Hybrid Carbon Film Electrodes for Electroanalysis

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Carbon materials have been widely used for electrochemical analysis and include carbon nanotubes, graphene, and boron-doped diamond electrodes in addition to conventional carbon electrodes, such as those made of glassy carbon and graphite. Of the carbon-based electrodes, carbon film has advantages because it can be fabricated reproducibly and micro- or nanofabricated into electrodes with a wide range of shapes and sizes. Here, we report two categories of hybrid-type carbon film electrodes for mainly electroanalytical applications. The first category consists of carbon films doped or surface terminated with other atoms such as nitrogen, oxygen and fluorine, which can control surface hydrophilicity and lipophilicity or electrocatalytic performance, and are used to detect various electroactive biochemicals. The second category comprises metal nanoparticles embedded in carbon film electrodes fabricated by co-sputtering, which exhibits high electrocatalytic activity for environmental and biological samples including toxic heavy metal ions and clinical sugar markers, which are difficult to detect at pure carbon-based electrodes.

Keywords Carbon film, sputter deposition, surface termination, metal nanoparticles, electroanalysis

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1 Introduction

In the electroanalytical chemistry field, carbon materials have been widely used because they have wider potential windows than those of other metals such as platinum and gold. Previous review articles have introduced various carbon materials used in electrochemistry.\(^2,3\) Glassy carbon (GC) electrodes have been employed for basic electrochemical measurements. In contrast, printed electrodes using carbon inks have been studied for use in practical biosensors and wearable sensing devices because of their low cost and simple fabrication process.\(^4,5\)

Since the 1990s, new carbon materials have been employed by several groups. Boron-doped diamond (BDD) electrodes\(^6-9\) have a wider potential window, a lower capacitive current and low adsorption of biomolecules than other electrodes. Thanks to their superior electrochemical performance, BDD electrodes have led to an increase in the number of analytes with high oxidation or reduction potentials.\(^10\) However, BDD electrodes have mainly been formed by using plasma chemical vapor deposition (CVD) or a thermal CVD process with high deposition temperature of 400 – 700°C, which has limited the available substrate materials.

More recently, various nanocarbons including carbon nanofibers, carbon nanotubes (CNT) and graphenes\(^9-11\) have been studied mainly for developing electrode materials for energy devices, since such nanocarbons have extremely large surface areas. This is also advantageous for immobilizing various biomolecules, such as enzymes, to develop sensitive biosensors\(^12-14\) and biofuel cells.\(^15\) However, their large surface area also increases the noise level when employing such electrodes for direct electrochemical measurements. In addition, the reproducible fabrication of nanocarbon electrodes is difficult because the materials have to be used after modification on solid electrodes.

In contrast, carbon films have advantages because they can be fabricated reproducibly into electrodes with a wide range of shapes and sizes.\(^16\) Various different carbon film fabrication processes have been proposed. The pyrolysis of deposited 3, 4, 9, 10-peryleneetetracarboxylic dianhydride\(^17,18\) or polymer\(^19,20\) has been used to fabricate the carbon films that are applied to electrochemical measurements. In contrast, various kinds of vacuum deposition processes have been used to fabricate carbon film electrodes. These processes include radio frequency (RF) magnetron sputtering,\(^21\) electron cyclotron resonance (ECR) sputtering,\(^22,23\) electron beam evaporation,\(^24\) and a filtered cathodic vacuum arc (FCVA) system.\(^25\) We have employed ECR and unbalanced magnetron (UBM) sputtering to fabricate nanocarbon film electrodes.\(^26,27\) The film electrodes consist of both sp\(^2\) and sp\(^3\) bonds, and the sp\(^3\) content can be controlled up to around 40% by changing ion acceleration voltage.\(^28\) The carbon film has a very smooth surface\(^29\) and has a much wider potential window than GC electrodes. We have been using these films to detect DNAs, including methylated DNAs,\(^29,31\) and to detect a neurotransmitter after modifying the film with enzymes.\(^32\)

Although the pure carbon film electrodes show excellent electrochemical characteristics as described above, film electrodes were required that offered improved performance such as higher electrocatalytic activity and controllable surface wettability. Doping or surface termination with other atoms or modifying the carbon electrode surface with electrocatalytic nanomaterials can be utilized to meet these requirements. In this review, we focus on two types of hybrid carbon film electrodes for application in the field of electroanalytical chemistry. The first examples are surface terminated or doped carbon film electrodes with nitrogen or fluorine atoms. We then move on to carbon film electrodes embedded or modified with metal nanoparticles (NPs) that offer improved electrochemical activity.

2 Surface Terminated or Doped Carbon Film Electrodes

It has been reported that the surface termination or doping of carbon materials with other atoms changes their electrochemical properties. The introduction of an oxygen containing group such as a hydroxyl group significantly changes surface wettability and has been applied when performing biochemical measurements.\(^33\) A hydrophilic surface is particularly effective in suppressing the adsorption of hydrophobic or large molecules on the electrode surface.

In contrast, Ozaki et al. reported the development of a carbon alloy that greatly reduces the overpotential of an oxygen reduction reaction (ORR) by doping carbon materials with boron and nitrogen.\(^34\) Dai et al. reported nitrogen-doped CNTs, which also exhibit high ORR activity.\(^35\) In contrast, the fluorination of carbon materials, including GC, CNTs, graphene and diamond, has been reported since fluorinated carbons provide interesting characteristics such as improved hydrophobicity and control of the surface electron transfer rate.\(^36-38\) Thanks to the above unique characteristics, surface terminated and doped carbon film electrodes have been developed mainly for electroanalytical applications.

2-1 Nitrogen terminated or doped carbon film electrodes

Several fabrication methods have been employed for nitrogen doped or terminated carbon film including pulsed laser-arc deposition,\(^40,41\) various sputtering methods,\(^39,44-50\) and CVD,\(^43\) as shown in Table 1. Yoo et al. used filtered cathodic arc and pulsed laser arc processes, respectively, to fabricate N-containing or doped tetrahedral amorphous carbon films.\(^39\) Honda et al. reported a hydrogenated amorphous carbon film fabricated with micro-wave assisted plasma-enhanced CVD.\(^46\) Various sputter deposition methods, including DC magnetron,\(^48\) ECR\(^42\) and
Ultraviolet and photosensitive (UBM) sputtering, have been used to fabricate nitrogen containing carbon films. The structure of N-containing carbon films such as the sp² concentration can be controlled by changing the nitrogen concentration.

N-containing carbon films exhibit unique electrochemical performance compared with pure carbon films. Several groups have reported that the potential window of carbon film electrodes is increased by increasing nitrogen concentration. The electrochemical activity when using Ru(NH₃)₆²⁺/³⁺ and Fe(CN)₆³⁻/⁴⁻ have also been studied. Swain et al. reported that the resistivity decreases with increasing nitrogen concentration. They also reported that the ΔE value of Fe(CN)₆³⁻/⁴⁻, which shows an inner sphere electron transfer, can be decreased significantly from 119 to 84 mV, and the electrochemical activity when using Ru(NH₃)₆²⁺/³⁺, Fe²⁺/³⁺ and Fe(CN)₆³⁻/⁴⁻ have also been studied. Swain et al. reported that the potential window and electron transfer behavior of Fe(CN)₆³⁻/⁴⁻ with low N concentration film improved electrochemical reaction of H₂O₂ and oligonucleotides. However, the absolute nitrogen concentration was not evaluated since it was controlled by the N₂ gas flow rate. Our group studied the effect of the nitrogen concentration on the structure and electrochemical properties of the carbon films. Unlike the results reported by Swain et al., the sp³ concentration is lower and the ΔE value of Fe(CN)₆³⁻/⁴⁻ is higher than the corresponding values for pure carbon film. The sp² concentration decreases and the ΔE of Fe(CN)₆³⁻/⁴⁻ increases when the nitrogen concentration increases from 8.89 to 30.39%. Therefore, we need to consider film with a nitrogen concentration lower than 8.89% and fabricate such films by carefully controlling the N₂ gas flow rate during sputtering. Similarly to the report by Swain et al., the ΔE of Ru(NH₃)₆²⁺/³⁺ depends on the nitrogen concentration. We prepared carbon films with lower nitrogen concentrations by employing UBM sputtering, which realizes a similar film structure to that formed by ECR sputtering. Similar to the report by Swain et al., the ΔE of Ru(NH₃)₆²⁺/³⁺ is almost unchanged at lower nitrogen concentrations less than 20% but increases significantly at concentrations exceeding 20%. In contrast, the ΔE of Fe(CN)₆³⁻/⁴⁻ decreases by incorporating nitrogen and shows its lowest value at concentrations below 9%, indicating that the carbon films reported by Swain et al. might contain relatively low concentrations of nitrogen.

Although the electrochemical activity of carbon films was improved by incorporating a low concentration of nitrogen, the electrochemical activity also depended upon the chemical structure of the nitrogen containing groups. As shown in Fig. 2, carbon films containing nitrogen also contain various functional groups, including graphite-like (GL), pyridine-like (PL), pyrrole-like and nitrogen-oxide structures. As regards the ORR activity, it has been reported that a carbon alloy with a larger quantity of GL structures exhibits higher ORR activity than those with a larger quantity of PL structures. Nakamura et al. studied the ORR activity of four types of nitrogen-doped highly oriented

| Film and fabrication process | Electroanalytical application |
|-----------------------------|-------------------------------|
| N-doped tetrahedral amorphous carbon film by filtered cathodic arc | Extended potential window and electron transfer rate of some redox species |
| N-doped DLC by DC magnetron sputtering | ASV analysis of heavy metal ions (Pb²⁺, Cd²⁺) |
| N-containing tetrahedral amorphous carbon film by pulsed laser-arc deposition | Wide potential window and electron transfer behavior of Ru(NH₃)₆²⁺/³⁺ and Fe(CN)₆³⁻/⁴⁻ |
| N-doped hydrogenated amorphous carbon films by microwave-assisted plasma-enhanced CVD | Propanol and hydrochlorothiazide oxidation in standard and synthetic biological fluid |
| N-doped carbon film by ECR sputtering | Measurements of electron transfer rates for Fe²⁺/³⁺, Fe(CN)₆³⁻/⁴⁻, Ce²⁺/³⁺ |
| N-doped carbon film by UBM sputtering | Potential window and electron transfer behavior of Ru(NH₃)₆²⁺/³⁺ and Fe(CN)₆³⁻/⁴⁻ |
| (1) Sputter deposited carbon by introducing Ar/N₂ gas | Improved electrochemical reaction of H₂O₂ and oligonucleotides |
| (2) Carbon film annealed with NH₃ at 300°C | Potential window and electron transfer behavior of Fe(CN)₆³⁻/⁴⁻ with low N concentration film |
| N-doped carbon film by UBM sputtering and then treated with NH₃ or N₂ plasma | Improved electrochemical reaction of H₂O₂ and oligonucleotides |

Fig. 1 Variation of ΔE for Fe(CN)₆³⁻/⁴⁻ and Ru(NH₃)₆²⁺/³⁺ dependent on nitrogen concentration in the carbon films
pyrolytic graphite (N-HOPG) model catalysts and reported that the PL-HOPG exhibits higher ORR activity than the other HOPGs. The ORR peak potential became more positive as PL bonding increased, which was similar to previously reported results. Due to improved activity and the wide potential window of carbon films containing nitrogen, they have been employed for measuring biochemical and environmental samples. Our group fabricated two types of carbon films, one rich in GL-like nitrogen-containing bonds and the other rich in PL-like bonds without changing their nitrogen concentration, sp²/sp³ ratio or surface flatness. The ORR peak potential became more positive as PL bonding increased, which was similar to previously reported results. Due to improved activity and the wide potential window of carbon films containing nitrogen, they have been employed for measuring biochemical and environmental samples. Zeng et al. reported the detection of Pb²⁺ and Cd²⁺, which are pollutants of drinking water. Swain’s group has used their N-containing tetrahedral amorphous carbon film, which they obtained by pulsed laser-arc deposition, for detecting a number of analytes including norepinephrine, tryptophan and tyrosine by combining the film with a flow injection analysis (FIA) system. Figures 3A and 3B show voltammograms of L-ascorbic acid and nicotinamide adenine dinucleotide (NADH) obtained with pure carbon film, PL-rich and GL-rich nitrogen containing carbon films. Pure carbon film (pure-UBM) was formed by UBM sputtering and PL-rich nitrogen containing carbon film (PL-UBM) was prepared by the NH₃ treatment of pure-UBM at 300 °C. In contrast, graphite-rich nitrogen containing carbon film (GL-UBM) was prepared by UBM sputtering with the addition of N₂ gas to the Ar sputtering gas. The peak potentials of both PL-UBM and GL-UBM films are lower for both L-ascorbic acid and NADH, and specifically the lowest peak potential was observed at PL-UBM. We also reported clear overpotential decreases when detecting mononucleotides, such as guanosine and adenosine.

For the electrochemical measurement of biochemical samples, the biocompatibility of the sensing electrode is very important. Yasukawa et al. studied the antifouling of the electrode surface using DLC film electrodes. They observed no significant change in the voltammogram of 0.1 mM Fe(CN)⁶³⁻/⁴⁻ after soaking in bovine serum albumin (BSA) solution at an oxygen plasma treated DLC film electrode. In contrast, a significant increase in the ΔE of Fe(CN)⁶³⁻/⁴⁻ was observed at the DLC without treatment. Recently, our group studied the effect of various types of plasma treatment on BSA adsorption and electrochemical response. Figure S1 (Supporting Information) compares voltammograms for 1 mM Fe(CN)⁶³⁻/⁴⁻ in the presence or absence of 100 mg/mL BSA during measurement at untreated, H₂O plasma treated, and NH₃ plasma treated UBM carbon film electrodes. Without treatment, ΔE increases from 0.162 to 0.843 V in a 0.1 M KCl solution with BSA, indicating the adsorption of BSA on the electrode surface. In contrast, ΔE increases were suppressed significantly at the electrodes with plasma treatment. In particular, the ΔE increase was negligible at the NH₃ plasma treated UBM carbon film, except for the current decrease in the BSA containing solution because the diffusion coefficient in this solution decreases due to a viscosity increase. We also observed the suppression of serotonin adsorption after its electrochemical oxidation after NH₃ plasma or NH₃/H₂O plasma treatment.

2-2 Fluorinated carbon film electrodes

Compared with nitrogen containing carbon films, there are fewer reports about fluorinated carbon film electrodes. Fluorinated carbon films have been formed by magnetron sputtering and their physical properties have been reported, but their electrochemical characterization has not been studied. Our group treated ECR nanocarbon films with CF₄ plasma, which is more stable than GC, also after CF₄ plasma treatment. The water contact angle increases after CF₄ plasma treatment and the electron transfer rates of various redox species such as Fe²⁺/³⁺ and Fe(CN)⁶³⁻/⁴⁻ were greatly suppressed in aqueous solutions. However, widely controllable electron transfer at fluorinated carbon film can be successfully applied to the electrode of an electrochemical biosensor. We developed an electrochemical lipopolysaccharide (LPS) sensor as shown in Fig. 4A. The

Fig. 2 Schematics of different nitrogen-containing groups. Reproduced with permission from Ref. 49.

Fig. 3 Cyclic voltammograms of the (a) PL-UBM, (b) GL-UBM and (c) pure-UBM, (N = 4 at %) electrodes for (A) 0.1 mM NADH in 0.1 M phosphate buffer (pH 8.2) at a scan rate of 0.01 V/s and (B) 1.0 mM L-ascorbic acid in 1.0 M NaNO₃ at a scan rate of 0.1 V/s. Reproduced with permission from Ref. 49.
fluorinated carbon film was modified with poly-ε-lysine (ε-PL), which captures LPS, and then with ferrocene labeled polymyxin B (FcPMB). The redox reaction of FcPMB was enhanced in the presence of Fe\(^{2+/3+}\) by the redox cycling of FcPMB. Although the current increased with increasing Fe\(^{2+/3+}\) concentration, the background current also increased because Fe\(^{2+/3+}\) was directly oxidized on the electrode surface. However, the redox reaction of Fe\(^{2+/3+}\) can be suppressed at the fluorinated carbon film as shown in Fig. 4B, while maintaining sufficient electrochemical activity for FcPMB. Figure 4C shows the relationship between the LPS concentration and the current obtained with ε-PL modified fluorinated electrodes and the original nanocarbon film electrodes. The fluorinated carbon film electrode has a good linear relationship over a relatively wide concentration range compared with pure carbon film due to the suppression of the direct electrochemical reaction of Fe\(^{2+/3+}\).

Fluorinated carbon films have been used to detect lipophilic antioxidants in foods such as olive oil. Kuraya et al. employed our fluorinated carbon film electrode to detect α-tocopherol (vitamin E) in olive oil samples by using a bicontinuous microemulsion (BME) solution and succeeded in selectively detecting lipophilic vitamin E\(^{60,61}\) with no interference of L-ascorbic acid, which is a well-known hydrophilic antioxidant.

3 Carbon Film Electrode Embedded with Metal Nanoparticles

3.1 Electrode fabrication process and structure

Compared with carbon electrodes, metal electrodes have advantages, including high electrochemical activity and the adsorption of heavy metal ions and thiol compounds. Metal NPs, such as platinum (Pt) NPs, have been extensively studied as a fuel cell catalyst. The high electrocatalytic activity was also advantageous when NPs were applied to electroanalysis as reviewed by Compton et al.\(^{62}\) They introduced silver (Ag), gold (Au), Pt, palladium (Pd), ruthenium (Ru), copper (Cu) and nickel (Ni) NPs, their fabrication methods, characterization and applications. Carbon materials are widely used as electrodes to modify metal NPs. Bulk electrodes such as GC\(^{63}\) and nanocarbons such as CNTs\(^{64}\) have been modified with metal NPs. In contrast, carbon film electrodes, suitable for practical analytical devices modified with NPs have also been developed by several groups.

Various processes, including electrochemical deposition, ion implantation, and co-sputtering, have been used for fabricating carbon film electrodes modified or embedded with metal NPs. Figure 5 shows the structures of such modified electrodes. Electrochemical deposition and self-assembly deposition are simple and can be used to realize the structure shown in Fig. 5(A). The NPs are physically adsorbed on a carbon substrate with a relatively large surface area. However, the NPs sometimes detach from the carbon surface depending on the measurement condition. In contrast, carbon films embedded with NPs as shown in Fig. 5(B) can be obtained by the pyrolysis of a metal complex and polymer composite, ion implantation, and co-sputtering processes. Metal NPs are distributed inside the carbon film with some of them appearing on the surface, and they only contribute to the electrochemical reaction. Since NPs were partially embedded in the stable carbon film, NP
detachment can be suppressed. In contrast, the surface area of NPs for an electrochemical reaction is smaller than that of NP modified electrodes. Our group has employed RF and UBM co-sputtering processes to fabricate carbon film electrodes embedded with metal NPs. Our sputtering equipment is shown schematically in Figs. S2(A) and S2(B) (Supporting Information). The RF sputtering system in Fig. S2(A) has one target system and co-sputtering was achieved by placing metal disks on the carbon target. This method was previously reported by Hayashi and Hirono et al. for fabricating carbon film embedded with magnetic cobalt (Co)NPs. The metal NP concentration can be controlled by changing the number of metal disks. However, this system is limited since the sputtering rate of metals is much higher than that of carbon. Figure S2(B) shows a UBM sputtering system that we are currently using to fabricate carbon films embedded with metal NPs. Since we employed a three-target system and each target faces the substrate, two metals and carbon can be sputter deposited simultaneously and even form metal alloy NPs (nano alloy) in the carbon film. The concentrations of each metal and the carbon can be varied by changing the power of each target, and so we can control the film structure more flexibly.

Table 2  Summary of the metal nanoparticle modified film electrodes for electrochemistry

| Metals | Fabrication process                                                                 | Applications                                                                 |
|--------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------|
| Pt     | Pyrolysis of polymer and Pt complex RF-co-puttering of carbon and Pt67,68           | Electrocatalytic reactions69,70                                               |
|        | UBM co-sputtering of carbon and Pt71                                                | Electrochemical reduction of O2,71 detection of H2O2, glucose by enzyme       |
|        |                                                                                     | modification and acetylcholine as an electrode of HPLC70                      |
| Co/Ru  | Pt/Ru incorporated N-doped DLC film by DC co-magnetron co-sputtering72               | Potential window and voltammetric behavior72                                  |
| Pd     | Urchin-like PdNPs were electrodeposited on a CNT thin film73                          | Selective detection of NO2,75                                                 |
| Pd     | RF-co-puttering of carbon and Pd74                                                   | Detection of H2O2,75                                                          |
| Au     | Electrodeposition of Au on a carbon film using porous alumina template75             | Structure characterization75                                                   |
|        | Au deposited on BDD film electrode76                                                 | Total inorganic As detection in water76                                       |
|        | Self-assembly deposition of AuNPs at amine-terminated BDD77                          | Detection of neuraminidase77                                                  |
|        | Electrodeposition of AuNPs on DLC films formed by filtered cathodic vacuum arc deposition78 | Non-enzymatic detection of glucose in NaOH solution78                         |
|        | UBM co-sputtering of carbon and Au79                                                | Detection of As79 in tap water samples by ASV and comparison with ICP-MS results79 |
| Ir     | RF-co-puttering of carbon and Ir81                                                  | Detection of H2O2 and application to l-glutamate enzyme biosensor81           |
| Ni     | Ion implantation of Ir into BDD electrode82                                          | Amperometric detection of As3+ ion82                                          |
| Ni     | RF-co-puttering of carbon and Ni81                                                   | Detection of sugars by combining with HPLC and improved detection limit83      |
| Ni/Cu  | Ion beam co-sputtering employing a 3-cm Kaufman ion source by attaching a Ni strip to the graphite target48 | Structure characterization (not for electrochemical analysis)84                |
| Ni/Cu  | Electrodeposition of NiNPs onto BDD electrode85                                       | Detection of adenine and DNA in alkaline solution86                            |
| Ni/Cu  | UBM co-sputtering of carbon, Ni and Cu and changing Ni/Cu ratio86                    | Improved sensitivity and stability for clinical sugar markers for intestinal disease86 |
| Cu     | RF-co-puttering of carbon and Cu88                                                   | Improved detection limit of clinical sugar markers by combining with HPLC87   |
| Bi     | Electrochemical deposition of Bi89                                                  | Detection of glucose in alkaline solution88                                   |
|        | Electrochemical deposition of Bi onto BDD electrode80                                 | Determination of Zn2+, Cd2+ and Pb2+ by square wave ASV89                     |

Fig. 5  Structures of carbon film electrodes modified and embedded with metal NPs (a) carbon film modified metal NPs, (b) carbon film with embedded metal NPs, (c) metal selectively deposited onto metal NPs embedded in carbon film.
Our group fabricated carbon film electrodes embedded with AuNPs by using UBM co-sputtering as shown in Fig. S2(B) and then selectively deposited Au only on the embedded AuNPs as shown in Fig. 5(C). This makes it possible to increase the surface area while suppressing NP detachment.66 The next section describes fabrication methods and the electroanalytical application of the modified carbon films embedded with metal NPs listed in Table 2 for each metal NP.

3.2 Electrochemical characterization and analytical applications

3.2.1 Carbon films embedded with Pt, Pd and IrNPs

Pt and PdNPs have been intensively studied to develop electrodes for fuel cells by using them to modify carbon materials with a higher surface area. In contrast, film electrodes modified with PtNPs have been reported for basic studies related to fuel cell electrodes or electroanalytical applications. McCreery et al. prepared a GC-like film electrode incorporating PtNPs by the pyrolysis of polymers with Pt, characterized aspects of its structure such as PtNP size and studied electrocatalytic activity.67,68 Our group employed RF co-sputtering as shown in Fig. S2(A) to fabricate carbon film electrodes embedded with PtNPs69,70 and PdNPs.74 Figure 6 shows TEM images of carbon film electrodes embedded with (A) PtNPs and (B) PdNPs. Since the compatibility between the noble metal and carbon is poor, PtNPs with an average diameter of 2.5 nm and PdNPs with an average diameter of 4.0 nm can be clearly observed in Figs. 6(A) and 6(B). The electrodes show high electrochemical activity for hydrogen evolution, dioxygen reduction69 and \( \text{H}_2\text{O}_2 \) oxidation.70 Figure 6(C) shows the glucose response at the glucose oxidase (GOx) modified carbon film electrodes embedded with PtNPs (above) and Pt bulk electrodes modified with GOx (below). A stable baseline can be obtained at the GOx modified carbon film electrode embedded with PtNPs, while the baseline decreases quickly at the Pt bulk electrode modified with GOx. In addition, the baseline current of a film electrode modified with 6.5% GOx and embedded with PtNPs is much lower than that of the Pt bulk electrode. The surface deactivation is particularly serious when we use the electrode to detect trace amounts of biochemicals, such as neurotransmitters, because it takes a long time to obtain a stable baseline current. We employed carbon film embedded with PtNPs to detect acetylcholine and choline by combining HPLC and an enzymatic reactor and obtained an improved detection limit and baseline stability compared with a Pt bulk electrode.70 We also fabricated a carbon film electrode embedded with IrNPs and achieved a low detection limit for L-glutamate, which is known to be a neurotransmitter, by modifying the electrode with glutamate oxidase.81

Carbon film electrodes embedded with PtNPs fabricated by UBM co-sputtering were employed for detecting geosmin, which is produced by certain species of fungi and blue-green algae and is known to have an earthy/musty odor.71 By applying the electrode as a detector for HPLC, an improved linear range of 0.1 - 1000 \( \mu \text{g/L} \) and an LOD of 100 ng/L were achieved while the Pt film electrode exhibited a linear range of 1 - 1000 \( \mu \text{g/L} \), and an LOD of 1.0 \( \mu \text{g/L} \). A co-sputtering method was also used to fabricate carbon film embedded with a metal nanoalloy by using carbon and Pt\(_{50}\)Ru\(_{50}\) alloy targets.72 The electrodeposition of metal NPs onto carbon film electrodes
template as a mask and a unique nanostructure was realized.\textsuperscript{75} Modify carbon film with AuNPs using a porous alumina applications. Electrochemical deposition has been employed to reported carbon films modified with AuNPs for electroanalytical electrodes. Figure 7(A) shows ASV results for various As\textsuperscript{3+} AuNPs are more stable than that of AuNP-deposited or carbon embedded in the carbon film with a wide potential window, the UBM co-sputtering of carbon and Au. Since AuNPs are toxicity. The BDD film electrode modified with AuNPs exhibits an electrode for metal ion analysis, is now avoided due to its electrodeposition and used it for detecting NO\textsubscript{2}.\textsuperscript{73} High electrocatalytic activity can be expected with such NPs because of their large surface area and very fine nanostructure.

\subsection*{3.2-2 AuNPs embedded carbon films}

Au electrodes have been widely used for electroanalytical chemistry because various electroactive species can be modified by employing a self-assembly technique using a thiol group.\textsuperscript{92} The unique catalytic activity of AuNPs has been developed by Haruta et al.\textsuperscript{93} As shown in Table 2, several groups have reported carbon films modified with AuNPs for electroanalytical applications. Electrochemical deposition has been employed to modify carbon film with AuNPs using a porous alumina template as a mask and a unique nanostructure was realized.\textsuperscript{75} Swain et al. fabricated BDD film modified with AuNPs by electrodeposition and applied it to detect inorganic arsenic (As\textsuperscript{3+}) in real water samples by anodic stripping voltammetry (ASV) analysis.\textsuperscript{76} Unlike other metal ions, such as Zn\textsuperscript{2+}, Cd\textsuperscript{2+} and Pb\textsuperscript{2+}, As\textsuperscript{3+} ions cannot be preconcentrated on a carbon electrode by applying a negative potential. Au and bismuth (Bi)-based electrodes have been used because the mercury electrode, which was the most suitable and traditionally used electrode for metal ion analysis, is now avoided due to its toxicity. The BDD film electrode modified with AuNPs exhibits a detection limit of 0.005 ppb, which is lower than that of a Au foil electrode (LOD = 0.23 ppb).

We developed carbon film embedded with AuNPs by the UBM co-sputtering of carbon and Au. Since AuNPs are embedded in the carbon film with a wide potential window, the AuNPs are more stable than that of AuNP-deposited or carbon electrodes. Figure 7(A) shows ASV results for various As\textsuperscript{3+} concentrations obtained with a UBM carbon film electrode embedded with AuNPs (Au = 17 at %). The peak current increased linearly as the As\textsuperscript{3+} concentration increased. As a result, an LOD of 0.55 ppb (S/N = 3) and a linear dynamic range of 1 - 100 ppb were obtained. This LOD is much lower than the concentration recommended by the WHO guideline (10 ppb).\textsuperscript{79} Figure 7(B) shows the variation in the stripping current for repetitive measurements of As\textsuperscript{3+} when using the UBM carbon film embedded with AuNPs (Au = 17 at %) and the UBM carbon film electrodes with electrodeposited AuNPs. The latter electrode shows a higher current at the first ASV measurement, but the current decreases significantly after 5 days. In contrast, the former electrode shows considerably superior reproducibility thanks to the embedded structure. The ASV results agreed well with those obtained by ICP-MS with tap water samples. Besides AuNP modified electrodes, electrodes modified with NPs of other metals have been used for detecting various metal ions. Einaga et al. fabricated a BDD electrode with embedded IrNPs by ion implantation and applied it to As\textsuperscript{3+} detection.\textsuperscript{82} Bi electrodeposited on carbon electrodes including BDD were developed to detect heavy metal ions including Cd\textsuperscript{2+} and Pb\textsuperscript{2+}.\textsuperscript{89,90}

The beneficial effects of selenium (Se\textsuperscript{4+}) on human health have been confirmed, and they include anticancer effects and enhancement of the immune response. However, it is also known that the Se\textsuperscript{4+} concentration range between a beneficial effect and a toxic level is very narrow (50 - 220 \textmu g/day). Therefore, the detection of Se\textsuperscript{4+} is also important. However, preconcentration by electrochemical reduction of Se\textsuperscript{4+} is slow compared with other metal ions. We enhanced the AuNP size by the selective deposition of Au ions only on the exposed AuNP parts of carbon film electrodes with embedded AuNPs, as illustrated in Fig. 5(C), by utilizing the difference between the overpotentials of the Au and carbon surfaces.\textsuperscript{46} The SEM images and ASV results before (A and C) and after (B and D) Au deposition are shown in Fig. S3. Since the average size of the embedded AuNPs is less than 5 nm from a TEM image, the increase in AuNP size can be clearly observed and the sensitivity of Se\textsuperscript{4+} also improved. In addition to metal ion detection, AuNP modified carbon film electrodes can be used for the non-enzymatic oxidation of glucose in a strong alkaline solution.\textsuperscript{79}

\subsection*{3.2-3 Carbon films embedded with Ni and CuNPs and their alloy}

Au bulk electrodes have been used in HPLC to detect sugar by the pulse amperometric method. As with Au, it is well known that Ni and Cu show high activity for oxidizing various organic chemicals in strong alkaline solution.\textsuperscript{44} The mechanism of a Ni electrode for glucose oxidation is illustrated in Fig. 8. When a positive potential is applied to the Ni electrode, the surface hydroxyl-groups are oxidized and form a super oxide group,
which exhibits strong oxidation activity for sugars including glucose. Our group fabricated carbon films embedded with CuNPs with different numbers of surface hydroxyl groups and found that an electrode with more surface hydroxyl groups exhibits a higher glucose oxidation current than one with fewer hydroxyl groups. We also compared the performance of a Ni bulk electrode and a carbon film electrode embedded with NiNPs when both electrodes were used to detect glucose, fructose, sucrose, and lactose as electrochemical detectors for HPLC. In a hydrodynamic voltammetry measurement, we found that the oxidation current of a carbon film electrode embedded with NiNPs starts to increase at 0.25 V (vs. Ag/AgCl), whereas it increases from 0.4 V at a Ni bulk electrode, indicating the higher electrocatalytic activity of the NiNPs embedded in the carbon film. The electrode embedded with NiNPs shows higher sensitivity for the above four sugars than a Ni bulk electrode. To improve sensitivity, we recently fabricated an electrode consisting of a Ni/Cu nanoalloy embedded in carbon film by UBM sputtering, as shown in Fig. S2(B). Since our UBM sputtering equipment has three independent targets, the Ni/Cu ratio in the nanoalloy and its concentration can be widely controlled by changing each target power. The carbon film embedded with Ni/Cu nanoalloy was employed to achieve an improved detection limit for D-mannitol, which is one of the sugar biomarkers for the diagnosis of severe intestinal disease. Figure 9(A) shows the compositional dependence of the Ni/Cu ratios of the nanoalloy on the electrocatalytic current of 500 μM D-mannitol. The electrode activity of some bimetallic nanoalloys was more pronounced than that of the corresponding monometallic NPs and bulk electrodes. We obtained the highest value with a Ni/Cu ratio of around 64/36, indicating the high electrocatalytic activity of nanoalloys. Figure 9(B) compares continuous FIA measurements of 300 μM D-mannitol at a carbon film electrode embedded with Ni64Cu36 nanoalloy and a Ni70Cu30 alloy film electrode (without carbon). The carbon film electrode embedded with the nanoalloy provided higher stability for D-mannitol oxidation (RSD: 4.6%, n = 60) than that obtained with the alloy film electrode (RSD: 32.2%). The signal currents of the carbon films embedded with Ni64Cu36 nanoalloy are larger than those of the Ni70Cu30 alloy films after 10 measurements in spite of the Ni/Cu nanoalloy having a smaller area than the alloy film. Figure S4(A) shows five
clinical sugar markers including D-mannitol and (B) shows a schematic representation of the principle for diagnosing normal and damaged intestines. When a human subject ingests five sugar markers with different molecular sizes orally, the smaller sugars easily permeate the intestinal wall. In contrast, a small amount of large sugars permeates the intestinal wall in healthy people. Therefore, the amounts of permeated sugars and the balance between large and small sugars are greatly changed at gastrointestinal diseases can be detected by measuring the amount of each sugar in a urine sample. However, a low LOD of below 1 μM is required due to the low concentration of each sugar marker in the urine. Figure S4(C) shows chromatograms of permeated sugars where the intestinal wall of a patient. Since all sugars are non-metabolic, intestinal diseases can be detected by measuring the metabolic, amount of each sugar in a urine sample. However, a low LOD limit were superior to those of a Ni electrode and a Au electrodeposition was successfully applied for the detection of adenine and DNA in an alkaline solution. 

4 Conclusions

In this review, we introduced hybrid type carbon film electrodes for electroanalytical applications, including carbon films doped or surface terminated with other atoms, and the films modified or embedded with metal NPs. In addition to the unique properties of carbon films such as their wide potential window and low background noise, hybrid type carbon film electrodes offer an expanded performance range that includes surface hydrophilicity, hydrophobicity and electrocatalytic activity. In the future, various materials such as conducting metal oxides and metal organic frameworks could be successfully combined with various carbon films to create advanced functional electrodes, which will help to extend the range of measurable targets in environmental and biochemical analyses.

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6 Supporting Information

This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/

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