

In situ polymerization of *p*-phenylenediamine on a bacterial cellulose matrix as a potential glucose biosensor

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Abstract

Bacterial cellulose (BC) is a biopolymer distinguished by its high purity, nanofibrillar structure, large specific surface area, and excellent mechanical and biocompatible properties, making it an attractive platform for biosensing applications. In this study, we report the *in situ* polymerization of *p*-phenylenediamine (PPD) on a bacterial cellulose matrix as a novel strategy for the fabrication of a functional composite material with potential biosensor applications. The polymerization process was carried out directly within the BC network, enabling uniform deposition and strong interfacial interactions between the polymer phase and the cellulose nanofibers. The resulting BC/PPD composite was characterized using spectroscopic, structural, and morphological analyses to confirm successful polymer formation and integration within the BC matrix. The modification led to noticeable changes in the physicochemical properties of bacterial cellulose, including enhanced electrical responsiveness and altered surface chemistry, which are critical parameters for sensor performance. Owing to the combination of BC's porous architecture and the functional properties of poly(*p*-phenylenediamine), the developed composite demonstrates promising potential as a flexible, biocompatible sensing platform for future biosensor and bioelectronic applications.

Keywords

bacterial cellulose, flexible biosensor, modification, polymerization

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1. Introduction

Cellulose is a ubiquitous natural polymer that plays a fundamental role in numerous industrial, biomedical, and technological applications. While plant-derived cellulose has traditionally dominated practical use, increasing attention has been directed toward BC, a structurally distinct form of cellulose biosynthesized by aerobic bacteria, primarily belonging to the genus *Komagataeibacter*. Despite its identical chemical composition to plant cellulose, bacterial cellulose exhibits a unique set of properties resulting from its biological mode of synthesis, which confers a highly ordered three-dimensional nanofibrillar architecture (Iguchi et al., 2000). The absence of lignin, hemicelluloses, and other plant-derived impurities gives bacterial cellulose exceptionally high chemical purity, uniformity, and reproducibility. It is a nanoscale fiber network that provides a large specific surface area, high crystallinity, and remarkable mechanical strength, while maintaining flexibility and excellent water retention capacity (Sozcu et al., 2025). These characteristics have driven extensive research into BC-based materials for biomedical engineering, including wound dressings, tissue scaffolds, and drug delivery systems, where biocompatibility and structural integrity are of critical importance (Gorgieva and Trček, 2019).

In recent years, the application scope of bacterial cellulose has expanded beyond passive biomedical materials toward active functional systems, particularly in the field of biosensing. The highly porous structure of BC enables efficient diffu-

sion of analytes, while the abundance of hydroxyl groups on cellulose chains allows for chemical modification and immobilization of functional components. These features make BC an attractive matrix for the fabrication of composite materials designed to exhibit electrical, electrochemical, or stimuli-responsive behaviour (Gabrys et al., 2022). One promising approach to impart such functionality involves the incorporation of redox-active or conducting polymers into the bacterial cellulose network. Among aromatic amine monomers, PPD has gained interest due to its ability to undergo oxidative polymerization, forming poly(*p*-phenylenediamine), a conjugated polymer with redox activity and electrical conductivity. PPD is a low-molecular-weight diamine characterized by a rigid aromatic backbone and reactive amino groups, which facilitate polymer growth and strong interactions with hydroxyl-rich substrates such as cellulose. The polymerization of *p*-phenylenediamine can be achieved via chemical or electrochemical oxidation, resulting in polymer chains capable of participating in electron transfer processes (Hasan et al., 2025). Materials based on poly(*p*-phenylenediamine) have been investigated in a range of sensing-related applications, including electrochemical sensors and chemically responsive coatings, due to their sensitivity to environmental changes and their ability to mediate charge transport. When combined with biopolymer matrices, such polymers can form hybrid systems that integrate mechanical flexibility, biocompatibility, and functional electrical properties. *In situ* polymerization of PPD within a bacterial cellulose matrix represents a particularly effective strategy for composite fabrication (Yu et



al., 2025). This approach enables uniform polymer deposition throughout the BC network, promotes strong interfacial interactions between the polymer phase and cellulose nanofibers, and minimizes phase separation. Similar cellulose-based composites incorporating conducting polymers such as polyaniline or polypyrrole have demonstrated enhanced sensing performance, confirming the suitability of cellulose as a scaffold for functional polymer systems.

The present study focuses on the synthesis and characterization of a bacterial cellulose–poly(*p*-phenylenediamine) (BC/PPD) composite obtained via *in situ* polymerization of PPD within the BC matrix. The work aims to evaluate the structural and physicochemical properties of the resulting material and to assess its potential as a platform for biosensor applications. By combining a natural, sustainable biopolymer with a functional aromatic polymer, this study contributes to the development of advanced bio-based materials for next-generation sensing technologies.

2. Experimental Work

2.1. Materials

The chemicals used in the experiment included: D-glucose ($\geq 99\%$), meat peptone and sodium hydroxide ($\geq 97\%$, pellets) all purchased from Sigma Aldrich (Darmstadt, Germany); yeast extract and micro agar purchased from Duchefa Biochemie (Haarlem, The Netherlands); disodium hydrogen phosphate dihydrate purchased from Merck (Darmstadt, Germany); citric acid purchased from Chempur (Piekary Śląskie, Poland); acetic acid (99.8%); and ethanol (96%) purchased from Honeywell (Charlotte, NC, USA); *p*-phenylenediamine and iron chloride (FeCl_3) purchased from Pol-Aura (Morąg, Poland); PBS tablets (Phosphate Buffered Saline tablets) purchased from Bionovo (Legnica, Poland).

2.2. Microorganisms

The bacterial strains used in this study were *Komagataeibacter Intermedius* (LMG 18909) purchased from Belgian Coordinated Collections of Microorganism, which were maintained and precultured in the laboratory of Faculty of Materials, Civil and Environmental Engineering, University of Bielsko-Biala.

2.3. Production of Bacterial Cellulose

The reinforced acetic ethanol culture medium (RAE) used for bacterial cellulose production was selected for the biosynthesis process. The liquid medium contained glucose (40 g/L), peptone from meat (10 g/L), yeast extract (10 g/L), disodium hydrogen phosphate dihydrate ($\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$, 3.38 g/L), citric acid (1.37 g/L), acetic acid (10 mL/L), and ethanol

(10 mL/L). The solid medium, used for bacterial revitalization and inoculation, included the same components with the addition of agar (10 g/L). All media were sterilized by autoclaving at 121°C for 15 minutes. Acetic acid and ethanol were added to the medium after autoclaving. The bioprocesses were carried out in 250 mL Erlenmeyer flasks equipped with membrane screw caps. Each flask, containing 50 mL of RAE medium, was inoculated with a single bacterial colony and incubated in a water bath for 7 days at 30°C . During the first 24 hours, the flasks were shaken; after this period, shaking was stopped and the bioprocess was continued under static conditions. In this manner, bacterial cellulose samples were obtained. The prepared BC samples were subsequently washed with distilled water. Next, the purification process, which allows the removal of bacterial cell residues, was initiated using 0.5 M NaOH. The purification was carried out in a water bath at 80°C for 1 hour with shaking. Then, they were washed again with distilled water up to neutral pH.

2.4. *In Situ* Polymerization of *p*-Phenylenediamine on a Bacterial Cellulose Matrix

The aim of the study was to carry out oxidative polymerization of PPD using the *in situ* method, i.e., directly inside the bacterial cellulose matrix to obtain BC/PPD composite. Due to the original form of bacterial cellulose as a typical hydrogel, the polymerization procedure was shown in Figure 1. In order for the polymerization process to proceed evenly throughout the entire volume of the sample, a previously prepared disc of moist and purified BC weighing 28 g was placed for 24 hours in a beaker containing 92.5 mL of a 0.1 M aqueous solution of PPD.

In Situ Polymerization of poly(*p*-Phenylenediamine) in BC matrix

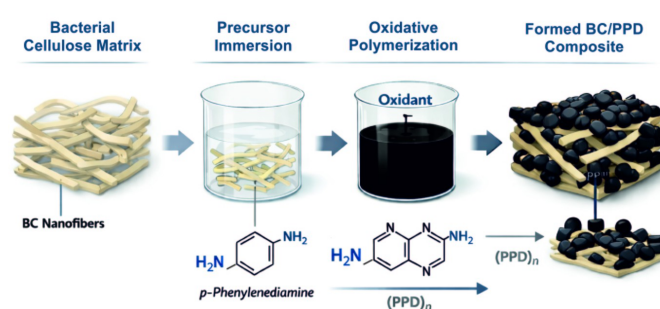


Figure 1. Scheme showing the modification process.

After this time, the beaker was placed in a water bath at a temperature of 10°C and 185 mL of a 0.1 M iron chloride solution, which acted as an oxidizing agent, was slowly added drop by drop. Polymerization was carried out for 3 hours at a temperature of 10°C , continuously adding the oxidizing agent and gently stirring the sample. This process allows for the gradual and equal penetration of the oxidizing agent into the BC matrix. After this time, the temperature was

increased to 30 °C, thus completing the polymerization process. The sample of BC/PPD was then rinsed several times with distilled water to remove any post-reaction residues.

The final stage was the protonation of the composite to increase its conductivity. The previously obtained composite was completely immersed for 30 minutes in 0.1 M HCl at a temperature of 21 °C. Then it was rinsed again with distilled water until the acid was neutralized. The samples prepared in this way were subjected to further testing. Figure 2 shows the experimental work.

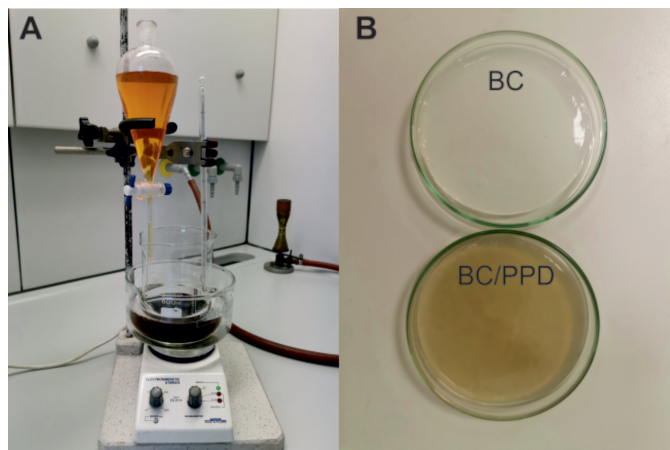


Figure 2. Experimental work (A); BC sample before and after polymerization of PPD.

2.5. Microscopy Analysis

The freeze-dried BC and BC/PPD samples were subjected to analysis using scanning electron microscopy JSM 5500 LV (Jeol, Japan), which enabled detailed observation of the surface morphology and internal structure of the materials. Prior to analysis, the samples were sputtered with gold in a low vacuum sputtering device JFC Fine Coater (Jeol, Japan).

Surface topography was examined using an optical microscope (Reichert, Vienna, Austria) equipped with an ARTCAM CCD camera (Olympus, Tokyo, Japan), controlled by the Motic Images Plus 2.0 computer program.

2.6. FTIR Analysis

Fourier Transform Infrared Spectroscopy (FTIR) measurements were taken using a Nicolet 6700 FT-IR spectrometer (Thermo Electron Corp., Madison, WI, USA) equipped with photoacoustics MTEC model 300 accessory. The following measurement parameters were used: resolution, 4 cm⁻¹; photoacoustic detector; number of scans, 128. For each sample, the spectra were collected over the range of 4000 to 500 cm⁻¹. Data collection and post-processing were performed using OMNIC software (v. 9.0, Thermo Electron Corp.).

2.7. Amperometry

The effectiveness of the developed BC/PPD composite as a biosensor for measuring glucose concentration in human body fluids was tested *in vitro* using amperometry. For this purpose, the composite obtained was placed on a graphite electrode, thus creating a working electrode with an area of 21 cm². The reference electrode was a platinum electrode (Eurosensor EPtP-301). The test was conducted in phosphate-buffered saline (PBS) imitating blood plasma, containing glucose in concentrations ranging from 2.5 mM to 40 mM at a temperature of 37 °C.

Measurements of changes in current intensity depending on glucose concentration, at a constant potential of 0.7 V, were performed using a Keithley 2410 Source Meter device (Tektronix, Oregon, USA). This method was used to construct a calibration curve and measure the selectivity of the biosensor.

3. Results and Discussion

3.1. Microscopy Analysis

Scanning electron microscopy revealed significant differences in the microstructure of native BC and the BC/PPD composite (Figure 3). The pristine BC exhibited a typical three-dimensional network composed of randomly oriented, interwoven nanofibers forming an open and highly porous structure. The fibrils were uniformly distributed, creating a homogeneous network, characteristic of bacterial cellulose synthesized under static culture conditions.

In contrast, the BC/PPD composite showed noticeable morphological changes after *in situ* polymerization of *p*-phenylenediamine. The fibrous network remained continuous; however, the presence of additional material deposited along the cellulose nanofibers was clearly observed. A similar effect has been described in an extensive study by other researchers (Nair et al., 2025).

As indicated by the arrows, localized thickening of fibrils and the formation of elongated, denser regions were evident throughout the matrix. These features suggest successful deposition and growth of poly(*p*-phenylenediamine) within the BC network rather than mere surface coating. The polymer phase appears to preferentially accumulate along cellulose fibers and at fiber junctions, likely due to strong interactions between amino groups of PPD and hydroxyl groups of cellulose during polymerization.

This resulted in partial reduction of pore size and increased structural anisotropy in selected regions, while preserving the overall interconnected architecture of the BC scaffold. Importantly, no evidence of large polymer agglomerates or phase separation was observed, indicating that the *in situ* polymerization approach enabled relatively uniform distribution of the polymer throughout the cellulose matrix.

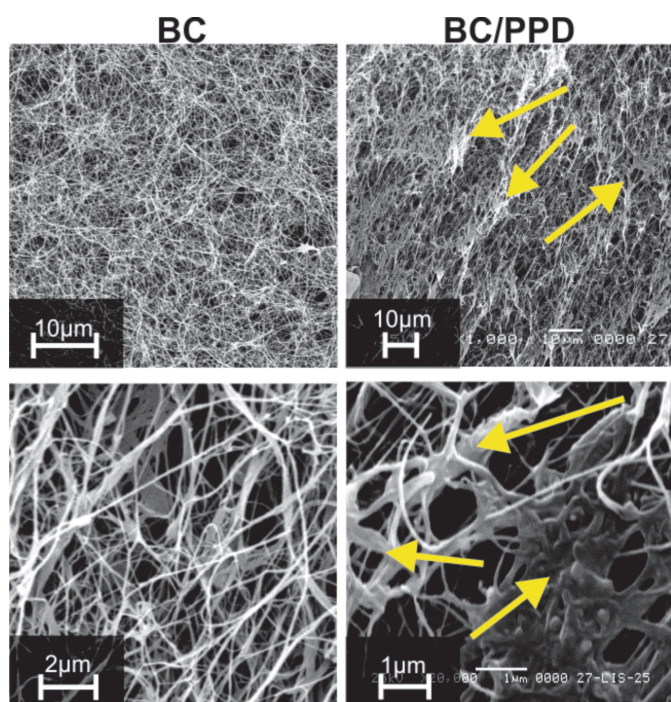


Figure 3. SEM micrographs of BC and BC/PPD at different magnifications. In the BC/PPD composite, localized polymer-rich regions are visible.

Such morphological modification is expected to enhance the functional properties of the composite, particularly with respect to electron transport pathways and interfacial area, which are critical parameters for biosensor performance. The retention of the fibrous network combined with the introduction of a conductive or redox-active polymer phase confirms the suitability of bacterial cellulose as an effective template for the fabrication of functional sensing composites.

Images of native BC and the BC/PPD composite obtained using a polarizing microscope (Figure 4) revealed differences between the morphology of pure bacterial cellulose and the morphology of cellulose after polymerization with *p*-phenylenediamine. In the case of pure BC, networks of densely arranged fibrous fibrils intertwined with each other and exhibiting a crystalline structure were observed, as indicated by the luminescence of the cellulose fibrils in the microscope's field of view.

In contrast, images obtained for the bacterial cellulose composite after polymerization with *p*-phenylenediamine (BC/PPD) showed a compact structure of the resulting material. Individual fibrillar structures, characteristic of pure bacterial cellulose, were not observed. As a result of the polymerization reaction, interactions likely occurred between the amine groups present in *p*-phenylenediamine and the hydroxyl groups of cellulose.

During the polymerization reaction, PPD penetrated deep into the cellulose fibrils, which arranged into wide, twisted ribbons, as observed in the images obtained using a polarizing microscope (Figure 4).

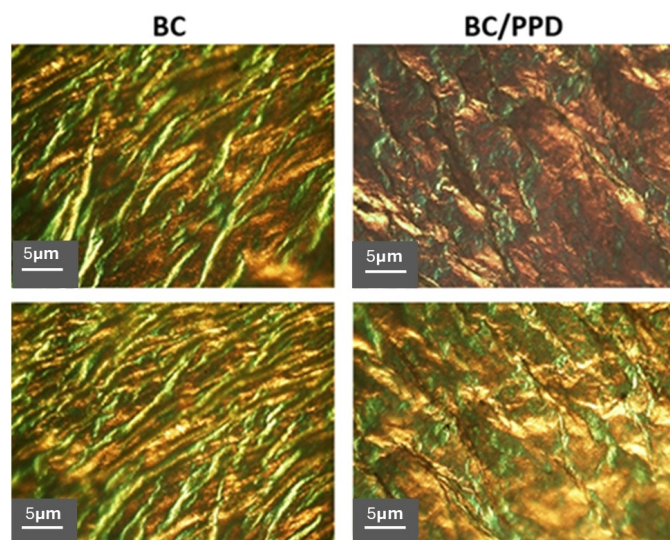


Figure 4. Micrographs of BC and BC/PPD surface morphology taken using a polarizing microscope.

3.2. FTIR Analysis

FTIR infrared spectroscopy was used to analyse the molecular structure of pure *p*-phenylenediamine (Figure 5) used in the polymerization process. The PPD spectrum features characteristic peaks at the band values of 3383 cm^{-1} , 3365 cm^{-1} , 3287 cm^{-1} , 3194 cm^{-1} , 3033 cm^{-1} , 1634 cm^{-1} , 1592 cm^{-1} , 1500 cm^{-1} , 1456 cm^{-1} , 1274 cm^{-1} , 1246 cm^{-1} , 1158 cm^{-1} , 1055 cm^{-1} , 927 cm^{-1} , 813 cm^{-1} , 750 cm^{-1} , 728 cm^{-1} and 536 cm^{-1} .

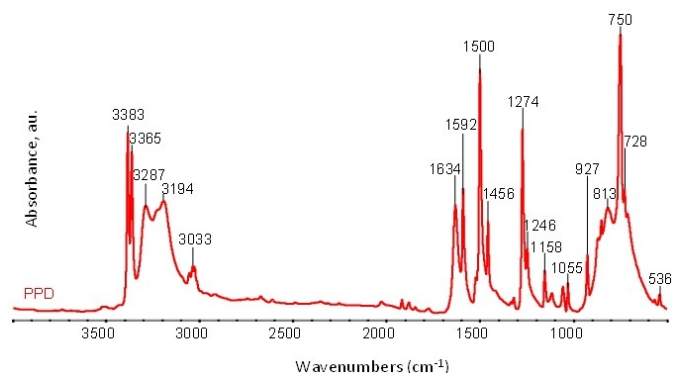


Figure 5. FTIR spectra of PPD at spectral range 4000–500 cm^{-1} .

The chemical structure of PPD contains two $-\text{NH}_2$ groups in the *para* position. In the FTIR spectrum obtained for PPD, we observe intense bands at maximum positions of 3383 cm^{-1} , 3365 cm^{-1} , 3287 cm^{-1} and 3194 cm^{-1} corresponding to the stretching vibrations of the $-\text{N}-\text{H}$ and $-\text{NH}_2$ groups (Georg, 2007; Kumar Trivedi et al., 2015).

We also observe intense bands at maximum positions of 1634 cm^{-1} and 1592 cm^{-1} corresponding to the stretching vibrations of the $-\text{C}-\text{N}-\text{H}$ functional groups. The band at a maximum of 3033 cm^{-1} is characteristic of the stretching

vibrations of the $-\text{CH}$ group present in the aromatic ring. The bands at maxima of 1500 cm^{-1} and 1456 cm^{-1} are characteristic of the stretching vibrations of the $-\text{C}-\text{C}-$ group present in the aromatic ring.

The band with a maximum at 1274 cm^{-1} is characteristic of $-\text{C}-\text{N}-\text{H}$ bending vibrations. The bands with maxima at 1158 cm^{-1} and 1055 cm^{-1} are characteristic of $-\text{C}-\text{H}$ in-plane deformational vibrations. The band at a maximum of 750 cm^{-1} is characteristic of $-\text{N}-\text{H}$ vibrations. The band at a maximum of 728 cm^{-1} is characteristic of $-\text{C}-\text{H}$ bending vibrations (out-of-plane) (Kumar Trivedi et al., 2015).

Spectroscopic studies were also performed on a bacterial cellulose (BC) sample and a bacterial cellulose composite following polymerization with *p*-phenylenediamine (BC/PPD) to observe differences between the tested samples (Figure 6).

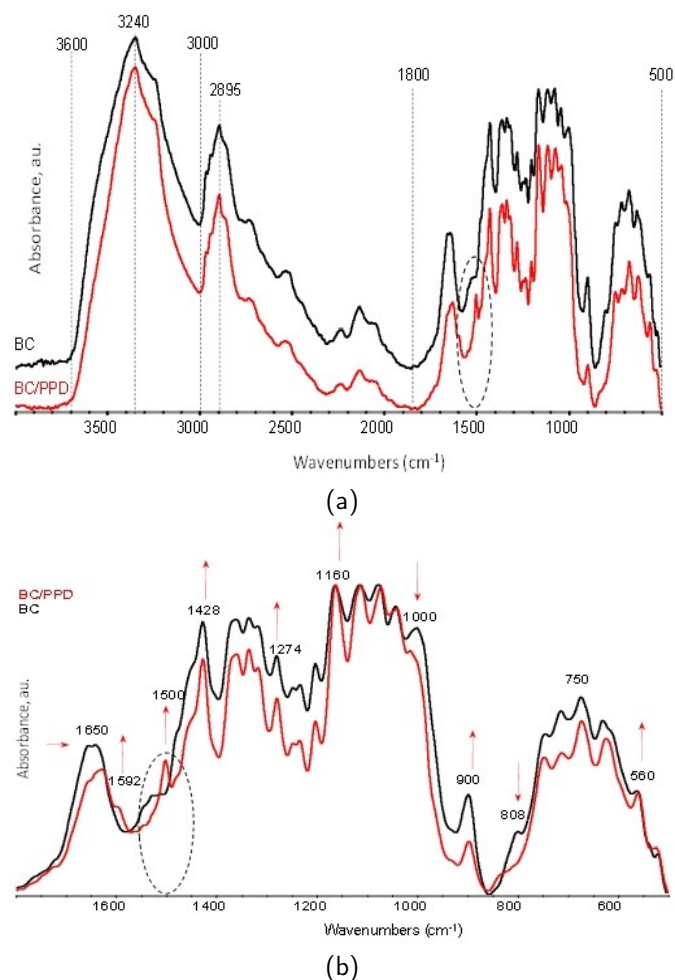


Figure 6. FTIR spectra of BC and BC/PPD beads at different spectral range: (a) $4000\text{--}500\text{ cm}^{-1}$; (b) $1800\text{--}500\text{ cm}^{-1}$.

In the FTIR spectra (Figure 6), it was observed that the spectra for the bacterial cellulose sample and the bacterial cellulose composite after polymerization with *p*-phenylenediamine re-

tained all the characteristic features of the bands for pure bacterial cellulose, with the exception of the band at a maximum of 1500 cm^{-1} . This band is characteristic of the stretching vibrations of the $-\text{C}-\text{C}-\text{H}$ bonds present in the aromatic ring of *p*-phenylenediamine. This may indicate a reaction between the amine groups present in *p*-phenylenediamine and the hydroxyl groups of cellulose.

In the spectra obtained for the tested samples, a characteristic broad absorption band of the stretching vibrations of the $\text{O}-\text{H}$ and $-\text{N}-\text{H}$ oscillators appears at wavenumbers in the range of approximately $3600\text{--}3000\text{ cm}^{-1}$ (Figure 6a). A characteristic band at a wavenumber of 2895 cm^{-1} is observed in the spectra, resulting from the absorption of radiation by $\text{C}-\text{H}$ oscillators.

In the $1800\text{--}500\text{ cm}^{-1}$ region (Figure 6b), slight shifts in the peak positions of the bands and a decrease or increase in their intensity are observed for the native BC and BC/PPD composite samples, as indicated by the red arrows. The region at $1160\text{--}500\text{ cm}^{-1}$ is associated with the vibrations of symmetric and asymmetric $\text{C}-\text{O}-\text{C}$ groups present in the glucopyranose ring and ether bridges (Gabrys et al., 2022).

3.3. Electrical Testing

The amperometry response of the BC/PPD composite toward glucose was investigated in PBS. Figure 7 presents the dependence of the measured current (I) on glucose concentration (C_M), demonstrating a clear and systematic increase in current with increasing analyte concentration.

The calibration curve exhibited a linear relationship over the studied concentration range, confirming the suitability of the BC/PPD composite for non-enzymatic glucose sensing. Linear regression analysis yielded an adjusted coefficient of determination ($R^2 = 0.94$), indicating good linearity and reliability of the amperometric response, which is consistent with previous reports demonstrating the applicability of BC-based materials in non-enzymatic glucose sensing systems (Yang et al., 2025).

The observed amperometric behavior can be attributed to the electrochemical oxidation of glucose occurring at the BC/PPD interface. Poly(*p*-phenylenediamine), incorporated into the bacterial cellulose network via *in situ* polymerization, provides redox-active sites that facilitate direct electron transfer between glucose molecules and the electrode surface. The possibility of modifying BC in a similar way and using it as an electrochemical sensor has been mentioned also by other scientists (Sheraz et al., 2025).

Furthermore, the intimate contact between the polymer phase and the cellulose nanofibers ensures efficient charge transport throughout the composite. The relatively low standard errors associated with the slope and intercept indicate good reproducibility and stability of the sensing response.

Unmodified BC exhibited only a negligible amperometric response toward glucose, with current values remaining close to the baseline over the investigated concentration range. Overall, the results demonstrate that the BC/PPD composite functions effectively as a non-enzymatic amperometry glucose sensor, combining the advantages of a natural, biocompatible matrix with the electrochemical activity of an aromatic amine polymer.

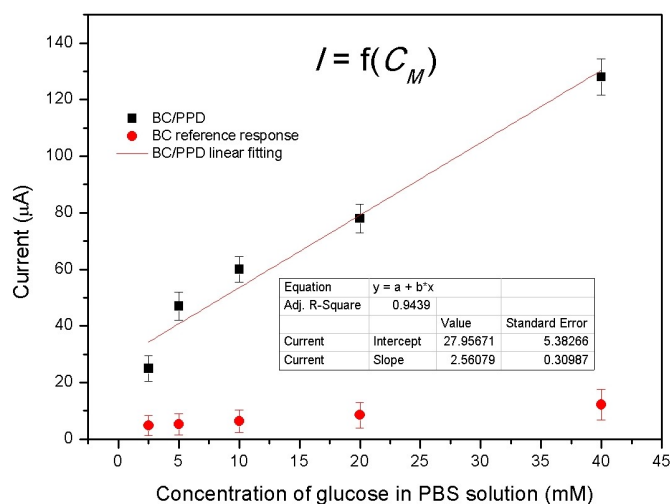


Figure 7. Amperometric response of the BC/PPD composite dependence of current on glucose concentration.

4. CONCLUSIONS

This study demonstrated the successful *in situ* polymerization of p-phenylenediamine within a bacterial cellulose matrix, resulting in a structurally integrated BC/PPD composite with enhanced functional properties. Spectroscopic and morphological analyses confirmed effective polymer incorporation and strong interactions between the polymer phase and cellulose nanofibers.

The composite exhibited a clear and reproducible amperometric response toward glucose, with good linearity, indicating its suitability for non-enzymatic sensing applications. The improved electrochemical performance is attributed to the presence of a redox-active polymer and efficient charge transport within the BC network.

Overall, the results highlight the potential of the BC/PPD composite as a flexible and biocompatible platform for biosensor development. Further work should address its long-term stability and performance in complex biological systems.

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