Synthesis and Morphological Studies of Cellulose and Cellobiose Conjugates of Para-Aminobenzoic Acid Hydrazide Using Scanning Electron Microscopy (SEM)

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ABSTRACT

Biopolymer conjugates have gained significant attention in the biomedical field due to their enhanced performance, particularly for drug delivery and antimicrobial treatments. This study focuses on the synthesis and characterization of conjugates of cellulose and cellobiose with para-aminobenzoic acid hydrazide (PABAH), employing scanning electron microscopy (SEM) for morphological analysis. SEM images revealed that cellulose conjugates exhibited a rough, highly porous surface, suggesting suitability for antimicrobial applications, while the smoother, more compact surface of cellobiose conjugates indicated potential for controlled-release drug delivery systems. This research provides insights into the relationship between surface morphology, porosity, and the potential biomedical applications of these conjugates.

Keywords: Biopolymer conjugates, Cellulose, Cellobiose, PABAH, Scanning electron microscopy

INTRODUCTION

Biopolymers such as cellulose and cellobiose have gained considerable attention due to their renewable, biodegradable, and non-toxic properties, making them ideal candidates for various biomedical applications, including drug delivery systems, antimicrobial coatings, and wound healing [1, 2]. Cellulose, a naturally abundant polysaccharide, has long been utilized in the pharmaceutical industry because of its biocompatibility and structural stability [3, 4]. Similarly, cellobiose, a disaccharide derived from glucose, plays a crucial role in enhancing the solubility and stability of therapeutic molecules, thereby improving drug efficacy [5].

Conjugating cellulose and cellobiose with bioactive molecules, such as para-aminobenzoic acid hydrazide (PABAH), offers additional benefits, including antimicrobial properties and the ability to facilitate controlled drug release, a critical feature for effective drug delivery [6, 7]. PABAH is particularly notable for its antimicrobial activity against drug-resistant pathogens like *Mycobacterium tuberculosis* [8, 9], making it an ideal candidate for conjugation with biopolymers. Conjugating these biopolymers with PABAH enhances their functional properties, including surface morphology, porosity, and structural integrity, all of which are critical factors in determining the effectiveness of their application in biomedical fields.

This study employs scanning electron microscopy (SEM) to evaluate the surface morphology and porosity of cellulose and cellobiose conjugates with PABAH. The goal of this research is to assess the potential of these

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conjugates in drug delivery and antimicrobial applications, providing valuable insights into their structure-function relationships.

METHODOLOGY

Materials and Reagents

Cellulose (Avicel), cellobiose, and para-aminobenzoic acid hydrazide (PABAH) were sourced from Sigma-Aldrich. The coupling reagent carbodiimide (EDC) and dimethyl sulfoxide (DMSO) were purchased from Merck. All chemicals were used as received without further purification.

Synthesis of Biopolymer Conjugates

Conjugation reaction

Cellulose (5.0 g) and cellobiose (5.0 g) were each dissolved in anhydrous dimethyl sulfoxide (DMSO) (100 mL) in separate round-bottom flasks. The reaction mixtures were stirred for 10 minutes at room temperature to ensure complete dissolution. To each mixture, carbodiimide (EDC, 1.2 equivalents to the hydroxyl groups of the biopolymer) was added to activate the hydroxyl groups for conjugation. Subsequently, para-aminobenzoic acid hydrazide (PABAH, 1.2 equivalents to cellulose or cellobiose) was introduced into the solution under anhydrous conditions. The reaction was allowed to proceed for 24 hours with constant stirring at room temperature (25 °C). During the reaction, the pH was maintained at approximately 7 by adding small amounts of dilute hydrochloric acid (HCl) or sodium hydroxide (NaOH), as necessary, to maintain the optimal conditions for EDC activation and conjugation. The reaction progress was monitored by Fourier Transform Infrared (FTIR) spectroscopy to confirm successful conjugation by the appearance of ester carbonyl bands.

After 24 hours, the reaction mixtures were purified by dialysis against deionized water for 48 hours to remove unreacted reagents and byproducts. The dialyzed solutions were then freeze-dried for 24 hours to yield the final conjugates.

Product Yield

- **Cellulose–PABAH conjugate**: The reaction yield is **6.3 g** of the conjugate, corresponding to a yield of **70%** based on the starting amount of cellulose.
- **Cellobiose–PABAH conjugate**: The reaction yield is **2.8 g** of the conjugate, corresponding to a yield of 56**%** based on the starting amount of cellobiose.

SEM Analysis

SEM Analysis

Sample preparation for SEM

For scanning electron microscopy (SEM) analysis, the cellulose–PABAH and cellobiose–PABAH conjugates were mounted on conductive carbon tape. The samples were then gold-coated using a sputter coater (SPI Module, USA) to enhance conductivity.

SEM Imaging Conditions

SEM imaging was performed with a JEOL JSM-6010LA scanning electron microscope at an operating voltage of 10 kV. Images were captured at magnifications of ×500, ×1000, and ×5000 to observe the surface features and microporosity of the conjugates. The imaging conditions were set at room temperature and atmospheric pressure, and the samples were analyzed once gold-coated.

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RESULTS

Surface Morphology and Structure

SEM images of cellulose conjugates revealed a rough surface with visible micropores, characteristic of cellulose's typical morphology. The highly porous structure, shown in suggests a high surface area that facilitates drug absorption and microbial interaction, beneficial for antimicrobial treatments and drug delivery applications [15]. These findings suggest that cellulose conjugates may be well-suited for applications in wound healing and topical antimicrobial treatments.

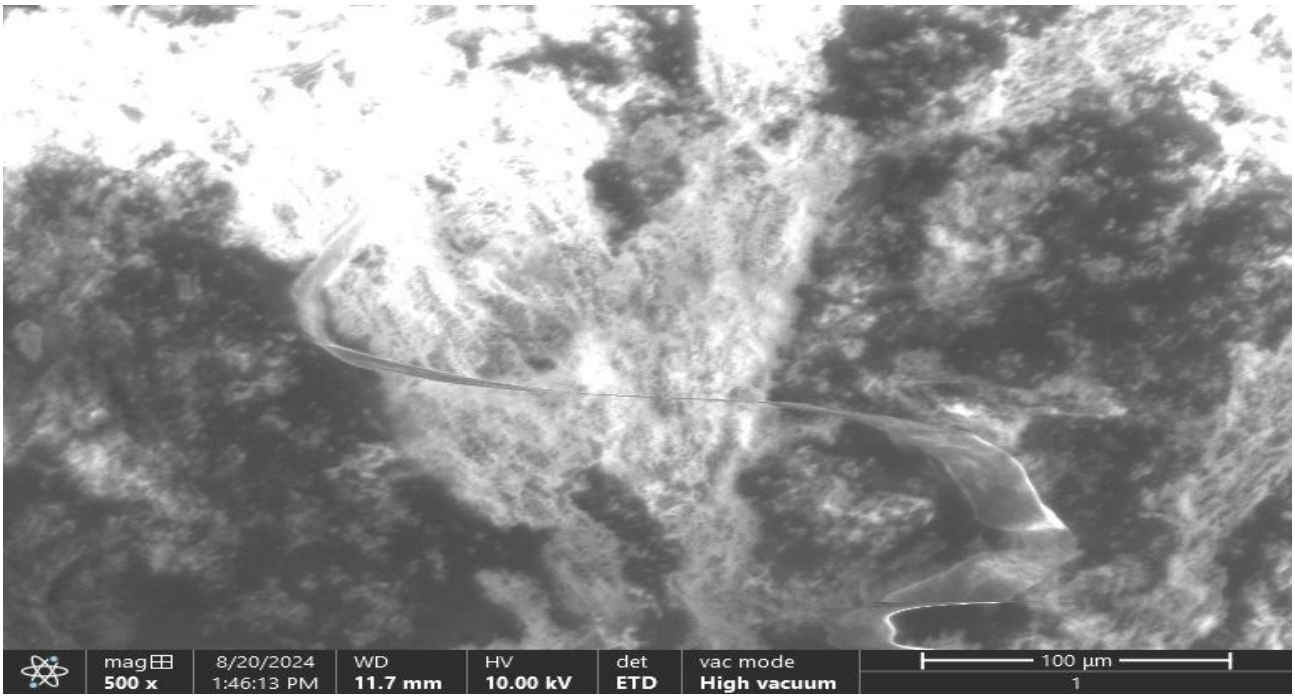

Plate 1: SEM Image of Synthesized Cellulose–Fatty Acid Methyl Ester in Methanol Using H_2SO_2

Conversely, cellobiose conjugates displayed a smoother, more compact surface, indicative of a stable structure ideal for controlled-release drug delivery systems. The lower porosity of cellobiose conjugates minimizes drug leakage and controls the rate of drug diffusion, making them suitable for sustained-release formulations [16].

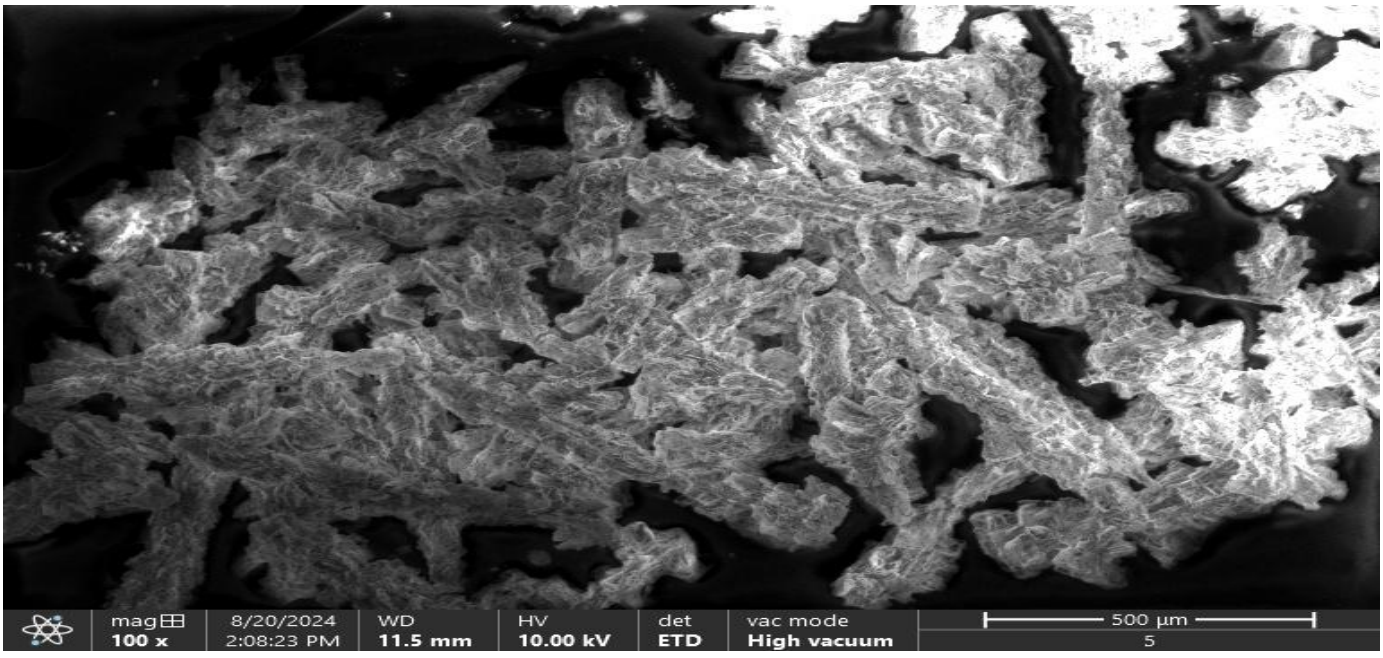

Plate 2: SEM Image of Synthesized Cellobiose–Fatty Acid Methyl Ester (Cellobiose–FAME)

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Porosity Analysis

SEM analysis indicated significantly higher porosity in cellulose conjugates compared to cellobiose conjugate. The larger pores in cellulose conjugates is shown in plate 3 below;

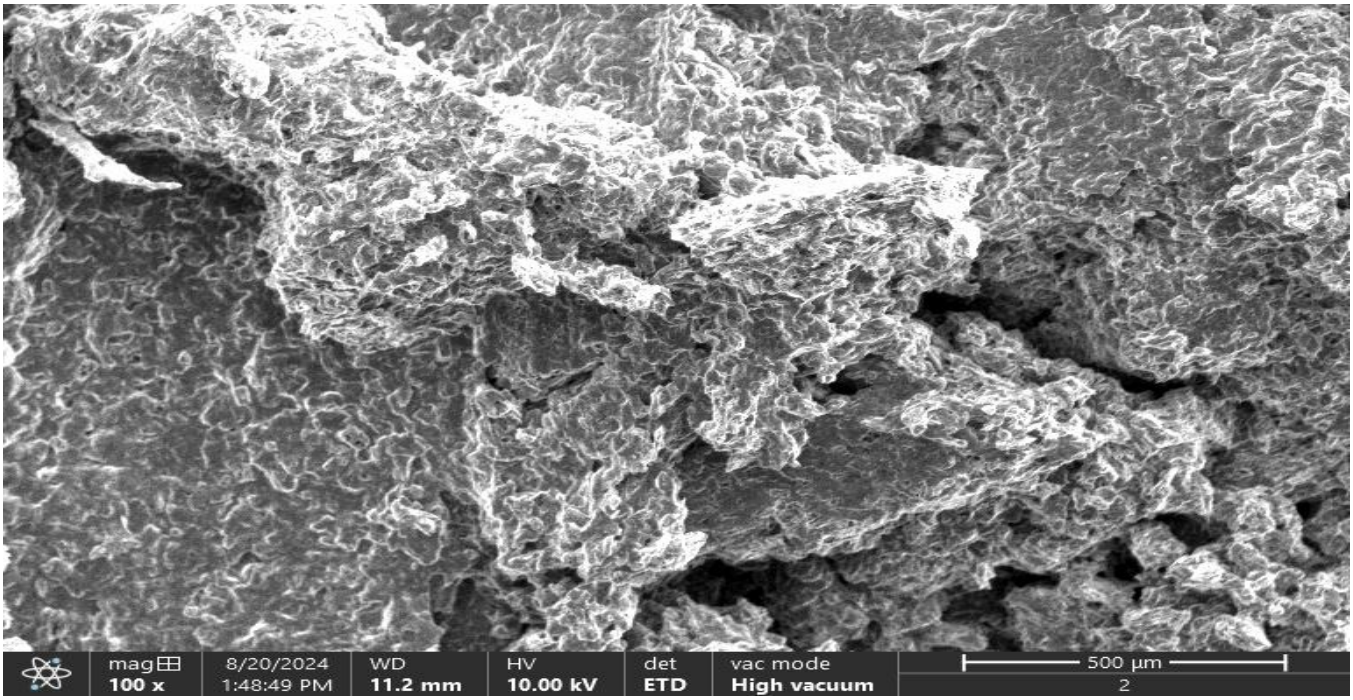

Plate 3: SEM Image of Synthesized Cellulose–Fatty Acid Methyl Ester (Cellulose–FAME)

Coupling provide greater surface area for drug loading, making them ideal for antimicrobial treatments requiring rapid drug release. In contrast, the lower porosity of cellobiose conjugates, with their smooth surface and compact texture, is more conducive to controlled drug release, essential for sustained therapeutic effects over time.

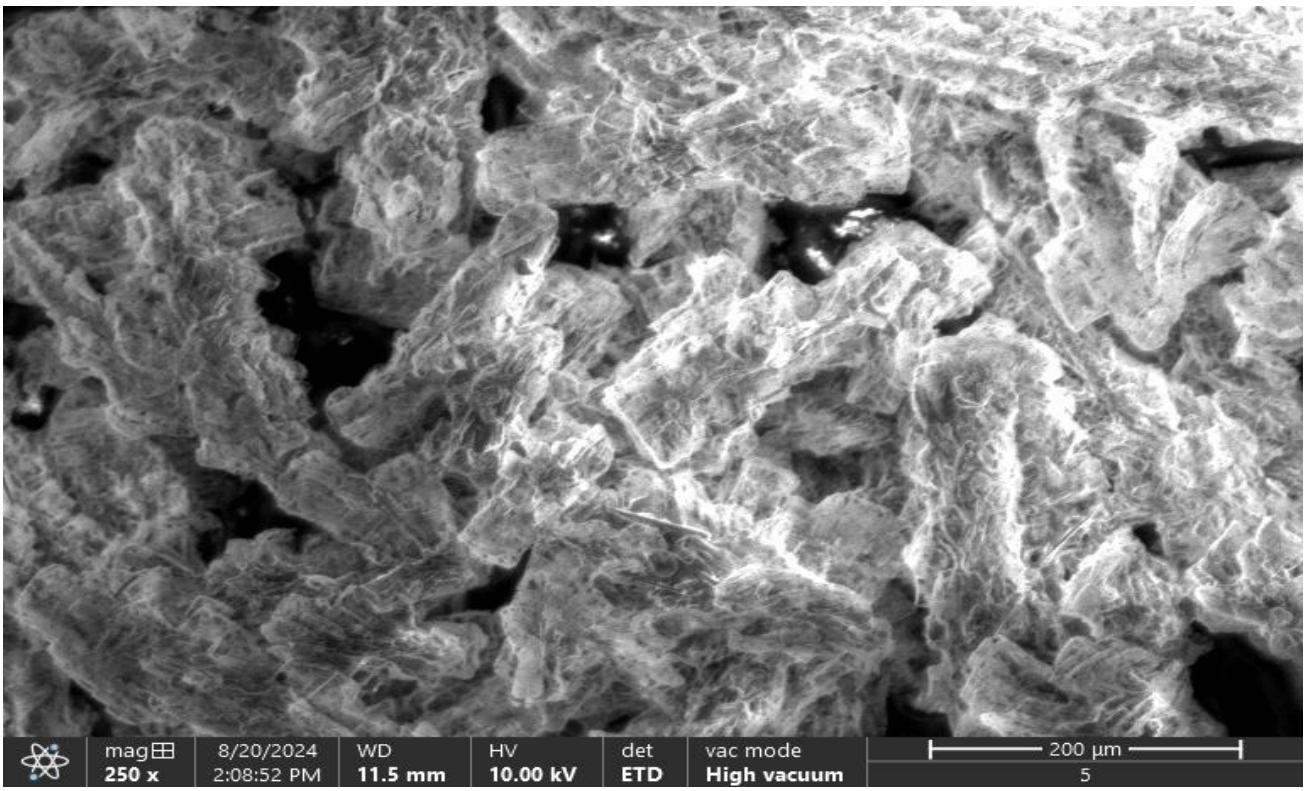

Plate 4: SEM Image of Synthesis of Cellulose–FAME and Double Coupling with Ethyl-PABA

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Biopolymer Interaction and Stability

Both conjugates demonstrated enhanced structural stability compared to their unmodified counterparts. The uniform distribution of PABAH on the cellulose surface, as seen in plate 5 below:

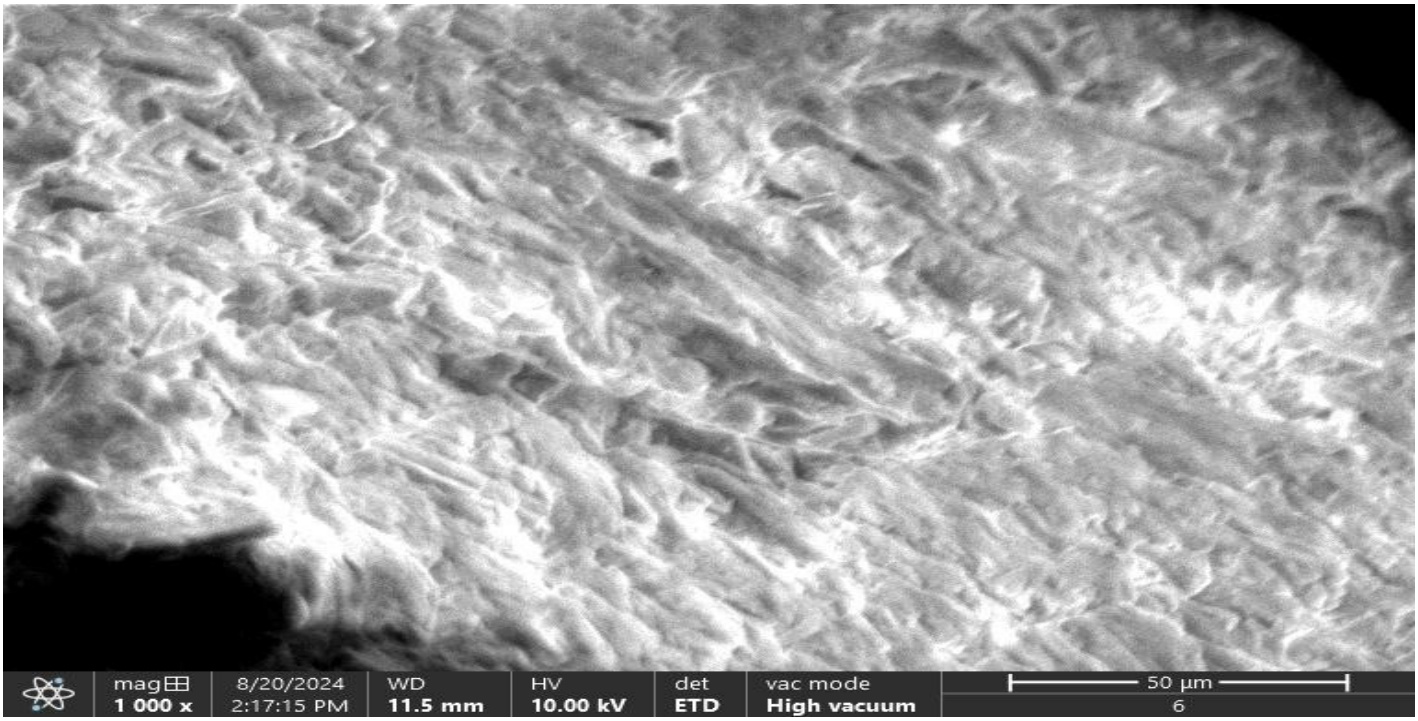

Plate 5: SEM Image of Synthesis of Cellobiose-FAME double Ethyl PABA Coupling

SEM Image of Synthesis of Cellulose-FAME with Ethyl PABA/Hydrazine Conjugate, improves both the biocompatibility and bioactivity of the conjugates. Similarly, this shows the successful integration of PABAH in cellobiose conjugates, indicating that the conjugation process did not compromise the structural integrity of the biopolymer.

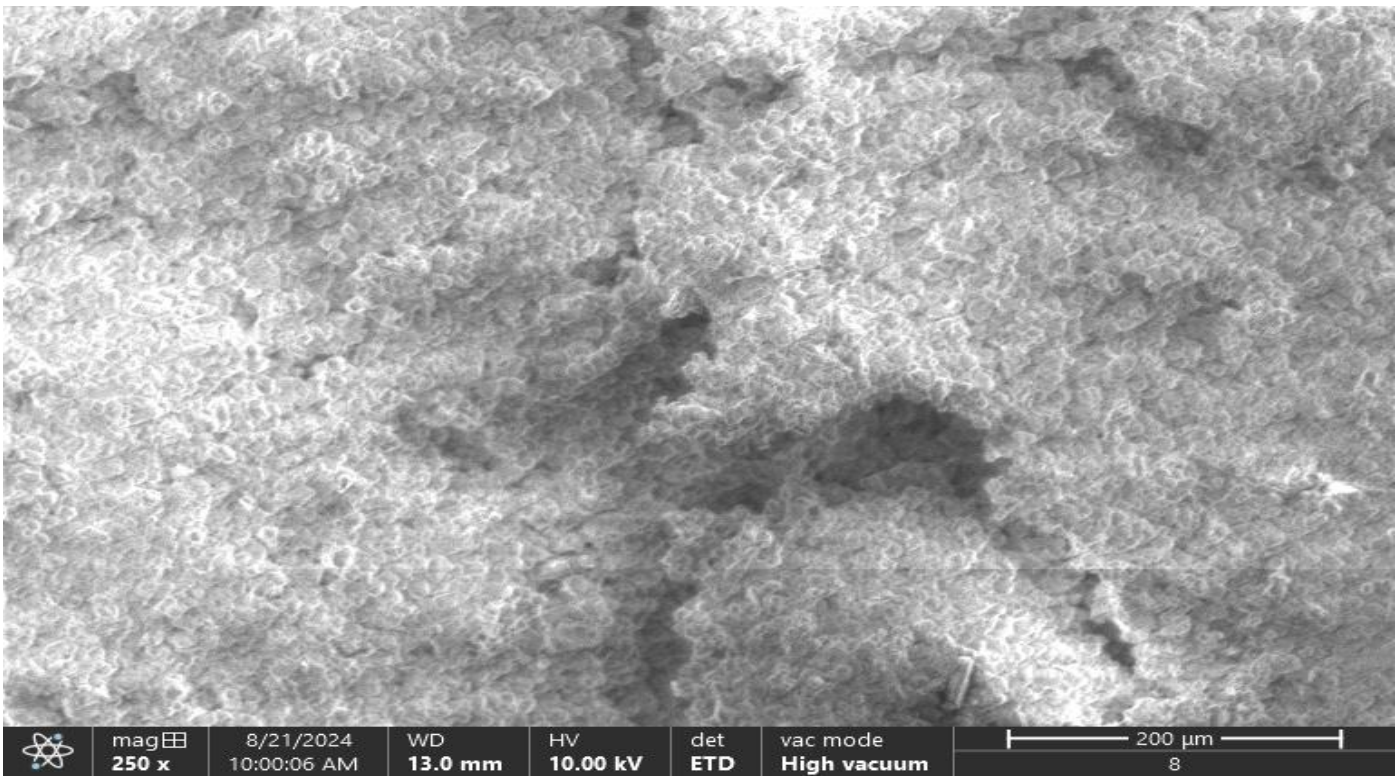

Plate 6: SEM Image of Synthesis of Cellulose-FAME with Ethyl PABA/Hydrazine Conjugate,

DISCUSSION

Surface Morphology and Applications

The surface morphology of the conjugates directly impacts their application in drug delivery and antimicrobial systems. The rough, porous surface of cellulose conjugates enhances their interaction with biological targets, making them ideal for antimicrobial coatings and rapid-release drug delivery systems [19]. Their high porosity increases drug loading capacity and enables quicker release, essential for immediate antimicrobial effects.

In contrast, the smooth surface of cellobiose conjugates, with lower porosity, supports controlled drug release, making them more suitable for sustained-release applications. Their compact structure minimizes leakage and ensures a gradual and sustained release of drugs, which is critical for long-acting drug formulations [20].

Porosity and Drug Release

Porosity plays a crucial role in the drug release profile of biopolymer conjugates. Cellulose conjugates, with their higher porosity, facilitate rapid drug diffusion, ideal for applications where quick drug release is needed, such as topical treatments [21]. Conversely, cellobiose conjugates, with their lower porosity, allow for slower drug release, making them suitable for oral drug delivery systems or implantable devices that require prolonged therapeutic effects [22].

Stability and Biocompatibility

The stability and biocompatibility of the conjugates were significantly enhanced through the successful conjugation with PABAH. The stable conjugation ensures that the antimicrobial properties of the conjugates are maintained during their application in long-term treatments [23]. The biodegradability of these conjugates further supports their safe use in biomedical applications, as they will break down harmlessly over time.

CONCLUSION

This study successfully synthesized cellulose and cellobiose conjugates with para-aminobenzoic acid hydrazide (PABAH) and evaluated their morphology and porosity using scanning electron microscopy (SEM). The SEM analysis revealed distinct differences in the surface morphology and porosity of the conjugates. Cellulose conjugates exhibited a rougher surface and higher porosity, making them suitable for antimicrobial applications and drug delivery systems requiring rapid release. In contrast, the smooth surface and lower porosity of cellobiose conjugates made them more suitable for controlled-release drug delivery systems. The successful conjugation of PABAH enhanced the biocompatibility, stability, and bioactivity of both conjugates, making them promising candidates for various biomedical and pharmaceutical applications. Further research is required to evaluate their antimicrobial efficacy and long-term stability in real-world applications.

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