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Electroanalysis with Carbon Film-based Electrodes

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1.1 Introduction

As electrode materials for analytical applications, carbon-based electrodes have been widely employed as detectors for high performance liquid chromatography (HPLC), capillary electrophoresis (CE) and various biosensors. Carbon materials usually shows wider potential window compared with those of novel metals such as platinum and gold electrode. These electrodes are chemically stable, highly conductive and low cost. A recent review article has well described the electrochemistry of certain carbon-based electrodes\([1]\). Glassy carbon (GC) and highly oriented pyrolytic graphite (HOPG) have been traditionally utilized for various electroanalytical methods. Later, carbon paste electrodes have been used mainly to develop enzymatic biosensors because carbon paste is low cost and the electrode can be fabricated only by printing and various biomolecules can be modified only by mixing with carbon ink.

In the last 20 years, electrochemical measurements using boron-doped diamond (BDD) electrodes have become more intensively studied by many groups\([2–4]\). A BDD electrode shows extremely wider potential window due to its chemical stability and lower background noise level than other electrode materials. Due to such unique performances, BDD electrodes are advantageous in terms of detecting various species including heavy metal ions (Pb\(^{2+}\), Cd\(^{2+}\))\([5]\), chlorinated phenols\([6]\), histamine and serotonin\([7, 8]\), and even nonmetal proteins\([9]\). The BDD electrodes have also been employed to fabricate modified electrodes including As\(^{3+}\) detection with iridium-implanted BDD\([10]\), DNA modified BDD\([11]\) and cytochrome c modified BDD\([12]\). In spite of excellent performance of BDD electrodes, high temperature between 400–700°C is needed for BDD fabrication, which limits the substrates only to inorganic materials such as silicon wafer, metals and glass plate.

More recently, nanocarbon materials including carbon nanotubes (CNTs), carbon nanofibers (CNFs) and graphene nanosheet have been more intensively studied with a view to using them as electrode materials for fuel and biofuel cells\([13–15]\). For electroanalytical application CNT and graphene have been employed to fabricate various
biosensors because nanocarbon electrodes have large surface area suitable to immobilize large amount of enzymes and antibodies [16–20]. The surface area of such nanocarbon film with immobilizing large amount of biomolecules can achieve sufficient sensitivity and longer stability. More recently, the graphene was modified onto interdigitated array electrode and applied for electrochemical immunoassay [21].

In spite of some works using nanocarbons as film electrode, the nanocarbon materials have been mainly used by modifying them on the solid electrode and larger surface area of nanocarbons also show large capacitive and background currents and reduce signal to noise (S/N) ratio when detecting trace amount of analytes.

In contrast, carbon film electrodes have been used for direct measurement of electroactive molecules such as neurotransmitters and nucleic acids. Various kinds of carbon film materials have been developed using various fabrication processes including pyrolysis of organic films, sputter deposition, chemical vapor deposition. However, carbon film electrodes are needed to improve the electron transfer rate of analytes in order to retain diffusion-limited electrochemical reactions because their smooth surface has fewer active sites than the surfaces of nanocarbon materials. Therefore, it is required to fabricate carbon films with better electroactivity. Another important advantage is that carbon film can be patterned to any shape and size with high reproducibility for use as platforms for chemical or biochemical sensors by utilizing conventional photolithographic process [22]. In this chapter, the fabrication processes of carbon film electrodes are introduced. Then, we described structure and electrochemical properties of various carbon film electrodes. Finally, we describe the application of carbon film electrodes for electroanalysis of mainly biomolecules.

1.2 Fabrication of Carbon Film Electrodes

In order to fabricate carbon film electrodes, the pyrolysis of organic films including various polymers and deposited aromatic compounds have been employed by many groups as summarized in Table 1.1.

Kaplan et al. deposited 3, 4, 9, 10-perylenetetracarboxylic dianhydride (PTDA) films on the substrate, pyrolyzed them above 700°C and obtained conducting carbon film [23]. The conductivity was comparable to that of a GC electrode. Rojo et al. obtained carbon film using a similar method to Kaplan et al. and employed it for electrochemical measurements of catechol and catecholamines [24]. Tabei and Niwa et al. employed this process to microfabricate interdigitated array electrodes by lithographic technique [25].

The conducting polymers are also suitable to make highly conducting carbon film because the film already has π–conjugated structure. Tabei et al. used poly(p-Phenylene Vinylene):PPV coated on the substrate and prepared carbon film electrode by the pyrolysis at 1100°C, then fabricated to microdisk array electrode [26]. The carbon films have been fabricated by pyrolyzing conventional polymers. Positive photoresist, which mainly consist of phenol resin was used as precursor polymer and pyrolyzed the film at high temperature because positive photoresist can be easily spin-coated into uniform films [27]. The resistivity was between $2 \times 10^{-2}$ to $2 \times 10^{-3}$ Ω cm depending on the pyrolysis temperature. The electrochemical performance of pyrolyzed photoresist films (PPF) has been intensively studied by McCreery and Madou’s groups [28, 29]. PPF film
has a lower O/C ratio than a GC electrode and relatively larger peak separations were observed from the voltammograms of Fe\(^{3+/2+}\) and DA. The carbon film obtained by photoresist has very smooth surface. In fact, Ranganathan et al. observed that the average roughness is less than 0.5 nm by the atomic force microscopy (AFM) measurement of PPF carbon film. The modification of PPF film by diazonium reduction was performed by Brooksby et al.\[30\]. The modification of such carbon films is very important to use them as platforms of various electrochemical biosensors. More recently, the relationship between fabrication processes of PPF such as types of resists, and heating programs, and their resistivity and surface roughness, were well summarized by Compton’s group\[31\]. Morita et al. carbonized polyimide (PI) film and fabricated IDA electrode\[32\]. The height of the electrode is ranging from 0.1 to 4.5 µm since PI is suitable to obtain thicker film.

On the other hand, carbon film electrodes have been developed by using various vacuum deposition techniques including magnetron or radio frequency (RF) or electron cyclotron resonance sputtering deposition, electron beam evaporation, plasma-assisted chemical vapor deposition (PACVD), radio-frequency plasma enhanced chemical vapor deposition (RF-PECVD). Most well known carbon film is diamond like carbon(DLC), which is very widely used for coating of drills and cutting tools because DLC is extremely hard. Smooth and inert surface of DLC is also suitable to improve biocompatibility and applied for the coating of medical devices. A Ternary phase diagram of amorphous carbons including DLC was reported by Ferrari et al.\[33\].
As an electrode materials, DLC shows high S/N ratio and low capacitance [34]. Blackstock et al. reported ultraflat carbon film (~0.1 nm) whose electrochemical response is similar to that of GC [35]. Swains’ group has been studied nitrogen-containing amorphous carbon films and their electrochemical performance as discussed in the later section [36]. Hirono et al. developed a very smooth and hard carbon film using electron cyclotron resonance (ECR) sputtering [37]. The film consists of sp\(^2\) and sp\(^3\) hybrid bonds with a nanocrystalline structure and the sp\(^2\) and sp\(^3\) ratio can be easily controlled by changing ion acceleration voltage from 20 to 85 V. Figure 1.1 shows surface image and line scan data of ECR sputtered carbon film obtained by AFM. The average roughness (Ra) is 0.07 nm, indicating atomic level flatness [38]. The film contains nanocrystalline graphite like structure different from amorphous carbon film, which contributes to improve electrochemical performance as described later. In fact, a parallel layered structure identified as a nano-order graphite crystalline structure can be observed at a low ion acceleration voltage, but a curved and closed nanostructure is dominant at a high ion acceleration voltage. More recently, Kamata et al. fabricated the

![AFM image of ECR sputtered carbon surface (a) and line profile (b).](image)

**Figure 1.1** AFM image of ECR sputtered carbon surface (a) and line profile (b). Reprinted with permission from [38]. Copyright 2006 American Chemical Society.

![Comparison of schematic diagram of UBM sputtering equipment (b) compared with conventional magnetron sputtering (a).](image)

**Figure 1.2** Comparison of schematic diagram of UBM sputtering equipment (b) compared with conventional magnetron sputtering (a).
carbon film with similar structure and electrochemical properties to those of ECR nanocarbon film by using unbalanced magnetron (UBM) sputtering [39]. Figure 1.1 shows schematic diagram of UBM sputtering equipment (Figure 1.2a) compared with conventional magnetron sputtering (Figure 1.2b).

The plasma is only distributed near the target in case of conventional magnetron sputtering. In contrast, the plasma is distributed near the substrate and the ion irradiation occurs onto the substrate, which can widely control the structure of carbon film including sp³ and sp² ratio.

1.3 Electrochemical Performance and Application of Carbon Film Electrodes

When fabricating carbon film based electrode, other atoms such as nitrogen and oxygen or even metal nanoparticles can be contained. For example, nitrogen doping can be performed in the presence of small amount of N₂ during vacuum process. Surface termination with other atoms such as hydrogen and nitrogen can be easily performed because the conducting carbon film contains certain amount of sp² bonds, which is chemically reactive. Metal nanoparticles which usually show better electrocatalytic performance for analytes have been developed by pyrolysis and vacuum technique. In this section, the electrochemical performance and applications of pure, surface terminated and hybrid carbon films are summarized.

1.3.1 Pure and Oxygen Containing Groups Terminated Carbon Film Electrodes

The carbon films prepared by pyrolyzing organic and polymer films usually contains graphite layers. Figure 1.3a is Raman spectra of the carbon film prepared by Niwa et al. [25] on the basis of the process reported by Rojo et al. [24].

The two relatively broad peaks were observed at 1590 and 1340 cm⁻¹, and assigned to disordered graphite structure. As an electrode material, Rojo et al. reported that the electrochemical response of catechol is irreversible, but became ideal after electrochemical treatment at 1.8 V. Figure 1.3b compared voltammograms of 100 μM dopamine (DA) at the carbon-based IDA electrode before (1) and after (2) electrochemical treatment. Carbon film-based IDA was fabricated by photolithographic technique. After electrochemical treatment, the current increases more rapidly compared with that before treatment. The electrochemical pretreatment increases surface area caused by etching the surface and introduces oxygen containing groups.

In contrast, carbon films prepared by vacuum process have wide variety of the structure as described above. Figure 1.4 shows relationship between potential window and sp³ [sp³/(sp²+sp³)] concentration of the UBM sputtered nanocarbon film. The width of potential window increases with increasing sp³ ratio [39]. However, the peak separations of Fe(CN)₆⁴⁻ and DA becomes larger when sp³ concentration is around 50%. The wide potential window of UBM sputtered nanocarbon film electrode is advantageous to measure biomolecules with high oxidation potential.

The flat surface of nanocarbon film also contributes to suppress the fouling of electrode surface. With a conventional electrode such as a GC electrode, the relatively rougher surface adsorbs the molecules. In contrast, the molecules easily desorbed from
the nanocarbon film electrode surface after electrochemical reaction because of its flat and chemically stable surface. For example, we achieved much better reproducibility and detection limit compared with GC when measuring 8-OHdG which is known as oxidative stress marker [40]. The suppression of fouling can be enhanced at hydrophilic surface. The electrochemical treatment simply introduces oxygen containing groups, which can be confirmed by reduction of contact angle and XPS measurements [41].
The electrochemical treatment of the carbon electrodes such as GC often make the surface very rough, but nanocarbon film still maintain smooth surface after electrochemical treatment. The electrochemical response of serotonin and thiol was greatly improved after electrochemical treatment at ECR nanocarbon film electrode. This performance is particularly advantageous when measuring biomolecules with large molecular weight since large biomolecules often strongly adsorb on the electrode surface and interfere with the electron transfer between the analytes and electrode. Simple electrochemical DNA analysis techniques such as DNA methylation [42] and single nucleotide polymorphism (SNP) [43] detection have been reported based on the quantitative measurement of all the bases by direct electrochemical oxidation. Figure 1.5 shows background-subtracted differential pulse voltammograms (DPVs) of 3μM of oligonucleotides (1: 5‘-CAG-CAG-CAG-3’, 2: 5’- CAG-CAA-CAG -3’, 3: 5‘- CAA-CAA-CAG -3’, 4: 5’- CAA-CAA-CAA-3’, the underline base represents a mismatch base) at the nanocarbon film electrode.

The peaks assigned by G oxidation decreases and A oxidation increases with increasing number of A in the oligonucleotide. However, the oxidation of C cannot be observed at GC electrode due to narrower potential window compared with those at ECR.

Figure 1.5 Background-subtracted differential pulse voltammograms (DPVs) of 3 mM of oligonucleotides (1: 5'-CAG-CAG-CAG-3', 2: 5'-CAG-CAA-CAG-3', 3: 5'-CAA-CAA-CAG-3', 4: 5'-CAA-CAA-CAA-3') at the (a) ECR nanocarbon film, GC, and BDD electrodes in 50 mM pH5.0 acetate buffer.
nanocarbon film and BDD electrodes. We also observed that oxidation current of oligonucleotide reduced rapidly by continuous measurement at GC, but not at ECR nanocarbon films due to their flat and hydrophilic surface. The response of each base is sharper at ECR nanocarbon film compared with BDD, indicating relatively rapid electron transfer. Furthermore, we also measured each base content of longer oligonucleotides (60mers) that constitute a non-methylated and a methylated CpG dinucleotide with some different methylation ratios [44].

1.3.2 Nitrogen Containing or Nitrogen Terminated Carbon Film Electrodes

It has been reported nitrogen containing carbon materials shows interesting electrocatalytic performances, particularly oxygen reduction reaction (ORR). Ozakis' group developed carbon alloy which enhances oxygen reduction activity by simultaneous doping of boron and nitrogen into carbon materials [45]. In particular, nitrogen doped carbon materials have been studied by many groups to apply as electrodes for fuel cell. In 2009, Dai et al. reported nitrogen-doped carbon nanotube arrays which show high ORR activity and long time stability [46]. Their group also developed nitrogen-doped graphene by thermally annealed with ammonia and realized n-type field-effect transistor at room temperature [47]. More recently, Uchiyama et al. observed hydrogen oxidation wave using glassy carbon electrode fabricated by stepwise electrolysis in ammonium carbamate aqueous solution and hydrochloric acid [48]. At holding the electrode at 0 V (vs Ag/AgCl), the oxidation current increases by bubbling hydrogen gas and decreases after stopping hydrogen gas supply.

Beside such bulk carbons and nanocarbon materials, nitrogen containing carbon film electrodes have been studied by many groups because the films have a wide variety of structure such as sp²/sp³ ratio and show improved electrocatalytic activity. Yoo et al. reported that nitrogen-incorporated tetrahedral amorphous carbon electrode shows more active charge transfer properties on a variety of systems relative to the H-terminated BDD and excellent stability [49]. Swain’s group reported the nitrogen-doped nanocrystalline diamond thin-film deposited by Gruen and co-workers using microwave-assisted chemical vapor deposition (CVD) from C₆₀/argon and methane/nitrogen gas mixtures consisted of hemispherical features about 150 nm in diameter with a height of 20 nm [50]. The film is active for redox species such as Fe(CN)₆³⁻/⁴⁻ and Ru(NH₃)₆²⁺/³⁺ without any conventional pretreatment and shows semimetallic electronic properties between 0.5 and –1.5 V (vs. Ag/AgCl). The same group also fabricated similar film electrode by plasma-enhanced CVD, which also shows high electrochemical activity [51]. Tanaka et al. fabricated nitrogen-doped hydrogenated carbon films also by plasma-enhanced CVD and studied their structure by XPS and basic electrochemical properties [52]. In contrast, Lagrini et al. used radio-frequency (RF) magnetron sputtering to fabricate amorphous carbon nitride electrode and studied their microstructure and electronic properties such as conductivity [53]. They also studied about correlation between the local microstructure and the electrochemical behavior by using XPS, FTIR, Raman spectroscopy, and electrochemical measurements [54]. The potential windows and voltammograms of Fe(CN)₆³⁻/⁴⁻ were changed by changing nitrogen partial pressure during deposition. Hydrogen and oxygen evolution at nitrogen doped amorphous carbon film electrodes formed with a filtered cathodic vacuum arc in a N₂ atmosphere were also studied by Zeng et al. [55].
Recently, Yang et al. [36] reported electrochemical responses of Ru(NH$_3$)$_6^{2+/3+}$ and Fe(CN)$_6^{3-/4-}$ at nitrogen-containing tetrahedral amorphous carbon thin-film electrodes by changing N$_2$ flow rate during deposition. The peak separation of former species was almost unchanged, but the latter shows lower peak separation when N$_2$ flow rate increases. The resistivity also decreases with increasing incorporation of nitrogen. Kamata et al. studied electrochemical properties of nitrogen-containing carbon film electrodes by widely changing nitrogen concentration. The carbon films were fabricated on boron doped silicon wafer by using ECR sputtering or UBM sputtering equipment [56, 57]. The nitrogen concentration was changed from 0 to 30.4\% characterized with XPS, and the surface image was obtained with AFM. The surface average roughness was almost unchanged when the nitrogen concentration was widely changed. The sp$^3$ concentration was 20\% for pure nanocarbon film and nanocrystalline layered structures can be observed by TEM as shown in Figure 1.6a. However, sp$^3$ concentration increases with increasing nitrogen concentration and became 53.8\% when nitrogen concentration was 30.4\%.

Circle and closed structures containing sp$^3$ bonds also increases with increasing nitrogen concentration as shown in Figure 1.6b. The potential window of the film becomes wider but the electrochemical activity for Fe(CN)$_6^{3-/4-}$ decreases with increasing nitrogen concentration from 9.0 to 30.4\%, although the film containing 9.0\% nitrogen shows smaller peak separation than that of pure nanocarbon film despite lower sp$^2$ concentration. The ORR peak of nitrogen containing nanocarbon film (9.0\%) is more positive than that of pure nanocarbon film, suggesting improved electrocatalytic activity (Figure 1.7).

Nitrogen-containing carbon films have been applied for electroanalysis including heavy metal ions and biomolecules. Table 1.2 summarizes examples of electroanalytical applications with nitrogen-containing carbon film electrodes.

Zeng et al. applied for the analysis of heavy metal ions including Pb$^{2+}$, Cd$^{2+}$, Cu$^{2+}$ by differential pulse anodic stripping voltammetry [58]. A linear dependence of lead concentration between $5 \times 10^{-7}$ to $2 \times 10^{-6}$ M was obtained. Swains' group mainly applied their nitrogen-incorporated tetrahedral amorphous carbon thin film electrodes for

![Figure 1.6](image-url)  
*Figure 1.6* Plain views of (a) pure-ECR and (b) N-ECR (N = 9.0 at. \%) (b) observed by TEM. N$_2$ gas contents during deposition are 0 for (a) and 2.5\% for (b). Scale bar = 5 nm. Reprinted with permission from [56]. Copyright 1994 American Chemical Society.
detecting small biomolecules. Norepinephrine [59] and tryptophan and tyrosine [60] were detected with their film electrode using flow injection analysis. They also applied to detect pharmaceuticals, propranolol (PROP) and hydrochlorothiazide (HTZ) by square wave voltammetry in standard and synthetic biological fluids [61]. PROP is a non-selective $\beta$–adrenergic antagonist drug (blocker) and HTZ is a diuretic drug belonging to the thiazide class. Low detection limits of $\sim 194$ng/ml for PROP and

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<th>Carbon film</th>
<th>Analytes and procedure</th>
<th>References</th>
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<tr>
<td>Nitrogen-doped Diamond-like carbon film by DC magnetron sputter</td>
<td>Differential pulse anodic stripping voltammetry (DPASV)</td>
<td>Zeng et al. 2002 [58]</td>
</tr>
<tr>
<td>Nitrogen containing nanocarbon film by ECR sputtering</td>
<td>Square wave voltammetry of guanosine and adenosine</td>
<td>Kamata et al. 2013 [56]</td>
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<tr>
<td>Nitrogen-incorporated tetrahedral amorphous carbon thin film</td>
<td>Flow injection analysis of norepinephrine</td>
<td>D’N. Hamblin et al. 2015 [59]</td>
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<td></td>
<td>Flow injection analysis of tryptophan and tyrosine</td>
<td>Jarosova et al. 2016 [60]</td>
</tr>
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<td></td>
<td>Propranolol and hydrochlorothiazide oxidation in standard and synthetic biological fluid.</td>
<td>Lourencao et al. 2014 [61]</td>
</tr>
<tr>
<td>Nitrogen containing nanocarbon film by ECR and UBMsputtering</td>
<td>Reductive Detection of hydrogen peroxide</td>
<td>Kamata et al. 2013 [56] 2015 [57]</td>
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~744 ng/ml for HTZ were obtained. The oxidation peak potentials for guanosine and adenosine were compared at pure ECR nanocarbon and nitrogen containing nanocarbon films by using square wave voltammetry [56]. Much sharper and larger oxidation peaks of both analytes were observed at more negative potential with nitrogen containing nanocarbon film compared with those with pure nanocarbon films as shown in Figure 1.8. In case of hydrogen peroxide detection, the larger reduction peaks can be obtained at more positive potential by containing nitrogen due to improved electrocatalytic activity.

1.3.3 Fluorine Terminated Carbon Film Electrode

Fluorination, one of the most attractive surface terminations, has been reported for various carbon-based electrodes including graphite, GC, carbon nanotube, graphene, and diamond [62–67]. These fluorinated carbon electrodes provide unique characteristics, such as improved hydrophobicity and a different electron transfer rate compared with the original carbon electrodes. However, some fluorinated carbon electrodes have serious problems, including lower stability due to loss of fluorine atoms and/or damage due to oxidation [41, 62, 65]. In contrast, fluorinated BDD electrodes exhibit better long-term stability [64, 67], suggesting that a fluorinated surface containing sp3 carbon experiences less oxidization and damage during anodic polarization than GC.

To fabricate electrochemically stable fluorine-terminated nanocarbon (F-nanocarbon) film electrodes, the surface of the nanocarbon films was shortly treated with CF4 plasma [65, 68–70]. The fluorinated surface is easily prepared without losing the surface conductivity and surface flatness of the nanocarbon film electrode as summarized in Table 1.3. After fluorination, the sp2 content decreased from 58.1 to 45.0 %. At the same time, the F/C ratio was 0.2 [65]. These results clearly indicate that the sp2 bond is selectively fluorinated by the CF4 plasma. The contact angle of the film surface increased after surface fluorination (Table 1.3 and Figure 1.9c).

Figure 1.8 Background-subtracted SWVs of 100 μM guanosine (dotted) and adenosine (solid) at pure-ECR and N-ECR (N = 9.0 at. %) electrodes measured in 50 mM acetate buffer (pH 5.0). Adapted with permission from [56]. Copyright 2013 American Chemical Society.
The fluorinated electrodes exhibited a $C^0$ value of 3.32 $\mu$F cm$^{-2}$, which was only one-quarter that of the original nanocarbon film electrode (11.1–12.3 $\mu$F·cm$^{-2}$) [65]. However, the GC electrode was treated with fluorination under the same conditions, and exhibited a larger $C^0$ value of 9.79 $\mu$F cm$^{-2}$. Extremely low electrochemical double layer capacity is highly advantageous in terms of the S/N ratio to rule measurement limits.

The electron transfer rates at the F-nanocarbon film electrodes was investigated by using some typical redox species such as Ru(NH$_3$)$_6$$^{3+/2+}$ and Fe$^{3+/2+}$. The responses of outer-sphere Ru(NH$_3$)$_6$$^{3+/2+}$ was almost unchanged after fluorination owing to the high electron transfer rate and its surface insensitivity [65]. On the other hand, the electron transfer rate of Fe$^{3+/2+}$ was greatly affected by surface fluorination. Figure 1.10a shows a CV of Fe$^{3+/2+}$ at the F-nanocarbon film electrode.

It is well known that inner-sphere Fe$^{2+/3+}$ is generally very sensitive to the presence of oxygen-containing functionalities on an sp$^2$ carbon electrode surface [65, 71]. The F-nanocarbon film electrode provided much larger $\Delta E_p$ at the original nanocarbon film electrode. The O/C ratio decreased after fluorination. These results clearly

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**Table 1.3** Surface properties of the O-nanocarbon and F-nanocarbon films.

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<th>Original</th>
<th>O-terminated</th>
<th>F-terminated</th>
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<tr>
<td>C 1s %$^{(a)}$</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>sp$^2$ content</td>
<td>58.1</td>
<td>45.0</td>
<td>45.0</td>
</tr>
<tr>
<td>sp$^3$ content</td>
<td>41.9</td>
<td>55.0</td>
<td>55.0</td>
</tr>
<tr>
<td>F/C $^{(a)}$</td>
<td></td>
<td></td>
<td>0.2</td>
</tr>
<tr>
<td>O/C $^{(a)}$</td>
<td>0.06</td>
<td>0.10</td>
<td>0.02</td>
</tr>
<tr>
<td>$R_a$ nm$^{-1}$</td>
<td>0.067</td>
<td>0.21</td>
<td>0.075</td>
</tr>
<tr>
<td>Contact angle$^*$</td>
<td>72–75</td>
<td>14</td>
<td>93</td>
</tr>
<tr>
<td>$C^0$ $\mu$F cm$^{-2}$$^{(b)}$</td>
<td>11.1–12.3</td>
<td>20.1</td>
<td>3.32</td>
</tr>
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</table>

$^{(a)}$ The chemical components of C, F, and O were obtained and analyzed using XPS analysis.
$^{(b)}$ The average roughness ($R_a$) were obtained by AFM measurements.
demonstrated that surface fluorination contributed to give very slow electron transfer of Fe$^{3+/2+}$ unlike that of surface oxygen functionalities.

The F-nanocarbon film electrode can be also used to selectively detect lipophilic antioxidant α-tocopherol (vitamin E), which is one of typical major antioxidant components in liquid foods such as olive oil, in combination with use of bicontinuous microemulsion (BME) [69, 70]. Figure 1.10b shows typical CVs of ascorbic acid (vitamin C), and α-tocopherol, and their mix solution using the F-nanocarbon film electrodes in the BME solution. Irreversible oxidation peaks for lipophilic α-tocopherol was observed. In contrast, electrochemical response for hydrophilic ascorbic acid was effectively suppressed. The oxidation peak observed with the F-nanocarbon film electrodes was proportional to the square root of the scan rate, but not to the scan rate, indicating the simple diffusion control of these species in the BME [69]. Moreover, even in the mixed solution, the peak current at the F-nanocarbon film electrode was in good agreement with that for the α-tocopherol alone. These results indicate that there is no interference. This is highly advantageous in terms of constructing a simple assay of antioxidant because an extraction process is usually required prior to the conventional assay [70]. Indeed, the F-nanocarbon film electrode could provide direct qualitative and quantitative electrochemical analysis of lipophilic antioxidants in some olive oil samples including extra virgin olive oils (EVOOs) as a novel methodology without an extraction process [71].

1.3.4 Metal Nanoparticles Containing Carbon Film Electrode

Although various kind of carbon films have been employed for electroanalysis, it is difficult to realize high electrocatalytic activity similar to novel metal electrodes such as Pt. Carbon-based electrodes (including carbon films) containing small amount of nitrogen atom have been reported to improve ORR or hydrogen peroxide reduction, but the activity is still not as high as that of Pt. It is well known that metal nanoparticles (NPs) such as Pt, Pd and Au have high electrocatalytic activity compared with metal bulk electrodes. Since carbon films usually have low background noise current compared
with metal electrodes, the metal NPs modified carbon electrodes realize not only high
electrocatalytic activity but also relatively low noise level compared with metal film
electrodes.

NPs are in general modified onto the carbon electrode by drop casting after their
chemical synthesis, or electrodeposition as summarized in Table 1.4. For example,
Compton’s group reported the GC electrode with AuNP for As(III) [72] and H$_2$O$_2$ [73]
detection. They also reported more sensitive As(III) [74] and tinidazole [75] detection
by co-modificatin of AuNP and CNT. Such sp$^2$ bond carbon materials has a problem of
low stability and reproducibility due to the passivation of the electrode surface [76],
which lead to nanoparticle desorption. BDD electrode has also been preferentially uti-
lized as a nanoparticle scaffold because of its excellent morphological and microstruc-
tural stability in addition to low and stable background current [77–81]. BDD thin film
electrode modified electrodeposited Au nanoparticles (AuNPs) have been reported by
Swain et al. [82] In detecting arsenic ion As(III), they co-electrodeposited not only Au
ion but also As(III) to form intermetallic Au-As nanoparticles, and then oxidized As(III)
component to obtain the reproducible and quantitative stripping peak currents. Similar
procedure was also reported by Compton et al. for Cd and Pd analysis [78, 79]. Einaga
et al. also reported AuNP modified BDD electrode. They modified AuNPs during pre-
concentration step of As(III) to improve preconcentration efficiency [83]. However,
since BDD surface is chemically inert, the adsorptive force between nanoparticle and
BDD thin film surface is weak. This could result in desorption of the NPs from the
electrode surface or their aggregation, in particular, in case of a hydrodynamic system.

In contrast, NPs embedded carbon film electrodes can be fabricated with much sim-
pler processes, also summarized in Table 1.4. In the 1990s, McCreery et al. reported the
small Pt cluster (~1 nm) dispersed glassy carbon film electrodes prepared by pyrolysis of
both carbon and metal precursors at 600$^\circ$ C, and demonstrating high electrocatalytic
activity toward the hydrogen evolution reaction and ORR [84]. This method was applied
to fabricate carbon nanofiber electrodes containing various metal NPs and nanoalloys
in order to apply them for electroanalysis of sugars and ORR. Ion implantation of metals
into BDD was reported to fabricate iridium NPs containing BDD electrode [10]. This
method can suppress the detachment of NPs from BDD because the most of the NPs
were embedded and only part of NPs appeared on the electrode surface. However, ion
implantation system is very expensive as equipment of electrode fabrication.

You and Niwa et al. reported the metal NPs embedded carbon film electrodes by
using radio-frequency co-sputtering by placing metal pellets on the carbon target. Due
to poor immiscibility of metals and carbon, various metals such as Pt, Ni, Cu can be
employed to fabricate such hybrid film electrodes [85–87]. These electrodes have atomi-
cally flat surface, which was characterized by AFM. The carbon matrix composed of
disordered graphite-like carbon with partially exposing top body of the NPs, observed
by TEM. The NPs of average diameter of 2.5 nm was embedded in the carbon film when
Pt was used and that of 4 nm was obtained by using palladium (Pd) as indicated in
Figure 1.11 [85, 88].

The former electrode shows high electrocatalytic performance for hydrogen peroxide
detection compared with bulk Pt electrode. Figure 1.12 shows amperometric response
of glucose at glucose oxidase (GOD) modified Pt-NPs embedded carbon film electrode
compared with that at GOD modified Pt bulk electrode. Due to low Pt amount
(1–7 at. %), the absolute current value at the GOD modified bulk Pt electrode is larger
Table 1.4  Summarization of the metal nanoparticle modified carbon electrode for electroanalysis.

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<tr>
<th>Metal NP</th>
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<td>NP modification onto the electrode</td>
<td></td>
<td></td>
<td></td>
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<td>Au-NP</td>
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<td>As(III)</td>
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<td>Au-NP</td>
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<td>Ni-Cu nanoalloy</td>
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Figure 1.11 Plain view of the co-sputtered (a) Pt and (b) Pd nanoparticle embedded carbon film electrode obtained by TEM. Adapted in part with permission from [88] and [87]. Copyright 2002 American Chemical Society and 2007 MYU KK, respectively.

than that at GOD modified Pt-NPs embedded carbon film electrode, but latter electrode shows much flatter baseline current compared with that at bulk electrode, indicating Pt-NPs embedded carbon film is extremely stable. The high electrocatalytic activity for oxygen reduction and hydrogen evolution was also observed at Pt-NPs embedded carbon film electrodes [89]. In contrast, non-noble Ni-NP and Cu-NP embedded graphite-like carbon film are applied to sugar detection [86]. Figure 1.13 shows the hydrodynamic voltammograms of glucose at Ni-NP embedded graphite-like carbon film compared with Ni bulk electrode. Electrocatalytic oxidation of glucose
Electroanalysis with Carbon Film-based Electrodes

begin at slightly negative potential (~0.15 V) than that of Ni bulk electrode, and exhibiting analytical performance such as high stability with relative standard deviation (RSD) of 1.75 % (n = 40) and low limit of detection (LOD) of 20–50 nM for glucose, fructose, sucrose and lactose.

Recently, UBM co-sputtering with multiple targets, which can independently control each target power of metal and carbon have been developed to widely control the metal NPs concentration and carbon film structure. In fact, sp² and sp³ ratio in the carbon film can be widely controlled by changing acceleration voltage between target and substrate [39]. Kato et al. recently reported Au-NP embedded carbon film electrode with a variety of Au-NP concentrations (from 13 to 21 at. %) fabricated by UBM co-sputtering, [90] This electrode was applied to As(III) detection, which demonstrates that Au-NP embedded electrode at the optimized Au concentration (17 at. %) exhibit not only lower LOD of 1 ppb than Au bulk electrode, but also sufficient long-term stability with RSD of 11.7 % (n = 15 / 5 days) as shown in Figure 1.14. This higher stability is ascribed to tightly embedded Au-NPs in carbon matrix. In fact, As stripping current with the Au-NPs electrodeposited on the sputtered carbon film is sharply decreased because of weak interactions between Au-NPs and carbon surface, resulting in desorption of Au-NPs.

More recently, bimetallic nanoparticles (nanoalloy) embedded carbon film electrode composed of small Ni-Cu nanoalloy (~3 m) are also fabricated by Shiba et al. (Figure 1.15) the composition of which can be dynamically changed without changing nanoalloy size [91]. The electrocatalytic activity for D-mannitol is significantly affected by Ni/Cu ratio (including monometallic Ni-NP and Cu-NP), resulting in getting maximum with Ni/Cu ratio of 64/36. Figure 1.16a and 1.16b show CVs comparing the electrocatalytic activities of the Ni₆₄Cu₃₆ nanoalloy embedded carbon film and the Ni₇₀Cu₃₀ alloy (without a carbon film matrix) for D-mannitol, which is one of the intestinal permeability indicators.
Figure 1.14 Stability of As measurement at the Au-embedded UBM carbon film (circle, Au = 17 at %) and the Au-electrodeposited UBM carbon film (triangle) electrodes. The average peak current density was obtained from repetitive As measurements (3 measurements per day). ASV parameters: deposition at −0.8 V vs Ag/AgCl for 60 s, potential scan rate of 1.5 V s⁻¹. Adapted in part with permission from [90]. Copyright 2003 American Chemical Society.

Figure 1.15 Nanostructural observation and elemental mapping obtained by HAADF-STEM and STEM-EDS measurements. (See color plate section for the color representation of this figure.)

At the Ni₆₄Cu₃₆ nanoalloy embedded carbon film, a smaller D-mannitol oxidation current (red solid line) and background current (black solid line) were obtained because of the smaller metal surface area. After correction with the surface metal concentration (15.6 at%, dotted line), a background-subtracted electrocatalytic current density (S–B current) of 1529 μA cm⁻² at 0.6 V for the Ni₆₄Cu₃₆ nanoalloy embedded carbon
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A film was obtained, which is 3.4 times larger than that of the Ni$_{70}$Cu$_{30}$ alloy film ($443\, \mu A\, cm^{-2}$). These results clearly demonstrate that the nanoalloys exhibit higher electrocatalytic activity due to the small size of the nanoalloys separated from each other by the carbon matrix. Similar to the electrode mentioned above, most of the nanoalloy body are embedded in carbon film demonstrated by AFM measurements, where convection size of the surface is less than nanoalloy radius (<1.5 nm, Ra of 0.21 nm). Therefore, the electrocatalytic activity and stability toward D-mannitol oxidation are extremely high as shown in Figure 1.16c (RSD: 4.6 %, n = 60), which is superior to Ni-Cu alloy film electrode with a similar Ni/Cu composition (32.2 %, n = 60) [91].

**Figure 1.16** CVs obtained with (red line) or without (black line) 300 μM D-mannitol in 0.1 M NaOH solution using (a) the Ni$_{64}$Cu$_{36}$ nanoalloy embedded carbon film and (b) the Ni$_{70}$Cu$_{30}$ alloy film before (solid line) and after (dotted line) correction by metal concentration. (c) Electrocatalytic oxidation currents after background subtraction with various compositions of nanoalloy embedded carbon films for 500 μM D-mannitol at 0.6 V during an anodic scan (n = 3). All the potential sweep measurements are conducted at a scan rate of 0.1 V s$^{-1}$ after a background scan at the same scan rate. Adapted in part with permission from [91]. Copyright 2016 Royal Society of Chemistry.

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**References**


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