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Brief Report

Hemoglobin–Polyaniline Composite and Electrochemical Field Effective Transistors

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Abstract: A composite of hemoglobin/polyaniline was prepared. The chemical structure of this obtained composite was confirmed using infrared absorption spectroscopy measurement. The luminol reaction of the composite manifested chemical emissions from the composite. Furthermore, electrochemical transistors using the composite were created. The hemoglobin/polyaniline-based electrochemical transistor could switch to external current flow via an electrochemical reaction. The color of the transistor surface changed from green to red upon applying electrochemical potential.

Keywords: electrochemical transistor; hemoglobin; polyaniline; direct polymerization-deposition method

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1. Introduction

Hemoglobin (Hb) is a protein found in the red blood corpuscle. This protein is essential for the bonding of oxygen molecules and transporting them throughout the body. Hb has high oxidation activity, redox properties, and stability [1–4]. Polyaniline (PANI) is a conductive polymer which can be prepared in water. PANI has good redox properties.

In this study, we synthesized a composite of Hb and PANI (Hb/PANI). The chemical structure of the composite was confirmed using infrared (IR) absorption spectroscopy measurement. Since PANI can be prepared in a water medium, PANI is biocompatible and can be expected to be used in vivo. We carried out direct deposition of Hb/PANI composite on an electrode during chemical preparation to create electrochemical transistors.

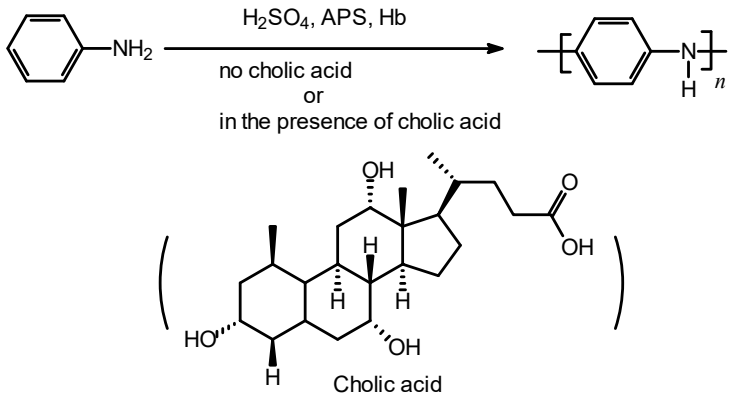
Moreover, acidic surfactants are used to tune the solubility and processability of PANI. Surfactants show liquid crystallinity. Applications of liquid crystals for reaction field can produce functional polymers [5–10]. Cholic acid was used as an acidic surfactant to prepare the composite to yield Hb/PANI/cholic acid. The chemical structure of the composite was characterized via IR spectroscopy.

2. Materials and Methods

2.1. Synthesis

2.1.1. Synthesis of Hb/PANI

Hemoglobin (1.023 g), aniline monomer (1.058 g), and sulfuric acid (1.046 g) were dissolved in water (200 mL) during cooling in an ice bath. Subsequently, ammonium peroxodisulfate (APS, 1.022 g) was added. A large volume of methanol was poured into the solution after 24 h to wash the polymer. The product was filtered, collected, and vacuum-dried to produce 1.696 g of Hb/PANI composites. During the reaction, a comb-shaped electrode was immersed for creating an electrochemical transistor. A comb-shaped electrode for a rain drop sensor YL-83 (weight: 13 g, dimensions: 54 mm × 40 mm (L × W)), glass and iron), purchased from Aitendo (Tokyo, Japan), was employed. Following polymerization, the composite was deposited onto the electrode (Scheme 1).



Scheme 1. Synthesis. APS: ammonium peroxodisulfate; Hb: hemoglobin.

2.1.2. Synthesis of Hb/PANI/cholic acid

During cooling in an ice bath, Hb (1.017 g), aniline monomer (1.11 g), sulfuric acid (1.023 g), and sodium cholic acid (1.016 g) as a biosurfactant produced by the liver were dissolved in water. Subsequently, 1.109 g of APS was added. A large volume of methanol was poured into the solution after 48 h to wash the polymer. The product was filtered, collected, and vacuum-dried to produce 2.902 g of the Hb/PANI/cholic acid composite.

3. Results and Discussion

3.1. pH Change

Figure 1 shows the change in pH during the polymerization process. Addition of aniline as a monomer to water gradually increased the pH value. Next, addition of sulfuric acid decreased the pH value to ca. pH = 2. Aniline sulfate was produced in the reaction medium by means of the addition of sulfuric acid. Subsequent addition of APS increased the pH value in the initial stage, and decreased the pH value in the polymerization. The gradual decrease in the pH value indicated the progression of the polymerization reaction.

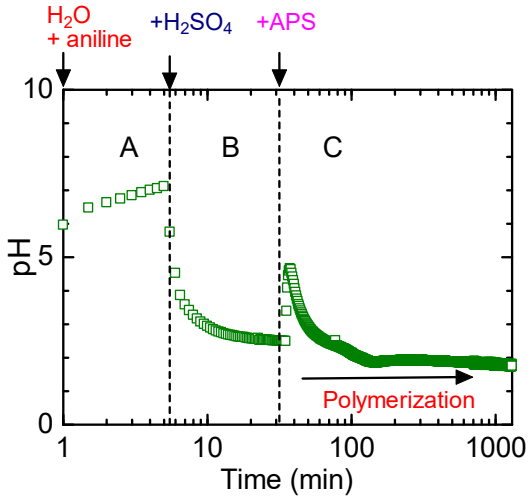


Figure 1. Change in pH during polymerization. Change in pH after the addition of aniline (A). Change in pH after the addition of sulfuric acid (B). Change in pH after the addition of ammonium peroxodisulfate (APS) (C).

3.2. IR

The absorption of Hb/PANI and Hb/PANI/cholic acid was measured using Fourier transform infrared (FT–IR) spectroscopy. Figure 2a depicts the entire absorption spectrum of Hb/PANI. The out-of-plane bending vibration of C–H in the aromatic ring (820 cm^{-1}), the in-plane bending vibration of C–H in the aromatic ring (1130 cm^{-1}), the stretching

vibration of the N atom adjacent to the benzene ring (1515 cm^{-1}), and the stretching vibration of the N atom adjacent to the quinoid ring (1650 cm^{-1}) were observed as characteristic peaks in polyaniline (Figure 2b,c). The entire absorption spectrum of Hb/PANI/cholic acid is shown in Figure 3. Furthermore, the out-of-plane bending vibration of C–H in aromatic ring (770 cm^{-1}), the in-plane bending vibration of C–H in aromatic ring (1100 cm^{-1}), the stretching vibration of two N atoms adjacent to the benzene ring (1500 cm^{-1}), and the stretching vibration of two N atoms adjacent to the quinoid ring (1610 cm^{-1}) were observed. In both cases, the presence of signals at 1300–1700 cm^{-1} in the spectrum was indicative of the absorption of the amide bonds. Therefore, the FT–IR spectra confirmed that the composites contain both hemoglobin and polyaniline as components.

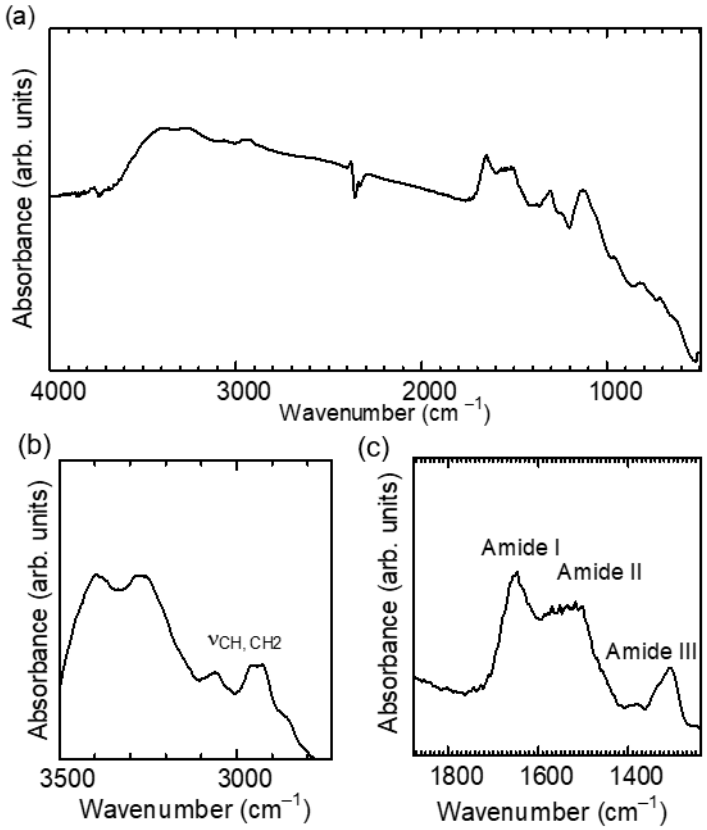


Figure 2. (**a**) Fourier transform infrared (FT–IR) absorption spectra of Hb/PANI. (**b**,**c**) Magnification of the IR absorption spectra of Hb/PANI. Hb: hemoglobin.

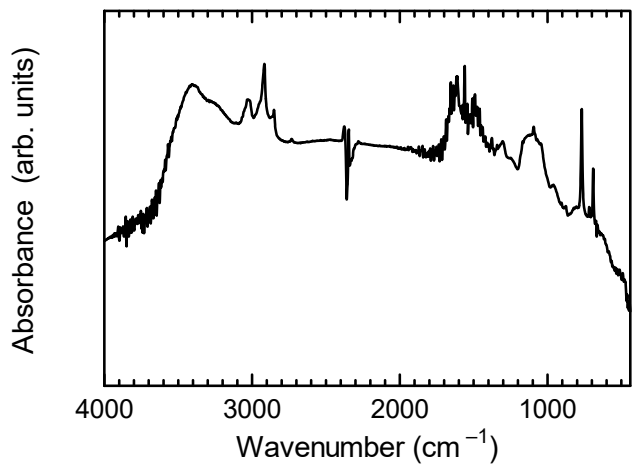


Figure 3. Fourier transform infrared (FT–IR) absorption spectra of Hb/PANI/cholic acid.

3.3. Luminol Emission

NaOH (0.6 g), luminol (0.02 g), and 3% hydrogen peroxide solution (30 mL) were added to distilled water (60 mL) to prepare a luminol solution. Hb/PANI and approximately ten milligrams of Hb/PANI/cholic acid were dissolved in luminol solution (5 mL). Light emission upon the irradiation of a UV light was observed. Photoluminescence (PL) spectroscopy measurements for the composite confirmed the emission, as shown in Figure 4. The PL spectra for the composites are shown in Figure 5. Spectral forms were replotted using the least-squares method from the original data. The luminol reaction allows the Hb/PANI to show intense blue emission and Hb/PANI/cholic acid a weak emission, as shown in Figure 4a. Both spectra of Hb/PANI and Hb/PANI/cholic acid showed PL signals at ~400 nm. This result demonstrates that both composites show luminol emission at the blue range. Hb/PANI is enveloped by the cholic acid in the form of a micelle. The outside cholic acid layer component depresses the contact of the Hb component with H_2O_2, resulting in a weak emission from Hb/PANI/cholic acid in the water solution. The possible structure of Hb/PANI/cholic acid as a colloid form in the luminol solution is shown in Figure 4b.

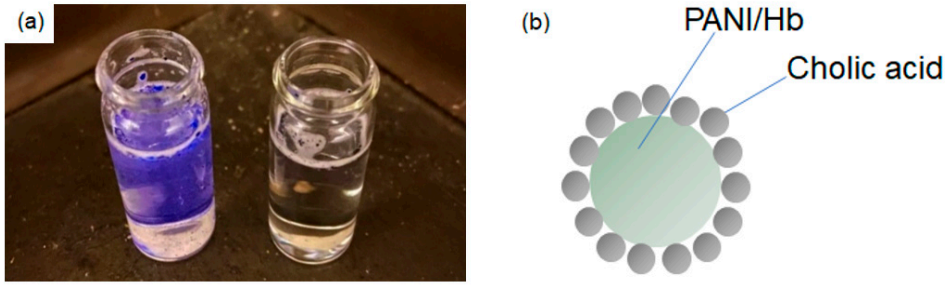


Figure 4. (**a**) Luminol emission from PANI/Hb. (Left) PANI/Hb. (Right) PANI/Hb/cholic acid. (**b**) Possible form of PANI/Hb/cholic acid in water.

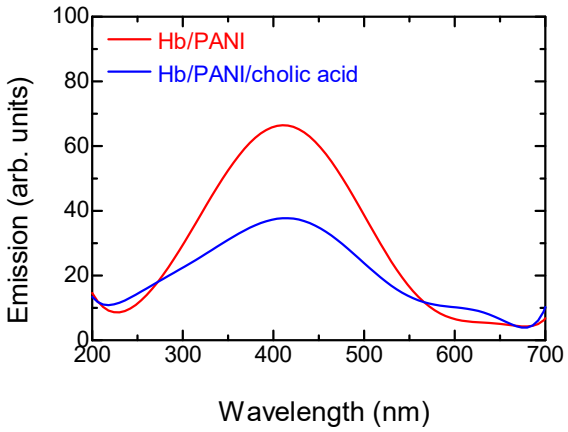


Figure 5. Photoluminescence (PL) spectra of Hb/PANI and Hb/PANI/cholic acid in luminol solution.

3.4. Transistor

The setup of a Hb/PANI-based electrochemical transistor was conducted. Hb/PANI was deposited on the comb-shaped electrode for the experiment. During the polymerization process of aniline, the reaction was carried out in the solution, and deposition of the resultant PANI on the wall of the reaction vessel simultaneously occurred. Therefore, immersion of the comb-shaped electrode in the polymerization solution allows the Hb/PANI to be deposited on the electrode surface. We refer to this method as direct polymerization–deposition, as a convenient method compared with the cast method, or the spray deposition method.

This circuit diagram is shown in Figure 6. First, 200 mL of 0.1 M sulfuric acid was prepared as an electrolytic solution in a beaker. Subsequently, a stainless-steel spring (5 cm in diameter and 20 cm in length) was placed in the beaker as a counter electrode. The comb type electrode was set in the center position of the spring.

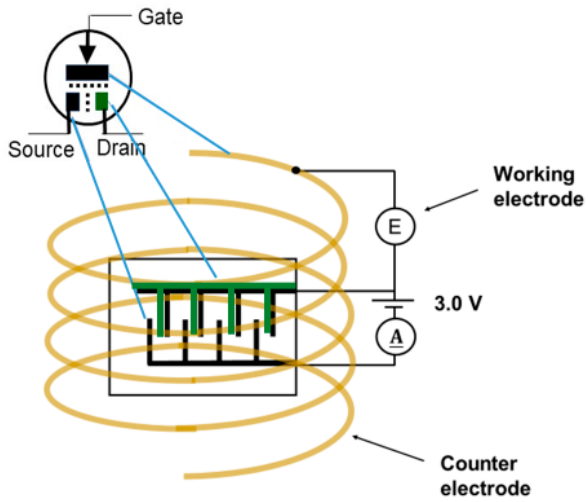


Figure 6. Hb/PANI-based electrochemical transistor.

Figure 7 shows the result of the change in the drain–source (D–S) current by changing the gate voltage from −3.0 to 1.0 V. No change was observed in the value from −3.0 to 0.5 V, whereas the D–S current increased linearly upon applying a gate potential greater than 0.5 V. This demonstrates that electrochemical doping–dedoping (oxidation–reduction) for the PANI component in the Hb/PANI composite drives the D–S current as a function of the electrochemical-field effect-type transistor (e-FET).

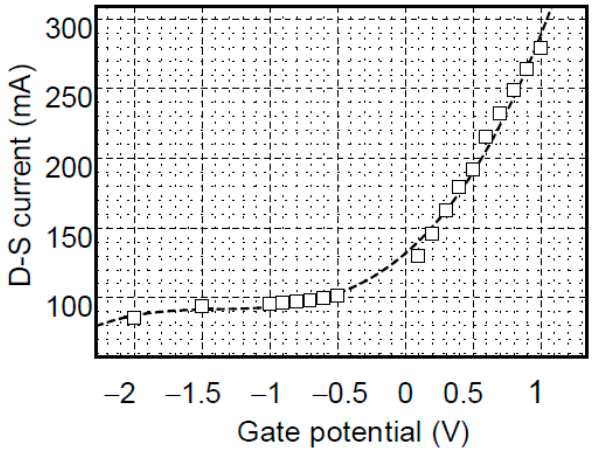


Figure 7. Change in drain–source current with gate potential for Hb/PANI as an electrochemical-field effect-type transistor (e-FET).

A change in the color of the surface of the comb-shaped electrode before and after applying the voltage was visually observed. Figure 8a presents an image of the Hb/PANI deposited on the interdigitated array electrode before the electrochemical application of voltage. Figure 8b presents an image of Hb/PANI after the application of voltage. The surface color changed from green to red upon the application of voltage accompanied by doping–dedoping. No such color change was observed for pure PANI with the electrochemical doping–dedoping. Hb/PANI in the dedoped (neutral) state and the PANI component reflect a purple (complete dedope) or green (mild dedope) color, mainly due to

the reflection of light from the PANI component in the composite (Figure 8a). Conversely, the PANI component in the doped state of the Hb/PANI manifested a pale color, inducing the reflection of a red color from the Hb component (Figure 8b). In this case, PANI is almost transparent, and Hb with a red color reflects red light.

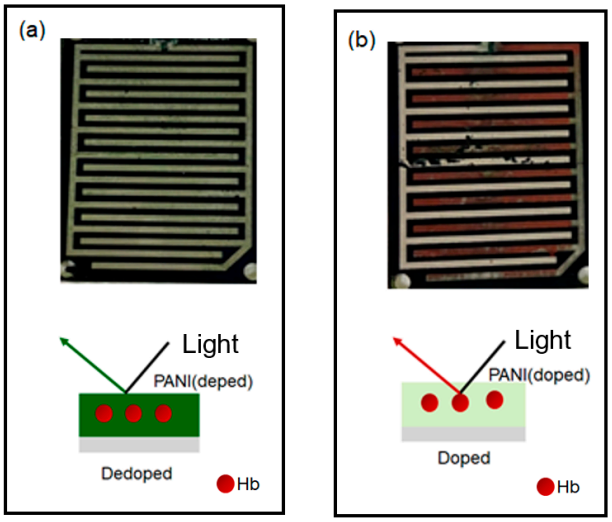


Figure 8. The appearance of Hb/PANI deposited on interdigitated array electrodes for an electrochemical transistor and the possible mechanism of the color change upon doping–dedoping. (**a**) Doping. (**b**) Dedoping.

4. Conclusions

We successfully synthesized a composite of hemoglobin and polyaniline. FT–IR measurements confirmed the chemical structure of polyaniline. Furthermore, the Hb/PANI composite demonstrated luminol activity. An e-FET based on the Hb/PANI composite was built. The combination of the oxygen capture function and the good redox properties of the composite may be of use for oxygen storage plastics or sensors.

5. Techniques

The FT–IR 4600 (JASCO, Tokyo, Japan) instrument used the KBr method. Reflection spectroscopy measurements were performed on an ARMN-735 (JASCO, Tokyo, Japan) equipment.

Author Contributions: M.I. synthesized the polymer and performed the analysis. M.I. and H.G. prepared the electrochemical transistors. Both authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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