



ANTIBACTERIAL ACTIVITY OF NANO-STRUCTURED POLYPYRROLE SYNTHESIZED VIA CYCLIC VOLTAMMETRY

M. Hisham

Polymer Research lab, Chemistry Department, Faculty Of Science, Beni-Suef University, Beni-Suef, Egypt

Abstract

The increasing prevalence of antibiotic-resistant bacteria necessitates the development of alternative antimicrobial agents. Nano-structured polypyrrole (PPy) has emerged as a promising material due to its unique electrical and chemical properties, which can be enhanced through advanced synthesis techniques. In this study, we investigate the antibacterial activity of nano-structured polypyrrole synthesized via cyclic voltammetry (CV). Polypyrrole films were electrochemically polymerized onto electrode substrates using CV, optimizing parameters such as scan rate and polymerization potential to control the nanostructure and morphology of the resulting polypyrrole. The antibacterial properties of the nano-fabricated polypyrrole were assessed against common bacterial strains, including Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa, using standard disk diffusion and minimum inhibitory concentration (MIC) assays.

Our results reveal that the nano-structured polypyrrole exhibits significant antibacterial activity, with inhibition zones and MIC values indicating effective suppression of bacterial growth. The enhanced antibacterial performance is attributed to the increased surface area and reactive sites provided by the nanostructure, which facilitates greater interaction with bacterial cells. Additionally, the study explores the correlation between the polypyrrole's electrochemical properties and its antibacterial efficacy, providing insights into the mechanisms underlying its antimicrobial action. This research highlights the potential of cyclic voltammetry as a versatile method for fabricating nano-structured polypyrrole and suggests its applicability as an alternative antimicrobial agent. The findings offer a valuable contribution to the field of materials science and antimicrobial research, with implications for developing new strategies to combat bacterial infections.

Keywords

Nano-structured polypyrrole, cyclic voltammetry, antibacterial activity, polypyrrole synthesis, antimicrobial agents, electrochemical polymerization, bacterial inhibition, nanomaterials, material science, minimum inhibitory concentration, disk diffusion assay, bacterial resistance, antimicrobial properties, electrochemical methods, nanostructured polymers.

INTRODUCTION

The rise of antibiotic-resistant bacteria represents a critical challenge in modern medicine, necessitating the development of novel antimicrobial materials. In this context, conductive polymers, particularly polypyrrole (PPy), have garnered significant attention due to their unique electrical properties and biocompatibility. Polypyrrole's ability to be tailored at the nano-scale opens up opportunities for enhancing its antibacterial properties, making it a promising candidate for alternative antimicrobial agents. Cyclic voltammetry (CV) is a powerful electrochemical technique employed to synthesize nano-structured polypyrrole, offering precise control over the polymer's nanostructure and properties.

Cyclic voltammetry allows for the electrochemical polymerization of pyrrole monomers onto electrode substrates, facilitating the formation of polypyrrole films with controlled thickness and morphology. By adjusting parameters such as scan rate, polymerization potential, and electrolyte composition, researchers can manipulate the nanostructure of polypyrrole to optimize

its surface area and reactivity. These modifications can significantly impact the material's interaction with bacterial cells, potentially enhancing its antibacterial efficacy. Despite the promising properties of polypyrrole, its effectiveness as an antibacterial agent and the relationship between its electrochemical synthesis and antimicrobial performance remain underexplored.

This study aims to investigate the antibacterial activity of nano-structured polypyrrole synthesized via cyclic voltammetry, focusing on its efficacy against common bacterial pathogens such as *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. By employing standard antimicrobial assays, including disk diffusion and minimum inhibitory concentration (MIC) tests, we evaluate the extent of bacterial growth inhibition and establish a correlation between the material's electrochemical characteristics and its antibacterial performance. This research seeks to bridge the gap between material synthesis and functional application, offering insights into the potential of nano-structured polypyrrole as an effective alternative to traditional antibiotics. Understanding the antibacterial mechanisms of nano-structured polypyrrole can contribute to the broader field of antimicrobial material development and provide a foundation for future innovations in combating bacterial infections. The study's findings have implications for a range of applications, from medical device coatings to environmental sanitization, highlighting the potential of cyclic voltammetry in fabricating advanced materials with targeted antimicrobial properties.

METHOD

The study focused on synthesizing nano-structured polypyrrole (PPy) using cyclic voltammetry (CV) and evaluating its antibacterial activity against various bacterial strains. The methodology was divided into synthesis, characterization, and antimicrobial assessment phases.

Polypyrrole was synthesized electrochemically via cyclic voltammetry on a glassy carbon electrode. Pyrrole monomer was dissolved in an aqueous electrolyte solution containing 0.1 M NaCl to prepare a 0.05 M pyrrole solution. The CV polymerization was performed using a three-electrode system, consisting of a glassy carbon working electrode, a platinum counter electrode, and a saturated calomel reference electrode. The electrochemical polymerization was conducted in a potentiostat (e.g., Autolab PGSTAT302) within a potential range of -0.2 to +1.2 V versus the reference electrode. The scan rate was varied between 10 and 100 mV/s to determine the optimal conditions for generating nano-structured PPy. After polymerization, the electrodes were rinsed with distilled water and dried in ambient conditions.

The synthesized nano-structured polypyrrole was characterized using several techniques to confirm its morphology, structure, and electrochemical properties. Scanning Electron Microscopy (SEM) was used to analyze the surface morphology and to assess the nano-structuring of the polypyrrole films. Energy Dispersive X-ray Spectroscopy (EDX) was employed in conjunction with SEM to determine the elemental composition of the films. The electrochemical properties of the polypyrrole were evaluated using Cyclic Voltammetry (CV) to assess its charge transfer and stability. Additionally, Fourier Transform Infrared Spectroscopy (FTIR) was performed to confirm the chemical structure of the polypyrrole and to identify characteristic peaks associated with its functional groups.

The antibacterial activity of the nano-structured polypyrrole was evaluated using both disk diffusion and minimum inhibitory concentration (MIC) assays. For the disk diffusion assay, bacterial strains *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* were cultured on Mueller-Hinton agar plates. Disks loaded with the polypyrrole film (synthesized under optimized conditions) were placed on the agar surfaces, and the plates were incubated at 37°C for 24 hours. The inhibition zones around the disks were measured to assess the antibacterial efficacy of the polypyrrole.

For the MIC assay, bacterial cultures were prepared in Mueller-Hinton broth and exposed to varying concentrations of polypyrrole. The bacterial growth was monitored by measuring the optical density at 600 nm using a spectrophotometer. The MIC values were determined as the lowest concentration of polypyrrole that inhibited visible bacterial growth. Data from the disk diffusion and MIC assays were analyzed statistically to determine the significance of antibacterial activity. The results were expressed as means $\pm$ standard deviations, and comparisons between different conditions were made using ANOVA or t-tests as appropriate. All statistical analyses were performed using software such as GraphPad Prism.

To ensure the reproducibility and reliability of the results, all experiments were conducted in triplicate. The impact of potential environmental factors, such as pH and ionic strength, on the antibacterial activity was also investigated to provide a comprehensive understanding of the material's performance under varying conditions. The study adhered to strict laboratory protocols to maintain consistency and minimize contamination. The study employed cyclic voltammetry for the precise synthesis of nano-structured polypyrrole, characterized the material to confirm its structural and electrochemical properties, and evaluated its antibacterial activity using standardized assays. This multi-faceted approach aimed to provide a thorough assessment of the polypyrrole's potential as an antimicrobial agent.

RESULTS

The study successfully demonstrated the antibacterial activity of nano-structured polypyrrole synthesized via cyclic voltammetry. The synthesized polypyrrole exhibited a distinct nano-structural morphology, characterized by uniform nanoparticle distribution as observed under scanning electron microscopy (SEM). The electrochemical analysis via cyclic voltammetry confirmed the stability and conductivity of the polypyrrole, which were essential for its antimicrobial efficacy.

The antibacterial assays revealed that nano-structured polypyrrole effectively inhibited the growth of all tested bacterial strains,

including *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The disk diffusion assay showed clear zones of inhibition around the polypyrrole-coated disks, with the largest inhibition zones observed for *S. aureus* and *P. aeruginosa*. Quantitative analysis of these zones indicated that the inhibition was significantly greater compared to control samples, underscoring the enhanced antimicrobial properties of the synthesized material.

In the minimum inhibitory concentration (MIC) tests, nano-structured polypyrrole demonstrated effective bacterial growth inhibition at relatively low concentrations. The MIC values were determined to be 25 µg/mL for *E. coli*, 15 µg/mL for *S. aureus*, and 20 µg/mL for *P. aeruginosa*, indicating that the polypyrrole has potent antibacterial activity against these pathogens. These results highlight the effectiveness of the polypyrrole as an antimicrobial agent and suggest that its nano-structuring plays a critical role in enhancing its antibacterial performance.

Statistical analysis confirmed that the differences in antibacterial activity were statistically significant, with p -values < 0.05 for comparisons between polypyrrole-treated and control samples. The findings align with the hypothesis that the nano-structured polypyrrole synthesized via cyclic voltammetry provides improved antibacterial activity due to its increased surface area and reactive sites, which facilitate greater interaction with bacterial cells. Overall, the results demonstrate that nano-structured polypyrrole synthesized by cyclic voltammetry holds significant potential as an effective antimicrobial material. This study provides valuable insights into the material's antibacterial properties and supports further exploration of its applications in medical and environmental contexts.

DISCUSSION

The results of this study underscore the promising antibacterial properties of nano-structured polypyrrole synthesized via cyclic voltammetry. The synthesis method employed effectively produced polypyrrole with a well-defined nano-structure, which was crucial for enhancing its antibacterial efficacy. The uniform distribution of nanoparticles, as evidenced by SEM, significantly increased the material's surface area and reactive sites, facilitating greater interaction with bacterial cells and thereby improving its antimicrobial performance.

The disk diffusion and minimum inhibitory concentration (MIC) assays provided strong evidence of the material's effectiveness against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The clear inhibition zones and low MIC values demonstrate that the nano-structured polypyrrole exhibits potent antibacterial activity, particularly against *S. aureus*, which is known for its clinical relevance and resistance to multiple antibiotics. The improved antibacterial activity can be attributed to the enhanced surface properties of the nano-structured polypyrrole, which likely disrupts bacterial cell membranes more effectively than its non-nano-structured counterparts.

The cyclic voltammetry-based synthesis not only ensured precise control over the polypyrrole's nanostructure but also contributed to its stability and conductivity, which are crucial for its antibacterial action. The electrochemical method allowed for reproducible and scalable production of the material, offering a viable approach for developing antimicrobial coatings or other applications where controlled material properties are essential.

However, while the study demonstrates significant antibacterial activity, there are several considerations for future research. The antibacterial mechanisms of nano-structured polypyrrole should be further investigated to understand how its structural properties interact with bacterial cells at a molecular level. Additionally, the effectiveness of polypyrrole in real-world applications, such as medical device coatings or environmental disinfectants, should be explored, including its performance under varying environmental conditions and potential cytotoxicity to human cells. This study provides substantial evidence that nano-structured polypyrrole synthesized via cyclic voltammetry is a promising candidate for antimicrobial applications. The successful synthesis and demonstrated antibacterial activity highlight the potential of this material to address challenges related to antibiotic resistance and offer new solutions in antimicrobial technology.

CONCLUSION

This study highlights the significant antibacterial activity of nano-structured polypyrrole synthesized via cyclic voltammetry. The electrochemical polymerization technique employed successfully produced polypyrrole with a well-defined nano-structure, which markedly enhanced its antimicrobial properties. The nano-structured polypyrrole demonstrated effective inhibition of bacterial growth, with particularly strong activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, as evidenced by substantial inhibition zones and low minimum inhibitory concentration (MIC) values. These findings validate the hypothesis that nano-structuring increases the material's surface area and reactivity, contributing to its potent antibacterial performance.

The successful integration of cyclic voltammetry for synthesizing nano-structured polypyrrole not only offers precise control over material properties but also presents a scalable method for developing advanced antimicrobial agents. The study's results suggest that nano-structured polypyrrole could serve as an effective alternative to traditional antibiotics, particularly in combating antibiotic-resistant bacteria.

Future research should focus on further elucidating the antibacterial mechanisms of nano-structured polypyrrole and exploring its applicability in various real-world scenarios, such as medical device coatings or environmental sanitization. Additionally, evaluating the biocompatibility and long-term stability of the material will be crucial for its practical implementation. Overall, the study provides a robust foundation for utilizing nano-structured polypyrrole as a promising candidate in antimicrobial applications, paving the way for innovations in addressing bacterial infections and resistance.

REFERENCES

1. L.U. Guiqian, W.U. Dingcai , D.Weimin and F.U. Ruowen, Studies on the synthesis and antibacterial activities of polymeric quaternary ammonium salts from dimethylaminoethyl methacrylate , *Journal of Reactive and Functional polymers*, 67, 2007, 355.
2. P. Nicolas, A .Mor, Peptides as weapons against microorganisms in the chemical defense system of vertebrates, *Journal of Annual Review of Microbiology*, 49, 1995, 277-304.
3. H.M. Lode, Clinical impact of antibiotic-resistant Gram-positive pathogens, *Journal of Clinical Microbiology and Infection*, 15, 2009, 212-217.
4. N.M. Milovic, J.Wang,K.Lewis, A.M. Klivanovm, Immobilized N-alkylated polyethylenimine avidly kills bacteria by rupturing cell membranes with no resistance developed, *Journal of Biotechnoogyl and Bioengineering*, 90, 2005, 715-722.
5. B. Dizman, M.O. Elasri, L.J. Mathias, ScienceSynthesis and antimicrobial activities of new water-soluble bis-quaternary ammonium methacrylate polymers, *Journal of Applied Polymer*, 94, 2004, 635.
6. G. Han, G.Shi, Conducting polymer electrochemical actuator made of high-strength three-layered composite films of polythiophene and polypyrrole, *Journal of Sensors and Actuators B*, , 99,2004, 525.
7. J.M. Pernaut, J.R. Reynolds, use of conducting electroactive polymers for drud delivery and sensing of bioactive molecules .A redox chemistry approach ,*Journal of Physical Chemistry B*, 104, 2000, 4080.
8. L.A.P.Kane-Maguire, G.G. Wallace, Communicating with the building blocks of life using organic electronic conductors, *Journal of Synthetic Metals*, 119, 2001, 39.
9. S. Kamalesh, P. Tan, J. Wang, T. Lee, E.T. Kang, C.H. Wang, Biocompatibility of electroactive polymers in tissues, *Journal of Biomedical Materials Research*, 52, 2000, 467.
10. T. Nonaka, Y. Uemura, K. Ohse, S. Kurihara, Preparation of resins containing phenol derivatives from chloromethylstyrenetetraethyleneglycol dimethacrylate copolymer beads and antibacterial activity of resins, *Journal of Applied Polymer Science*, 66, 1997, 1621.
11. G.Thenmozhi,D. J. Kumar, M. Gopalswamyb and J. R. Santhi. . Synthesis, characterisation and biological applications of conducting poly (p- amino phenol) and its nano compound ,*Journal of Der Pharma Chemica*, 3 (4), 2011, 116-126
12. G. Arslan, B. Yazici, Me. Erbil, The effect of pH, temperature and concentration on. electrooxidation of phenol, *Journal of Hazardous Materials B124* , 2005,37-43.
13. M.Kong, X. G. Chen, K. Xing, H. J. Park, Antimicrobial properties of chitosan and mode of action: A state of the art review, *Journal of Food Microbiology*, 144, 2010, 51–63
14. T. Nonaka, Y. Uemura, K. Ohse, S. Kurihara, Preparation of resins containing phenol derivatives from chloromethylstyrenetetraethyleneglycol dimethacrylate copolymer beads and antibacterial activity of resins, *Journal of Applied Polymer Science*, 66, 1997, 1621.
15. N. A. Mohamed, M. W. Sabaa, A. H. El-Ghandourb, M. M. Abdel-Azizc and O.F. Abdel-Gawad , Quaternized N-substituted carboxymethyl chitosan derivatives as antimicrobial agents, .*International Journal of Biological Macromolecules*, 60, 2013, 156– 164.