

Article

Polypyrrole/ α -Fe₂O₃ Hybrids for Enhanced Electrochemical Sensing Performance towards Uric Acid

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Abstract: Uric acid, a metabolite formed by the oxidation of purines in the human body, plays a crucial role in disease development when its metabolism is altered. Various techniques have been employed for uric acid analysis, with electrochemical sensing emerging as a sensitive, selective, affordable, rapid, and simple approach. In this study, we developed a polymer-based sensor (PPy/ α -Fe₂O₃) for the accurate determination of uric acid levels. The PPy/ α -Fe₂O₃ hybrids were synthesized using an uncomplicated in situ growth technique. Characterization of the samples was performed using scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FT-IR), X-ray powder diffraction (XRD), and X-ray photoelectron spectroscopy (XPS). The electrochemical sensing performance towards uric acid was evaluated through cyclic voltammetry (CV) and differential pulse voltammetry (DPV). The obtained results demonstrated that the sensor exhibited excellent sensitivity towards uric acid detection within a wide range of 5–200 μ M with a limit of detection (LOD) as low as 1.349 μ M. Furthermore, this work elucidated the underlying sensing mechanism and highlighted the pivotal role played by PPy/ α -Fe₂O₃ hybrids in enabling efficient uric acid sensing applications using electrochemical sensors.

Keywords: polypyrrole; α -Fe₂O₃; uric acid; electrochemical sensor; differential pulse voltammetry



Citation: Wang, R.; Liu, S.; Song, X.; Jiang, K.; Hou, Y.; Cheng, Q.; Miao, W.; Tian, L.; Ren, Y.; Xu, S. Polypyrrole/ α -Fe₂O₃ Hybrids for Enhanced Electrochemical Sensing Performance towards Uric Acid. *Coatings* **2024**, *14*, 227. <https://doi.org/10.3390/coatings14020227>

Academic Editor: Kamila Malecka-Baturo

Received: 16 January 2024

Revised: 28 January 2024

Accepted: 29 January 2024

Published: 15 February 2024



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1. Introduction

Uric acid (UA) is a metabolite generated through the oxidation of purines within the human body. Elevated levels of serum UA (hyperuricemia) have been implicated in the pathogenesis of various diseases, including gout, nephrolithiasis, and chronic kidney disease [1,2]. Therefore, the precise and expeditious detection of UA is imperative for the diagnosis and management of these ailments. Currently, there are several methods for determining serum UA levels, including high-performance liquid chromatography (HPLC) [3], colorimetric methods [4,5], and enzymatic methods [6,7]. However, these techniques require expensive equipment or complicated operating procedures, which limit their widespread use in clinical laboratories.

Electrochemical detection has gained significant attention in recent years due to its high sensitivity, selectivity, affordability, rapid response time, and simplicity when compared to other methods [8,9]. Electrochemical-based sensors have been extensively employed for the detection of environmental pollutants such as heavy metals (e.g., arsenic, cadmium, mercury, lead, and chromium) [10–15] as well as organic pollutants, including toxins, pesticides, antibiotics, phenolics, etc. [16–19]. With technological advancements, electrochemical biosensing technology is gradually replacing traditional biological sensing techniques for various applications like dopamine and glucose detection. Currently, the limit of detection (LOD) achieved through electrochemical techniques is approximately 1–25 nM [20–24] and 0.1–0.3 μ M [25–27] for dopamine and glucose, respectively. Notably, fruitful results have also been obtained in utilizing electrochemical detection for UA research [28–32].

Sensor materials play a crucial role in electrochemical sensing technology due to their large surface areas, excellent electrochemical activity, and ability to promote electron transfer reactions. Transition metal oxides, such as ZnO [33,34], CuO [35], NiO [36], and Fe₂O₃ [37,38], are extensively utilized for electrochemical sensors owing to their remarkable properties. Among them, α -Fe₂O₃ is considered the most promising material due to its non-toxicity; its biological, economical, and chemical inertness; as well as its outstanding electrical and catalytic characteristics. However, the easy aggregation, poor dispersibility, and conductivity limitations of transition metal oxides significantly hinder their electrochemical performance. To address this issue effectively, extensive research has focused on incorporating conductive materials to enhance the electrochemical sensing capabilities. Noble metals like Au and Ag have been doped into transition metal oxide sensors to improve their electrochemical performance [39,40]. Additionally explored are conductive carbon materials such as graphene and carbon nanotubes that possess high surface areas along with excellent electron mobility while being environmentally friendly and non-toxic. These have been widely investigated as substrates for electrochemical sensors [16,32,41,42]. However, the preparation process of graphene and carbon nanotubes is intricate and prone to agglomeration. In comparison with these options, conductive polymer materials offer simpler preparation processes, better formability, and greater suitability for wearable devices; hence, they represent an important research direction in the field of electrochemical sensing at the present time. Among various extensively studied conductive polymers, polypyrrole (PPy) stands out due to its facile synthesis, high electrical conductivity, and good stability.

In conclusion, α -Fe₂O₃ displays remarkable potential as a sensitive material for UA detection, while PPy has the ability to enhance electron mobility. Furthermore, both materials are harmless to the environment and non-toxic. Taking into account these factors, we fabricated nano- α -Fe₂O₃ decorated PPy hybrids in this study to detect UA. The utilization of in situ synthesis techniques effectively prevented the agglomeration of α -Fe₂O₃ particles and the incorporation of PPy significantly improved their electron mobility. The electrochemical sensing performance of UA was comprehensively characterized through the use of diverse analytical techniques. Moreover, the mechanism underlying UA sensing was also elucidated.

2. Experimental Section

2.1. Materials and Solvents

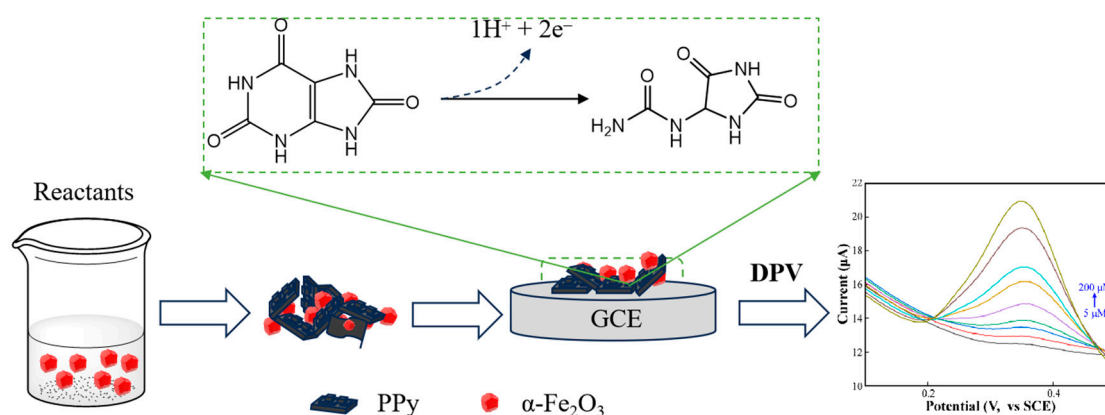
Pyrrole (Py) was obtained from Ron's Reagent. Ferric chloride hexahydrate (99%, FeCl₃·6H₂O) and uric acid (99%, UA) were both purchased from Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Ammonia was obtained from Huateng Chemical Co., Ltd. (Beijing, China). Nafion was purchased from DuPont Inc. Phosphate buffer saline (0.1 M, PBS) was purchased from Solarbio Science & Technology Co., Ltd. (Beijing, China). High-purity nitrogen was provided by Yingzhong Chemical Products Technology Co., Ltd. (Zhengzhou, Henan, China). Absolute ethanol and methanol were purchased from Damao Chemical Reagent Factory (Tianjin, China). All chemical reagents used in this study were of analytical grade and were used without further treatment after purchase.

2.2. The Fabrication of PPy/ α -Fe₂O₃ Composite

The pure α -Fe₂O₃ nanoparticle (NP) was synthesized through a hydrothermal reaction using FeCl₃·6H₂O and ammonia as raw materials. Firstly, a certain amount of FeCl₃·6H₂O was dissolved in deionized water. Then, NH₃·H₂O was added drop by drop and the mixture was continuously stirred for 1 h. The resulting emulsion was then transferred to a reactor and placed in an oven for the complete reaction. After the reaction, the solution was centrifuged, washed with distilled water several times, and dried in a vacuum oven at 60 °C. The dried sample was ground into a fine powder and then subjected to calcination at 350 °C in a Muffle furnace with a heating rate of 10 °C/min for 30 min, resulting in the formation of α -Fe₂O₃.

The pure PPy was prepared by a simple polymerization reaction. Firstly, an appropriate amount of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ was weighed and dissolved in deionized water to prepare an aqueous solution. Then, a certain quantity of pyrrole was added to deionized water, followed by the gradual addition of the FeCl_3 solution while maintaining stirring at room temperature for 10 h, resulting in the formation of a black slurry. The obtained black precipitate was washed multiple times with methanol and distilled water, then dried at 60°C in a vacuum oven to obtain pure PPy.

The PPy/ $\alpha\text{-Fe}_2\text{O}_3$ composites were prepared by a simple in situ growth technology, as shown in Scheme 1. Firstly, pyrrole droplets were added to deionized water and stirred magnetically to form a pyrrole suspension. Different amounts of $\alpha\text{-Fe}_2\text{O}_3$ were added to the pyrrole suspension and sonicated for 1 h. Secondly, a certain amount of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ was dissolved in 100 mL of deionized water. After complete dissolution, it was added dropwise to the above solution to obtain a black emulsion, which was fully reacted by magnetic stirring for 10 h. Thirdly, at the end of the reaction, the precipitate was collected and washed successively with methanol and distilled water. The products were dried in a vacuum dryer. A series of samples were prepared and recorded as PPy/ $\alpha\text{-Fe}_2\text{O}_3$ -X, where X corresponds to the molar ratio of nano- $\alpha\text{-Fe}_2\text{O}_3$ to polymer precursors. In this study, pure PPy was used as a control sample.



Scheme 1. Procedure for the fabrication of the PPy/ $\alpha\text{-Fe}_2\text{O}_3$ hybrids and the detection mechanism of UA.

2.3. Characterization

The microstructure and morphology of the pure PPy, $\alpha\text{-Fe}_2\text{O}_3$, and PPy/ $\alpha\text{-Fe}_2\text{O}_3$ composites were observed using scanning electron microscopy (SEM, INSRECT F50, FEI, USA). The chemical structure of the composite was characterized by Fourier transform infrared spectroscopy (FT-IR, Nicolet Is-20, ThermoFisher, China). The chemical composition of the samples and the existence state of these elements were analyzed with reference to the C1s signal peak (~ 284.8 eV) by using X-ray photoelectron spectroscopy (XPS, ThermoFisher, ESCALAB Xi+, China). The crystal structure of the synthesized composites was determined through X-ray diffraction (XRD, Bruker D8 ADVANCE, Germany). Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were measured by an electrochemical workstation (CHI 760E, China) in a standard three-electrode configuration with Pt wire as the counter electrode and Hg/HgCl as the reference electrode. The electrolyte solution was 0.1 M PBS solution saturated with N_2 .

2.4. Preparation of Working Electrodes

The exposed surface of the glass carbon electrode (with an inner diameter of 3 mm and an outer diameter of 6 mm) was polished with aluminum oxide polishing powder ($0.05\ \mu\text{m}$). The polished, bare electrode was sonicated in ethanol and deionized water for 2 min and dried at room temperature. The 5 mg sample was dispersed in a mixture of 1 mL deionized water, 0.5 mL anhydrous ethanol, and 10 μL 0.5 wt% nafion solution, and a

uniform mixture was formed after sonication for 3 h. PPy/ α -Fe₂O₃/GCE can be obtained by coating 6 μ L of the sample suspension uniformly on a glass carbon electrode and drying at room temperature. Using the PPy/ α -Fe₂O₃/GCE nanocomposite sensor prepared after drying, the UA was qualitatively and quantitatively analyzed by adjusting and optimizing the suitable voltage.

2.5. Biosensing Performance Measurements

Whether the modified electrode reacted with UA was determined by performing CV tests in 0.1 M of nitrogen-saturated PBS electrolyte. The working electrode, reference electrode, and counter electrode were immersed in a PBS electrolyte containing 5 mM UA, and CV tests were conducted on the working electrode after the electrochemical workstation was stabilized. The voltage range in the test was controlled within $-1.6 \sim 0.5$ V, and the scanning rate was $50 \text{ mV} \cdot \text{s}^{-1}$. Activation was required before accurate testing, and the CV test was performed after the working electrode was stabilized. The REDOX peak, peak current position, and peak height were observed to determine whether the working electrode had a response to UA and the best response. After selecting the best response ratio, CV tests were conducted on the working electrode at different scanning rates, and linear fitting was performed according to the data. The UA reaction mechanism was determined by fitting the curve.

DPV tests were performed on PBS electrolytes containing different concentrations of UA. By linear fitting the peak current value, the limit of detection (LOD) of UA was calculated according to the equation $LOD = 3\sigma/K$. LOD is the detection limit; σ is the standard deviation of the blank sample measurement, that is, the determination of 0.1 mM UA-free saturated nitrogen PBS solution with PPy/ α -Fe₂O₃ composite material; and the K value is the slope of the DPV simulation curve.

3. Results and Discussion

3.1. Characterizations of Samples

The FT-IR spectrum of α -Fe₂O₃, PPy, and PPy/ α -Fe₂O₃ is presented in Figure 1a to elucidate the material composition. As depicted in the figure, distinct characteristic absorption peaks of pure PPy are prominently observed at 3445.6 cm^{-1} , 1536 cm^{-1} , 1451.1 cm^{-1} , 1303.2 cm^{-1} , 1170.1 cm^{-1} , 1040.9 cm^{-1} , and 901.6 cm^{-1} , which correspond to the symmetric stretching mode of the imino group (N–H), the symmetric stretching mode of C=C, the stretching mode of C–C, the bending mode of C=N, the stretching mode of C–N, the bending mode of =C–H, and the stretching mode of =C–N⁺–C, respectively [43,44]. These peaks provide evidence for the formation of PPy and its effective oxidation by FeCl₃. In the α -Fe₂O₃ NP spectrum, a weak peak is observed at 1630.5 cm^{-1} due to O–H stretching vibrations from water molecules, while peaks at 561.2 cm^{-1} and 480 cm^{-1} correspond to the vibration of the chemical bond of Fe³⁺–O^{2–}, which are basically in accordance with the literature values [45,46]. Comparatively lower wavelength shifts are observed in all characteristic peaks when comparing the spectrum obtained for PPy/ α -Fe₂O₃ with that for pure PPy, indicating synergistic electron interactions between the PPy chain and α -Fe₂O₃ NP. Notably, no significant peak corresponding to α -Fe₂O₃ NP is observed in the PPy/ α -Fe₂O₃ spectrum, suggesting its encapsulation within the PPy matrix (as confirmed by SEM images).

The crystal structures of pure α -Fe₂O₃, PPy, and the PPy/ α -Fe₂O₃ composite materials were analyzed using X-ray diffraction (XRD) patterns, as depicted in Figure 1b. For pure α -Fe₂O₃, the seven characteristic peaks observed at 24.2° , 33.5° , 36.3° , 43.3° , 51.4° , 53.5° , and 64.1° corresponded to the diffraction planes of (012), (104), (110), (113), (024), (116), and (300), respectively. Similarly, the XRD pattern of the PPy sample exhibited an amorphous structure with a broad peak centered around 24° , indicative of its non-crystalline nature [47]. Upon combining these components in the composite material formulation, apart from the broad peaks attributed to PPy's amorphous phase presence, distinct peaks corresponding to α -Fe₂O₃ were also detected by XRD analysis, confirming

the successful compounding of PPy/ α -Fe₂O₃ composites. Furthermore, the study revealed that the surface functionalization of PPy did not induce any changes in either the crystal structure or phase characteristics of α -Fe₂O₃.

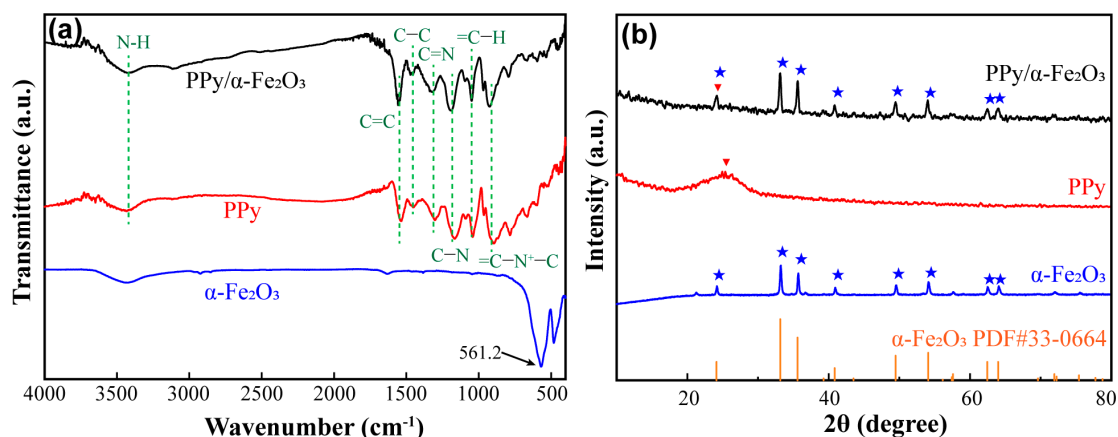


Figure 1. FT-IR spectra (a) and XRD patterns of α -Fe₂O₃, PPy, and PPy/ α -Fe₂O₃ (b) were obtained. The characteristic peaks of α -Fe₂O₃ and PPy are represented by blue stars and red triangles, respectively. The orange lines correspond to the standard PDF card of α -Fe₂O₃ (Color figure online).

The microstructure and morphology characteristics of PPy, α -Fe₂O₃, and PPy/ α -Fe₂O₃ composites were investigated using scanning electron microscopy (SEM), as depicted in Figure 2. The bare PPy exhibited a layered structure with each layer resembling several irregular particles adhered together (Figure 2a). The presence of the five-membered ring structure in Py contributed to the stiffness of PPy, resulting in its layered morphology. Additionally, the flexibility observed in the irregular particle morphology was attributed to the C-C bonds connecting the structural units of PPy. The morphology of α -Fe₂O₃ consisted of uniformly distributed nanoparticles with an average particle size of approximately 70 nm (Figure 2b). Upon composite formation with α -Fe₂O₃, SEM analysis revealed that apart from retaining its layered morphology, small dispersed α -Fe₂O₃ particles were also observed on the surface of PPy (indicated by yellow squares and arrows in Figure 2c,d). Notably, some α -Fe₂O₃ nanoparticles were encapsulated within the PPy matrix. These SEM images confirmed the successful fabrication and distribution of α -Fe₂O₃ NPs on the surface as well as within the matrix of PPy. Furthermore, EDS investigation provided additional evidence for the presence of both PPy and α -Fe₂O₃ within the composite material (Figure S1).

XPS spectroscopy was employed to investigate the surface chemical composition and the chemical state of α -Fe₂O₃, PPy, and PPy/ α -Fe₂O₃ composites. As shown in Figure S2, the XPS spectra reveal the presence of C, O, and N elements in the pure PPy samples; Fe, C, O, and N elements in the PPy/ α -Fe₂O₃ composites; and Fe, O, and C (impurity) elements in the bare α -Fe₂O₃ samples. These findings are consistent with the EDS and XRD results. Importantly, the XPS results also confirmed that the lower content of Fe in the composite was due to the encapsulation of α -Fe₂O₃ within the PPy matrix observed by SEM and FT-IR analysis. A representative XPS spectrum illustrating the structure of the PPy/ α -Fe₂O₃ nanocomposite is shown in Figure 3. The core-level C 1s spectra are curve-fitted into three groups: C–C (284.79 eV), C–N (286.12 eV), and C=N (288.39 eV) from PPy (Figure 3a). In Figure 3b, the N 1s peaks at 397.5 eV, 399.6 eV, and 401.4 eV for PPy/ α -Fe₂O₃ are assigned to the imine group (–NH–), the charged polaronic nitrogen group (=HN⁺–), and the neutral pyrrolylium nitrogen group (=N–) in PPy, respectively. The presence of these functional groups confirms the existence of PPy within the composite material. The core-level O 1s spectrum is deconvoluted into three peaks corresponding to the O–H species (531.9 eV) and surface adsorbed oxygen (533.2 eV) of the PPy and the Fe–O bonds (530.9 eV) of the α -Fe₂O₃ (Figure 3c). The Fe 2p peaks are separated into Fe

2p_{1/2} (724.1 eV) and Fe 2p_{3/2} (710.9 eV) for α -Fe₂O₃ in Figure 3d, which further supports its existence.

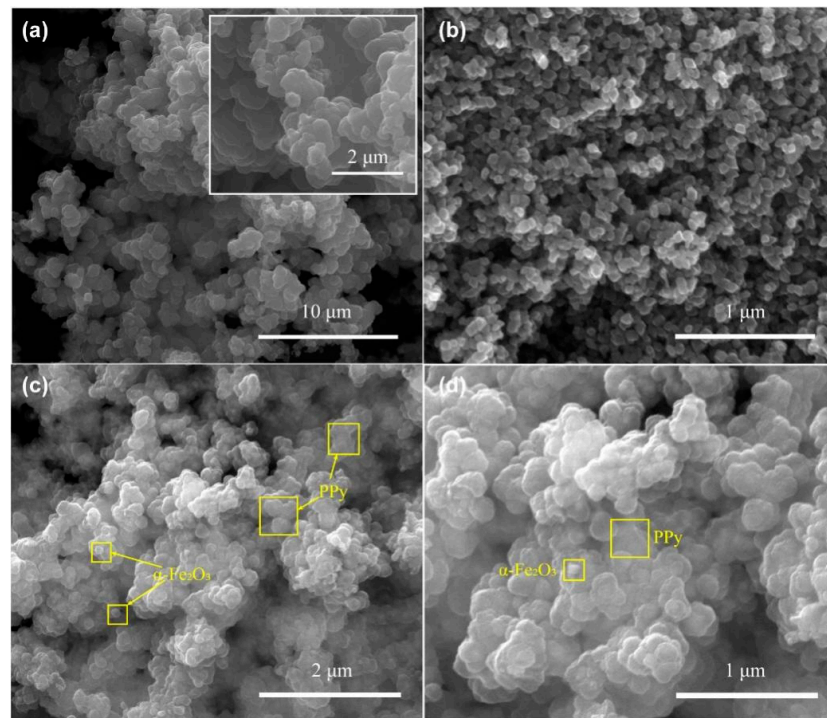


Figure 2. The microstructure and morphology of PPy (a), α -Fe₂O₃ (b), and PPy/ α -Fe₂O₃ (c,d). The white horizontal lines in each figure represent the scale bars.

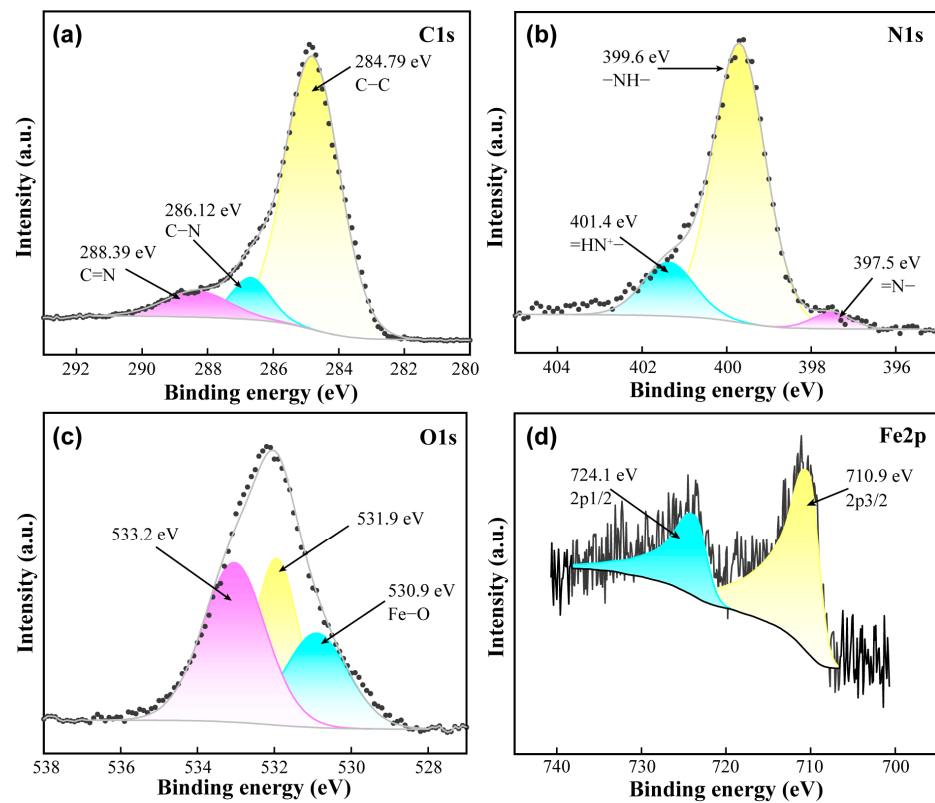


Figure 3. The XPS spectra of the C 1s (a), N 1s (b), O 1s (c), and Fe 2p (d) elements in the PPy/ α -Fe₂O₃ composite.

3.2. Electrochemical Performance of PPy/ α -Fe₂O₃ Composite

The electrochemical properties of pure PPy, α -Fe₂O₃, and PPy/ α -Fe₂O₃ hybrid electrodes were investigated by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) in a KCl solution (0.1 mol/L) containing 5 mM [Fe(CN)₆]^{3−/4−}. As depicted in Figure 4a,b, the PPy/ α -Fe₂O₃ hybrids exhibit enhanced electrochemical activity compared to bare PPy and α -Fe₂O₃, particularly for sample PPy/ α -Fe₂O₃–2. From Figure 4c,d, it can be observed that the charge transfer resistance (R_{ct}) is lowest for the PPy/ α -Fe₂O₃–2 sample, indicating an increase in the conductivity of α -Fe₂O₃ after loading with PPy. The electroactive surface area (ESA) was calculated using the Randles–Sevcik equation [48]:

$$I_p = 2.69 \times 10^5 A D^{1/2} n^{3/2} \nu^{1/2} C \quad (1)$$

where A represents the ESA, ν denotes the scan rates, D refers to the diffusion coefficient, n represents the electron transfer number, and C signifies the concentration of [Fe(CN)₆]^{3−/4−}. The peak current for all electrodes, as shown in Figure S3b,d,f,h,j,l, exhibits a strong linear relationship with the square roots of the scan rate. Equation (1) can be used to calculate the ESA by determining the slope of the I_p - $\nu^{1/2}$ curve. The PPy/ α -Fe₂O₃–2 hybrids demonstrate a significantly higher ESA value (0.242 cm²) compared to pure PPy (0.206 cm²) and α -Fe₂O₃ (0.215 cm²), indicating an abundance of active sites on the PPy/ α -Fe₂O₃–2 hybrids.

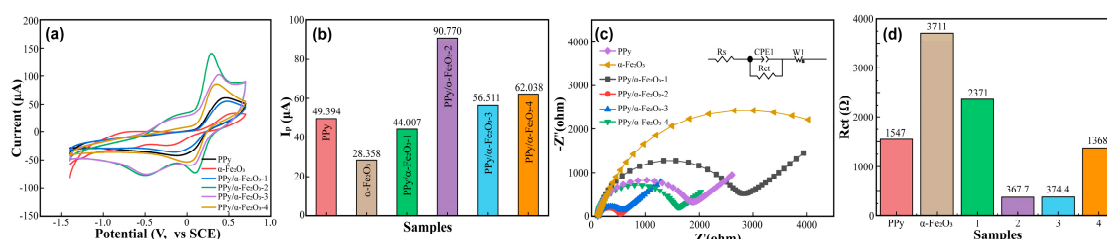


Figure 4. CV curves (scan rate of 50 mV/s) (a), the oxidation peak current (b), the Nyquist plots (c), and the resistance of charge transfer (R_{ct}) (d) of PPy, α -Fe₂O₃, and PPy/ α -Fe₂O₃ hybrids recorded in 0.1 mol/L KCl containing 5 mM [Fe(CN)₆]^{3−/4−}. R_s , R_{ct} , CPE1, and W1 in (c) represent the solution resistance, charge transfer resistance, constant-phase element, and diffusion impedance, respectively.

To investigate the sensing performance of the PPy/ α -Fe₂O₃ composite towards UA, the electrochemical responses of UA on various modified electrodes were examined by using cyclic voltammetry (CV). CV scans were performed with different modified electrodes as working electrodes in nitrogen-saturated PBS containing 0.5 mmol/L UA, employing a scanning rate of 50 mV/S. The CV responses of PPy, α -Fe₂O₃, and PPy/ α -Fe₂O₃ electrodes towards UA are shown in Figure 5a. As depicted in the figure, pure PPy and α -Fe₂O₃ exhibited a low response within the voltage range 0.2–0.6 V, corresponding to the response towards UA. Most of the PPy/ α -Fe₂O₃ composites displayed a significantly higher current response with a pair of REDOX peaks. However, when a small amount of α -Fe₂O₃ was added to modify PPy (PPy/ α -Fe₂O₃–1), a weaker current response was observed. This could be attributed to the encapsulation of most of the α -Fe₂O₃ into PPy at low content levels, resulting in fewer exposed active sites for interaction with UA. In addition, the recombination of a small amount of α -Fe₂O₃ also reduced the charge transport performance of PPy to a certain extent. We hypothesized that the composite should exhibit an enhanced response compared to pure PPy due to the presence of the polyvalent iron element as an active center in α -Fe₂O₃. Actually, with the increasing content of α -Fe₂O₃, particularly in sample PPy/ α -Fe₂O₃–2, a distinct peak response was observed within the range of 0.2–0.6 V, further confirming that the incorporation of α -Fe₂O₃ effectively enhances UA detection capability. However, as the content of α -Fe₂O₃ exceeded an optimal level, we noticed a relative weakening in response, which could be attributed to agglomeration caused by excessive amounts of α -Fe₂O₃ leading to the reduced exposure of active sites on

the composite surface. Furthermore, excess amounts of $\alpha\text{-Fe}_2\text{O}_3$ were found to negatively impact the charge transport performance and subsequently reduce its responsiveness towards UA detection.

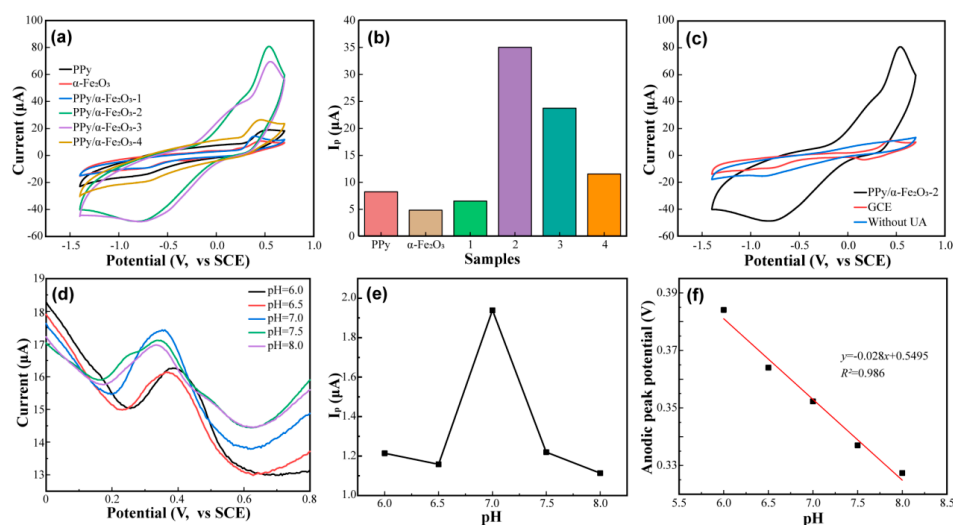


Figure 5. CV curves of various modified electrodes in the presence of UA (a) and the impact of different proportions of PPy and $\alpha\text{-Fe}_2\text{O}_3$ in the modified materials on the redox peak current of UA (b); comparison of cyclic voltammograms of the PPy/ $\alpha\text{-Fe}_2\text{O}_3$ -2 electrode and blank electrode before and after adding 0.5 mM UA (c); DPV curves of the PPy/ $\alpha\text{-Fe}_2\text{O}_3$ -2 electrode collected in 0.1 mol/L PBS containing 50 $\mu\text{mol/L}$ UA with a pH value ranging from 6.0 to 8.0 (d); anodic peak current (e) and the calibration curve of the potential versus the pH (f).

Figure 5b demonstrates that an optimized composition ratio between PPy and $\alpha\text{-Fe}_2\text{O}_3$ leads to enhanced electrocatalytic activity for UA detection compared to the individual materials alone. When the content of $\alpha\text{-Fe}_2\text{O}_3$ is low, the current response of UA closely resembles that of pure PPy and $\alpha\text{-Fe}_2\text{O}_3$. However, as the $\alpha\text{-Fe}_2\text{O}_3$ content increases to PPy/ $\alpha\text{-Fe}_2\text{O}_3$ -2, the current response of UA reaches its maximum value, which is approximately five times higher than that observed for bare PPy. Nevertheless, a further increase in the $\alpha\text{-Fe}_2\text{O}_3$ content results in a decrease in the current response of UA. These findings suggest that the presence of $\alpha\text{-Fe}_2\text{O}_3$ facilitates an increased electrochemical surface area and promotes more active sites for electrocatalysis, thereby synergistically enhancing the oxidation reaction of UA. In summary, based on our observations, an optimal composition ratio for maximizing UA oxidation can be achieved with the composite PPy/ $\alpha\text{-Fe}_2\text{O}_3$ -2.

The CV curves of the PPy/ $\alpha\text{-Fe}_2\text{O}_3$ electrode with and without 0.5 mM UA, as well as the bare glass carbon electrode (GCE), are presented in Figure 5c. Upon the addition of 0.5 mM of UA, an oxidation peak was observed near 0.2 V and 0.4 V in the CV curve, demonstrating a significantly enhanced current signal compared to the oxidation peak at 0.4 V observed without UA. This observation suggests that the primary reaction predominantly occurs at this potential, which is consistent with the expected response of UA [28].

The effect of pH on the electrochemical response of UA was investigated through DPV measurements in a buffer solution with a pH range from 6.0 to 8.0. As depicted in Figure 5d, the anodic peak potentials shifted negatively as the pH value increased from 6.0 to 8.0. Furthermore, Figure 5f illustrates a linear correlation between the anodic peak potential (E_{ap}) and pH value, with a linear regression equation of $y = -0.028 \text{ pH} + 0.5495$ ($R^2 = 0.986$). The slope value is half that calculated from the Nernst equation, which is 0.0592 V/pH, indicating that twice as many as electrons are involved in oxidation compared to protons ($2e^-$ and $1H^+$) [49]. The electrochemical oxidation process can be expressed by the equation in Figure S4. Due to the electrical absorption of the carbonyl group, the unpaired electrons

on the intermediate tertiary amine of the two carbonyl groups may become electron donors. UA donates an electron from the intermediate tertiary amine of both carbonyl groups, resulting in the formation of a cation radical. Upon proton loss, this cation radical transforms into a radical species. Subsequently, this radical species loses an electron and undergoes hydrolysis to form a secondary amine. The aforementioned prediction was consistent with earlier reports on the oxidation of similar structures [31]. Additionally, as shown in Figure 5e, the anodic peak current reached its maximum at a pH of 7.0; therefore, all electrochemical measurements were conducted at this pH.

In order to further investigate the reaction mechanism of UA at the electrode interface, we examined the mechanism by varying the scanning rate in a nitrogen-saturated PBS solution containing 0.5 mmol/L of UA. Figure 6a illustrates the cyclic voltammograms of 0.5 mmol/L of UA at the PPy/ α -Fe₂O₃ modified electrode interface under different scan rates from 30 mV/s to 100 mV/s. The voltammograms exhibited an increase in the oxidation current peak with higher scan rates. The relationship between the square root of the scan rate and the redox peak current values is depicted in Figure 6b. The redox peak current values demonstrate a strong linear correlation with the square root of the scanning rate within the range of 30 mV/s to 100 mV/s, following $I(\mu A) = 4.47V^{1/2} (mV^{1/2}/s^{1/2}) + 14.48 (R^2 = 0.99)$, where I represents the peak current and V represents the scanning rate. The direct proportionality observed between the square root of the scan rate and the oxidation current peaks indicates that the UA oxidation is diffusion-controlled at the electrode interface. Furthermore, through Figure 6a, it can be observed that there is partial inconsistency between the oxidation and reduction processes, suggesting that UA undergoes a quasi-reversible, rather than completely reversible, redox process.

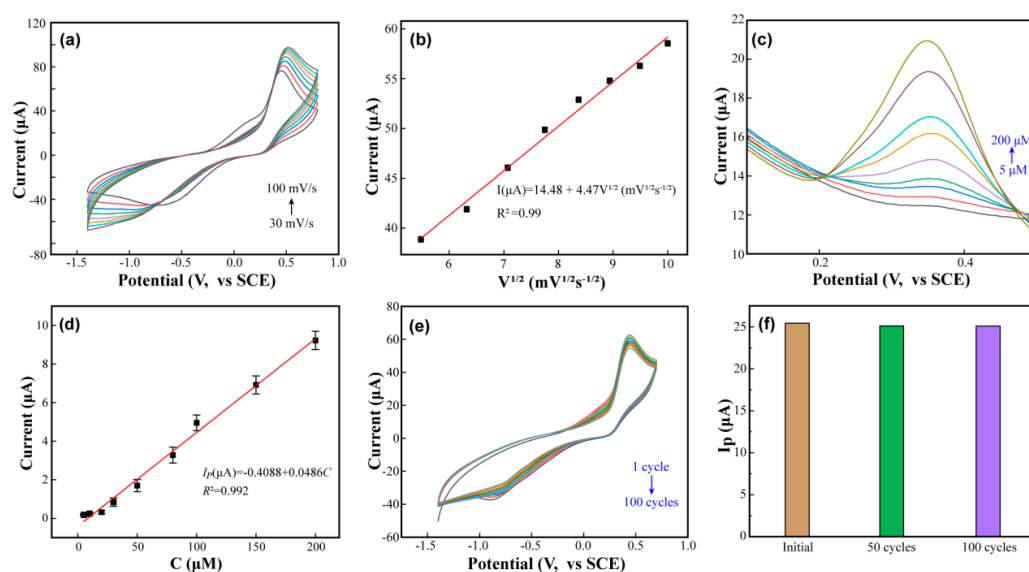


Figure 6. Cyclic voltammograms of PPy/ α -Fe₂O₃–2 recorded at various scanning rates in a UA solution (a), and the linear correlation between the anodic peak current and the square root of the scanning rate (b); successive DPV responses of PPy/ α -Fe₂O₃–2 with varying concentrations of UA in PBS buffer (c), and the linear correlation between the peak current and UA concentration (d); 100 repeated CV scans of the PPy/ α -Fe₂O₃–2 hybrids in 0.5 mM of UA at a scan rate of 50 mV/s (e), and the stability of the PPy/ α -Fe₂O₃–2 hybrids (f).

The sensitivity of the developed electrode towards UA detection was evaluated using the differential pulse voltammetry (DPV) technique, and the results are presented in Figure 6c,d. Figure 6c illustrates the DPV voltammograms obtained for a wide range of UA concentrations ranging from 5 μ mol/L to 200 μ mol/L. The DPVs exhibited an oxidation potential peak at approximately 0.32–0.35 V. Additionally, Figure 6d demonstrates the relationship between UA concentration and its corresponding oxidation current peak. The

findings revealed that as the concentration of UA increased, so did the oxidation current peak. A strong linear correlation was observed between the oxidation peak current and UA concentration, indicating that the PPy/ α -Fe₂O₃-2 modified electrode can be effectively employed for the quantitative detection of UA. Based on the fitted linear correlation equation $I_p (\mu A) = 0.0486C (\times 10^{-6} M) - 0.4088$ ($R^2 = 0.992$), where I represents the oxidation peak current and C denotes the UA concentration, it can be inferred that this modified electrode has a limit of detection (LOD) for UA determined to be approximately 1.349 μM using DPV technique according to the $LOD = 3\sigma/K$ formulae calculation method applied herein. The performance of the PPy/ α -Fe₂O₃ sensor was compared with that of other sensors, as summarized in Table 1. In terms of UA detection, the PPy/ α -Fe₂O₃ electrode exhibited a significantly lower LOD compared with the W-ZIF-67, the Au/Ni-MOF, the UOx/ZnONFs, the GP5AuNPs5, the holey MoS₂, and the Co_{0.01}Ni_{0.99}Fe₂O₄ electrodes. In addition, the LOD achieved by our fabricated sensors is two orders of magnitude lower than the normal physiological levels found in healthy individuals, thus highlighting great potential value for PPy/ α -Fe₂O₃ as a low-cost electrocatalyst in detecting UA.

Table 1. Detection performance comparison with other sensors.

Matrix	Detection Range	LOD	LOQ	References
W-ZIF-67	20–1000 μM	3.04 μM	9.12 μM	[50]
Au/Ni-MOF	10–500 μM	5.6 μM	16.8 μM	[51]
UOx/ZnONFs	5–750 μM	130 μM	390 μM	[52]
RTIL-NiHCF-NP-Gel	1.0–2600.0 μM	0.33 μM	1 μM	[53]
GP5AuNPs5	20–500 μM	1.47 μM	4.41 μM	[41]
Holey MoS ₂	200–700 μM	5.62 μM	16.86 μM	[54]
Co _{0.01} Ni _{0.99} Fe ₂ O ₄	4–5280 μM	6.38 μM	19.14 μM	[55]
PPy/Fe ₂ O ₃	5–200 μM	1.349 μM	4.407 μM	This work

The stability of the PPy/ α -Fe₂O₃-2 hybrid decorated electrode was evaluated by CV over 100 cycles within a potential window ranging from -1.4 V to 0.7 V. As illustrated in Figure 6e,f, only a marginal decrease of 1.31–1.33% in the redox peak current was observed even after 50 or 100 cycles, indicating excellent stability. Additionally, Figure S5a illustrates the long-term stability of the PPy/ α -Fe₂O₃-2 hybrid electrode by showcasing its current density responses over a period of 180 days. Remarkably, even after this extended duration, the electrode exhibits an impressive retention rate of 97.7% in response to UA. The interference of dopamine (DA) and ascorbic acid (AA) in clinical analysis is significant. Figure S5b presents the selectivity test against UA of the PPy/ α -Fe₂O₃-2 hybrid sensor in PBS at a pH of 7.0. It is evident that there exists a potential difference of 0.399 V between the oxidation potentials of DA and UA. Based on this observation, the oxidation peak of UA is sufficiently separated from that of DA, indicating the excellent selectivity of the hybrid sensor for UA detection at a specific operating voltage.

3.3. The Detection Mechanism

The sensor detection is based on the REDOX reaction of the UA molecule on the sensor surface. As illustrated in Figure 7, the oxidation of UA occurs through a double electron transfer process involving one proton. In the anode reaction, Fe³⁺ ions on the working electrode undergo reduction to Fe²⁺, while UA is oxidized to form allantoin, resulting in the release of one proton and two electrons. The liberated electrons generate an induced current on the electrode surface, whose magnitude depends on the concentration of UA. The charged polaronic nitrogen (=HN⁺–) and neutral pyrrolylium nitrogen (=N–) in PPy also possess the capability to participate in a two-electron, one-proton exchange reaction with UA. This explains why bare α -Fe₂O₃ and pure PPy exhibit a certain level of sensing response towards UA. According to previous test results, it can be observed that compounding PPy and α -Fe₂O₃ significantly enhances the electrical signal of the UA REDOX reaction. This enhancement can be attributed to the hydrogen bond formation

between the —NH and C=O functional groups of UA with the —NH functional groups of PPy, which strengthens the interaction between UA and the composite material and promotes its REDOX reaction on the surface of PPy/ $\alpha\text{-Fe}_2\text{O}_3$ electrodes. Additionally, the introduction of PPy greatly improves the charge transport performance of $\alpha\text{-Fe}_2\text{O}_3$, thereby enhancing the REDOX reaction efficiency for UA. Furthermore, combining PPy with $\alpha\text{-Fe}_2\text{O}_3$ effectively reduces the agglomeration of $\alpha\text{-Fe}_2\text{O}_3$ NPs and increases active centers within the composite material; this provides more active sites for REDOX reactions involving UA. The synergistic effect between PPy and $\alpha\text{-Fe}_2\text{O}_3$ significantly enhances the sensing performance for detecting UA using PPy/ $\alpha\text{-Fe}_2\text{O}_3$ electrodes.

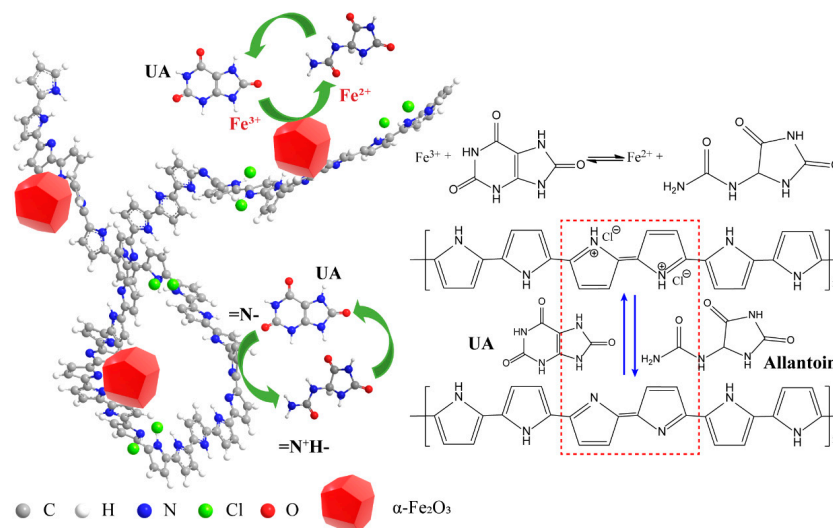


Figure 7. The principles of UA sensing by the PPy/ $\alpha\text{-Fe}_2\text{O}_3$ composite.

4. Conclusions

In summary, we present the fabrication and characterization of PPy/ $\alpha\text{-Fe}_2\text{O}_3$ composites, which exhibit promising potential as UA sensors. The incorporation of PPy/ $\alpha\text{-Fe}_2\text{O}_3$ not only enhances the charge transport performance but also increases the number of active sites for the UA reaction, thereby improving the sensing capability towards UA. By optimizing the ratio between PPy and $\alpha\text{-Fe}_2\text{O}_3$ in the hybrids, an optimal proportion was determined (the sample PPy/ $\alpha\text{-Fe}_2\text{O}_3$ –2). The modified electrode had an *LOD* of the UA, based on the DPV technique, of approximately $1.349\ \mu\text{M}$, with a linear concentration range spanning from $5\ \mu\text{M}$ to $200\ \mu\text{M}$. Although the fabricated sensor in this paper was prepared based on cost-effective materials, it had a lower *LOD* than some recently reported UA sensors. Our findings validate its potential as a sensor for detecting ultra-low concentrations of UA in real samples.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/coatings14020227/s1>, Figure S1. EDS mapping of PPy/ $\alpha\text{-Fe}_2\text{O}_3$ composite. Figure S2. The XPS spectra of $\alpha\text{-Fe}_2\text{O}_3$, PPy, and PPy/ $\alpha\text{-Fe}_2\text{O}_3$ composite. Figure S3. CV curves and the dependence plots of anodic peak current vs. square root of scan rate of (a,b) PPy, (c,d) $\alpha\text{-Fe}_2\text{O}_3$, (e,f) PPy/ $\alpha\text{-Fe}_2\text{O}_3$ –1, (g,h) PPy/ $\alpha\text{-Fe}_2\text{O}_3$ –2, (i,j) PPy/ $\alpha\text{-Fe}_2\text{O}_3$ –3, and (k,l) PPy/ $\alpha\text{-Fe}_2\text{O}_3$ –4 in $0.1\ \text{M}$ KCl solution containing $5\ \text{mM}$ $[\text{Fe}(\text{CN})_6]^{3-/4-}$. Figure S4. The probable reaction mechanisms of UA on PPy/ $\alpha\text{-Fe}_2\text{O}_3$ electrode. Figure S5. (a) Storage stability of the PPy/ $\alpha\text{-Fe}_2\text{O}_3$ electrode tested by CV for 180 days. (b) DPV curves of the PPy/ $\alpha\text{-Fe}_2\text{O}_3$ electrode were obtained in a solution containing $0.5\ \text{mM}$ UA, and $0.3\ \text{mM}$ DA their mixtures, respectively.

Author Contributions: Validation, S.L., X.S., K.J., Y.H., Q.C., W.M., L.T., Y.R. and S.X.; Formal analysis, Y.H. and Q.C.; Investigation, S.L., X.S. and K.J.; Resources, W.M., Y.R. and S.X.; Data curation, X.S., K.J. and L.T.; Writing—original draft, R.W. and S.L.; Writing—review & editing, R.W., Q.C., W.M., L.T., Y.R. and S.X.; Supervision, R.W.; Funding acquisition, R.W. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Natural Science Foundation of China (32000388), the Natural Science Foundation of Henan University of Technology (2021BS006), the Natural Science Project of the Science and Technology Department of Henan Province (232102310010), and the Cultivation Programme for Young Backbone Teachers at the Henan University of Technology (21421210).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article and supplementary materials.

Conflicts of Interest: The authors declare that they have no conflict of interest.

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