanging mercury drop electrodes (HMDE) in neutral phosphate buffer (pH 7.2), adding further evidence to the reversibility of the electron transfer steps. The peak potential separation value of 30 mV indicated that the electron transfer is reversible. The first-order rate constant for the chemical reaction was calculated to be $1.39 \times 10^3 \text{s}^{-1}$ by CV. [181]

![Figure 12. Direct oxidation pathway of ascorbic acid at neutral pH.](image)

The dissociative adsorption pathway involves, as the name suggests, the adsorption of AA before it is oxidized. The adsorption leads to the formation of CO on the Pt surface, which is thought to block the active surface sites resulting in the high overpotential of AA oxidation at Pt compared to other electrode materials [182, 183]. The adsorption is sensitive to the Pt surface structure and is dependent on potential at neutral pH. [182] The dissociative adsorption of AA is followed by the direct oxidation of AA yielding DKG as the main oxidation product as described above in the direct oxidation pathway (Figure 12). However, the adsorbed CO also undergoes oxidation leading to the formation of CO$_2$ as observed in in situ Fourier transform infrared spectroscopy (FTIR) and Fourier transform infrared reflection-absorption spectroscopy (FTIRRAS) [176, 183]. Xing et al. argued that the dissociative adsorption takes place via an interaction between the side-chain of the AA molecule and the Pt surface [176] Climent et
al. also proposed the possibility that the oxidation products of AA or residues of the AA dissociative adsorption can also undergo oxidation, producing CO₂. [183]

While the direct oxidation pathway has been reported at Hg [174], Au [173], Pt [175] and GC [177] electrodes, the dissociative adsorption pathway has only been observed at Pt electrodes [176, 182, 183]. Xing et al. reported that the oxidation of AA is faster on single-crystal Au faces than Pt, because no dissociative adsorption occurs and the electron transfer proceeds without any surface blocking. They observed, however, a second oxidation peak assigned to the oxidation of DHA (or a species following the hydrolysis) that is structure-sensitive and depends strongly on the anionic species of the electrolyte. [184] Climent et al. observed that an adsorbed Bi layer on Pt catalyzes AA oxidation. The authors suggested that a high surface coverage of Bi suppresses the dissociative adsorption in favour of the direct electrochemical oxidation of AA. This was confirmed by a lower oxidation potential (300 mV cathodic shift) and slightly increased peak current compared to an unmodified Pt electrode. [183] Takamura & Sakamoto also showed that metal ions such as Bi³⁺, Pb²⁺, Hg²⁺, Ti⁺, Ag⁺ and Sb³⁺ adsorbed on Pt catalyze the oxidation of AA by preventing AA adsorption on the surface. However, not all adsorbed metal ions catalyzed the reaction (Cu, Cd, In, Sn and Mo). They also found that the extent of surface coverage affected the oxidation potential and was lowest at a submonolayer coverage. [185]

4.5 Uric acid

Functions in the body

Uric acid (UA) is a cellular waste product formed by the oxidation of purine derivatives hypoxanthine and xanthine. UA plays an important role along AA as an antioxidant to protect the body from oxidative stress, which has been linked to several neurodegenerative disorders. It has been suggested that plasma and CSF UA levels may modulate the outcome of neurologic diseases such Alzheimer’s disease [169], Parkinson’s disease [186] and multiple sclerosis [187]. At physiological pH, UA exists as the urate monoanion (pKₐ = 5.4) that is an effective oxygen scavenger and prevents lipid peroxidation (leading to cell membrane damage and formation of carcinogenic or mutagenic compounds [188]) by readily reacting with reactive oxygen species such as hydroxyl radicals, hydrogen peroxide and peroxyl radicals [163, 164, 189]. In the scavenging reaction, urate is oxidized in a 1e⁻ process to a urate free radical that has been observed by electron spin resonance (ESR) and that can be reduced back to urate by ascorbate [164, 190, 191]. Another important antioxidant effect of urate is its ability to chelate (i.e. form stable coordination complexes with) metal ions such as copper and iron, effectively preventing the oxidation of ascorbate and inhibiting lipid peroxidation [192]. This reciprocal protection and regeneration between urate and ascorbate indicates a possible synergy between the two compounds to maintain antioxidant capacity in the body [169, 190, 192].

In vivo concentration

The concentration of uric acid in the CSF is around 20 μM in healthy controls and about 2 times higher in patients with neurological diseases such as MS, myelopathy, stroke, epilepsy and viral meningitis. [193] This agrees well with the CSF concentrations measured by Kuracka et al. in a similar study comparing controls and patients with different neurological disorders.
Dopamine and Its Electrochemical Detection

Reiber et al. reported a CSF UA concentration of 26 μM but found smaller average concentrations in patients with MS (15 μM) [170]. Bowman et al. measured a concentration of 17.7 μM in human CSF extracted by lumbar puncture from patients with mild to moderate Alzheimer’s disease and analyzed with HPLC coupled to electrochemical detection [169]. Other reported values for the UA CSF levels are 17.25 μM [194] and 29 μM [168] for healthy adults, which are in agreement with the previous results.

**Electrochemical oxidation**

The electrochemical oxidation mechanism of UA has been investigated at graphite and gold electrodes by cyclic voltammetry, double potential step chronoamperometry and thin-layer spectroelectrochemistry [195-197]. At neutral pH, UA exists as the urate monoanion that undergoes a 2e⁻ 2H⁺ oxidation to an unstable and readily reducible anionic diimine species as shown in Figure 13. The diimine species can be observed by cyclic voltammetry at very fast scan rates (typically > 1-20 V/s), but the rate depends on the electrode material. Owens et al. suggested that appearance and magnitude of the reduction peak is related to the electrode surface roughness and thus to the availability of adsorption sites. [196]

![Figure 13. The electrochemical oxidation pathway of uric acid at neutral pH.](image)

The diimine species is then attacked by water to form an anionic imine alcohol that is further hydrated to eventually yield allantoin and a small amount of urea as the end products at neutral pH. It has been proposed that the imine alcohol can also undergo a 2e⁻ 2H⁺ reduction before further chemical reactions, which has been observed in cyclic voltammograms at graphite electrodes. The last chemical reactions following the formation of imine alcohol are not well known but the final products have been identified and several mechanisms have been proposed for their formation depending on pH and phosphate (H₂PO₄⁻) concentration [197, 198] At low pH,
alloxan and urea are formed, whereas at neutral pH allantoin and urea are the main products. At intermediate pH a mixture of the allantoin and alloxan is produced.

### 4.6 Other Interferents

**DOPAC**

DOPAC (3,4-dihydroxyphenylacetic acid) is a DA metabolite. DOPAC is an anion at physiological pH and can be discriminated with a negatively charged ion-exchange membrane such as Nafion. [113, 115] Gonon et al. have measured the concentration of DOPAC in rat neostratium to be $22 \pm 4 \, \mu M$ (mean ± standard deviation, $N = 33$) using DPV with electrochemically pre-treated 8 μm thick carbon fiber electrodes implanted in anaesthetized rats. [199] Parsons & Justice reported a concentration of 5.7 μM in the NA of rats [121] and Smith et al. a concentration of 17-30 μM in the striatum of rats [122] using microdialysis in both cases. Abercrombie et al. obtained average values of 3 μM, 7 μM and 0.5 μM in the ECF of the rat striatum, nucleus accumbens and frontal cortex, respectively, measured by microdialysis and HPLC [200]. These values are 2-3 orders of magnitude higher than the basal concentrations of DA in the same brain areas.

As with other substituted catechols that we studied in publication IV, the oxidation mechanism of DOPAC follows an EC mechanism in which it oxidizes in a 2e⁻ 2H⁺ process yielding an o-quinone. Deakin has proposed that the electron and proton transfer sequence at neutral pH is $H^+, e^-, H^+, e^-$, according to the scheme of squares [147, 148]. The o-quinone may then undergo a dimerization [156] or hydroxylation reaction [154] like was observed for catechol and methylcatechol in publication IV.

**Homovanillic acid**

HVA is another DA metabolite found in the central nervous system. Smith et al. measured the average extracellular concentration of HVA to be 26.6 μM in rat striatum using microdialysis [122], whereas Abercrombie et al. measured a concentration of 2 μM in the same area. Abercrombie et al. also measured the average ECF concentration to be 4 μM in the nucleus accumbens and 0.6 μM in the frontal cortex of rats by microdialysis and HPLC. [200]

The concentration of HVA seems to be lower in CSF than in ECF, since Blennow et al. reported a concentration gradient from 75 to 450 nM in CSF of humans taken with lumbar puncture and analyzed with gas chromatography mass spectrometry and HPLC [201]. Holst et al. reported values of 170 nM and 770 nM in lumbar and central CSF, respectively, of control patients by means of mass fragmentography [202].

At neutral pH, the carboxylic acid group is deprotonated ($pK_a = 3.4$) and the oxidation of HVA proceeds through a 2e⁻ 1H⁺ oxidation followed by decarboxylation and attack by water to form vanillin or vanillic alcohol. Vanillin is then further oxidized in a similar 2e⁻ 1H⁺ process to yield a product that is hydrolyzed leading to the loss of methanol and formation of the substituted o-quinone species. [203, 204] The voltammetric features of the o-quinone may be seen on the reverse and subsequent scans [203]. This oxidation process is similar to that of substituted methoxyphenols reported in literature [203, 205].
Table 14. Comparison of DA and its main interferents. The concentrations refer to in vivo concentration measured by several methods (see section 4.2). Charge of the molecule at physiological pH. Oxidation potential measured in publication V using CV (100 μM DA, 1 mM AA and 50 μM UA, separately measured in PBS pH 7.2, 50 mV/s).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (μM)</th>
<th>Charge</th>
<th>$E_{pa}$ (V vs Ag/AgCl) 50 nm ta-C</th>
<th>$E_{pa}$ (V vs Ag/AgCl) 50 nm ta-C+CNT</th>
</tr>
</thead>
<tbody>
<tr>
<td>DA</td>
<td>0.005-0.5a</td>
<td>+</td>
<td>0.38</td>
<td>0.17</td>
</tr>
<tr>
<td>AA</td>
<td>130-500</td>
<td>-</td>
<td>0.67</td>
<td>0.00</td>
</tr>
<tr>
<td>UA</td>
<td>17-30</td>
<td>-</td>
<td>0.64</td>
<td>0.37</td>
</tr>
<tr>
<td>DOPAC</td>
<td>3-30</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HVA</td>
<td>1-25</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a basal concentrations are 0.005-0.060 μM and transient concentrations 0.1-0.5 μM

4.7 Sensitive and Selective Detection of Dopamine

In publication V, we investigated the modification of ta-C with multi-walled carbon nanotubes (MWCNTs) for fabricating a hybrid carbon electrode as a potential in vivo sensor for the detection of DA in the presence of AA and UA at physiological concentrations. We compared ta-C thin films of 15 and 50 nm, because the thickness affects the electrochemical properties of the electrodes as was shown in publication III. MWCNTs were grown on both type of ta-C films for comparison. The biocompatibility of ta-C and ta-C+CNT electrodes was assessed with cell cultures of mouse neural stem cells (mNSCs).

The ta-C electrodes were modified with carbon nanotubes, because of their unique properties such as high aspect ratio, conductivity, thermal stability, flexibility, mechanical strength and reactivity. Carbon nanotubes have been shown to confer very high sensitivity and selectivity towards different analytes [39, 206, 207] and thus they present an interesting material for the surface modification of ta-C. Our initial idea was to combine an inert, biocompatible substrate with a low background current such as ta-C with a highly reactive carbon allotrope to combine their best properties.

Individual nanotubes have metallic or semiconducting electronic properties depending on the number of carbon hexagons around the circumference of the tube. [208] Most electrochemical applications using nanotubes involve large numbers of tubes with different diameters and DOS distribution, so the effective DOS will be broadened by the combination of many different DOS profiles [10]. Banks et al. have shown that the edge-plane like sites at the open tube ends are responsible for the electrocatalytic properties of CNTs. [209] However, it is debated whether the electrochemical reactivity of CNTs is determined by the special properties of the tubes themselves, the amount of oxides and defects, graphitic impurities or metal catalysts that were not completely removed after synthesis. [10, 210, 211]

We modified 15 and 50 nm thick ta-C films with MWCNTs by directly growing them on ta-C by a chemical vapour deposition (CVD) process with a multilayer catalyst film of 0.2 nm Al, 3 nm Co and 3 nm Fe. The SEM images (Figure 14A and B) showed that a mostly uniform mesh of intertwined and several micrometers long MWCNTs had grown on both 15 and 50 nm ta-C films. The diameter of the MWCNTs was 20–40 nm.
A few clear differences were observed in the cross-sectional TEM micrographs shown in Figure 14C and D. The Ti interlayer was intact and around 25 nm thick in the 15 nm ta-C+CNT sample. The ta-C film was completely consumed in the growth process and the MWCNTs had grown directly from the Ti interlayer. In the 50 nm ta-C+CNT sample, the Ti interlayer was continuous but thinner (around 15 nm) and a 50–60 nm thick disordered graphite-like carbon layer was observed instead of the ta-C film. This carbon layer contained MWCNT-type structures and numerous metal nanoparticles that were confirmed with EDS to be a mixture of Fe and Co catalysts. Both samples showed dark regions in the interfacial region that were identified as Fe silicides with dissolved Co (indicated as (FeCo)Si in the micrographs), but these areas were more numerous in the 50 nm ta-C+CNT sample. In both samples, metal nanoparticles were observed also at the end of the MWCNTs. The relatively high $I_D/I_G$ ratios observed by Raman spectroscopy indicated that the MWCNTs were highly defective.

The sensitivity and limit of detection (LOD) were determined using CV, since its high temporal resolution makes it particularly well suited for in vivo measurements as was discussed previously. The linear range, sensitivity and LOD values are tabulated in Table 15 and the corresponding CVs and calibration curves for 50 nm ta-C and 50 nm ta-C+CNT are shown in Figure 15A and B, respectively. The ta-C electrodes had a higher sensitivity than the MWCNT-modified electrodes, but the values of LOD were better for the electrodes with 50 nm ta-C than 15 nm. The different morphology and surface chemistry of ta-C and MWCNTs may explain the difference in sensitivity, since these properties play a key role in the adsorption and oxidation of DA. In particular, the adsorption of DA to the electrode surface is necessary before the oxidation process initiates [95, 96]. Even if the MWCNTs offer a higher surface area compared to bare ta-C, we see from the magnitude of the current responses that the electrochemically active surface area is actually smaller at nanotubes.
The limit of detection was calculated using the equation: 
\[
\text{LOD} = 3.3 \times \frac{\sigma}{S}
\]
(where \(\sigma\) is the standard deviation of the blank CVs and \(S\) the sensitivity). The 50 nm ta-C and 50 nm ta-C+CNT electrodes had a lower LOD compared to the electrodes with 15 nm ta-C. This was attributed to the 50 nm ta-C film that has a lower lower double layer capacitance than the 15 nm ta-C due to its higher fraction of sp\(^3\)-bonded. This improved the signal-to-noise ratio. In addition, the standard deviation of the blank CVs was smaller at the 50 nm ta-C film, which improved the calculated LOD. The carbon layer that was formed at interface between Ti and MWCNTs after the CVD process could play a similar role in lowering the background current as the thicker ta-C film.

In a ternary solution containing 1 mM AA, 50 \(\mu\)M UA and varying amount of DA, only one oxidation peak is observed at the 15 and 50 nm ta-C electrodes in Figure 15C. Looking at the separate CVs produced by each analyte separately, it is easy to see that their current responses overlap. However, at the MWCNT-modified electrodes, three clear peaks are seen in the ternary solution corresponding to the oxidation of each analyte. The oxidation potentials of each analyte are given in Table 14 for the 50 nm ta-C and ta-C+CNT electrodes. The modification with MWCNTs shifts the oxidation peak of AA from 0.67 V at bare ta-C to 0.0 V (vs Ag/AgCl) at the modified electrode, enabling the simultaneous detection of each analyte.
The shift in the oxidation potential of AA is widely reported in literature and has been observed at most modified electrodes that can simultaneously detect DA, AA and UA using CV [212-215]. This phenomenon is generally attributed to the catalytic properties of the modifying compound, or in the case of carbon allotropes to the edge planes and defective sites. More recently, several authors have shown that metal nanoparticles used in the growth of many carbon nanomaterials and nanographitic impurities due to the growth process are actually the cause of the catalytic effect [210, 211, 216]. Furthermore, when the modified electrode contains a porous layer of conductive material, a change in diffusion regime from planar to thin film diffusion can occur leading to a shift in oxidation potential. This was demonstrated computationally and experimentally by Henstridge et al., who used a MWCNT-modified GC electrode. [217] The oxidation peak potential shifted in the cathodic direction while the reduction peak potential shifted in the opposite direction decreasing the peak-to-peak separation. However, thin layer diffusion is indistinguishable from peak-to-peak separation caused by adsorption, which was also noted by the authors. [217]

Table 15. Comparison of sensor performance of ta-C and ta-C+CNT electrodes. Sensitivity and LOD values were calculated from CVs (shown in Figure 15 for 50 nm ta-C and 50 nm ta-C+CNT).

<table>
<thead>
<tr>
<th>Electrode</th>
<th>Linear range (μM)</th>
<th>Sensitivity (A M⁻¹ cm⁻²)</th>
<th>LOD (nM)</th>
<th>Selectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 nm ta-C</td>
<td>0.01-100</td>
<td>0.28</td>
<td>84.3 ± 14</td>
<td>no</td>
</tr>
<tr>
<td>50 nm ta-C</td>
<td>0.01-100</td>
<td>0.52</td>
<td>39.8 ± 5.9</td>
<td>no</td>
</tr>
<tr>
<td>15 nm ta-C+CNT</td>
<td>0.01-5</td>
<td>0.08</td>
<td>76.7 ± 4.6</td>
<td>AA+UA+DA</td>
</tr>
<tr>
<td>50 nm ta-C+CNT</td>
<td>0.05-10</td>
<td>0.07</td>
<td>41.4 ± 0.4</td>
<td>AA+UA+DA</td>
</tr>
</tbody>
</table>

In our case, we observed that cycling to high enough a potential or a HNO₃-pretreatment was necessary to induce the lowering of the AA oxidation peak. In a 1 mM AA solution, as the upper potential was slowly shifted to the oxygen evolution region, the oxidation peak of AA gradually shifted to lower potential. In our opinion, this indicates a change in surface chemistry or structure rather than a change in diffusion regime. It has been shown that HNO₃-treatment of CNTs results in the increase of oxygen functional groups and induces defects that affect particularly the oxidation of AA [218]. Similar chemical and structural changes have been observed by Pumera et al. after electrochemical oxidation of CNTs [219]. However, we cannot rule out the effect of metal nanoparticles in the oxidation reaction, since catalyst films of Al, Co and Fe were used as in the MWCNT growth process.

Tetrahedral amorphous carbon films exhibit good biocompatibility [220] and thus the MWCNT-modified films were compared to them. Mouse neural stem cells (mNSCs) cultured on the films showed a typical morphology with no major differences between ta-C and ta-C+CNT samples. Cell viability determined from fluorescence images indicated no statistically significant differences in cell count. Therefore, both ta-C and ta-C+CNT films exhibited good biocompatibility for mNSCs.

The biocompatibility and electrochemical results showed that ta-C and ta-C+CNT electrodes have potential as in vivo sensor materials. Their detection limit is in the physiologically meaningful range to detect basal and transient concentrations. The modification with MWCNTs did not improve LOD, which was more dependent on the substrate material, but did confer the necessary selectivity to simultaneously detect DA, AA and UA.
5. Summary

The main objectives in this thesis were to 1) deepen the knowledge on the electrochemical properties of a-C and ta-C, and 2) to fabricate an amorphous carbon based sensor for the sensitive and selective detection of dopamine. For these purposes, we deposited several types of a-C and ta-C thin films using CFUBMS and FCVA, respectively, and carried out detailed physical, chemical and electrochemical characterization of the films. We also investigated the oxygenation of a-C and MWCNT-modification of ta-C and compared their electrochemical properties to as deposited films.

The physical and electrochemical properties of a-C and ta-C could be heavily varied by adjusting the deposition parameters to obtain thin films with a wide range of properties. The undoped a-C and ta-C films showed wide potential windows, low background currents and low double-layer capacitance values but still had relatively fast electron transfer kinetics for several OSR and ISR probes. The electrochemical properties of ta-C were very close to those of diamond films due to the high sp³ fraction, whereas those of a-C were closer to graphitic materials due to the significant sp² fraction.

The a-C films deposited by CFUBMS resulted in conductive films with good electrical properties, but in the case of highly oxygenated films, the conductivity decreased significantly. Both oxygenation and variation of power density of a-C films affected the oxidation of DA and AA. In the case of oxygenated a-C films, the surface oxygen functional groups played a role in the oxidation of DA and AA, but as the amount of oxygen increased, it became increasingly difficult to make a distinction between the effect of surface chemistry and increase in electrical resistance on electron transfer. The increasing peak potential separation of DA at highly oxygenated films was assigned to the poor electron transport through the films. In the case of a-C thin films deposited with increasing power density, the clustering of the sp² phase and corresponding changes in DOS correlated with the decreasing oxidation potential of DA and AA. The observed electrochemical behavior of AA and DA still need to be further investigated to better understand the underlying causes, but in the future, it could be possible to tailor-make a-C thin films with a specific set of properties for a specific application such as the detection of dopamine or other biomolecules.

The electrochemical properties of ta-C were dependent on film thickness. Our results indicated that an increase in ta-C film thickness leads to a decrease in the sp² fraction and increase in the mobility gap. This correlated with the increase in charge transfer resistance observed by EIS and increase in peak potential separation for OSR probes as the film thickness grew from 7 to 100 nm. We proposed that this behavior is related to the electron transport through the film as opposed to electron transfer from a solution species to the surface of ta-C. In addition, the inclusion of Ti interlayer at the Si/ta-C interface lowered charge transfer resistance, which provided further support to the idea that the overall reaction kinetics of OSR probes are controlled by the electron transport through the ta-C films.
Finally, we demonstrated the applicability of highly sp³-bonded ta-C in electroanalytical applications by detecting physiologically relevant dopamine concentrations (40-85 nM) using cyclic voltammetry. The modification of ta-C with MWCNTs conferred the electrode the necessary selectivity to detect dopamine in the presence of its major interferents, ascorbic acid and uric acid, at physiological concentrations without significantly changing the limit of detection. Both the ta-C and the modified electrodes showed good biocompatibility, further emphasizing their potential as in vivo sensor materials.
References


References


