**In situ** synthesis of MoS2 on a polymer based gold electrode platform and its application in electrochemical biosensing

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Bulk layers of MoS2 were synthesized in situ on a polymer substrate at low temperature. The negative charges carried by the layered MoS2 are used to immobilize horseradish peroxidase conjugated IgG via the electrostatic attraction, forming an Au–MoS2/HRP hybrid. Trace H2O2 released from IgG-horseradish peroxidase was successfully evaluated in the linear range of 0–20 ng mL⁻¹.

**Introduction**

Two-dimensional (2D) nanomaterials have attracted increasing attention because of their advantageous physical and chemical properties.1 In particular, 2D transition metal dichalcogenides (TMD) show a wide range of electronic, optical, mechanical, chemical, and thermal properties. In contrast to zero-band gap graphene, TMD molybdenum disulfide (MoS2) has an indirect band gap of 1.29 eV in the bulk state and it can be tuned from indirect to direct band gap (1.90 eV) through layer control. Moreover, in some cases MoS2 is utilized for the hydrogen evolution reaction (HER) as a catalyst.

**References**


**Acknowledgements**

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photoelectron spectroscopy (XPS), high resolution transmission electron microscopy (HR-TEM) and Raman scattering spectroscopy. Then the as-prepared MoS₂ modified on Au electrode was used to immobilize IgG-HRP, and catalytic activity of the resultant HRP electrode to H₂O₂ reduction was observed with help of hydroquinone (HQ).

**Experimental**

**Chemicals and materials**

Phosphate buffer solution (pH = 7.0) was purchased from Sigma Aldrich (P4417, USA). IgG-HRP (NCI1430KR) was purchased from Thermoscientific. Hydroquinone (h3660) and hydrogen peroxide solution were purchased from Sigma Aldrich (P4417, USA). IgG-HRP (NCl1430KR) was used to immobilize IgG-HRP, and catalytic activity of the HRP electrode during electrochemical cyclic voltammetry (CV) measurements.

**Fabrication of the Au electrode on PCB**

We fabricated a layer of gold for all three working, counter, and reference electrodes to simplify fabrication process. Each gold (Au) electrode chip was fabricated by the semiconducting processes including positive photoresist (PR) coating, patterning, lift off and passivation in Scheme 1a. Approximately 250 three-electrode Au chips were fabricated on a glass wafer (4 inches in diameter). Au electrode chip with sensing PCB was fabricated by an electroplating method and used in further experiment. Each Au electrode chip was diced and glued on PCB. The center of Au electrode (diameter, 2 mm) was used further for the in situ synthesis of MoS₂. The other two side Au electrodes were masked during synthesis. The centre Au electrode with MoS₂ (Au–MoS₂ electrode) was used as a working electrode during electrochemical cyclic voltammetry (CV) measurements.

In situ synthesis of layered MoS₂ on the Au electrode PCB

Due to low carrier mobility, MoS₂ itself is not suitable as an electrode material for device applications. There have been some studies on MoS₂ FET based biosensor by transfer of MoS₂ onto a metal electrode, which may be susceptible to contact resistance related issue. Thus, in the present research, was deposited by implementing PECVD in situ synthesis. MoS₂ was synthesized using PECVD at 300 ºC with H₂S gas as a precursor on a prefabricated gold (Au) electrode on a glass wafer. The synthesis of MoS₂ on a Si/SiO₂ wafer under various process parameter conditions was reported previously. The process for the in situ synthesis of MoS₂ is shown in Scheme 1b. The synthesis of MoS₂ begins with the deposition of Mo metal on Au(111) herringbone surface by e-beam evaporation. The thickness of the molybdenum metal layer was 1 nm using a deposition rate below ~0.1 Å s⁻¹ under high vacuum conditions. During the deposition process, some of the Mo atoms were deposited on the surface of the Au electrode and some penetrated into the Au, forming an Au–Mo composite structure. This also facilitated a reduction of the contact resistance, which was opposite of that seen with the transfer of Mo on Au electrodes. During the PECVD process, the Au–Mo components reacted with the combination of H₂S (source gas) and Ar (carrier gas) plasma in an ultra-high vacuum (UHV) at 300 ºC. The synthesized MoS₂ thin films were characterized by HR-TEM (JEOL-JEM ARM 200F), Raman spectroscopy (Alpha300 M+, WITec GmbH, Germany) wavelength and power of laser are 532 nm and 2 mW respectively and X-ray photoelectron spectroscopy (XPS, ESCA2000 (VG Microtech Inc.)).

Evaluation of H₂O₂ with IgG-HRP by cyclic voltammetry

For the H₂O₂ biosensor, HRP-conjugated IgG was immobilized on the Au electrode modified with MoS₂ to form an Au–MoS₂/HRP electrode via the drop casting method (Scheme 1c). The Au–MoS₂ electrodes were washed with deionized water to eliminate unbound Igs. The concentration of IgG-HRP immobilized on each Au–MoS₂ electrode in these experiments were 0, 0.1, 0.5, 1, 5, 10 or 20 ng mL⁻¹. Electrochemical immunoassay measurements were performed on a VersaSTAT 3 potentiostat/galvanostat (Ametek, USA) with three electrodes comprising a platinum wire as the auxiliary electrode, Ag/AgCl (sat’d KCl) as the reference and a modified Au–MoS₂ as the working electrode. The Au–MoS₂/HRP electrodes were placed in an electrochemical cell containing 5.0 mL pH 7.0 PBS buffer, 0.5 mM hydroquinone (HQ) and 5.0 mM H₂O₂ where
hydroquinone worked as a diffusional mediator. Eqn (1)–(4) shows the overall electron relay mechanism for hydrogen peroxide reduction via the immobilized HRP enzyme.\textsuperscript{25}

\begin{align*}
\text{H}_2\text{O}_2 + \text{HRP reduction} & \rightarrow \text{H}_2\text{O} + \text{HRP oxidation} \quad (1) \\
\text{HRP oxidation} + \text{HQ reduction} & \rightarrow \text{HRP reduction} + \text{HQ oxidation} \quad (2) \\
\text{HQ oxidation} + 2\text{H}^+ + 2e^- & \rightarrow \text{HQ reduction} \quad (3) \\
\text{H}_2\text{O}_2 + 2e^- + 2\text{H}^+ & \rightarrow 2\text{H}_2\text{O} \quad (4)
\end{align*}

In the presence of HRP and hydroquinone (HQ), the addition of \( \text{H}_2\text{O}_2 \) triggered the oxidation of HQ to benzoquinone (BQ) which is subsequently reduced giving a current response of the electrode.

**Results and discussion**

**Preparation and characterization of in situ Au–MoS\(_2\)**

In the plasma phase, which is an ionized gas phase, the \( \text{H}_2\text{S} \) source gas is easily dissociated at low temperature. By taking advantage of this property, we synthesized wafer-scale layered MoS\(_2\) at 300 °C on the Au electrode deposited PCB, which is a polymeric substrate.

XPS was used to determine the chemical bonding composition of the surface. Fig. 1a and b show the XPS spectra of the MoS\(_2\) nanomaterial on Au electrodes. The Mo 3d spectrum (Fig. 1a) exhibited two well-separated peaks at binding energy levels of 229.3 eV and 232.6 eV respectively. The S 2p spectrum (Fig. 1b) shows two merged peaks at 162.5 eV and 163.4 eV. The XPS scans for other synthesis temperature conditions confirmed the chemical bonding states of the MoS\(_2\) layers. The calculated atomic concentration of Mo and S from XPS was 34.4% and 65.6%, yielding a ratio of 1 : 1.90. The ratio from our PECVD-produced MoS\(_2\) is the same as the ratio of the CVD-synthesized MoS\(_2\).\textsuperscript{26}

Fig. 1c shows the morphology of the MoS\(_2\) thin film as obtained by analysing HR-TEM, transferred to the TEM grid (TED PELLA, INC, 200 nm silicon nitride membrane). The measured grain size at 300 °C ranged from 5–7 nm and was of a crystalline nature. Raman spectra (Fig. 1d) show that the MoS\(_2\) synthesized at 300 °C had \( \text{E}_{1g}^{\text{lg}} \) and \( \text{A}_{1g} \) modes with observed Raman peaks at 380.15 and 405.15 cm\(^{-1}\). The 15 cm\(^{-1}\) difference between these peaks indicates the presence of about 6 to 7 layers of MoS\(_2\).\textsuperscript{27}

**Electrochemical investigation**

The effect of scan rate on the anodic and cathodic peak currents at the Au–MoS\(_2\) electrode/HRP was investigated in 10 mM PBS (pH 7.0) using cyclic voltammetry (CV). As shown in Fig. 2a, it depicts the CV response of the Au–MoS\(_2\) electrode with different scan rate. The current value was observed in the absence of
H₂O₂ which resulted from surface defects at 10 mV s⁻¹ (curve 1). However, after the addition of 5 mM H₂O₂ at 10 mV s⁻¹, the center of the reduction peak slightly increased to −0.25 V (curve 2). The redox peaks were clearly observed with a scan rate ranging from 10 to 100 mV s⁻¹ demonstrating that the reduction and oxidation peak currents increased as the scan rate increased. When the scan rate was changed 10 to 100 mV s⁻¹, the reduction peak was proportional to the voltage from −0.25 to −0.29 V (curves 2, 3, and 4). The shift of the potential and the increase of the current indicated a notable enhancement of the electrocatalytic activity of the Au/MoS₂ electrode toward the reduction of H₂O₂. Fig. 2b depicts the cyclic voltammetry (CV) response of the Au–MoS₂ electrode conjugated with IgG-HRP with a scan rate of 100 mV s⁻¹. Fig. 2b, curve 1, shows the CV response of the Au–MoS₂ electrode in 0.1 M PBS, while curve 2 is response with the addition of 5 mM H₂O₂ and curve 3 shows the response of Au–MoS₂/HRP (1 ng mL⁻¹) in the presence of H₂O₂. The H₂O₂ reduction was promoted by the electrocatalysis from IgG-HRP immobilized on the Au–MoS₂ electrode.

As shown in Fig. 3, well-characterized redox peak currents were observed at an IgG-HRP concentration ranging from 0 to 20 ng mL⁻¹ with a scan rate of 100 mV s⁻¹. The reduction current increased noticeably with the increase of IgG-HRP concentration. The inset indicated the linearity of the current level measured against the concentration of IgG-HRP from 0 to 20 ng mL⁻¹ at a corresponding potential of −0.8 V. Moreover it was found that reduction peak currents were proportional to the concentrations of HRP, indicating typical electrochemical behaviour of immunoassays as well as a much larger peak current, which was observed because of the direct electron transfer between the HRP and underlying electrode, which was enhanced by the MoS₂ thin film. In comparison with previous reported work the presented H₂O₂ sensor has lower detection limit²⁸ and linear response.²⁹ From these observations, the sensing mechanism of this sensor is the conjugation of the IgG target molecules and antibodies to IgG immobilized on Au-MoS₂/immuno-substances by adsorption of HRP-conjugated IgG. By utilizing well-known sandwich based immunoassay, the detection efficiency of the targeted sample can be enhanced by the competitive reaction. Furthermore, the current level caused by antibody-target-detection can be increased by reducing the possibility of non-specific binding of the antibodies after pre-treatment of the bovine serum albumin (BSA) blocking step, this setup is able to detect nothing but targeted conjugation.

Conclusions

In summary, an in situ synthesis method for bulk layer of MoS₂ was developed to fabricate H₂O₂ sensors. MoS₂ was successfully synthesized at 300 °C on a polymeric PCB substrate. The resultant Au–MoS₂/IgG-HRP electrode shows good electrocatalytic performance toward the reduction of H₂O₂ when HQ is used as mediator. The cyclic voltammetry results showed that the sensor of Au–MoS₂ conjugated with IgG-HRP thus exhibited excellent analytical responses to H₂O₂ with a wide linear range. In particular, the linear range of IgG-HRP was measured to be 0–20 ng mL⁻¹ with an R² value of 0.998. The aim of this study to immobilize on Au–MoS₂/immuno-substances, which can be used as an application in electrochemical biosensor. Our findings show that in situ synthesis process of MoS₂ can be used as biocompatible matrix for the enzyme immobilization and construction of electrochemical biosensor. Moreover it can be expected to have the potential for commercial applications because of its low temperature in situ synthesis process and the ease at which other chemical and biosensors could be developed using this sensing platform.

Acknowledgements

This study was supported by the research fund of the Ministry of Trade, Industry, and Energy of Korea (grant no. 10045220). This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIP) (no. 2009-0083540).

Notes and references