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Glutamate detection by amino functionalized tetrahedral amorphous carbon surfaces

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Abstract

In this paper, a novel amperometric glutamate biosensor with glutamate oxidase (GlOx) immobilized directly on NH$_2$ functionalized, platinum doped tetrahedral amorphous carbon (ta-C) film, has been successfully developed. First, we demonstrate that direct GlOx immobilization is more effective on amino-groups than on carboxyl- or hydroxyl-groups. Second, we show that anodizing and plasma treatments increase the amount of nitrogen and the proportion of protonated
amino groups relative to amino groups on the aminosilane coating, which subsequently results in an increased amount of active GIOx on the surface. This effect, however, is found to be unstable due to unstable electrostatic interactions between GIOx and NH$_3^+$. We demonstrate the detection of glutamate in the concentration range of 10 µM–1mM using the NH$_2$ functionalized Pt doped ta-C surface. The biosensor showed high sensitivity (2.9 nA µM$^{-1}$ cm$^{-2}$), low detection limit (10 µM) and good storage stability. The electrode response to glutamate was linear in the concentrations ranging from 10 µM to 500 µM. In conclusion, the study shows that GIOx immobilization is most effective on aminosilane treated ta-C surface without any pre-treatments and the fabricated sensor structure is able to detect glutamate in the micromolar range.

**Keywords:** Glutamate oxidase; tetrahedral amorphous carbon; self-assembled monolayers; immobilization; electrochemical detection

1. Introduction
Glutamate is the most abundant neurotransmitter in the brain and its imbalance and dysfunction are linked to several neurodegenerative diseases such as schizophrenia and Parkinson’s disease [1]. For diagnostic and therapeutic purposes, the accurate measurement of glutamate level in situ would be desirable. Currently, there are no chronically implantable glutamate sensors available. For the long term in vivo success of biosensors, critical issues, such as sensitivity, specificity and stability need to be assessed.

Glutamate is not electrochemically active and therefore an enzyme is utilized for the electrochemical detection of glutamate [2, 3, 4]. Recently, an enzyme-free method for the detection of glutamate was developed [5], but, unfortunately, this approach requires highly alkaline conditions for proper function. Therefore, the use of an enzyme is still required for glutamate detection in physiological pH.

The requirements for biosensor materials include electrical conductivity, high stability and the ability to resist attack from different biological molecules. Noble metals, including platinum and its alloys, gold, and palladium, have been experimented as candidates for glutamate detection [6]. Unfortunately, the metal electrodes suffer from severe biofouling, have tendency to exhibit high background current and are easily attacked in physiological conditions by chloride ions. Therefore, metal electrodes cannot be used in direct electrochemical detection of neurotransmitters. However, they are commonly used in enzyme based applications where the sensor surface is coated with polymers or other materials [2]. This approach induces mass transport effects and subsequent decreases in the response time. Therefore, direct coupling of the enzyme on the surface of the electrode material is desired.

Diamond-like carbon coatings can potentially be used as antifouling surfaces against microbial and protein attachment [7]. These materials are very stable and have a good biocompatibility [8,9,10] and they are able to resist bacterial adhesion [11]. Tetrahedral amorphous carbon (ta-C) is the form
of diamond-like carbon, which is the hardest, strongest, and slickest. ta-C has a water window of 3.7 V; making it an attractive sensor material as a wide water window enables a large operational range for analyte detection in water based solutions [12]. Moreover, we have recently demonstrated improved sensitivity towards dopamine with ta-C electrodes [13, 14]. However, carbon based materials are relatively insensitive to hydrogen peroxide and therefore we need Pt alloyed ta-C films.

This paper presents an effective method to immobilize glutamate oxidase (GlOx) directly on ta-C surface. As discussed above, enzymatic approach is required for glutamate detection in physiological pH. GlOx specifically catalyses the oxidative deamination of glutamate in the presence of water and oxygen leading to the formation of electrochemically detectable hydrogen peroxide. The functional groups of proteins suitable for covalent binding under mild conditions include the alpha amino groups of the chain and the alpha carboxyl group of the chain. Certain other functional groups are also suitable for covalent binding, such as sulfhydryls of cysteine, but in the structure of GlOx there are no such amino acids in the heads of the amino acid chains [15]. Creating amino or carboxyl functionalities on the surface should therefore enable the feasible immobilization of GlOx. Here the focus is on immobilization without the aid of cross-linking or covalent coupling methods. For example, glutaraldehyde cross-linking may result in loss of activity due to the distortion of the active enzyme conformation and the chemical alterations of the active site during cross-linking [16]. The usage of coupling agents such as N-hydroxysuccinimide (NHS) and 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) may result in unwanted polymerization between the enzymes. Furthermore, many of the cross-linkers and coupling agents have toxic side-effects and are potential allergens. Trace amounts remain after the treatment and are often difficult to remove. Self-assembled monolayers (SAMs) are considered for surface
functionalization since they are easy to form, versatile, stable due to their ordered arrangement and highly reproducible.

2. Materials and Methods

2.1 Substrate fabrication

2.1.1 ta-C coating

Two different kinds of samples, an undoped ta-C layer and Pt-doped ta-C layer were used as electrode material. Both types consisted of underlying Ti layer and top ta-C layer.

In samples with undoped top ta-C layer, the substrate was highly doped n++ Si <111> wafer (Okmetic). Samples were chemically etched in buffered hydrofluoric acid solution and argon ion beam cleaning in the deposition chamber. The samples were mounted by hanging in a rotating carrousel (2.4 rpm), with the axis of rotation perpendicular to the direction of the plasma plume. A 20-nm-thick layer of titanium was obtained by continuous current (55 A) filtered cathodic vacuum arc (FCVA) deposition to enhance the ta-C layer adhesion. ta-C was deposited using CVA deposition, during which the capacitor bank of 2.6 mF capacitance was discharged (from 200 V) yielding a current pulse with a frequency of 1 Hz, a maximum current of about 3 kA and a half width of 150 µs. An accumulation of about \(1.4 \times 10^{15}\) atoms cm\(^{-2}\) during each pulse was obtained.

In the samples with Pt-doped ta-C, p-type silicon (100) wafers with 0.001–0.002 Ohm-cm resistivity were used as a substrate. All wafers were cleaned by standard RCA-cleaning procedure before the deposition. Samples were placed in a horizontally rotating holder (20 rpm). A Ti layer of 20 nm thickness was deposited by direct current magnetron sputtering (100W, 350 s, 0.67 Pa of Ar atmosphere). Composite cathodes, which consisted of 6.35 mm carbon rode with two embedded Pt wires of 1 mm in diameter, was used to obtain Pt-doped ta-C (7 nm thick) via FCVA. The ratio of carbon to platinum area was 95 : 5. During the deposition, the 2.6 mF capacitor bank was charged
to 400 V. The arc current pulses had 0.7 kA amplitude and 0.6 ms pulse width. Each pulse was triggered at 1 Hz frequency. The number of pulses was 360. Total pressure during the deposition process was no less than 1.3×10⁻⁴ Pa. The distance between the substrate holder and the filter was approximately 20 cm.

2.1.2 Self-assembled monolayers

The activity and adhesion of GlOx was first studied on silicon surfaces with three different terminations: -COOH, -NH₂ and -OH. The surface chemistries were fabricated by using silane SAMs in a protocol similar to Toworfe et al. [17]. The substrates were polished P-type <100> silicon wafers that were cleaned by hydrofluoric acid and RCA 1 solutions and activated by oxygen plasma prior to SAM deposition. All SAMs were deposited from anhydrous toluene at room temperature. After the SAM deposition, the surfaces were rinsed in toluene and acetone and ultrasonicated in deionized water. The carboxylic acid (-COOH) terminated surfaces were fabricated by first depositing triethoxysilylpropyl succinic anhydride (TESPSA; Geniosil GF 20, Wacker Chemie, München, Germany; 10% solution) and subsequently hydrolysed in 10 mM HCL for 30 min at 32.4°C and rinsed in deionized water. The amino (-NH₂) terminated surfaces were fabricated by deposition of (3-aminopropyl)triethoxysilane (APTES, Sigma-Aldrich, St. Louis, MO, USA, 5% solution). The hydroxyl (-OH) terminations were achieved by depositing (3-glycidoxypropyl)methyldiethoxysilane (GPTMS; Sigma; 20% solution) and subsequently immersing the resulting epoxy terminated surfaces in 70mM mercaptoethanol in phosphate buffered saline (PBS) for 16 hours in room temperature and rinsing in deionized water. Uncoated, oxygen plasma treated silicon surfaces were used as controls. The surface chemistry of the unmodified chips was mainly oxidized silicon.
The NH$_2$ surface chemistry was found to be the most effective for the enzyme immobilization and was therefore studied further. In order to deposit NH$_2$ SAMs on ta-C surfaces, the samples were pre-treated either by oxygen plasma or anodizing. For anodizing, conducting wires were attached on the back sides of the silicon chips with silver epoxy. Anodizing was performed in 0.1 M H$_2$SO$_4$ (pH 1.0) at 2.5 V vs. Ag/AgCl (Sarissa Biomedical Ltd., Coventry, U.K.) for 60 minutes. All samples were washed with deionized water in ultrasonic bath and dried with air. To induce NH$_2$ SAM deposition on the pre-treated and the unmodified ta-C surfaces APTES treatment was performed following the previously described protocol.

2.1.3 Glutamate oxidase coating

L-Glutamate Oxidase (Sigma or Cosmo Bio Co., Ltd., Tokyo, Japan) was diluted into PBS and stored at -70°C. 100 µl of GlOx-solution was placed on the surfaces for 4 h at room temperature. A concentration of 500 mU/ml of GlOx in PBS was used for adsorption testing and a concentration of 100 mU/ml for activity testing and electrochemical measurements. After the incubation, the samples were washed three times in DI-water and dried with N$_2$ gas (adsorption measurements) or washed three times in PBS and stored in PBS at 4°C (activity measurements, electrochemical measurements).

2.2 Sample characterization

2.2.1 Cross sectional transmission electron microscopy of ta-C

The samples were prepared for cross-sectional transmission electron microscopy (TEM) by grinding and polishing until a thickness of less than 10 µm, followed by Ar ion milling using a
PIPS Ionmiller (Gatan USA). High-resolution transmission electron microscopy (HRTEM) was performed using a double-aberration corrected JEOL 2200FS (JEOL, Japan) microscope equipped with a field emission gun (FEG) operated at 200 kV. The TEM was equipped with an energy dispersive X-ray (EDX) spectrometer for elemental analysis. A Gatan 4kx4k UltraScan 4000 CCD camera was used for recording HRTEM images.

2.2.2 Contact angles

An optical goniometer (Theta, Attension, Espoo, Finland) was used to measure the contact angles by the sessile droplet method. The volume of the droplet was 3 µl. The reported values are averages of two measurements. The standard deviations were less than 1° for all samples, but in general the uncertainty of the contact angle measurement is a few degrees. The contact angles were measured immediately (within 1 hour) after the deposition of the silane monolayer.

2.2.3 X-ray photoelectron spectroscopy (XPS)

The elemental composition of the ta-C surfaces was measured with ESCALAB 250Xi X-ray photoelectron spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) equipped with monochromatic Al Kα (1486.6 eV) X-ray source. Data was collected using 900-µm X-ray spot size and the spectrometer was operated in constant energy analyzer (CAE) mode. Wide energy range survey spectra and high-resolution elemental spectra of oxygen, nitrogen, and carbon were collected from each sample. The survey spectra were collected by setting the detector pass energy to 150 eV and using the step size of 1 eV. The elemental spectra were recorded with the pass energy of 20 eV and the step size of 0.1 eV.
The XPS data was analyzed by using Thermo Avantage XPS data analysis software (Thermo Fisher Scientific, Waltham, MA, USA).

2.3 Evaluation of glutamate oxidase immobilization

GlOx adsorption was determined with Plasmos SD 2300 ellipsometer (PLASMOS GmbH, Munich, Germany). The reflective indices of SiO2 (n=1.467) and silane SAMs (TESPSA: n=1.441, APTES: n=1.423, and GPTMS: n=1.429) are approximately the same. Moreover, the refractive indices of proteins are usually in the same range and fall between 1.35 and 1.55 [18,19,20,21]. Therefore, to simplify the model, the thickness of adsorbed layers was calculated using a planar isotropic model that assumed a refractive index of 3.865 for silicon and 1.450 for all the other layers. The effect of error in the assumed refractive indices on the calculated thickness is described in detail in ref [22].

The adsorbed amount ($\Gamma$) at the air/solid interface can be calculated as

$$\Gamma = \rho d,$$

where $\rho$ is the density of the protein and $d$ the thickness of the layer [23]. For GlOx (140 kDA), it is estimated that $\rho = 1.41 \text{ g/cm}^3$ [24]. The reported values are averages of ten measurements on four replicate samples.

2.4 Measurement of glutamate oxidase activity

GlOx activity was determined using a Glutamate Oxidase Assay Kit (ab138885, Abcam, Cambridge, UK) according to the manufacturer's instructions. The activity was measured using an excitation wavelength of 544 nm and emission wavelength of 590 nm with microplate reader (Plate CHAMELEON V, Hidex, Turku, Finland or FLUOstar Optima, Ortenberg, Germany). Four
surfaces of each sample type were studied. All numerical results are expressed as the mean ± the standard deviation. The statistical significance of the observed differences between groups was evaluated using the Mann-Whitney U test.

2.5. Electrochemical measurements

H$_2$O$_2$ solutions (0 – 10 mM) were freshly prepared from 30 % H$_2$O$_2$ (Merck KGaA, Darmstadt, Germany) by dilution in phosphate buffered saline (PBS, pH 7.4). Glutamate solutions (0-10 mM) were freshly prepared from glutamic acid (Sigma) by dilution in PBS. Cyclic voltammetry was performed with a Gamry Reference 600 potentiostat and Gamry Framework software (Warminster, PA, USA). Prior to H$_2$O$_2$ measurements, the samples were cycled in nitrogen-purged 0.15 M H$_2$SO$_4$ for 500 cycles with 1 V/s and rinsed with deionized water to clean the samples. The experiments were conducted by immersing the sample in nitrogen purged H$_2$O$_2$ solution and cycling three times between -0.4 V and 1.3 V vs. Ag/AgCl with a cycling rate of 50 mV s$^{-1}$. Glutamate solutions were not purged with nitrogen since the enzymatic reaction requires the presence of oxygen and cycling was done between -0.4 V and 1 V vs. Ag/AgCl. Glutamate concentrations were measured using chronoamperometry (potential step 600 mV vs. Ag/AgCl).

3. Results and discussion

3.1 Structure of the ta-C coating

Figure 1 shows a bright-field TEM image of a cross-section from the fabricated Si/Ti/ta-C sample. Based on the micrograph, the structure of the ta-C layer is amorphous and there are no visible crystalline areas inside the layer. Moreover, the Si/Ti and Ti/ta-C interfaces both show an additional
phase with a light contrast. Based on the EDX analyses (not shown here) both phases show traces of oxygen. This indicates the presence of an amorphous SiO$_x$ at the Si/Ti interface and an amorphous Ti[O,C]$_x$ solid solution layer at the Ti/ta-C interface.

3.2 SAMs on silicon

3.2.1 Contact angles

The contact angles measured immediately after the silane deposition on silicon were $\approx 0^\circ$ for the plasma oxidized silicon, $33^\circ$ for TESPSA (−COOH), $23^\circ$ for APTES (−NH$_2$) and $32^\circ$ for GPTMS (−OH) (Table 1). All of the materials are clearly hydrophilic, the unmodified surface being the most hydrophilic, followed by NH$_2$ and then OH and COOH-modified surfaces. The contact angle values are in agreement with those previously reported for silane SAMs [17, 25] and therefore indicate a successful SAM coating.

3.2.2 Glutamate oxidase adsorption and activity on SAMs on silicon

Ellipsometry is not a highly sensitive technique and a higher concentration of GlOx had to be used in the adsorption experiment than in the activity experiment. Still, the amount of GlOx on the COOH- and OH-terminated surfaces was barely detectable. On the other hand, the amount of adsorbed GlOx on the NH$_2$-terminated surface was clearly larger than on any of the other surfaces. The amount of adsorbed GlOx is presented in Table 1.

The activity measurements confirmed that GlOx preserved its functionality on the NH$_2$-modified surface (Figure 2). The GlOx activity was approximately 5-fold higher on the NH$_2$-terminated
surface than on the unmodified or COOH- or OH-terminated surfaces (p<0.03). Importantly, the enzyme coating remained stable for 7 weeks when stored in PBS at 4°C.

Both the amount and activity of the enzyme were largest on the NH$_2$-terminated surface. The NH$_2$-terminated surface supports the immobilization of GlOx and preserves the functionality of GlOx. When comparing the remaining three surfaces, the amount of GlOx is largest on the unmodified surface and smallest on the COOH-terminated surface. However, immediately after the enzyme immobilization the activity is approximately same on the unmodified surface and on the COOH-terminated surface and higher on these surfaces than on the OH-terminated surface. This indicates that the unmodified and the OH-terminated surfaces have a negative effect on the functionality of GlOx. Noteworthily, there was a large variation in both the amount and activity of the enzyme on the unmodified surface. This is expected, since the surface chemistry on the unmodified surface is more heterogenic than on the SAM-modified surfaces. Although COOH-terminated surface did not show promising results, it is possible that immobilization of GlOx to carboxyl group could be enhanced by treating the surface with NHS and EDC to activate carboxyl groups to yield amine reactive esters.

In conclusion, NH$_2$-terminated surface seems attractive for GlOx coated glutamate sensors.

3.3 APTES modified ta-C

3.3.1 XPS

The elemental composition of the ta-C surfaces after the pre-treatments and APTES coating was measured with XPS. Prior to the analysis, the energy scale of the spectra was charge corrected by using the binding energy of the sp3 component of the ta-C films (C1s = 285.5 eV). The survey
spectra measured from the samples showed traces of carbon, oxygen, nitrogen, titanium, and silicon as expected.

Figure 3 presents the relative amounts of the elements. The pre-treatments increased surface oxidation by 60% and after APTES treatment the amount of nitrogen was increased by 50% on the pre-treated surfaces compared to the reference.

The XPS of N 1s spectrum after APTES deposition is shown in Figure 4. In addition to the NH$_2$ scissor mode (peak at 400 eV), NH$_3^+$ deformation mode was present (peak at 401.8 eV). The intensity of the latter increased with the pre-treatments. When samples are exposed to air after synthesis, water molecules are weakly bonded to the NH$_2$ groups, thereby allowing for the protonation of the amine [26]. The pre-treatments increase the stability of this protonated amine and this may influence the enzyme immobilization.

### 3.3.2 Glutamate oxidase activity on ta-C

After APTES treatment the amount of nitrogen was increased by 50% on the pre-treated surfaces compared to reference sample without pre-treatments. This resulted in a 20% increase in the amount of active GlOx on the surface immediately after GlOx immobilization (Figure 5). However, the pre-treatments significantly decreased the stability of the glutamate oxidase. This is logical as the amount of protonated amine was higher on these surfaces. The protonated amine interacts with GlOx via electrostatic interaction as the isoelectric point of GlOx is pH 6.2 [27]. Consequently, the electrostatically adhered GlOx is washed away in the PBS washes and the activity reduced with time. Another possibility is that the activity is reduced due to the denaturation of the structure of GlOx when bound to NH$_3^+$. However, this denaturation would likely happen during the immobilization and the activity would already be low in the initial measurement and not reducing as
a function of time. PBS contains phosphate and chloride ions that compete with glutamate on electrostatic interactions with NH$_3^+$. In conclusion, GlOx immobilization is most effective on APTES coated ta-C surface without the tested pre-treatments.

### 3.3.3 Electrochemical measurements

Undoped ta-C surfaces were not able to detect H$_2$O$_2$ (results not shown) and therefore electrochemical measurements were performed for APTES coated Pt-doped ta-C surface only. The Pt/ta-C/APTES/GlOx electrode showed concentration dependent response to glutamate (Figure 6). Cyclic voltammetry showed clear peak at approximately 500 mV vs. Ag/AgCl. Chronoamperometry at 600 mV vs. Ag/AgCl verified the concentration dependent response to 10 µM–1 mM glutamate levels (Figure 6 B). Linear relationship between current and glutamate concentration was observed in concentration range of 10 and 500 µM (Figure 6 C). The performance of the electrode is comparable to other carbon based glutamate sensors (Table 2). The sensitivity of the electrode is 2.9 nA µM$^{-1}$ cm$^{-2}$. When we measured H$_2$O$_2$ on Pt/ta-C and Pt/ta-C/APTES, it was obvious that the APTES coating blocked some of the H$_2$O$_2$. Therefore, an alternative method for creating NH$_2$ surface functionalities needs to be considered to increase sensitivity. Limits of detection for most electrochemical carbon based glutamate sensors are typically in the range 200 nM – 2.5 µM. However, these are typically calculated values and therefore measured concentration of 10 µM is comparable. Storage stability of the Pt/ta-C/APTES/GlOx sensor was excellent as demonstrated by the GlOx stability (up to 7 week, Figure 2). Unfortunately, the operational stability of the electrode is limited by the GlOx stability at 37°C as already 30 minutes in 37°C decreases the GlOx activity to about 90% of maximum [32]. We tested the stability of GlOx in PBS (without any immobilization), when stored at 37°C, and the recovery of GlOx was only 24% over a period of 7 days. For our sensor, analytical recovery of added glutamate (10-500 µM) was 80% over a period of 3 days. Battra et al. have demonstrated
good operational stability for glutamate oxidase based sensor limited to measuring high glutamate concentrations (5-10 mM) [33]. However, this was only possible when the electrode was stored at 4 °C in-between measurements.

In this article the focus was on investigation of GlOx immobilization on electrode surfaces with different surface functionalities. We demonstrated NH₂ surface chemistry to be more effective than COOH or OH functionalities and demonstrated detection of glutamate in micromolar concentration range. Advantageous features of the proposed platform include simplicity which may enable fast response times and CMOS compatibility.

4. Conclusions

In conclusion, an effective method to immobilize GlOx on ta-C surface was demonstrated, which enabled the realization of a simple sensor structure capable of detecting glutamate in micromolar concentration range. A significantly larger amount of GlOx was adsorbed on the NH₂-terminated surface than on the unmodified or COOH- or OH-terminated surfaces. The enzyme also preserved functionality on the NH₂ surface for 7 weeks when stored in PBS. The NH₂-terminated surface is thus attractive for GlOx based glutamate sensors. The composition of APTES treated ta-C surface is most favourable for GlOx immobilization without pre-treatments, since both anodizing and plasma-treatments were found to increase the amount of protonated NH₃⁺ groups, which results in unstable electrostatic interactions between the GlOx and the surface. The Pt/ta-C/APTES/GlOx biosensor showed high sensitivity (2.9 nA µM⁻¹ cm⁻²), low detection limit (10 µM) and good storage stability. The electrode response to glutamate was linear in the concentrations ranging from 10 µM to 500 µM.

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References


Figure 1: Cross-sectional TEM micrograph from the Si/Ti/ta-C thin film stack after deposition. The clearly distinct and even layers can be seen in the micrograph. The cross-sectional TEM micrograph with higher magnification from the Si/Ti/ta-C thin film stack after deposition clearly shows the presence of oxygen at the Si/Ti and Ti/ta-C interfaces. The amorphous nature of the ta-C film is also readily seen.
Figure 2: The effect of self-assembled monolayers on glutamate oxidase activity immediately after adsorption and 3 and 7 weeks later.
Figure 3: The pre-treatments influenced the functional groups of nitrogen present on the surface. The pre-treated surfaces had larger amount of NH$_3^+$ (peak at 401.8 eV) functionalities than NH$_2$ (peak at 400 eV), whereas the reference sample had more NH$_2$ than NH$_3^+$. 
**Figure 4:** A) The pre-treatments increased the amount of oxygen on the surface compared to reference. B) The pre-treatments increased APTES binding on the surface compared to non-pre-treated reference.
**Figure 5:** The effect of pre-treatments on glutamate oxidase activity on APTES modified ta-C surfaces.

**Figure 6:** Glutamate detection on Pt/ta-C/APTES/GlOx surface. A) Cyclic voltammogram of different glutamate concentrations, B) amperometric detection of glutamate at 600 mV vs. Ag/AgCl, C) showing linear response in the concentration range from 10 µM to 500 µM.
Table 1: Contact angles of water and the thickness of enzyme coating.

<table>
<thead>
<tr>
<th>Surface</th>
<th>θ (°)</th>
<th>Γ (ng/mm²)</th>
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</thead>
<tbody>
<tr>
<td>Plasma oxidized Si</td>
<td>≈0 ± 1</td>
<td>1.4 ± 1.3</td>
</tr>
<tr>
<td>-COOH</td>
<td>33 ± 1</td>
<td>0.0 ± 0.4</td>
</tr>
<tr>
<td>-NH₂</td>
<td>23 ± 1</td>
<td>3.2 ± 0.4</td>
</tr>
<tr>
<td>-OH</td>
<td>32 ± 1</td>
<td>0.5 ± 0.3</td>
</tr>
</tbody>
</table>

Table 2: Comparison of the performance of some amperometric glutamate biosensors constructed based on a carbon component and glutamate oxidase.

<table>
<thead>
<tr>
<th>Material</th>
<th>Sensitivity (nA µM⁻¹ cm²)</th>
<th>Detection limit (µM)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
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<td>CNT</td>
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<td>2</td>
<td>[28]</td>
</tr>
<tr>
<td>GC/Pt-GNPs/GlOx</td>
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<td>0.75</td>
<td>[29]</td>
</tr>
<tr>
<td>GC/Pt/GlOx</td>
<td>91</td>
<td>2.4</td>
<td>[29]</td>
</tr>
<tr>
<td>BDD/Pt/GlOx</td>
<td>24</td>
<td>0.35</td>
<td>[29]</td>
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<tr>
<td>GlOx/cMWCNT/AuNP/CHIT</td>
<td>155</td>
<td>1.6</td>
<td>[30]</td>
</tr>
<tr>
<td>GlOx/cMWCNT/AuNPs/CHIT/Au</td>
<td>486</td>
<td>2.32</td>
<td>[31]</td>
</tr>
</tbody>
</table>

BDD Boron doped diamond; CHIT chitosan; cMWCNT carboxylated multi walled carbon nanotube; CNT Carbon nanotube; GC glassy carbon; GNP graphene nanoplatelet; NP nanoparticle.