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Coating and Characterization of Plant-Based Antibiotics via Layer-By-Layer (LBL) on Commercial Bandage

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Abstract
This study investigated the extraction of Curcumin, a plant-based antibiotic and the coating ability on commercial bandage by the use of a layer-by-layer (LBL) technique for the purpose of harnessing the antibacterial properties of the plant-based antibiotics to facilitate targeted wound therapy. 2 mg/mL of solutions admix of amylase and extracted curcumin, chitosan, and carboxy-methyl cellulose (CMC) were prepared at room temperature. The coating procedure involves dipping of the commercial bandage (2cm x 6cm) in the solution of chitosan for 180 seconds for 10 LBL, washed in aqua dest for 3 seconds, the treated bandage is further coated with CMC for 180 seconds for another 10 LBL. The treated bandage was washed again in aqua dest for another 3 seconds before finally coated with admix of amylase and curcumin. The coated bandage was again washed in aqua dest after which was allowed to be air dried. Extracted chitosan and carboxy-methyl cellulose (CMC) served as the positive and negative charges for the deposition and the barrier layers respectively. Scanning electron microscopy (SEM) analysis was employed to characterize the structure of the deposited layers using SEM-EDX Joel-JSM-7600K. LBL deposition successfully coated the commercial bandage with curcumin, as confirmed by SEM and EDX analysis. This study confirmed the potential of LBL technique for introducing plant-based antibiotics into existing wound dressings. This study is aimed at making a scholarly contribution to the field of wound care by employing nanotechnology and natural antimicrobial agents to enhance patient outcomes and alleviate the challenges posed by antibiotic resistance in the healthcare sector.

Keywords: *Deposition, LBL, plant-based antibiotics, commercial bandage, wound-dressing.*

1.0 Introduction

The management of wounds has presented a longstanding difficulty in the field of healthcare (Smith, 2020). Infections and the subsequent delay in healing have emerged as substantial threats to the overall health and welfare of patients (Li *et al.*, 2022). In recent years, there has been significant interest within the medical research and pharmaceutical communities about the advancement of innovative approaches for wound care (Anderson and White, 2022).

Recent scientific advancements have provided valuable insights into the potential of plant-based antibiotics. Plant-based antibiotics, also known as phytobiotics or plant-derived antimicrobials, are a class of natural chemicals produced by plants that exhibit antibacterial properties (Singh *et al.*, 2021). These chemicals play a crucial role in the plant's defense mechanisms, helping them fight off bacterial infections (Sharma *et al.*, 2020). Plants and plant products are organic and biodegradable with little or no side effects compared with their synthetic counterparts (Mohammad *et al.*, 2023). These findings suggest that plant-based antibiotics hold promise as effective treatments for wounds. Recent works in biotechnology and pharmaceutical science have widened novel frontiers in biomedical fields which require materials with biocompatibility and, at most times, transient existence (Kumar *et al.*, 2023). Any material intended for implantation must be biocompatible such that a second surgical intervention is avoided (Reddy *et al.*, 2020). Biomaterials are purposed to work directly with body systems to mend, care for, or replace any organ or body function (Patil *et al.*, 2021).

The rising global worry regarding the incidence of antibiotic-resistant bacterial strains has led to the imperative need for the exploration of alternative therapeutic techniques (Sharma *et al.*, 2020). Natural products, such as those obtained from botanical sources, have become a valuable reservoir of antibacterial compounds that possess distinct modes of action (Nguyen *et al.*, 2021). These compounds hold promise in addressing the issue of antibiotic resistance (Choudhary *et al.*, 2021). Furthermore, the utilization of plant-derived antibiotics aligns with the worldwide movement towards sustainable and environmentally conscious healthcare approaches (Chen *et al.*, 2023).

The use of thin films in drug delivery systems (DDS) has been widely utilized in biomedical applications, including drug delivery and tissue engineering. This nanotechnology-based approach offers great versatility in its implementation (Zhou *et al.*, 2022). The application of this method enables the precise arrangement of thin films with nanometer-scale accuracy, providing a modifiable framework for the integration and continuous discharge of therapeutic substances (Olaniran *et al*., 2021). Through the utilization of layer-by-layer (LBL) deposition, it becomes feasible to develop wound dressings capable of delivering plant-based antibiotics in a localized and sustained manner, enhancing their therapeutic effectiveness while mitigating adverse reactions (Huang *et al*., 2023).

In other words, the increasing problem of antibiotic resistance calls for the exploration of alternative treatment approaches in wound management (Ahmed et al., 2023). In response to this urgent need, this study investigates the potential of plant-based antibiotics through a novel application of layer-by-layer (LBL) deposition on commercial bandages. By leveraging the antibacterial properties of natural compounds, the research aims to enhance localized drug delivery for wound healing. Through the integration of biocompatible materials with nanotechnology, this work introduces a sustainable and potentially more effective approach to wound therapy, offering a promising solution to key challenges posed by antibiotic resistance. The potential for localized wound treatment is further strengthened by combining plant-based antibiotics with LBL deposition technology (Wu *et al.*, 2024).

2.0 Materials and Methods

The materials used in this study include commercial bandage, Turmeric (Curcuma longa), amylase, carboxyl methyl cellulose (CMC), crab shell, and distilled water (aqua dest). A dipping machine was designed and fabricated using locally available materials to facilitate the precise and controlled deposition of polyelectrolyte solutions onto the commercial bandage. This machine played a crucial role in achieving uniform coating layers and ensuring reproducibility during the layer-by-layer (LBL) coating process. The primary purpose of the dipping machine was to immerse the commercial bandage into the different polyelectrolyte solutions consistently and precisely, enabling the sequential deposition of layers to form multilayer coatings of the desired thickness (Martinez *et al*., 2021).

2.1 Construction of Dipping Machine

The dipping machine was constructed using a basic pulley system, chosen for its simplicity, cost-effectiveness, and ease of operation. The machine frame was made of wood and light metal due to their lightweight nature and availability. The main components of the machine included a rod serving as both the wheel and the axle and bearings at both ends to facilitate smooth rolling during the upward and downward movements of the bandage during the coating process (Chen *et al*., 2022).

2.2 Extraction of Curcumin from Turmeric (Curcuma longa)

Conventional extraction was carried out using the Soxhlet method. Turmeric rhizomes were dried in an oven at 105°C for 3 hours, then ground using a mortar and sieved to achieve a uniform powder with a particle size of 0.18 mm. The turmeric powder was stored in a refrigerator to prevent moisture absorption. For Soxhlet extraction, 33.4 g of turmeric powder was placed in a thimble and extracted with acetone at 60°C for 15 hours. After extraction, acetone was removed using a rotary evaporator under vacuum at 35°C. The residue (oleoresin) was dried, weighed, and dissolved in methanol for curcumin content analysis (Rahman *et al*., 2021).

2.3 Synthesis of Chitosan from Crab Shell

The synthesis of chitosan involved three stages: deproteination, demineralization, and deacetylation, using established protocols (Hossain *et al*., 2022).

Deproteination: Fine crab shell powder was mixed with NaOH (2.0 N) in a 1:6 ratio (sample: solvent), heated to 80°C while stirring for 1 hour, and then washed with distilled water until neutral pH was achieved. The sample was then dried in an oven at 80°C for 24 hours.

Demineralization: The deproteinated crab shell powder was treated with HCl (1.5 N) in a 1:12 ratio, stirred for 1 hour, washed to neutral pH, and dried in an oven at 80°C for 24 hours.

Deacetylation: The demineralized chitin was treated with 50% NaOH in a 1:10 ratio (sample:solvent) at 90°C for 2 hours, washed to neutral pH, and dried at 80°C for 24 hours. The final product was stored in a desiccator until use (Nguyen *et al*., 2023).

2.4 Methodology

A solution of CMC (2 mg/mL) and a mixed solution of amylase and curcumin (2 mg/mL) were prepared. Chitosan (67 mg/mL) was dissolved in warm distilled water (60°C) to overcome its limited solubility (Ali *et al*., 2024). A commercial bandage was mounted on the fabricated dipping machine for polyelectrolyte deposition.

The dipping process involved immersing the bandage sequentially in different solutions placed in separate 250 mL beakers. Each 2 × 6 cm bandage piece was attached to the machine's string. The wheel was manually rotated at 120° per second to release and retract the bandage into the solutions.

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Chitosan Deposition: The bandage was immersed in the chitosan solution (positively charged) for 180 seconds to allow sufficient deposition, followed by retraction and re-immersion for 10 LBL cycles. The bandage was washed twice in distilled water for 10 seconds per dip.

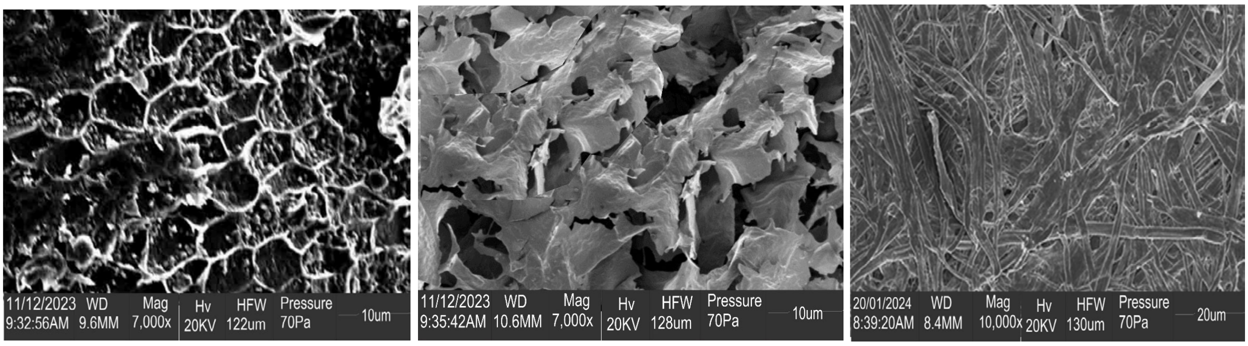
CMC Barrier Layer: The bandage was then immersed in the negatively charged CMC solution for 180 seconds per dip, followed by another round of washing.

Curcumin-Amylase Deposition: The process was repeated with the curcumin-amylase mixture for 10 LBL cycles, followed by washing and subsequent CMC layering.

The assembled system was allowed to air dry. The coated bandage underwent physico-chemical analysis using Scanning Electron Microscopy (SEM) to evaluate its multilayered structure (Khan *et al*., 2023). All depositions were carried out at room temperature in a dust-free environment to prevent substrate contamination (Yin *et al*., 2022).

3.0 Results and Discussion

The SEM (Scanning Electron Microscopy) and EDX (Energy-Dispersive X-ray Spectroscopy) investigations revealed important information about the morphological and elemental features of the chitosan compound, curcumin extract, and coated material on the commercial bandage. According to the SEM analysis, the generated images presented in Figures 1a, 1b and 1c provided useful information on the morphological properties of the chitosan compound, curcumin extract and the coated material (Chen *et al*., 2020). These qualities correspond to the surface's optical appearance, structure, and texture as examined through a scanning electron microscope. This is consistent with prior studies on similar biopolymers and plant-based materials, emphasizing the importance of shape and elemental content in affecting material performance (Zhang *et al*., 2022).



(a) (b) (c)

Figure 1a: SEM image of curcumin solution
Figure 1b: SEM image of chitosan compound
Figure 1c: SEM image of the coated bandage at 10,000x magnification

The curcumin solution, extracted using the Soxhlet method, indicated successful preparation, as evidenced by EDX data in Figure 2a revealing peaks for carbon and oxygen, which are key components of curcumin. The presence of expected elements like carbon and oxygen aligns with the presence of curcumin as well as the peak intensities which corresponds with the standard indicator that the deposition on the bandage was successful. The successful deposition of curcumin and chitosan via the LBL technique highlights the potential clinical application of these coated bandages. Their biocompatibility and antibacterial properties suggest that they could serve as a sustainable alternative to conventional wound dressings. Furthermore, the scalability of this LBL technique is promising for large-scale production. By modifying the dipping machine for automated processes, large batches of coated bandages can be efficiently produced for clinical use. This observation corroborates with the study documented by Mohan *et al*. (2021), who depicted the effectiveness of Soxhlet extraction in isolating bioactive chemicals with minimum degradation.

Silicon, however, raises concerns about potential contamination probably due to contamination during the grinding process of the turmeric into fine powder or Soxhlet extraction process. This showed the important requirement for tight handling practices to reduce contamination hazards, which agreed with Nasution *et al*. (2023) report on maintaining purity in bio-extraction procedures. The presence of silicon in the curcumin extract suggests potential contamination during the grinding. To mitigate this issue, future studies could implement stricter contamination control measures, such as using high-purity extraction solvents, ensuring proper sterilization of equipment, and working in clean-room conditions during sample preparation. These precautions would help improve the purity of the plant-based antibiotics and enhance the overall biocompatibility of the coated bandage.

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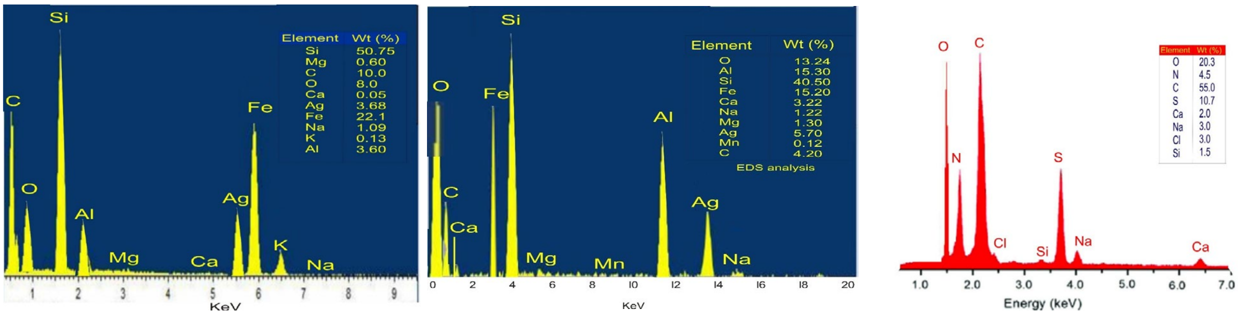

(a) (b) (c)

Figure 2a: EDX graph showing peak of elements present in curcumin compound.
Figure 2b: EDX graph showing peak of elements present in chitosan compound.
Figure 2c: EDX graph showing peak of elements present in the coated nylon bandage.

The EDX results showed the presence of key components like carbon and oxygen, as well as trace levels of aluminum, silicon, calcium, and magnesium. Trace amounts of other elements like magnesium, calcium, iron, sodium, and potassium are often present in plant-based materials and might not necessarily indicate contamination. This current result is consistent with those reported by Li *et al*. (2020) and Kumar *et al*. (2019), who found trace elements in natural biopolymers that were often derived from raw materials or extraction residuals. Such trace elements are generally deemed harmless if they do not significantly change the chemical or functional properties of the biopolymer.

In the case of the chitosan, the presence of varying surface morphology and the elements presented by the EDX result presented in Figure 2b indicates that the chitosan contains the basic elements that should be present in the compound such as carbon and oxygen. Aluminum, silicon, iron, calcium, sodium, and magnesium are often present in trace amounts in chitosan. Trace elements including magnesium, calcium, and iron are common in natural biopolymers and are frequently attributed to inherent plant material impurities or extraction residuals (Kumar *et al*., 2019). These findings are consistent with recent research by Li *et al*. (2020), which investigated trace element profiles in biopolymer matrices and their little impact on targeted material qualities.

From the SEM analysis presented in Figure 1c, the presence of small particle-like formations on the surface indicates that drug solutions were deposited onto the bandages. These particles have different sizes, shapes, and arrangements. Some particles are unique and distinct, while others come together to form groupings or patterns. This alters the texture of the bandage. This observation is in agreement with the study reported by Singh *et al*. (2021), who discovered that differences in surface shape can affect the release kinetics and bioavailability of bioactive chemicals in wound healing applications.

Results from the EDX analysis is presented in Figure 2c. The result shows the elemental composition of the coated sample, indicating the presence of carbon, oxygen, nitrogen, and sulfur comprising over 50% of the elements present in both the curcumin and chitosan compounds. The EDX spectra showed typical peaks for these elements, showing their successful deposition on the surface of the commercial bandage during the coating process. Chen *et al*. (2020) have similarly reported the effective integration of biopolymers and plant-derived compounds in medical textiles. Furthermore, this current study is consistent with research documented by Li *et al*. (2020), who enumerated the relevance of biocompatible and biodegradable materials in producing effective wound care coverings (Chen *et al*., 2020; Li *et al*., 2020).

The EDX analysis of the uncoated and coated samples reveals significant changes in elemental composition due to the LBL deposition process. Before coating, the primary elements detected in the chitosan and curcumin compounds were carbon, oxygen, sodium, and calcium, along with trace amounts of silicon and magnesium. After coating, the EDX spectrum of the coated bandage shows an increase in the percentage of carbon and oxygen, which can be attributed to the successful deposition of curcumin and chitosan layers, both rich in these elements. Additionally, the presence of nitrogen and sulfur in the coated bandage indicates the incorporation of amylase and curcumin during the final deposition steps (Singh et al., 2021; Zhang et al., 2022).

The increase in carbon content aligns with the organic nature of curcumin and chitosan, while the detection of nitrogen confirms the successful incorporation of biopolymers during coating (Mohan et al., 2021; Li et al., 2020). Trace amounts of silicon and calcium observed in both coated and uncoated samples suggest minimal contamination from the handling and preparation processes (Nasution et al., 2023; Yin et al., 2022). Despite the demonstrated effectiveness of LBL deposition in producing coated bandages, further research is required to validate these findings in clinical settings. In vivo testing on animal models could offer valuable insights into the biocompatibility, antibacterial efficacy, and wound healing performance of the coated bandages. Additionally, optimizing the dipping machine for high-throughput, automated production would enhance scalability, facilitating clinical trials and eventual commercialization.

Amylase + Curcumin Solution: Amylase and Curcumin are organic compounds and primarily composed of Carbon, Oxygen, and Nitrogen.

CMC (Carboxyl Methyl Cellulose) Solution: CMC is a cellulose derivative, primarily made up of Carbon, Oxygen, and Hydrogen.

Chitosan Solution: Chitosan is a biopolymer derived from chitin, which is primarily composed of Carbon, Hydrogen, and Oxygen.

Table 1: Elemental composition comparison of chitosan, curcumin, and the coated bandage

Chitosan Elemental Composition	Curcumin Elemental Composition	Coated Bandage Elemental Composition
Carbon	Carbon	Carbon
Oxygen	Oxygen	Oxygen
Sodium	Sodium	Sodium
Calcium	Calcium	Calcium
Silicon	Silicon	Silicon

4.0 Conclusion

The EDX analysis and SEM imaging confirmed successful deposition of drug solutions onto the commercial bandage via the layer-by-layer dipping method. The presence and distribution of fine particles across the bandage surface indicated effective drug loading. These findings suggest the LBL coating approach is promising for drug delivery in wound treatment. This process will enable target and controlled drug release at the wound site, offering a regulated and efficient pathway for loading bandages with required drugs. Overall, the results highlighted the LBL technique's potential as a viable strategy for developing cutting-edge wound care.

This experiment highlighted the LBL coating technique's potential as a viable strategy for creating advanced wound dressings with controlled drug delivery. Further research and process optimization are necessary to fully unlock its potential in clinical applications.

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