# SYNTHESIS AND CHARACTERIZATION OF POLYPYRROLE FOR BIODETECTION

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***Abstract:*** *This work involves the synthesis of polypyrrole (PPy) and its application as a non-enzymatic electrochemical sensor for biologically relevant analytes such as creatinine, urea, glucose, and uric acid. A low-cost and readily available material was used to prepare a conductive polymer (polypyrrole) that was used in biodetection using a non-enzymatic method. This is a novel approach that gave positive results compared to other studies conducted using an enzymatic method or the presence of nanomaterials. PPy was prepared using the chemical oxidative polymerization of pyrrole. Ammonium peroxodisulphate and hydrochloric acid were used as an oxidizing and doping agent, respectively. The resulting PPy was deposited onto ITO glass substrates to fabricate modified electrodes. The structural, morphological, and chemical properties of PPy were characterized using FTIR spectroscopy, X-ray diffraction (XRD), and scanning electron microscopy (SEM). Electrochemical characterization and analytical performance evaluation were carried out using cyclic voltammetry. The PPy-based electrodes exhibited a strong and distinct electrochemical response toward creatinine and urea, demonstrating their promising sensing capability. In contrast, no significant voltammetric response was observed for uric acid and glucose under the same conditions, indicating selective sensitivity. This novel, enzyme-free sensing approach demonstrates advantages over conventional enzymatic and nanomaterial-based methods, presenting an efficient and cost-effective route for biomolecular detection.*
***Keywords****: polypyrrole, creatinine, urea, glucose and uric acid*
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## 1. Introduction

Polymers are often regarded as very favorable substances for biomedical purposes [1-3]. Polymers have been widely used in many forms, such as bulk scaffolds or hydrogels, as well as colloidal nanoparticles or nanogels, to meet a wide range of medical requirements [4, 5]. From their inception, polymers have been recognized as electrical insulators, and their initial applications reflected this property. Nevertheless, this concept faced opposition when electrically conductive polymers were discovered in the late 1970s by Shirakawa et al. [6]. Conducting polymers are thought of as macromolecules with a completely conjugated bond sequence throughout their backbone that can undergo oxidation or reduction to acquire a positive or negative charge. The conducting polymers are extremely vulnerable to chemical or electrochemical oxidation or reduction. They contain an extended conjugated π electron system [7, 8]. Consequently, by carefully regulating the oxidation and reduction process, conducting polymers' electrical and optical properties could be exactly changed. Because these reactions are frequently reversible, transitioning from a highly conductive state to a semiconducting state and ultimately to an insulating state allows for systematic and extremely precise control of the electrical and optical properties [9-15]. In addition, conducting polymers can be nanostructured, chemically grafted with functional groups, or combined with other functional materials, like nanoparticles, to greatly enhance the biosensor's sensitivity, selectivity, stability, and repeatability in response to a range of bioanalytes [16].

Polypyrrole (PPy) is a naturally occurring conducting polymer that is frequently used as a biomaterial because of its exceptional qualities, which include stability, electrical conductivity, and exceptional biocompatibility [17]. Due to its affordability and strong electrical characteristics, PPy is the most extensively used in the biomedical sector, as well as in applications like sensors, actuators, and energy generators (batteries and solar cells) [18]. PPy-based nanocomposites have emerged as a significant class of bioconductive material due to numerous tissues of the body reacting to electrical fields. PPy nanocomposites, which are created by combining it with other biopolymers or nanomaterials, exhibit notable enhancements in their mechanical, biological, and physicochemical characteristics [19]. Physical-chemical and spectral analysis techniques were used to ascertain the structure and content of the resultant polymer [20].

The aim of this study is to synthesize polypyrrole (PPy) through chemical oxidative polymerization and evaluate its performance as a low-cost, non-enzymatic sensing material for the detection of biomolecules such as creatinine, urea, glucose, and uric acid. The work focuses on developing PPy-modified ITO electrodes and assessing their electrochemical behavior and analytical sensing capabilities using cyclic voltammetry, with the goal of determining the potential of PPy as an efficient and accessible alternative to enzymatic and nanomaterial-based biosensing systems.

## 2. Experimental part

**2.1. Instrumentations.** All electrochemical measurements were conducted with a potentiostat apparatus. A three-electrode system was used comprising a modified ITO electrode with ppy as the working electrode, a saturated calomel electrode (SCE) as the reference electrode, and a platinum wire as the counter electrode. The following techniques were used to determine the structure of PPy: field emission scanning electron microscopy (FE-SEM) (Axia Chemi SEM), FTIR spectroscopy (Shimadzu FT-IR-8400), and X-ray diffraction (XRD) (Malvern Panalytical).

**2.2. Chemicals.** Pyrrole was purchased from Sigma-Aldrich (USA); acetone and ethanol from Scharlau (Spain); ammonium persulfate from Prolabo (France); hydrochloric acid from CDH (India); and creatinine was procured from Alpha Chemika (India).

**2.3. Methods. Polypyrrole preparation.** PPy was produced through the chemical oxidative polymerization of pyrrole (Py). Initially, 0.2 M of Py monomer solution was prepared by dissolving 0.71 mL (0.687 g) of Py in 50 mL of deionized water and agitating for 15 minutes. Ammonium persulphate (APS) 0.25 M was prepared via adding 50 mL of hydrochloric acid (0.2 M) to 2.85 g of APS, which was then added dropwise to a Py solution over a period of 30 minutes. The reaction mixture was thereafter agitated for 2 hours at room temperature to finalize the polymerization. Upon complete reaction, the black resultant was filtered and subsequently rinsed multiple times with deionized water and ethanol. Finally, the resulting PPy was dried [21, 22]. The yield of polypyrrole in the polymerization reaction is 58.2%.

## 3. Results and discussion

**3.1. FT-IR spectrum of PPy.** Fourier spectrophotometry was used to analyze the spectrum in the 400–4000 $cm^{-1}$ range in order to find distinctive absorption bands associated with the functional groups in the sample [23] (Fig. 1).

The FT-IR spectrum of polypyrrole exhibited a band at 3440 $cm^{-1}$ of the (NH) group. The (=C-H) group caused absorption bands at 3014.53 $cm^{-1}$. The absorption band at 1629.74 $cm^{-1}$ is attributed to (N=C). The band of (C=C) at 1554.52 $cm^{-1}$ and (N-H) bending appeared at (1461.94) $cm^{-1}$, while the band at 1298 $cm^{-1}$ is attributed to the C–N group [24]. All these locations and bands are shown in Fig. 1.

**3.2. X-ray diffraction of PPy.** The X-ray diffraction pattern of PPy is depicted in Fig. 2. The extensive diffraction peak is located at $2\theta$ = 24.8564. X-ray diffraction measurements indicate that the polypyrrole powder is amorphous [25].

**3.3. FESEM of polypyrrole.** The FESEM image illustrated the morphology of polypyrrole synthesized using ammonium persulfate solution. According to the FESEM image, the average diameter ranges from 139.1 to 442.3 nm, and the images exhibit a cauliflower-like shape composed of microspherical grains with an average size of 53.05-93.32 [26], as illustrated in

Fig. 3.

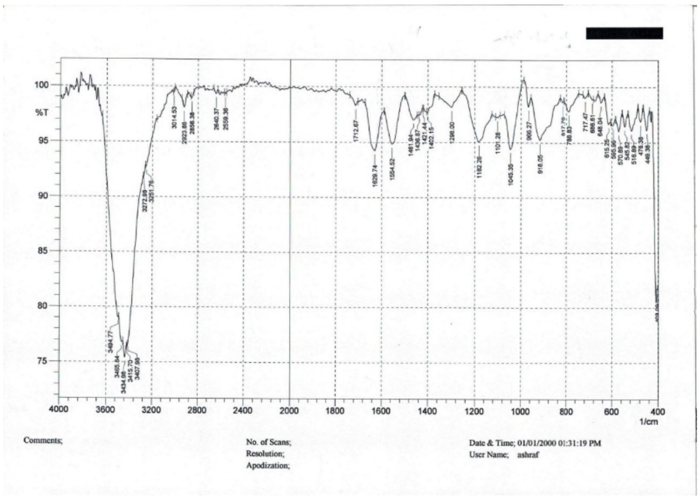



**Fig. 1.** FT- IR spectrum of polypyrrole

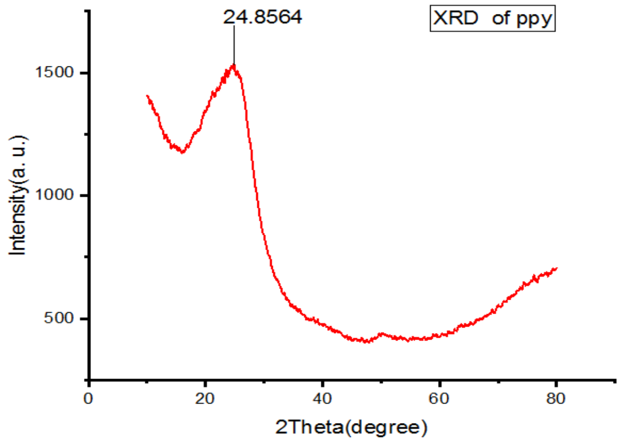
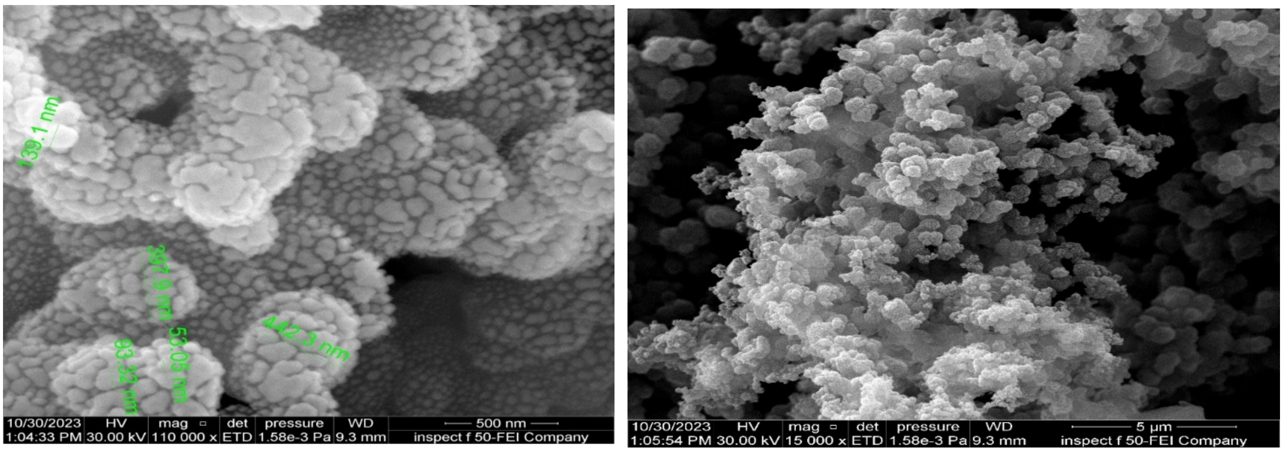



**Fig. 2.** XRD pattern of polypyrrole




**A** **B**

**Fig. 3.** FESEM Images of polypyrrole

**3.4. Biosensing study.** Cyclic voltammetry (CV) is widely employed to characterize redox-active systems because it provides essential information regarding the number of redox processes, their reversibility, and stability [27]. Electrochemical sensing performance in this study was evaluated using a three-electrode configuration consisting of a fabricated ITO electrode as the working electrode (WE), a saturated calomel electrode (SCE) as the reference electrode, and platinum wire serving as the counter electrode.

Fig.s 4A–D present the CV responses of pure polypyrrole (PPy)-modified electrodes in the presence of creatinine, urea, glucose, and uric acid. Fig. 4A shows the cyclic voltammogram of creatinine (1 mg/dL) recorded in an acidic medium using a PPy film, demonstrating the appearance of two distinct redox peak pairs. The anodic peak current reaches 3.44 µA at −0.075 V, whereas the cathodic peak corresponds to −4.22 µA at −0.425 V. The presence of these peak pairs confirms redox interaction between creatinine molecules and the PPy-modified electrode.

When a conducting polymeric material interacts with a gaseous or liquid analyte, electron transfer occurs between the analyte and the sensing surface. Such electron exchange induces changes in electrical resistance, charge transfer characteristics, and the work function of the sensing material, thereby enabling electrochemical detection [28].

The cyclic voltammograms (CVs) of urea (1 mg/dL) in an acidic medium demonstrate a noticeable increase in peak current values, indicating clear oxidation and reduction processes. The anodic peak current reaches 0.828 mA at a potential of 195 mV, while the cathodic peak current reaches −1.4041 mA at −160 mV, as shown in Fig. 4B.

In contrast, the CVs of glucose (1 mg/dL) and uric acid (1 mg/dL) under the same conditions exhibit no observable oxidation or reduction peaks, indicating that the electrode shows no electrochemical response toward these analytes. This behavior is illustrated in Fig.s 4C and 4D.

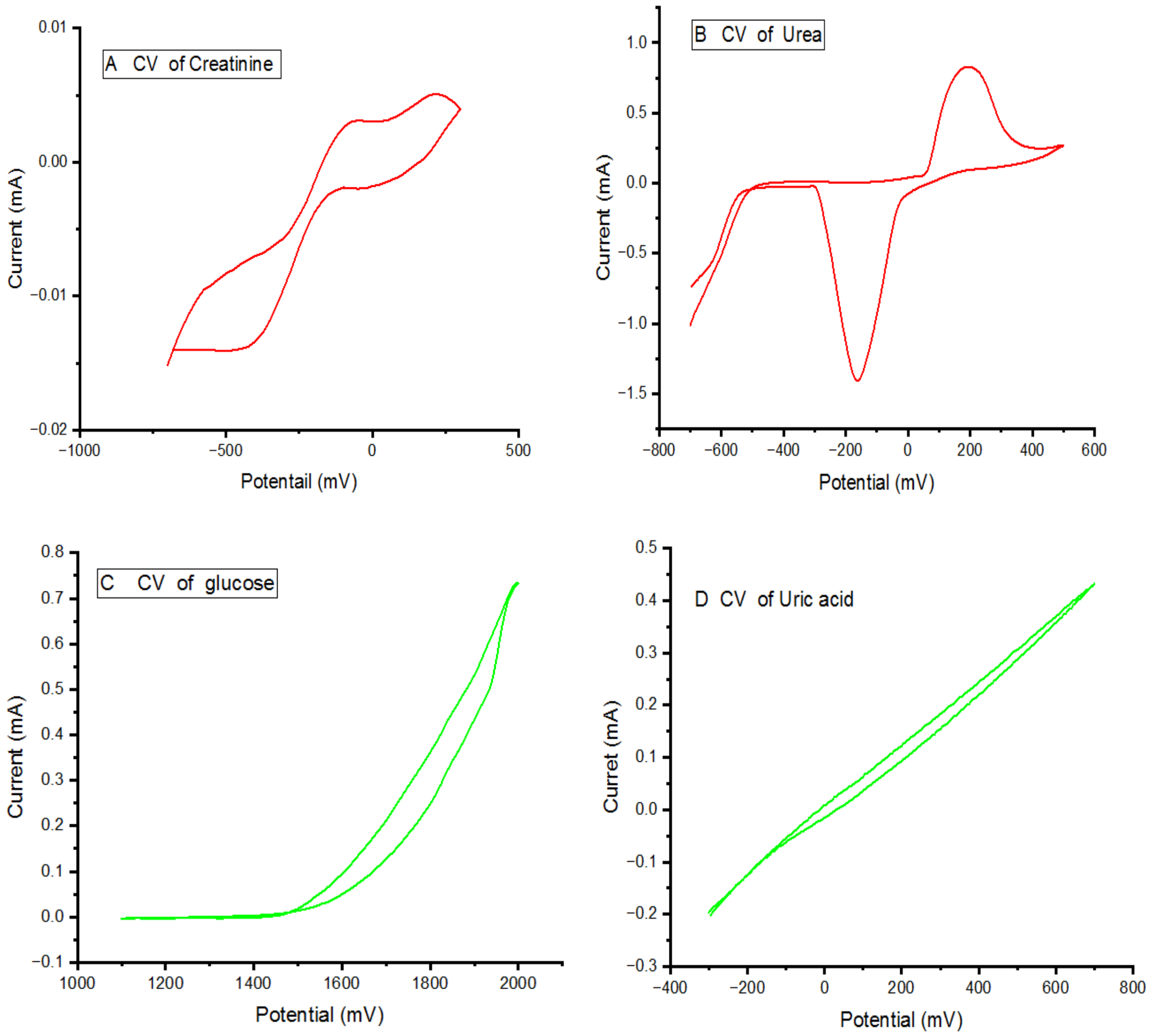



**Fig. 4. (A)** CVs of Creatinine (1mg/dl); **(B)** CVs of Urea (1mg/dl); **(C)** CVs of Glucose (1mg/dl); **(D)** CVs of Uric acid (1 mg/dl)

## Conclusion

Polypyrrole (PPy) was synthesized by chemical oxidative polymerization using ammonium persulfate as the oxidizing agent and hydrochloric acid as a dopant. The resulting polymer was deposited onto electrodes and employed for biodetection applications. The structural and morphological properties of PPy were characterized using FTIR, XRD, and FESEM techniques. The fabricated PPy-based electrodes were evaluated as biosensors for the detection of creatinine, urea, uric acid, and glucose. The electrodes exhibited a strong electrochemical response toward creatinine and urea, while no significant response was observed for uric acid and glucose.

This study introduces a novel, non-enzymatic sensing approach utilizing an inexpensive and readily available conductive polymer material. Compared to conventional enzymatic detection methods or sensors incorporating nanomaterials, the developed PPy-based electrodes demonstrated promising analytical performance, highlighting their potential for low-cost biosensing applications.

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