

Comparative Study of Poly(4-vinylpyridine) and Polylactic Acid-block-poly(2-vinylpyridine) Nanocomposites on Structural, Morphological and Electrochemical Properties

(Kajian Perbandingan Nanokomposit Poli(4-vinilpiridin) dan Polilaktik Asid-blok-poli(2-vinilpiridin)
ke atas Sifat Struktur, Morfologi dan Elektrokimia)

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ABSTRACT

Polymer-based nanocomposites have attracted a lot of attention for amperometric biosensor development due to their general physical and chemical properties including biocompatibility, film-forming ability, stability and different functional groups that can be bonded with other biomolecules. In this study, poly-4-vinylpyridine homopolymer (P4VP) and polylactic acid-block-poly(2-vinylpyridine) block copolymer (PLA-b-P2VP) were used to hybridize with gold precursors (Au^{3+}) based on the association between the nitrogen of the pyridine group of P4VP or P2VP block with gold precursors. P4VP/ Au^{3+} and PLA-b-P2VP/ Au^{3+} nanocomposites were prepared with ratio of gold to P2VP or P4VP (10:1). The Au^{3+} in both polymers was reduced to gold nanoparticles (AuNPs) via in-situ approach by using hydrazine. Fourier transform infrared spectroscopy (FTIR), ultraviolet-visible spectroscopy (UV-vis), transmission electron microscopy (TEM) and cyclic voltammetry (CV) were used to characterize the structural, morphological and electrochemical properties of the nanocomposites. The peak currents of P4VP/AuNPs and PLA-b-P2VP/AuNPs nanocomposites modified electrode were 6.685 nA and 69.432 nA, respectively, which are much lower than bare electrode (205.019 nA) due to the non-conductivity of P4VP and PLA-b-P2VP. In order to improve the electron transfer capability of electrode, graphene oxide (GO) was blended and electrochemically reduced to obtain P4VP/AuNPs/rGO and PLA-b-P2VP/AuNPs/rGO nanocomposites. After immobilization of these two nanocomposites on electrode through drop casting method, the peak currents of P4VP/AuNPs/rGO and PLA-b-P2VP/AuNPs/rGO nanocomposites modified electrode were 871.172 nA and 663.947 nA, respectively, which are much higher than bare electrode (205.019 nA) and shown good capability to accelerate electron transfer. Based on these characterizations, P4VP/AuNPs/rGO has potential as the nanocomposite to modify the electrode for enzymatic biosensor development.

Keywords: Block copolymers; cyclic voltammetry; enzyme biosensor; graphene; Poly-4-vinylpyridine

ABSTRAK

Nanokomposit polimer bagi pembangunan biosensor amperometrik menarik banyak perhatian kerana sifat fizikal dan kimia am mereka termasuk keserasian-bio, keupayaan pembentukan filem, kestabilan dan bergantung kepada kumpulan berfungsi yang berkemampuan untuk terikat dengan biomolekul lain. Dalam kajian ini, poli-4-vinilpiridin (P4VP) dan polilaktik asid-blok-poly (2-vinilpiridin) (PLA-b-P2VP) telah digunakan untuk dihibriddkan dengan prekursor emas berdasarkan gabungan antara nitrogen kumpulan piridin daripada P4VP atau P2VP blok dengan prekursor emas (Au^{3+}). Nanokomposit P4VP/ Au^{3+} dan PLA-b-P2VP/ Au^{3+} telah disediakan dengan nisbah emas kepada P2VP atau P4VP (10:1). P4VP/AuNPs dan PLA-b-P2VP/AuNPs nanokomposit telah disediakan melalui pendekatan in-situ dengan menggunakan hidrazin. Spektroskopi transformasi Fourier inframerah (FTIR), spektroskopi ultraviolet boleh dilihat (UV-vis), mikroskop elektron penghantaraan (TEM) dan voltammetri berkitar (CV) telah digunakan untuk mencirikan sifat struktur, morfologi dan elektrokimia nanokomposit tersebut. Arus puncak untuk bahan P4VP/AuNPs dan PLA-b-P2VP/AuNPs pengubah suai elektrod adalah 6.685 nA dan 69.432 nA, yang jauh lebih rendah daripada nilai elektrod (205.019 nA) disebabkan sifat tidak kekonduksian daripada P4VP dan PLA-b-P2VP. Dalam usaha untuk meningkatkan keupayaan pemindahan elektron elektrod, grafin oksida (GO) telah dicampurkan dan diperoleh dengan kaedah elektrokimia bagi penyediaan P4VP/AuNPs/rGO dan nanokomposit PLA-b-P2VP/AuNPs/rGO. Selepas proses immobilasi oleh bahan nanokomposit pada elektrod melalui teknik saliran titisan dengan puncak elektrod arus untuk P4VP/AuNPs/rGO dan PLA-b-P2VP/AuNPs/rGO nanokomposit masing-masing diubah suai menunjukkan nilai 871.172 nA dan 663.947 nA. Nilai tersebut jauh lebih tinggi daripada permukaan elektrod (205.019 nA) serta menunjukkan kemampuan yang baik untuk mempercepatkan pemindahan elektron. Berdasarkan pencirian ini, P4VP/AuNPs/rGO mempunyai potensi sebagai komposit nano untuk mengubah suai elektrod bagi pembangunan enzim biosensor.

Kata kunci: Blok kopolimer; enzim biosensor; grafin; poli-4-viniliridine; voltammetri berkitar

INTRODUCTION

It is widely acknowledged that nanomaterials-modified electrode of an enzymatic biosensor play an important part in improving the performance of biosensor. Biosensor provides an attractive means to detect and analyze the content of a biological sample such as glucose in microalgae and microbial culture. Gold nanoparticles (AuNPs) and polymers based nanocomposites are among nanomaterials that have gained considerable attention based on their excellent conductivity and catalytic properties. AuNPs introduce many advantages to electrochemical sensors, including their ability to provide biocompatibility and efficient loading sites for immobilizing enzymes and further improving electron transfer between the active site and electrode (Putzbach & Ronkainen 2013). Certain properties of polymers, including stability, ease of synthesis, excellent tailorability, and good processability, also attracted many researchers' attention (Wang & Uchiyama 2013). Vinyl pyridines such as P2VP and P4VP are an important class of synthetic polymer that attract many attentions due to the nitrogen atom in the aromatic ring (Özyalçın et al. 1997). Poly(4-vinylpyridine) (P4VP) as a synthetic water soluble polymer and coordinative reagent for transition metals has been paid much attention in recent years, which is often used to extract heavy metals in waste water treatment and also as a host ligand of metal-containing chromospheres (Li et al. 2008, 2007). P4VP and P2VP are also frequently co-polymerized with other polymer for block copolymers (BCPs) synthesis which made up of blocks of different polymerized monomers, where they are linked by a covalent bond at their ends (Ku et al. 2014). BCPs made of blocks of different polymerized monomers linked by covalent bonds at their ends likewise attracted interest based on their structure, size and chemical composition. Self-assembly of BCPs can generate nanostructured particles with tunable internal structures, shapes and surface properties, which provide potential application in particle-based technology (Smart et al. 2008).

The polymer chains grafted/coated on the surfaces of AuNPs not only enhance the stability of gold cores intensively, but also functionalize the gold core due to the special properties of outside polymer layers (Li et al. 2009). Furthermore, it is well acknowledged that incorporating nanoparticles into a polymer matrix can have a significant influence in the properties of the binding matrix, including mechanical strength, conductivity, permeability and catalytic activities (Chiu et al. 2005). Normally, metal nanoparticles/polymer nanocomposites can be synthesized through *ex-situ* and *in-situ* approaches. In the *ex-situ* approach, metal nanoparticles are first produced by soft-chemistry routes and then dispersed into polymeric matrices, while for *in-situ* approach, metal nanoparticles are generated inside a polymer matrix through chemical reduction of a metallic precursor dissolved into the polymer (Kim & Yi 2015; Sarkar et al. 2012).

Over the past few years, graphene attracted a lot of attention due to its one-atom-thick planar sheet of sp^2

bonded carbon atoms that are densely arranged into a 2D honeycomb-structure with superior electrical conductivity, excellent chemical stability and high surface to volume ratio (Pumera 2011). Its ability for facilitating electron transfer, excellent biocompatibility, large specific surface and low cost make graphene an ideal material for the construction of sensors and biosensor-based devices in various transduction modes, from electrical and electrochemical transduction to optical transduction (Dreyer et al. 2010; Pumera 2011). The electrochemical reduction of graphene oxide (GO) to produce reduced graphene oxide (rGO) was frequently applied in graphene based nanocomposites (Pei & Cheng 2012; Unnikrishnan et al. 2013).

To the best of our knowledge, no study has been conducted to utilize the P4VP homopolymer and PLA-b-P2VP block copolymer for enzymatic biosensor, in which the preliminary research towards the application should be conducted. In this research, polylactic acid (PLA)-block-poly(2-vinylpyridine) (P2VP) (PLA-b-P2VP) and poly-4-vinlyridine (P4VP) were used for hybridization with Au^{3+} by taking advantages of the association between the long-pair nitrogen of the pyridine and various metal irons (Lin et al. 2010). The *in-situ* synthesis of gold nanoparticles in BCP and P4VP was accomplished via *in-situ* synthesis by using hydrazine hydrates as reducing reagent and characterized through FTIR, UV-vis, TEM and CV. After blending with GO, BCP/AuNPs/GO and P4VP/AuNPs/GO nanocomposites were electrochemical reduced in order to prepare BCP/AuNPs/rGO and P4VP/AuNPs/rGO for electrode modification and characterized through CV.

MATERIALS AND METHODS

REAGENTS AND APPARATUS

Polylactic acid-block-poly(2-vinylpyridine) (PLA-b-P2VP) with molecular weight (M_w) 38400 g mol⁻¹ and $f_{PLA} = 0.36$, poly-4-vinlyridine (P4VP) with molecular weight (M_w) 24000 g mol⁻¹ was synthesized and obtained from Polymer Nano Electronics Laboratory, KAIST, South Korea. Gold (III) chloride trihydrate ($\geq 99.9\%$ trace metals basis, $HAuCl_4 \cdot 3H_2O$), hydrazine solution (35 wt. % in H_2O , $N_2H_4 \cdot H_2O$) and dichloromethane (CH_2Cl_2 , anhydrous, $\geq 99.8\%$) were all purchased from Sigma-Aldrich. Potassium ferricyanide ($K_3Fe(CN)_6$) was purchased from R&M Chemicals, Selangor, Malaysia.

All electrochemical experiments to characterize BCP/AuNP composites were performed using a conventional three-electrode cell and a pocketSTAT (IVIUM Technologies, Eindhoven, Netherlands) as potentiostat/galvanostat/impedance analyzer. A modified glassy carbon electrode (GCE, inner diameter of 3.0 mm) was used as the working electrode (WE), platinum (Pt) was used as the counter electrode (CE) and silver/silver chloride (Ag/AgCl) in 3.0 M saturated sodium chloride (NaCl) was used as the reference electrode (RE). All electrodes were purchased from ALS Co.,

Ltd., Tokyo, Japan. Prior to surface modifications, the GCE was polished with diamond ($0.1\text{ }\mu\text{m}$) and alumina slurry ($0.05\text{ }\mu\text{m}$) purchased from ALS Co., Ltd., Tokyo, Japan, ultrasonicated in ethanol for 1 min followed by deionized (DI) water for 1 min and then dried in a desiccator for 30 min before use. UV-vis spectroscopy measurements (350–650 nm) were performed using UviLine 9400 (SECOMAN, France) at room temperature with a 10 mm optical length quartz cuvette. The Fourier transform infrared spectroscopy (FTIR) investigations were carried out with the Nicolet iS50 FT-IR Spectrometer (Thermal Scientific, USA). The morphology of nanocomposites was characterized via Transmission Electron Microscopy (JEOL JEM-2100 using an accelerating voltage of 200 kV). For phosphate buffer solution preparation, sodium phosphate dibasic heptahydrate ($\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$), sodium phosphate dibasic (Na_2HPO_4), sodium phosphate monobasic (NaH_2PO_4) and potassium phosphate monobasic (KH_2PO_4) were purchased from Sigma-Aldrich. Potassium chloride (KCl), potassium ferricyanide ($\text{K}_3\text{Fe}(\text{CN})_6$) and sodium chloride (NaCl) were obtained from R&M, Chemicals, Selangor, Malaysia. Distilled water (DI) water obtained from ultrapure water purification system (SARTORIUS, Arium 611) was used throughout all experiments.

HYBRIDIZATION OF GOLD PRECURSORS WITH POLYMERS

A 5 wt. % of BCP (PLA-b-P2VP) solution and 5 wt. % P4VP were separately prepared in dichloromethane (DCM) and ethanol solutions, stirred for 14 h. The BCP/Au³⁺ and P4VP/Au³⁺ nanocomposite were prepared based on the specific mole ratio (Au³⁺ : BCP = 10:1 and Au³⁺ : P4VP = 10:1) through 24 h stirring. BCP/Au³⁺ and P4VP/Au³⁺ nanocomposites and control (BCP in DCM, P4VP in ethanol) were characterized via FTIR for investigation of association between the nitrogen of pyridine group and Au³⁺.

IN-SITU SYNTHESIS OF GOLD NANOPARTICLES

AuNPs in P4VP and BCP were *in-situ* synthesized using hydrazine hydrate ($\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$) reduction method. The color of the Au³⁺/polymer solutions changed from yellow to purple after addition of N_2H_4 . The formation of nanoparticles is confirmed by UV-vis spectroscopy where the peak is formed within the measurement range.

CHARACTERIZATION OF BCP/AuNPs AND P4VP/AuNPs NANOCOMPOSITES

The Fourier transform infrared spectroscopy (FTIR) investigations were carried out with the Nicolet iS50 FT-IR Spectrometer (Thermal Scientific, USA). UV-vis spectroscopy measurements (350–650 nm) were performed using UviLine 9400 (SECOMAN, France) at room temperature with a 10 mm optical length quartz cuvette. The morphology of nanocomposites was characterized via Transmission Electron Microscopy (JEOL JEM-2100 using an accelerating voltage of 200 kV). Cyclic voltammetry (CV) was performed to evaluate the electron-transfer

capabilities of the modified layer based on the oxidation peak current in 0.05 M potassium ferricyanide ($\text{K}_3\text{Fe}(\text{CN})_6$) solution at a scan rate of 50 mV/s.

PREPARATION AND CHARACTERIZATION OF BCP/AUNPS/GRAPHENE AND P4VP/AUNPS/GRAPHENE COMPOSITES

GO was blended into these two nanocomposites based on 1:1 ratio of GO: BCP/AuNPs or P4VP/AuNPs. After 30 cycles of electrochemical reduction in 0.01 M PBS (pH 5), the resulting BCP/AuNPs/rGO and P4VP/AuNPs/rGO nanocomposites were characterized via CV in order to evaluate the electron-transfer capabilities of the modified layer on electrode.

RESULTS AND DISCUSSION

Figure 1 shows FTIR spectrum of BCP, BCP/Au³⁺ and BCP/AuNPs nanocomposite. The two peaks at 1435 and 1473 cm⁻¹ were attributed to C-C stretching of the pyridine ring. The peak at 1753 cm⁻¹ was related to the C=O stretching of PLA block (Yuen et al. 2005). Another two peaks from 1550 to 1600 cm⁻¹ were related to C=N stretching of the pyridine group. Whereas, FTIR spectrum of P4VP, P4VP/Au³⁺ and P4VP/AuNPs nanocomposites is shown in Figure 2. The two peaks around 1418 and 1451 cm⁻¹ were attributed to C-C stretching of the pyridine ring (Yuen et al. 2005). Another two peaks from 1600 to 1700 cm⁻¹ were also related to C=N stretching of the pyridine group.

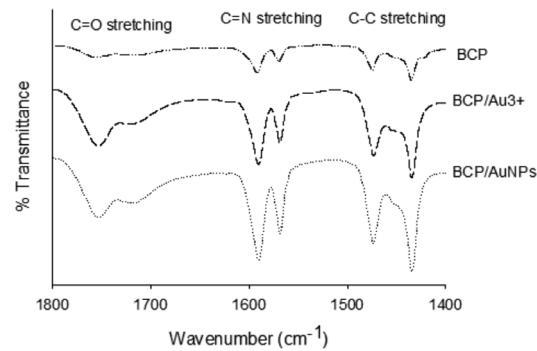


FIGURE 1. FTIR spectrum of BCP, BCP/Au³⁺, BCP/AuNPs composites in the range from 1400 to 1800 cm⁻¹

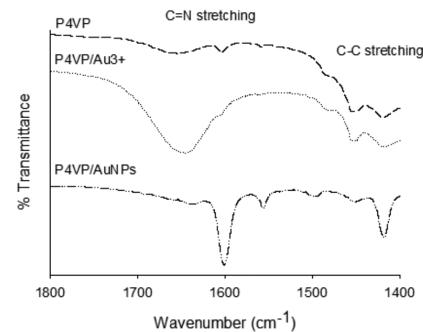


FIGURE 2. FTIR spectrum of P4VP, P4VP/Au³⁺, P4VP/AuNPs composites in the range from 1400 to 1800 cm⁻¹

Table 1 summarized the comparison of C=N stretching between BCP and P4VP related nanoparticles. The wavenumber of C=N stretching of BCP/Au³⁺ was shifted to 1569 and 1590 cm⁻¹ from 1570 and 1592 cm⁻¹ of BCP, respectively. On the other hand, there is no shift of wavenumber for BCP/Au³⁺ into BCP/AuNPs nanocomposites. The wavenumber of C=N stretching of P4VP/Au³⁺ however was slightly shifted to 1652 cm⁻¹ from 1653 cm⁻¹ of P4VP. On the other hand, slight shift were observed for P4VP/Au³⁺ and P4VP/AuNPs nanocomposites from 1604 and 1652 cm⁻¹ to 1602 and 1651 cm⁻¹, respectively. This blue shift (decrease in wavelength) is important to study the hybridization between Au³⁺ and pyridine groups from P2VP block. The shift attributes to the association of the gold precursors with the long pair nitrogen of P2VP which cause the increase of binding energy associated with C=N stretching (Lin et al. 2010). In addition, the blue shift of P4VP with gold precursors is slightly stronger than that of BCP which could be related to the different arrange of pyridine groups from P2VP block and P4VP.

After *in-situ* synthesis of AuNPs in Au³⁺/polymers nanocomposites by using hydrazine, the characteristic plasmon resonance absorption band for AuNPs occurred between 500 and 600 nm in the visible region of the electromagnetic spectrum, in which confirmed the formation of nanoparticles in these two nanocomposites

TABLE 1. The comparison of C=N stretching of BCP and P4VP related nanocomposites

Samples	Wavenumber of C=N stretching from pyridine group (cm ⁻¹)
BCP	1570, 1592
BCP/Au ³⁺	1569, 1590
BCP/AuNPs	1569, 1590
P4VP	1604, 1653
P4VP/Au ³⁺	1604, 1652
P4VP/AuNPs	1602, 1651

(Johan et al. 2012). The successful synthesis of gold nanoparticles through hydrazine reduction of HAuCl₄•3H₂O was proved via UV-vis spectra.

Based on Figure 3, the wavelength of peak AuNPs absorbance in BCP was 536 and 548 nm of P4VP, which indicate the size of AuNPs in P4VP was larger than AuNPs in BCP (Sasha & Hadi 2008). This result was confirmed through TEM images as shown in Figure 4. The dark spots were AuNPs and the bright parts were polymer (BCP or P4VP). TEM study of AuNPs in BCP and P4VP confirmed that the particles are almost spherical in shape with size ranging from 10 to 40 nm (BCP) and 10 to 70 nm (P4VP).

The electrochemical properties of the BCP/AuNPs and P4VP/AuNPs nanocomposites were identified through CV. Figure 5 shows CVs of bare GCE and GCE modified with BCP/AuNPs, P4VP/AuNPs nanocomposites. The peak currents of BCP/AuNPs (69.432 μA) and P4VP/AuNPs (6.685 μA) modified GCE were much lower than that for a bare GCE (205.019 μA) owing to the non-conductive property of the BCP and P4VP.

In order to improve the conductivity of the modified layer on the GCE and accelerate electron transfer, highly conductive reduced graphene oxide was introduced to prepare BCP/AuNPs/rGO and P4VP/AuNPs/rGO nanocomposites. After blending GO with BCP/AuNPs and

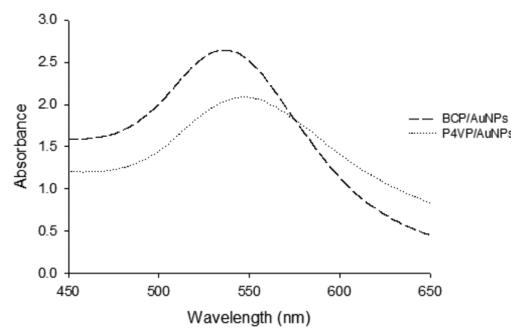


FIGURE 3. UV-vis spectrum of BCP/AuNPs, P4VP/AuNPs composites in the range between 450 and 650 nm

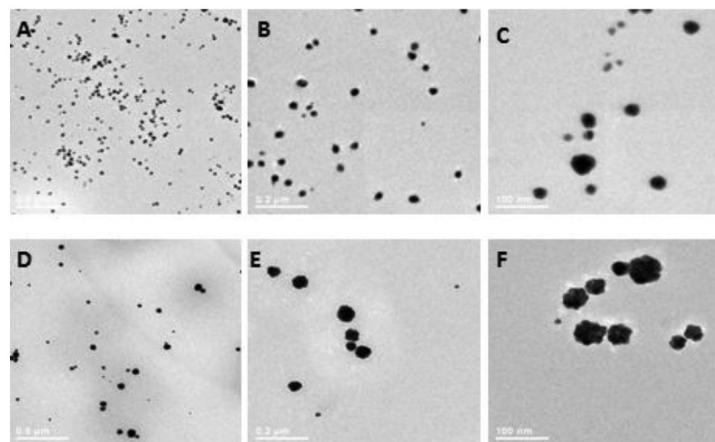


FIGURE 4. TEM images of BCP/AuNPs (A, B, C) and P4VP/AuNPs composites (D, E, F): A and D: 0.5 um, B and E: 0.2 um, C and F: 100 nm

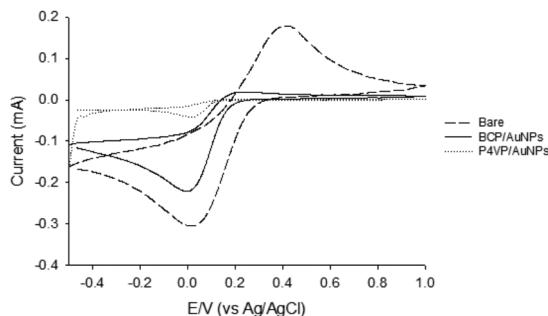


FIGURE 5. CVs of bare GCE, and GCEs modified with BCP/AuNPs and P4VP/AuNPs nanocomposites in 0.05 M $K_3Fe(CN)_6$ solution at a scan rate of 50 mV/s

P4VP/AuNPs, electrochemical reduction was applied to reduce GO to rGO in 0.01 M PBS (pH5) (Pei & Cheng 2012). The electrochemical properties of the BCP/AuNPs/rGO and P4VP/AuNPs/rGO nanocomposites were identified through CV, which is shown in Figure 6. The peak currents of bare GCE, BCP/AuNPs/rGO and P4VP/AuNPs/rGO were at 205.019 μ A, 663.947 μ A and 871.172 μ A, respectively. This indicates that the high conductivity of P4VP/AuNPs/rGO has potential to be used as transducer modification and enzyme immobilization for enzymatic biosensor.

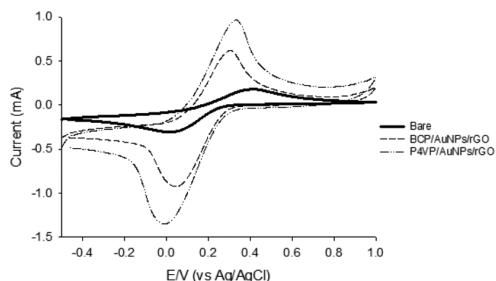


FIGURE 6. CVs of BCP/AuNPs/rGO and P4VP/AuNPs/rGO composites in 0.05 M $K_3Fe(CN)_6$ solution at a scan rate of 50 mV/s

CONCLUSION AND FUTURE WORK

In this research, the comparative study of P4VP/AuNPs and PLA-b-P2VP/AuNPs nanocomposites on structural, morphological and electrochemical properties were accomplished. After adding graphene and electrochemical reduction, the conductivity and ability for electron transfer of BCP/AuNPs/rGO and P4VP/AuNPs/rGO nanocomposites was improved and P4VP/AuNPs/rGO will be selected as the optimum nanocomposite to modify the electrode for further enzymatic biosensor research. These nanocomposites have potential to be used for the targeted application.

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