Introduction

Carbon materials have attracted growing interest in recent decades because of their various allotropes of carbon, such as graphene, fullerene, carbon nanotubes (CNT), diamond, diamond-like carbon (DLC), graphite, and their wide variety of structurally dependent electronic and electrochemical properties.\(^1\)\(^\text{--}\)\(^7\) In addition, the electrochemical activation of carbon surfaces has been used for applications including electroanalytical chemistry and electrocatalysis.\(^8\)\(^\text{--}\)\(^10\) Recently, it has been revealed that nitrogen atoms containing functional groups can be easily introduced onto the surfaces of glassy carbon (GC) electrodes by the electrochemical oxidation of ammonium carbamate in an aqueous medium at a highly positive electrode potential.\(^11\) We named this electro-oxidized GC electrode aminated GC electrode.\(^11\) In this electrochemical modification procedure, not only the amino group but also other nitrogen-containing functional groups can be introduced onto the GC electrode.\(^12\) In the immediate past, the redox waves between hydrogen molecule (H\(_2\)) and hydrogen ion at highly positive potential range were obtained by using long-term electrode reduction of the aminated GC electrode in a strong acid electrolyte. We named this electro-reduced aminated GC electrode: electrodeposited platinum particles on glassy carbon electrodes modified with nitrogen-containing functional groups (Pt-NGC). Furthermore, we reported that the H\(_2\) redox wave that appeared is related to the alternate redox reactions between hydrazino, diazene and diazo groups attached to the electrode surface.\(^13\) In general, the electrode oxidation of H\(_2\) is observed only when a bulk platinum electrode is used, and the electrode oxidation wave of H\(_2\) at a conventional carbon electrode has not been reported to our knowledge except for in Ref. 13.

Oxidative stress occurs from the strong cellular oxidizing potential of excessive reactive oxygen species (ROS) and free radicals.\(^14\)\(^\text{--}\)\(^18\) H\(_2\) selectively reduced the hydroxyl radical, the most cytotoxic of ROS, and effectively protected cells.\(^19\) H\(_2\) has the potential as an antioxidant in preventive and therapeutic applications, and it can be used as an effective antioxidant.
therapy. Owing to such biological importance of H₂, the measurement of H₂ dissolved in medium provides useful information for this purpose.

The conventional measuring technique of H₂ dissolved in medium was used in gas chromatography. However, the rapid, simple and inexpensive measurement of H₂ is still difficult, since this method requires a highly sensitive thermal conductivity detector (TCD)-based apparatus, which is expensive. This has led to the need to develop simpler measurement systems that allows for rapid operation for use in practical applications.

Electrochemical methods are very simple and inexpensive; therefore, this technique has been successfully coupled with a flow injection analysis (FIA).\textsuperscript{20,21} Excellent detection performance can be achieved with small sample volumes by FIA with electrochemical detection.\textsuperscript{22-24} The introduction of novel electrode materials, such as carbon electrode with the electrocatalytic activity for the electrode oxidation of H₂ to FIA, offers the potential to construct a simple, rapid and low-cost electrochemical FIA system. We have previously developed a batch-type coulometric method for measuring the concentration of H₂ dissolved in medium.\textsuperscript{25} However, accurate and precise determination becomes difficult because the background current is large compared to the signal current, causing serious error in background correction.

In this paper, we report that the electrocatalytic oxidation wave of H₂ can be observed by using a Pt-NGC electrode fabricated by the electrode reduction of the aminated GC electrode in sulfuric acid electrolyte. The properties of the electrocatalytic activity for H₂ oxidation reaction have been investigated by employing hydrodynamic voltammetric measurements with a rotating disk electrode. Moreover, we first developed an amperometric sensor based on FIA of dissolved H₂ using a Pt-NGC electrode as the working electrode.

**Experimental**

**Reagents and chemicals**

Ammonium carbamate was purchased from Merck (Germany) and used without further purification. Sulfuric acid (H₂SO₄) and sodium hydroxide (NaOH) were supplied by Wako Pure Chemical Industries, Ltd. (Japan). The test stock solution used was prepared by bubbling of the H₂ gas into 0.10 M (M = mol dm⁻³) phosphate buffer (pH 7.0). H₂ gas was continuously generated using a hydrogen generator (A9150-100, Parker, USA).

**Fabrication of Pt-NGC electrode**

A potentiostat/galvanostat (HA-151B, Hokuto Denko Co. Ltd., Japan) was used to perform a controlled potential electrolysis. An aqueous Ag/AgCl (3 M NaCl electrolyte) electrode and a platinum spiral wire were used as a reference and a counter electrode, respectively. The Pt-NGC electrode was fabricated by stepwise electrolysis as follows. First, a bare GC electrode was electro-oxidized in ammonium carbamate aqueous solution at +1.1 V (vs. Ag/AgCl) for 60 min. Next, the electro-oxidized GC electrode was electro-reduced in 1.0 M sulfuric acid (electrolyte volume: 100 mL, distance of WE-CE: 2 cm) at −1.0 V (vs. Ag/AgCl) for 20 h at room temperature. During the electro-reduction of the electro-oxidized GC electrode in sulfuric acid electrolyte, platinum ion dissolved from platinum wire counter electrode is electrodeposited on the surface of nitrogen-containing functional groups introduced glassy carbon electrode.

**SEM observations of Pt-NGC electrode surface**

The size and properties of the Pt particles were measured by SEM (JIB-4500, JEOL, Ltd., Tokyo, Japan). We used an acceleration voltage of 10 kV for the SEM.

**Electrochemical measurements**

Hydrodynamic voltammetric measurements with a rotating glassy carbon disk electrode were carried out by using a rotating disk electrode system (rotating disk electrode apparatus: RRDE-1, motor speed controller: SC-5, Nikko Keisoku Corp., Japan) and an automation polarization system (HZ-3000, Hokuto Denko Corp., Japan) with a three-electrode cell consisting of a working Pt-NGC electrode, an aqueous Ag/AgCl (3 M NaCl electrolyte) reference electrode, and a platinum wire counter electrode. To observe the electrode oxidation of H₂, H₂ gas was bubbled into electrolyte continuously during hydrodynamic voltammetric measurement at a flow rate of 50 mL/min. By employing an oxygen electrode, we confirmed that the dissolved oxygen was almost removed by bubbling into H₂ gas continuously. Hydrodynamic voltammetric measurement for the electrode oxidation of H₂ was carried out by using the GC electrode (6 mm diameter, Nikko Keisoku Corp., Japan) activated by the electrochemical oxidation/reduction procedures described above.

**Amperometric flow injection analysis (FIA)**

Amperometric measurements of H₂ were performed using an electrochemical measurement apparatus in connection with a potentiostat (NPOT-2501, Nikko Keisoku Corp., Japan), and a digital recorder (GR-3500, KEYENCE, Japan). The FIA system consisted of a carrier reservoir, sample injector with 10 μL loop, interconnecting PTFE tubing, HPLC pump (L-6000, Hitachi, Japan) and the electrochemical detector. A working Pt-NGC electrode (6 mm diameter, BAS Inc., Japan), an aqueous Ag/AgCl (3 M NaCl electrolyte) reference and a platinum counter electrode were integrated on a radial flow cell (BAS Inc., Japan). Phosphate buffer (0.10 M, pH 7.0) was used as the carrier solution. The test solution used was 0.1 M phosphate buffer (pH 7.0) to which the bubbling of the H₂ gas was performed.

**Results and Discussion**

**SEM image of Pt-NGC electrode surface**

Figure 1 shows an SEM image of the Pt-NGC electrode surface. The light spots correspond to Pt particles. The Pt particles are dispersed on the faulty part of the GC surface corresponding to dark spots on this image. The faulty GC surface is generated by electrochemical oxidation/reduction procedures of the GC electrodes. The area around the fault on the GC surface must be modified by nitrogen-containing functional groups, and Pt particles are expected to be electrodeposited on this area. The size of the particles is smaller than 1 μm in diameter (submicrometer particles).

**Electrochemical characterization of Pt-NGC electrode**

Figure 2 shows the hydrodynamic voltammogram (HDV) of bubbling H₂ gas in 0.10 M phosphate buffer electrolyte (pH 7.0) obtained by using various electrodes. When the potential was scanned in the cathodic direction from +0.8 V, the oxidation wave began from +0.4 V and continued to −0.5 V when the Pt-NGC electrode was used as the working electrode (Fig. 2(a)). In contrast, when the potential was scanned in the anodic direction from −0.65 V, the oxidation wave began from −0.5 V and continued to +0.5 V. These results mean that an active site
has electrocatalytic activity in each of the above-mentioned potential ranges. The wave heights of the oxidation/reduction processes were stable, that is, they were constant regardless of the number of potential sweeps. This behavior obtained by the Pt-NGC electrode is typical as regards the electrocatalytic redox properties of H₂ dissolved in medium. As a reference, we examined the current response of H₂ using the aminated GC electrode, but no current response of dissolved H₂ was obtained (Fig. 2(b)). This can be considered to show that the electrodeposited platinum in collaboration with the nitrogen-containing functional groups introduced by stepwise electrolysis in ammonium carbamate aqueous solution and sulfuric acid are functioning as active sites of the specific electrocatalytic current for H₂. Furthermore, we confirmed that the electrocatalytic activity of the Pt-NGC electrode for H₂ oxidation did not decrease after ultrasonication for 5 min. This fact indicates that Pt particles on nitrogen-containing functional groups are tightly immobilized.

The HDVs for H₂ dissolved in medium obtained with various electrode rotation speeds are shown in Fig. 3. The oxidation wave height of H₂ increased with the increase in the electrode rotation speed from 500 to 5000 rpm. The limiting current measured during the hydrodynamic voltammetry experiments was plotted against the square root of the electrode rotation speed as shown in Fig. 4 (Levich plot). The limiting current of H₂ oxidation at –0.4 V (vs. Ag/AgCl) was proportional to the square root of the electrode rotation speed (with a slope of 0.620 FACD²ν⁻¹/₆) and the line intercepts the vertical axis at zero. This fact indicates that the electrochemical reversible electron transfer catalytic site of H₂ exists at the electrode surface.
Figure 5 shows the HDVs of bubbling H₂ in 0.5 M H₂SO₄ solution (pH 0.0), 0.10 M phosphate buffer solution (pH 7.0) and 0.1 M NaOH solution (pH 13). In case of the HDV in 0.5 M H₂SO₄ (pH 0.0), when the potential was scanned in the cathodic direction from +1.2 V, the oxidation wave began from +0.8 V and diminished to –0.2 V. In contrast, when the potential was scanned in the anodic direction from –0.25 V, the oxidation wave began from –0.2 V and diminished to +0.9 V. On the other hand, the HDV in 0.1 M NaOH (pH 13) denoted the same tendency of the HDV in the acidic medium. These results indicate that the oxidation wave of H₂ observed by using the Pt-NGC electrode is strongly dependent on the pH of the electrolyte.

Analytical performance

We constructed an amperometric sensor based on a FIA system of dissolved H₂ using a Pt-NGC electrode as the working electrode. In order to achieve the rapid measurement of dissolved H₂ with high sensitivity, we examined the effect of applied potential to the Pt-NGC electrode at a radial flow (–0.5 to +0.5 V vs. Ag/AgCl) on the peak current. Figure 6 shows the relationship between the applied potential vs. the peak current for the electrode oxidation of H₂ dissolved in 0.10 M phosphate buffer (pH 7.0) and background current. The current responses of the electrode oxidation of H₂ increased with an increase of the negative applied potentials (Fig. 6(a)). On the other hand, the background current noise also increased when the electrode potential exceeded +0.1 V (Fig. 6(b)). Therefore, the optimal potential to measure the peak current was determined to be +0.05 V.

Panel A of Fig. 7 shows the typical current vs. time curves in each flow rate of dissolved H₂ water. (B) Effect of the flow rate on current response obtained for the measurement of dissolved H₂ using FIA system.

Figure 8 shows the current vs. time curve obtained for the repetitive measurement of dissolved H₂.

Figure 9 Relationship between the current response and the dilution ratio of the dissolved H₂. The ratio of 1 corresponded to the concentration of the prepared dissolved H₂ without dilution.

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Panel A of Fig. 7 shows the typical current vs. time curves obtained by the FIA measurement under the various flow rates of carrier solution. When the flow rate is 5.0 mL min⁻¹,
the measurement of dissolved H₂ was fully completed in a short time (~15 s). Panel B of Fig. 7 shows the relationship between the flow rate of sample solution and the peak current of the oxidation of H₂. When the flow rate was 5 mL min⁻¹, the current response was the maximum and 5 mL min⁻¹ was then used for the next measurements.

Typical current vs. time curve obtained by the repetitive measurement of the H₂ dissolved in medium are shown in Fig. 8. This curve indicates that the determination of dissolved H₂ is completed in a very short time (~15 s), and no detectable residual current fluctuation appears after the electrolysis is completed. The relative standard deviation (RSD) for eleventh successive measurements was 12.4%. The concentration of H₂ is so unstable because the H₂ dissolved in medium gradually escaped from the solution to air, and the improvement of the stability of the concentration of dissolved H₂ water is now in progress.

Figure 9 shows the relationship between the peak current for electrode oxidation of H₂ dissolved in medium and the dilution ratio (DR) of the dissolved H₂. The dilution ratio was calculated according to the formula (1):

$$\text{DR} = \frac{C_o}{C_r}$$  \hspace{1cm} (1)

Where, C denotes the concentration of dissolved H₂ water prepared by bubbling of H₂ gas and C denotes the concentration of dissolved H₂ water diluted with arbitrary rates. As shown in Fig. 9, good linearity ($r^2 = 0.995$) of the current responses was observed in the range of dilution ratio from 0.1 to 1. Our proposed FIA system described here offers promise for use as an analytical method because the simple and rapid measurement of the dissolved H₂ concentration in water is easily performed.

Conclusion

The Pt-NGC electrode was prepared by electrode reduction in 1.0 M sulfuric acid followed by electrode oxidation in ammonium carbamate aqueous solution. The Pt-NGC electrode exhibited an electrocatalytic activity of H₂ oxidation reaction at highly positive potential range in the hydrodynamic voltammogram. The oxidation wave of H₂ is observed by using the Pt-NGC electrode that possesses electrocatalytic activity that is strongly dependent on the electrode potential. The typical current vs. time curve was obtained by the repetitive measurement of H₂ dissolved in medium. This result shows that the concentration of the dissolved H₂ can be easily monitored by using the Pt-NGC electrode, and it can be expected that the electrochemical modification of a carbon electrode can open the way for a convenient measurement method of H₂ dissolved in water.

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