Research Article

Improvement of Amperometric Biosensor Performance for H₂O₂ Detection based on Bimetallic PtM (M = Ru, Au, and Ir) Nanoparticles

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Novel bimetallic nanoparticles have been synthesized via rapid microwave irradiation, leading to an improved sensitivity and a highly anti-interference property for amperometric biosensor in H₂O₂ detection. The material characterizations were performed by TEM, XRD, and EDX, which show the bimetallic formation of Pt-based catalysts and well-dispersed nanoparticles of 2–5 nm. The sensitivities for the detection of H₂O₂ of PtRu, PtAu, and PtIr as the biosensor working electrode catalysts are 539.01 (R² = 0.99), 415.46 (R² = 0.99), and 404.52 (R² = 0.97) μA·mM⁻¹·cm⁻², respectively, nearly twice higher than the pure Pt catalyst (221.77 μA·mM⁻¹·cm⁻², R² = 0.98), at a low applied potential of +0.25 V versus Ag/AgCl. Furthermore, Pt-Ru and Pt-Ir show a highly sensitive response and a promising anti-interference capability to ascorbic acid, a major interferent, by reducing the interferent current as low as 7-8% significantly lower than that of Pt (30% change). The enhancement of both sensitivity and selectivity in the bimetallic catalysts can be found practical applications in biosensing.

1. Introduction

Platinum (Pt), a well-known and traditional heterogeneous catalyst, plays an important role in thermo, photo and electrocatalysis [1–3], and it is widely used in advanced optical, electronic, magnetic, and sensing devices [4–7]. The reactivity and selectivity of heterogeneous catalysts usually depend on their surface active sites, and therefore the synthesis of nanoparticles with controlled shapes and sizes is critical for tuning the surface active sites and important for improving their applications [8–10]. Well-dispersed Pt nanoparticles with different morphologies, such as polyhedral, truncated cubic, and cubic nanocrystals have been controllably synthesized and applied to biosensors [11–13]. In selected enzymatic reactions, the substrate (analyte) is oxidized, yielding intermediates (e.g., adsorbed CO and OH species), which tend to cover the active sites on the Pt catalysis surface [14, 15]. Consequently, the efficiency of the catalyst becomes limited, resulting in low sensibility and poor selectivity for biosensing applications.

By using Pt with adatoms on the surface or Pt-containing bimetallic nanocrystals, the catalysts resistance to poison can be enhanced [16–18]. Xiao et al. synthesized AuPt alloy nanoparticles that deposited ionic liquid-chitosan composite film and used nonenzymatic H₂O₂ detection [19]. The results reflected a remarkable synergistic effect, and the presence of Au could reduce the strength of Pt–OH formation providing needed adsorption sites for –OH species [19]. Furthermore, not only the resistance to poison can be improved by adopting bimetallic materials, the sensitivity and stability of biosensors were also enhanced. Muraviev et al. synthesized polymer-stabilized monometallic Pt (Pt-PSMNP) and bimetallic Pt/Cu core-shell nanoparticles (Pt/Cu-PSMNP) as sensing elements to detect H₂O₂ [20].
The results showed that the electrocatalytic activity of Pt/Cu-PSMNPs was far higher than that of monometallic Pt-PSMNPs \([20]\). Rajkumar et al. fabricated rhodium-palladium (Rh-Pd) particles modified, on glassy carbon electrode (GCE) and successfully used it for detecting \(H_2O_2\) in a real samples (10–340 \(\mu M\)), which was stable for 2 days, and gradual decreases occurred in the reduction and oxidation peak potentials \([21]\).

Many measurements of biosensors were conducted for the detection of enzymatically generated \(H_2O_2\) based on the enzymatic reaction. For example, the detection of the bile acids was based on the enzymatically formed nicotinamide adenine dinucleotide (NADH). By using an additional enzyme, NADH could be converted to \(NAD^+\) and generates \(H_2O_2\) \([22]\). Higher concentrations of the bile acids would result in greater concentrations of enzymatically produced \(H_2O_2\), which can be used to quantify bile acids in the testing medium. And there are also many other biomarkers, such as adenosine deaminase, glucose, pyruvate, lactate, and cholesterol, that are able to generate \(H_2O_2\) in corresponding enzymatic reaction \([23–26]\). In this study, we describe the synthesis and characterization of different Pt-containing bimetallic nanoparticles and demonstrate their electrocatalytic properties by catalytic oxidation of \(H_2O_2\). Consequently, the electrochemical oxidation current of \(H_2O_2\) can then be used to quantify the analyte. The sensitivity of the Pt-based bimetallic nanocatalysts to the oxidation of \(H_2O_2\) was investigated, and the interference from ascorbic acid (AA), a potentially major interference species, was also evaluated.

## 2. Experimental

### 2.1. Rapid Synthesis of Pure Pt and Bimetallic Nanoparticles

Pure Pt and Pt-based bimetallic nanoparticles were prepared by microwave heating of a highly dielectric solvent, ethylene glycol, (EG, Fisher, AR) solutions of Pt, and the other metal precursor salts. As a typical process for the synthesis of Pt nanoparticles, 0.75 mL of an aqueous solution of 0.05 M \(K_2PtCl_6\cdot6H_2O\) (Aldrich, ACS Reagent) was mixed with 25 mL of EG and 0.75 mL of 0.4 M KOH in a 300 mL beaker. The beaker was then placed in the center of a household microwave oven (Panasonic, 1250 W) and heated for 36 s. For the preparation of PtRu and PtIr bimetallic nanoparticles, the starting mixture contained 25 mL of EG, 0.75 mL of 0.05 M \(K_2PtCl_6\cdot6H_2O\) aqueous solution, 0.75 mL of 0.05 M RuCl\(_3\) (Aldrich, ACS Reagent) or 0.05 M IrCl\(_3\) (Aldrich, ACS Reagent) aqueous solution, respectively, and 0.75 mL of 0.04 M KOH solution. In the preparation of PtAu nanoparticles, the concentration of Pt and Au precursors was reduced to 0.025 M, the other reagents were maintained the same. The resulting pure Pt and bimetallic solutions (pure Pt: 11.60 mL, PtRu: 10.97 mL, PtIr: 5.96 mL, and PtAu: 11.76 mL) were mixed with 16 mg of carbon powder (Vulcan XC 72R, Cabot, USA) by ultrasound for 2 hours, respectively. These Pt-based bimetallic nanoparticles were mixed with the carbon powder, producing a printable ink to be evaluated and used for electrochemical characterization. The nanoparticle metal and carbon mixtures were washed with acetone and ethanol in sequence three or four times. After centrifuging and drying at 60°C under vacuum for 12 h, the mixed electrocatalysts were obtained and were ready to be used.

### 2.2. Characterization and Measurements

The morphology, size, and compositions of pure Pt and bimetallic nanoparticles were characterized by transmission electron microscopy (TEM, Techai F30, using an accelerating voltage of 300 kV) and energy-dispersive X-ray spectroscopy (EDX). The crystal phase of as-synthesized products was identified by powder X-ray diffraction (XRD) analysis (Cu\(K\alpha, \lambda = 1.54056 \text{Å},\) Scintag X-1, USA), and the XRD data were collected at a scanning rate of 0.02\(^\circ\) s\(^{-1}\) for 2\(\theta\) in a range from 10\(^\circ\) to 80\(^\circ\).

### 2.3. Electrochemical Characterization

Typically, 8 mg of the electrocatalysts was dispersed in ethanol (200 \(\mu L\)) and Nafion solution (100 \(\mu L\), 5% wt.) in an ultrasonic bath for 30 minutes. Eight microliters of the mixture (the ink) was deposited onto a glassy carbon working electrode (0.5 cm in diameter, AFE2M050GC, Pine Instrument) and dried in ambient air for 3 minutes. The working electrode was operated at the rotational speed of 900 rpm with Ag/AgCl reference and Pt mesh counter electrode (1 cm \(\times\) 1 cm). The sensing characteristics of all the electrocatalysts were investigated with various concentrations of \(H_2O_2\) in a phosphate buffer solution (PBS) of pH 7.4. Cyclic voltammetric and amperometric measurements (Electrochemical Workstation CHI 660A) were performed to determine the electrochemical response of \(H_2O_2\).

## 3. Results and Discussion

### 3.1. Structure and Morphology

The morphology and size of pure Pt and bimetallic nanoparticles were characterized by TEM (shown in Figure 1). The as-prepared particles are well dispersed and substantially uniform in size. By controlling the time of microwave irradiation and the concentration of metal precursors, different metals in the same size range can be obtained. When the Pt precursor mixed with KOH and EG solution was placed into the microwave and heated for 36 s, Pt nanoparticles with diameter about 3–5 nm were obtained (Figure 1(a)). Upon microwave irradiation for 28 s, bimetallic PtRu nanoparticles with a diameter of about 2–3 nm were formed (Figure 1(b)). Decreasing the concentration of Pt and Au precursor to 0.025 M yielded nanoparticles with a diameter of about 2–4 nm (Figure 1(c)). The particles size of PtIr is about 2–3 nm, which was obtained by heating the precursor solution for 20 s (Figure 1(d)).

Figure 2 is the EDX analysis of pure Pt and bimetallic nanoparticles. In the spectra of PtRu, PtIr, and PtAu, the peaks of Pt, Ru, Ir, and Au confirm the coexistence of two metals in each sample, respectively. Cu and C peaks presented in the samples come from the copper grid used to support the samples, and Fe, Co peaks are caused from the column in TEM instrument.

XRD measurements were used to identify the prepared pure Pt and bimetallic nanoparticles. It can be seen from Figure 3 that all the peaks in the XRD pattern of pure Pt...
were well indexed to Pt (JCPDS No. 87-0640, \(a = b = c = 0.3923\) nm) with high crystallization. As the bimetallic nanoparticles are formed, all the peaks in the XRD patterns of PtRu and PtIr are shifted slightly to larger \(2\theta\) values (right-shift) with respect to the pure Pt, indicating the decreased d-spacings and contraction of the lattice constant, due to the incorporation of increasing amounts of the smaller metal atoms into the Pt fcc lattice [27]. On the other hand, the XRD peaks of PtAu are shifted slightly to smaller \(2\theta\) values (left-shift) pattern, implying that the incorporation of larger Au atoms into the Pt fcc lattice. No Pt or other metal peaks appear, indicating that bimetallic nanostructures or alloys were formed.

### 3.2. Biosensing Characterization

#### 3.2.1. Amperometric Determination of Pure \(H_2O_2\)

Because many enzymatic reactions that are related to the key enzyme in a biological system generate \(H_2O_2\) as a by-product, therefore, the detection of \(H_2O_2\) is important to biosensing. The performance biosensing of the pure Pt and the bimetallic catalysts was assessed by electrocatalytic oxidation of \(H_2O_2\). First, cyclic voltammogram was assessed by electrocatalytic oxidation of \(H_2O_2\). First, cyclic voltammogram was carried out at an ambient temperature (22.0°C) in the presence and absence of 0.25 mM \(H_2O_2\) in a 75 mL test medium. Figure 4 shows the cyclic voltammograms of pure Pt, PtRu, PtAu, and PtIr from −0.2 to +0.6 V versus the Ag/AgCl reference electrode, at 50 mV/s scan rate. While the oxidation of \(H_2O_2\) for the pure Pt catalyst starts at +0.23 V, all bimetallic catalysts begin even before this potential. For instance, the oxidation of \(H_2O_2\) was started from 0.20, 0.20, and 0.15 V versus Ag/AgCl in the voltammograms of bimetallic PtRu, PtAu, and PtIr, respectively, and these results clearly demonstrated the enhanced electrocatalytic ability of the bimetallic nanoparticles toward the detection of \(H_2O_2\) at relatively low electrochemical potentials. The performance of bimetallic nanoparticles in this work is compared with other catalysts as shown in Table 1, suggesting superior performance in terms of onset catalytic potential. The selection of the applied potential to oxidize \(H_2O_2\) could be based on the results as shown in Figure 4, and also the relatively higher potential of pure Pt toward the detection of \(H_2O_2\) should be considered to oxidize other coexisting electroactive species. Hence, an applied potential of +0.25 V versus the Ag/AgCl reference electrode was then chosen in order to minimize the potential interference from the oxidation of the other species in the test medium.

The amperometric detection of different concentration of \(H_2O_2\) was then tested. Different concentrations of \(H_2O_2\) solution ranging from 0.25 to 3.25 mM were prepared,
Figure 2: EDX spectra of pure Pt (a) and bimetallic PtRu (b), PtAu (c) and PtIr (d) nanoparticle. (TEM instrument, carbon, and copper grid spectra corresponding to olive green asterisk: Fe, violet asterisk: Co, pink asterisk: C, and gray asterisk: Cu).

Figure 3: XRD spectra of pure Pt and bimetallic nanoparticles.

and the amperometric measurement technique was used to record the change in the current as a function of time. The applied potential was +0.25 V versus the Ag/AgCl reference electrode. Figure 5 shows the real time responses of different metal to different concentration of H₂O₂. The current increase was observed with multiple injections of H₂O₂. All the four metals demonstrated a stepwise increase in current corresponding to an increase in the supplemental concentration of H₂O₂. Also, we find that the current increase of pure Pt is much lower than bimetallic PtRu, PtAu, and PtIr nanoparticles, especially at higher H₂O₂ concentration.

Figure 6 shows the current concentration plot of bimetallic compounds as compared to the pure Pt to detect H₂O₂ from 0.25 mM to 3.25 mM. In particular, the sensitivities of PtRu, PtAu, and PtIr are estimated to be 539.01, 415.46, and 404.52 μA·mM⁻¹·cm⁻², respectively, twice as high as that of the pure Pt (221.77 μA·mM⁻¹·cm⁻²). Not only did both PtRu and PtIr have a comparable sensitivity, but also showed a high degree of repeatability as indicated by the
## Table 1: Comparison of the performance of various H$_2$O$_2$ sensors based on electrochemical measurement.

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Sensitivity (μA·mM$^{-1}$·cm$^{-2}$)</th>
<th>% RSD</th>
<th>LOD (μM)</th>
<th>Applied potential versus Ag/AgCl (V)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pt NPs</td>
<td>221.77</td>
<td>10.5</td>
<td>3.3</td>
<td>+0.25</td>
<td>This work</td>
</tr>
<tr>
<td>PtRu NPs</td>
<td>539.01</td>
<td>0.5</td>
<td>1.7</td>
<td>+0.25</td>
<td>This work</td>
</tr>
<tr>
<td>PtAu NPs</td>
<td>415.46</td>
<td>9.1</td>
<td>2.0</td>
<td>+0.25</td>
<td>This work</td>
</tr>
<tr>
<td>PtIr NPs</td>
<td>405.52</td>
<td>2.0</td>
<td>0.8</td>
<td>+0.25</td>
<td>This work</td>
</tr>
<tr>
<td>Hb/GNPs/Hb/MWNT</td>
<td>205.41</td>
<td>3.5</td>
<td>0.08</td>
<td>−0.3</td>
<td>[28]</td>
</tr>
<tr>
<td>Ag NPs/GO</td>
<td>13.93</td>
<td>3.9</td>
<td>1.9</td>
<td>−0.3</td>
<td>[29]</td>
</tr>
<tr>
<td>Pt/TiO$_2$/CNT</td>
<td>0.85</td>
<td>5.5</td>
<td>4</td>
<td>+0.3</td>
<td>[30]</td>
</tr>
<tr>
<td>CNT/Pt NPs</td>
<td>$1.8 \times 10^3$</td>
<td>3.5</td>
<td>1.2</td>
<td>+0.55</td>
<td>[31]</td>
</tr>
<tr>
<td>MP-Pt-ME</td>
<td>$2.8 \times 10^3$</td>
<td>no</td>
<td>4.5</td>
<td>+0.6</td>
<td>[32]</td>
</tr>
</tbody>
</table>

NPs: nanoparticles, Hb: hemoglobin, GNPs: gold nanoparticles, MWNT: multiwall carbon nanotubes, GO: graphene oxide, MP: mesoporous, ME: microelectrode, CNT: carbon nanotube RSD: relative standard deviation, LOD: limit of detection, RSD of Hb/CNT/Au NPs: $n = 9$ (0.1 mM H$_2$O$_2$), RSD of Ag NPs/GO: $n = 5$ (1 mM H$_2$O$_2$), RSD of GC/CNT/Pt NPs: $n = 8$ (1 mM), RSD of Pt/TiO$_2$/CNT: $n = 8$ (0.2 mM H$_2$O$_2$), negative applied potential: reduction, and positive applied potential: oxidation.

![Figure 4: Cyclic voltammetric responses of different metallic catalysts at 0.25 mM H$_2$O$_2$ in PBS (pH = 7.4), at 50 mV/s scan rate.](image-url)
50 100 150 200 250 300 350

0 1 2 3 4 5
×10⁻⁴

0 50 100 150 200 250 300 350 400

[H₂O₂] (mM)

Current density (μA·mM⁻¹·cm⁻²)

Table 2: Comparison of oxidation current change at 0.5 mM H₂O₂ detection with a presence of AA.

<table>
<thead>
<tr>
<th>Metal compound</th>
<th>Only 0.5 mM H₂O₂</th>
<th>0.5 mM H₂O₂ + 5 mg/L AA</th>
<th>Percentage change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pt</td>
<td>11.70</td>
<td>15.14</td>
<td>29.40</td>
</tr>
<tr>
<td>Pt-Au</td>
<td>114.19</td>
<td>115.86</td>
<td>1.46</td>
</tr>
<tr>
<td>Pt-Ir</td>
<td>115.00</td>
<td>123.73</td>
<td>7.59</td>
</tr>
<tr>
<td>Pt-Ru</td>
<td>105.54</td>
<td>97.20</td>
<td>7.90</td>
</tr>
</tbody>
</table>

3.2.2. Ascorbic Acid Interference. Further investigation of interference toward H₂O₂ detection was also carried out for biosensing. It has been known that ascorbic (AA) is one of the chemical species in physiological fluid and is commonly oxidized at +0.3 V versus Ag/AgCl. Therefore, in many biosensing applications, AA interferes the sensor performance. As shown in Figure 7, the head-to-head comparison between pure H₂O₂ and mixture of H₂O₂ with high concentration AA (5 mg/L) for each catalyst was presented. The oxidation current at 0.5, 1.0, and 1.5 mM H₂O₂ of the pure Pt shows the effect of AA at +0.3 V as shown by the red color data points. All three bimetallic compounds exhibit an improved selectivity. Especially for the PtIr, the difference in both currents resulted by the pure H₂O₂ and the mixture is nearly negligible for the detection below 1 mM of H₂O₂. At 0.5 mM H₂O₂ with 5 mg/L AA, only 7-8% of oxidation current change will occur by using PtRu and PtIr. A minute interference effect by the ascorbic acid was found to be only 1-2% for PtAu catalyst whose linearity was poorer than the other bimetallic catalysts in this study. By comparison, the single Pt catalyst shows approximately 30% of interference by the ascorbic acid oxidation in H₂O₂ detection as summarized in Table 2. In addition to highly selective property, at +0.3 V, PtRu and PtIr have the sensitivity value of 1236.4 and 1359.7 μA·mM⁻¹·cm⁻², R² = 0.99, respectively, approximately one order higher than the pure Pt (136.1 μA·mM⁻¹·cm⁻², R² = 0.99). Hence, both PtRu and PtIr nanoparticles suggest an excellent sensitive and
highly capable of anti-interference catalyst, which is superior to the pure Pt. Both bimetallic catalysts can minimize the AA interference and provide better biosensing performance.

4. Conclusions

Bimetallic Pt-Ru, Pt-Au, and Pt-Ir nanoparticle catalysts was successfully synthesized via rapid microwave irradiation with EG and KOH. The structure and morphology was illustrated by TEM images with the average particle size less than 5 nm in diameter. The elemental composition and existing of bimetallic interaction were also confirmed by EDX and shift of XRD peaks, respectively. Furthermore, electrocatalytic properties of bimetals, particularly for PtRu and PtIr show superior performance in both sensitivity and selectivity than the pure Pt at a low applied potential, providing an anti-interference catalyst platform for future biological applications.

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References


