MACEIÓ - ALAGOAS
EBA 15
15° ENCONTRO BRASILEIRO SOBRE ADSORÇÃO
15th Brazilian Meeting about Adsorption

The effect of the degree of deacetylation of chitosan on the surface properties of layer-by-layer polymeric films

Edgar Siebeneichler Branco[a,*], José Diego Magalhães Soares[a,b], Dybson Manoel da Silva Nascimento[a], Eduarda Baggio Paglia[c], Vânia de Lourdes das Graças Teles[a], Sérgio Paulo Campana Filho[d], Anderson Fiamingo[e], João Inácio Soletti[a], Marisa Masumi Beppu[c], João Batista Maia Rocha Neto[a]

[a] *Federal University of Alagoas, Center of Technology, Lourival Melo Mota Avenue, Maceió, 57072–900, Brazil*
[b] *Federal Institute of Education, Science and Technology of Alagoas, 350 Ferroviário Avenue, Maceió, 57020-600, Brazil*
[c] *University of Campinas, School of Chemical Engineering, Albert Einstein Avenue 500, Campinas, 13083-852, Brazil*
[d] *University of São Paulo, São Carlos Institute of Chemistry, Trabalhador são-carlense Avenue 400, 13566-590, São Carlos, Brazil*
[e] *Paula Souza State Center for Technological Education, Lúcio Sarti Street 809, 14706-120, Bebedouro, Brazil*

Abstract

This study investigates the effect of the degree of deacetylation (DD) of chitosan (CHI) on the surface properties of alginate (ALG)/CHI films produced via adsorption using the layer-by-layer (LbL) technique. CHI solutions were prepared with DD levels of 40%, 65%, and 85%. The LbL films were created by alternating immersions of glass substrates in ALG and CHI solutions at pH 3.0 for 15 minutes each, followed by rinsing twice with ultrapure water to ensure proper layering and removal of excess material. The resulting ALG/CHI coatings were characterized using Atomic Force Microscopy (AFM), Fourier-Transform Infrared Spectroscopy (FTIR), and contact angle measurements. AFM analysis revealed that increasing DD in chitosan led to greater roughness in the ALG/CHI films, as higher DD chitosan requires less material for charge compensation and forms films with isolated islands. This was confirmed by FTIR, which showed peaks in the 1300-1600 cm^{-1} range indicating fewer free amine radicals in high DD chitosan. Contact angle measurements demonstrated that all ALG/CHI coatings are hydrophilic. Overall, the study indicates that DD plays a crucial role in controlling chitosan-based materials, impacting their use in various biotechnological applications.

Keywords: Layer-by-Layer; chitosan; surface properties.

1. Introduction

Biomaterials are structures that interact with biological systems and represent a significant portion of the materials used in the healthcare sector [1]. They can be composed of natural or synthetic molecules, in which the use of natural polymers in the synthesis of nanostructured biomaterials offers advantages such as non-toxicity, biocompatibility, and biodegradability. Furthermore, the production techniques employed are crucial for ensuring the material's efficiency, quality, and applicability in biotechnological processes [2,3].

Chitosan (CHI) is a cationic natural polymer obtained through the deacetylation of chitin, a compound found in the shells of arthropods and the exoskeletons of insects. This polymer has been studied for its antimicrobial and wound-healing properties in the biomedical field [4].

Another significant compound is Alginate (ALG), an anionic natural polymer extracted from brown algae. It is recognized for its high biocompatibility and its ability to control the diffusion of encapsulated compounds, making it highly valuable in the pharmaceutical industry [5].

These polymers possess electrical charges in solution and characteristic functional groups, enabling the creation of polymeric coatings with multilayer combinations using the layer-by-layer (LbL) technique. This purely adsorptive technique involves the formation of a nanometric-order film through sequential adsorption of layers. The LbL technique includes alternating immersion—via dipping, spraying, or spinning—of the material in

solutions of electrolytes with opposite electrical charges [6]. The advantages of the LbL technique include precise control over the functionality, composition, and properties of the resulting materials, making it particularly valuable in the healthcare sector.

In recent decades, there has been growing interest in the development of natural polymeric coatings [7,8,9]. However, access to these technological innovations remains limited to a small group. There is a need for the development of more cost-effective materials with diverse compositions to enable their use in a broader range of applications.

Considering their potential functionality as biomaterials, the comprehension of physicochemical properties—such as roughness, hydrophobicity, and composition—is crucial in the synthesis of nanostructured coatings. This study investigates the effect of the number of bilayers and degree of deacetylation on the surface properties of ALG/CHI films produced via adsorption using the LbL technique.

2. Methodology

CHI and ALG solutions were prepared by dissolving the polymers at a concentration of 0.1% (w/v). The CHI solution was prepared using 100 mM glacial acetic acid. All solutions were stirred overnight and adjusted to a pH of 3.0 using 1.0 M HCl or 1.0 M NaOH solutions.

The glass substrates (26 x 76 mm) were cleaned by immersion in a commercial detergent aqueous solution for 15 minutes, followed by two rinsing steps with Milli-Q water for 1 minute and 30 seconds each.

The adsorptive film production processes using the LbL technique were carried out under different DD conditions (40%, 65%, and 85%). The films were assembled by alternating immersions of glass substrates in ALG and CHI solutions at pH 3.0 for 15 minutes each, followed by two rinsing steps with Milli-Q water (1 minute and 30 seconds each) between each polyelectrolyte solution.

The LbL procedure was repeated for eight cycles for all films, with ALG as the outer layer. The notation $(ALG/CHI_y)_z$ was used for each film produced, where y refers to the average degree of deacetylation and z represents the number of bilayers. The samples were stored at room temperature before characterization.

All characterizations were performed on dried films. The LbL films were characterized by AFM, contact angle measurement, and FTIR spectroscopy to evaluate the effect of chitosan deacetylation on the roughness and composition of the coatings. The AFM data were processed using Gwyddion software, while the contact angle and FTIR data were analyzed using Origin software.

3. Results

The contact angle measurements for each film are shown in Figure 1. Analysis of the data reveals that all ALG/CHI coatings exhibit a hydrophilic behavior, with all contact angles being less than 60°.

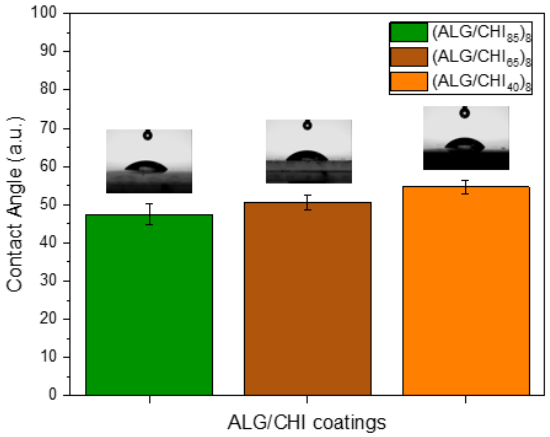

Fig. 1. Representation of the contact angle and variation of the contact angle for the ALG/CHI coatings.

Although no significant differences were observed between the contact angles of the ALG/CHI coatings analyzed, a trend toward lower contact angles for coatings with higher DD can be seen. This behavior enhances their suitability for various applications, such as wound dressings. Materials used for wound dressings must be hydrophilic to allow selective permeability to water vapor, carbon dioxide, and oxygen, as well as to possess hemostatic properties and contribute to cell regeneration [10].

The topography of the coatings, as shown in Figure 2, indicates that an increase in the CHI degree of deacetylation results in rougher and less uniform films.

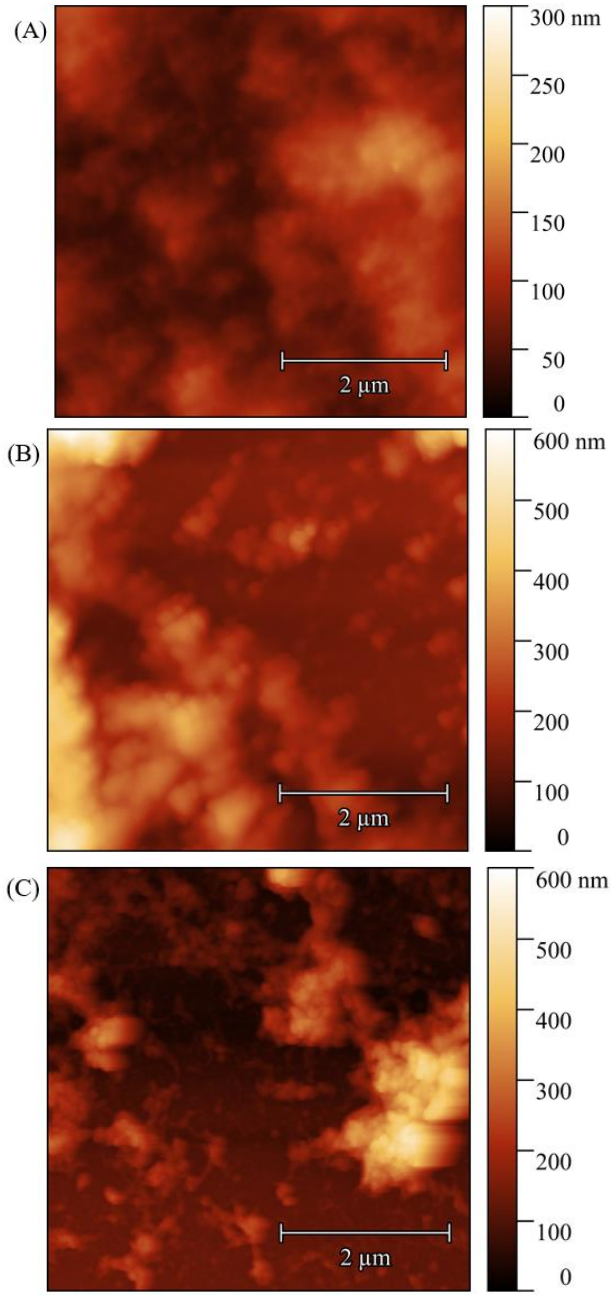

Fig. 2. AFM topography images for (A) $(ALG/CHI_{40})_8$, (B) $(ALG/CHI_{65})_8$ and (C) $(ALG/CHI_{85})_8$. The root-mean-square (RMS) roughness values for these films are 30 nm, 80 nm and 81 nm, respectively.

This result may be a direct consequence of the films assembly, which occurs in an isolated island regime. The use of chitosans with a higher degree of deacetylation (DD), and consequently greater cationic potential, provides a smaller quantity of these molecules for electrical compensation [11], which hampers the assembly of a large number of isolated islets, increasing the film roughness and decreasing its uniformity.

The FTIR spectra from the coating analysis are shown in Figure 3. The characteristic bands of the ALG/CHI coatings are listed in Table 1.

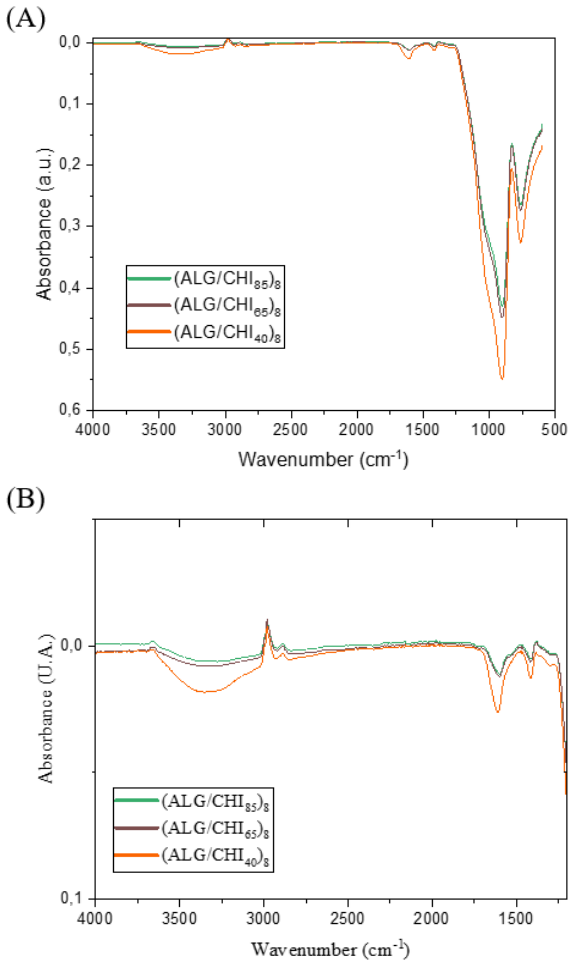

Fig. 3 (A) and (B). FTIR graphic from the ALG/CHI coatings.

The characteristic peaks related to the bonding of molecular chains between chitosan and alginate occur in the range of 1300-1600 cm^{-1}, representing the overlap of C=O and C-N-H bonds, as well as amide stretching and vibration. An increase in peak absorbance indicates a higher amount of free amine groups and a lower amount of complexed amine groups with a decrease in chitosan deacetylation degree [12,13]. This supports the observation that less chitosan is required for electrical compensation as the chitosan degree of deacetylation increases.

Table 1. Band spectra from characteristic groups of chitosan and alginate.

Spectra	Characteristic Group	Substance
700 to 1200	C-N C-O C-O-C	ALG/CHI
1600	C=O (amide I)	
1300	C-N (amide II)	CHI
1520	N-H (amine I)	
1370 to 1430	CH_3 and CH_2	ALG/CHI
2800 to 3600	C-H N-H O-H	ALG/CHI

However, peaks in other absorption ranges, specific to the skeletal saccharide vibrations of the electrolytes, suggest that a higher DD of chitosan results in shorter polymer chains.

4. Conclusion

The DD strongly affects the properties and characteristics of chitosan-based films, such as roughness and chain length. As the DD increases, the amount of chitosan required for charge compensation results in rougher and more hydrophilic films. The ability to generate different types of coatings through DD modification increases its versatility in the biotechnology field, allowing the production of efficient biomaterials through significant changes in hydrophilicity, chemical composition, roughness and surface topography. Therefore, it is understood that adsorption for the formation of multilayer films is a promising strategy to promote the design of modular biomaterials with physicochemical properties on a nanometric scale.

Acknowledgements

We thank the National Council for Scientific and Technological Development (CNPq, grant n. 408176/2023-7) and the Federal University of Alagoas (UFAL/Brazil) for their financial support.

References

[1] Matichescu, A., Ardelean, L. C., Rusu, L.-C., Craciun, D., Bratu E. A., Babucea, M., Leretter, M. Advanced biomaterials and techniques for oral tissue engineering and regeneration—a review. Materials, vol. 13, n.22, pp. 1–37, nov. 2020.

[2] Rocha Neto, J. B. M., Taketa, T. B., Bataglioli, R. A., Pimentel, S. B., Santos, D. M., Fiamingo, A., Costa, C. A. R., Campana-Filho S. P., Carvalho, H. F., Beppu, M. M. Tailored chitosan/hyaluronan coatings for tumor cell adhesion: Effects of topography, charge density and surface composition. Appl Surf Sci, vol. 486, pp. 508–518, aug. 2019.

[3] Ul-Islam M., Alabbosh K. F., Manan S., Khan S., Ahmad F., Ullah M. W. Chitosan-based nanostructured biomaterials: synthesis, properties, and biomedical applications. Advanced Industrial and Eng. Polymer Res., vol. 7, n.1, pp. 79–99, jan. 2024.

[4] Kulka K.; Sionkowska A. Chitosan based materials in cosmetic applications: a review. Molecules, vol. 28, n.4, feb. 2023.

[5] Hurtado A., Aljabali A. A. A., Mishra V., Tambuwala M. M., Serrano-Aroca Á. Alginate: enhancement strategies for advanced applications. Int J Mol Sci, vol. 23, n.9, may 2022.

[6] Taketa T. B., Bataglioli R. A., Rocha Neto J. B. M., Beppu M. M. Probing axial metal distribution on biopolymer-based layer-by-layer films for antimicrobial use. Colloids Surf B Biointerfaces, vol. 199, mar. 2021.

[7] Iñiguez-Moreno M., Ragazzo-Sánchez J. A., Calderón-Santoyo M. An extensive review of natural polymers used as coatings for postharvest shelf-life extension: trends and challenges. Polymers (Basel), vol. 13, n.19, oct. 2021.

[8] Díaz-Montes E., Castro-Muñoz R. Trends in chitosan as a primary biopolymer for functional films and coatings manufacture for food and natural products. Polymers, mar. 2021.

[9] Sergi R., Bellucci D., Cannillo V. A review of bioactive glass/natural polymer composites: State of the art. Materials, vol. 13, no. 23, pp. 1–38, dec. 2020.

[10] Niculescu, A.-G., Grumezescu, A. M. An up-to-date review of biomaterials application in wound management. Polymers (Basel), vol. 14, n.3, feb. 2022.

[11] Rocha Neto, J. B. M.; Lima, G. G.; Fiamingo, A.; Germiniani, L. G. L.; Taketa, T. B.; Bataglioli, R. A.; da Silveira, G. A. T.; da Silva, J. V. L.; Campana-Filho, S. P.; Oliveira Jr, O. N.; Beppu, M. M. Controlling antimicrobial activity and drug loading capacity of chitosan-based layer-by-layer films. Int J Biol Macromol, vol. 172, pp. 154–161, mar. 2021.

[12] Marques, C. F. Desenvolvimento de filmes assimétricos de quitosana. Universidade Federal do Rio Grande do Sul, Porto Alegre, 2023.

[13] Taketa, T. B. Obtenção e caracterização de recobrimentos de quitosana/ácido hialurônico e quitosana/alginato de sódio pela técnica layer-by-layer para aplicações antimicóticas. Universidade Estadual de Campinas, Campinas, 2013.