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ORIGINAL ARTICLE

Lysozyme-Mediated Degradation and Mucoadhesive Evaluation of Low-Molecular- Weight Chitosan for Dental Applications

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ABSTRACT
Chitosan has emerged as a global scientific interest due to its bulk availability and unique biomedical and dental properties with less toxicity. Low-molecular-weight chitosan (LMWC) is generally used for dental and biomedical applications due to its biocompatibility, biodegradability and mucoadhesive nature. The present study deals with the lysozyme-mediated biodegradation and mucoadhesive evaluation of low-molecular-weight chitosan. Chitosan samples were incubated with lysozyme at 37°C for 7 and 15 days. Degradation was analysed by the weight loss method and structural modifications were confirmed using Fourier-transform infrared spectroscopy (FTIR). Mucoadhesive behaviour was assessed using mucin interaction studies. LMWC showed progressive degradation with 12.20 ± 1.00% degradation on day 7 and 18.97 ± 0.65% on day 15. FTIR analysis demonstrated a reduction in peak intensity and structural changes in the glycosidic linkage region after enzymatic treatment. Mucoadhesive capacity increased from 23.1% to 74.3% with increasing concentration, indicating strong interaction with mucin glycoproteins. These findings suggested that low molecular weight chitosan has biodegradation and mucoadhesive properties, supporting its potential application as a biodegradable dental biomaterial.

Keywords: *Low-molecular-weight chitosan, Lysozyme, Biodegradation, Mucoadhesion, FTIR.*

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INTRODUCTION

Natural biomaterials such as chitosan, collagen, hyaluronic acid etc., are serving as promising alternatives in dentistry for controlling oral diseases. Chitosan has considerable role in dental and biomedical research because of its biodegradability, biocompatibility, less toxic and mucoadhesive properties [1, 2]. Chitosan is a naturally-available polysaccharide obtained through deacetylation of chitin, which contains amino and hydroxyl groups responsible for its various biological activities [3]. Compared to high-molecular-weight chitosan (HMWC), low-molecular-weight chitosan (LMWC) exhibits better solubility, enhanced penetration and improved biological interaction, making it appropriate for oral applications [4, 5]. In dentistry, biomaterials should possess controlled biodegradation, adequate retention and compatibility with oral tissues [6]. Low-molecular-weight chitosan has been investigated for periodontal therapy, oral drug delivery, wound healing and antimicrobial properties [7]. Its cationic nature promotes interaction with negatively charged mucosal surfaces and bacterial cell walls, thereby improving retention and therapeutic efficiency [8].

Lysozyme is a hydrolytic enzyme naturally there in saliva and oral tissues [9]. It plays an important role in the degradation of different polysaccharides and contributes to innate oral defence mechanisms [10]. Evaluation of lysozyme-mediated degradation helps to understand the stability and behaviour of biomaterials [11]. Controlled biodegradation is essential for temporary dental biomaterials as it reduces long-term accumulation and supports safe clinical application [12, 13]. Chitosan degradation mainly occurs through cleavage of glycosidic linkages, resulting in depolymerisation of the polymer backbone [14]. Figure 1 explains the schematic representation of biodegradability and mucoadhesive evaluation of

LMWC. In general, Fourier-transform infrared spectroscopy (FTIR) is used to analyse structural modifications associated with enzymatic degradation [15].

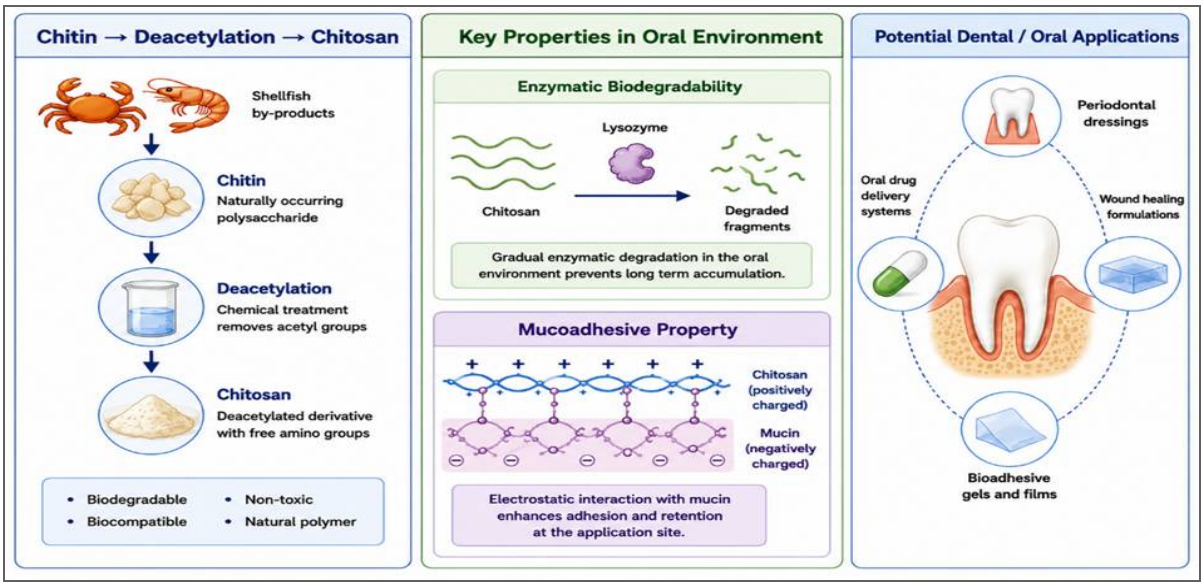

Figure 1. Schematic illustration of biodegradability and mucoadhesive evaluation of low-molecular-weight chitosan for dental applications.

Mucoadhesion is another significant characteristic for oral drug delivery and dental biomaterials [16]. Chitosan exhibits strong mucoadhesive behaviour due to its electrostatic interaction between protonated amino groups and negatively charged mucin glycoproteins [17]. Increased mucoadhesion improves retention time and therapeutic efficacy within the oral cavity [18].

Although several studies have reported the biomedical applications of chitosan, limited literature is available regarding both lysozyme-mediated degradation and mucoadhesive evaluation of LMWC. The present investigation deals with the biodegradation and mucoadhesive evaluation of low-molecular-weight chitosan using the weight loss analysis, FTIR spectroscopy and mucin interaction studies.

MATERIAL AND METHODS

Low-molecular-weight chitosan was available commercially (HiMedia, India) which was prepared using shrimp cells.

Lysozyme degradation assay

The lysozyme degradation of chitosan was measured by following the method described by Lončarević et al. [11] and Reay et al. [19] with slight modifications. LMWC was dissolved in 1% acetic acid to prepare a stock solution (10 mg/mL). Lysozyme solution (1 mg/mL) was prepared in phosphate-buffered saline (PBS, pH 7.4). Two millilitres of chitosan solution were mixed with 10 mL of lysozyme solution and incubated at 37°C under gentle shaking conditions. Degradation was evaluated after 7 and 15 days. Samples were centrifuged at 3500 rpm for 20 minutes, and the residual chitosan pellet was dried at 40°C until constant weight was achieved.

Weight loss analysis

Percentage degradation was calculated using: Degradation (%) = (Wi -Wf)/Wi) × 100

Where:

- (Wi) = Initial weight of chitosan
- (Wf) = Final dried weight after degradation

FTIR analysis

FTIR spectroscopy was performed before and after lysozyme treatment using a Thermo Nicolet Nexus 670 spectrometer within the range of 4000-500 cm^{-1} using the modified method of Islam et al. [15]. Samples were mixed with potassium bromide and compressed into pellets for analysis. Structural changes associated with enzymatic degradation were evaluated by comparing spectral peaks.

Mucoadhesive evaluation

The mucoadhesive behaviour of low-molecular-weight chitosan was evaluated using a turbidimetric assay based on the method described by Guerini et al. [20] with slight modifications. Mucin was dispersed

in PBS (pH 7.4) and mixed with varying concentrations of low-molecular-weight chitosan. After incubation under shaking conditions, absorbance was measured at 500 nm using a UV-visible spectrophotometer.

Adhesive capacity was calculated as: Adhesive capacity (%) = $(A_m\text{-}A_c)/A_m \times 100$

Where:

- (Am) = Absorbance of mucin solution
- (Ac) = Absorbance of chitosan-mucin mixture

RESULTS AND DISCUSSION

Lysozyme-mediated degradation

Weight loss analysis

Low-molecular-weight chitosan demonstrated progressive enzymatic degradation under simulated oral conditions. The degradation percentage increased from 12.20 ± 1.00% on day 7 and increasing to 18.97 ± 0.65% on day 15, indicating gradual cleavage of the chitosan polymer backbone by lysozyme. The findings indicate that LMWC possesses controlled biodegradation behaviour, which is desirable for temporary oral biomaterials and sustained drug delivery systems.

FTIR analysis

FTIR spectra of low-molecular-weight chitosan before lysozyme treatment showed characteristic absorption bands of chitosan. A broad peak observed at 3350 cm^{-1} corresponded to O–H and N–H stretching vibrations, while the peak at 2920 cm^{-1} represented C–H stretching vibrations (Figure 2). Characteristic amide bands were observed at 1640 cm^{-1} and 1570 cm^{-1} Peaks at 1150 cm^{-1} and 1060 cm^{-1} corresponded to saccharide structure and glycosidic linkages of the chitosan backbone, while the peak near 895 cm^{-1} indicated β-glycosidic linkage vibrations.

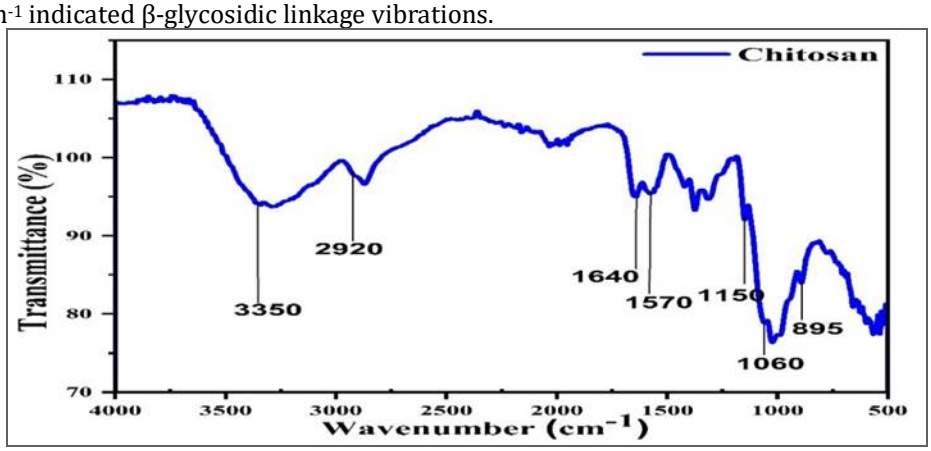

Figure 2. FTIR spectrum of low-molecular-weight chitosan showing characteristic functional groups.

After lysozyme treatment, reduction in peak intensity and slight band broadening were observed, particularly in the region between 1150 and 1000 cm^{-1} (Figure 3). Reduced transmittance at 1150 cm^{-1} and 1060 cm^{-1} indicated partial cleavage of glycosidic linkages and structural modification of the chitosan backbone following enzymatic degradation.

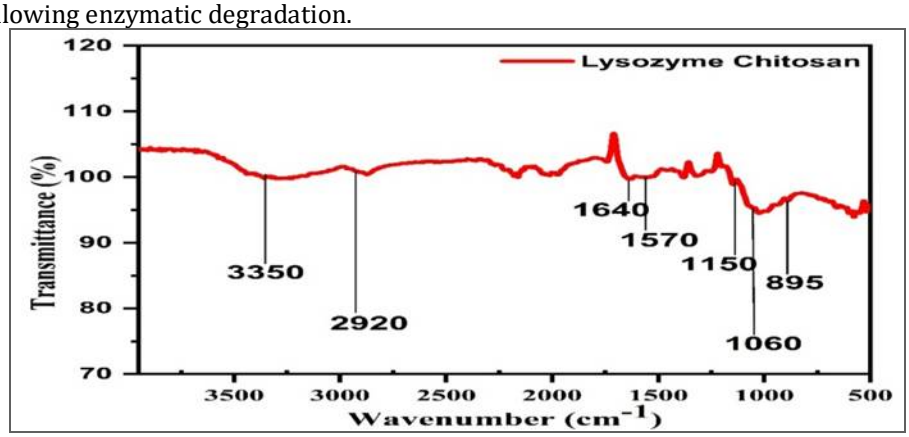

Figure 3. FTIR spectrum of lysozyme treated low-molecular-weight chitosan showing characteristic functional groups.

Minor changes in the amide region also suggested alteration of the polymer structure after lysozyme exposure. These FTIR findings confirmed lysozyme-mediated degradation of low- molecular-weight chitosan under simulated oral conditions. Overall, pattern of FTIR of LMWC before and after lysozyme degradation was mentioned in the Figure 4.

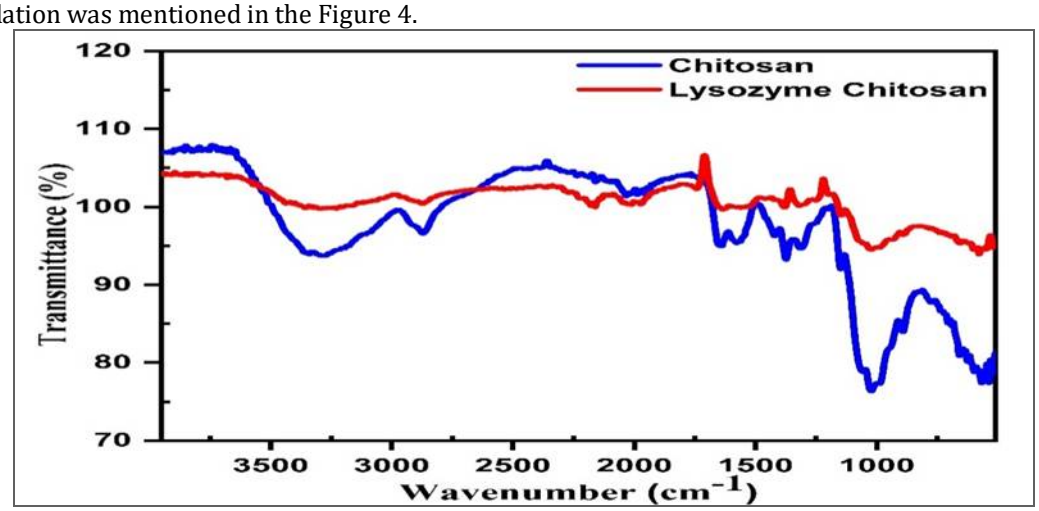

Figure 4. Overall pattern of FTIR spectra of low-molecular-weight chitosan before and after lysozyme-mediated degradation.

The current gradual lysozyme-mediated degradation of low-molecular-weight chitosan increased from day 7 to day 15, indicating gradual breakdown of the chitosan structure by lysozyme. Similar enzymatic cleavage of chitosan glycosidic linkages findings was reported by Lončarević et al. [11]. Zainol et al. [21] also reported faster degradation of irradiated chitosan membranes, with weight loss of 46%, 50% and 90% after 7–35 days due to improved lysozyme accessibility.

Mucoadhesive evaluation

Low-molecular-weight chitosan exhibited concentration-dependent mucoadhesive behaviour, with adhesive capacity increasing progressively as the chitosan concentration increased from 1 to 10 mg/mL. The adhesive capacity was 23.1% at 1 mg/mL, which increased to 27.7%, 36.9%, 45.0% and 61.9% at concentrations of 2, 3, 4 and 5 mg/mL, respectively (Figure 5). The highest adhesive capacity of 74.3% was observed at 10 mg/mL. These results indicated good interaction between chitosan and mucin glycoproteins at higher concentrations. It demonstrates low-molecular-weight chitosan's mucoadhesive potential for oral and dental applications.

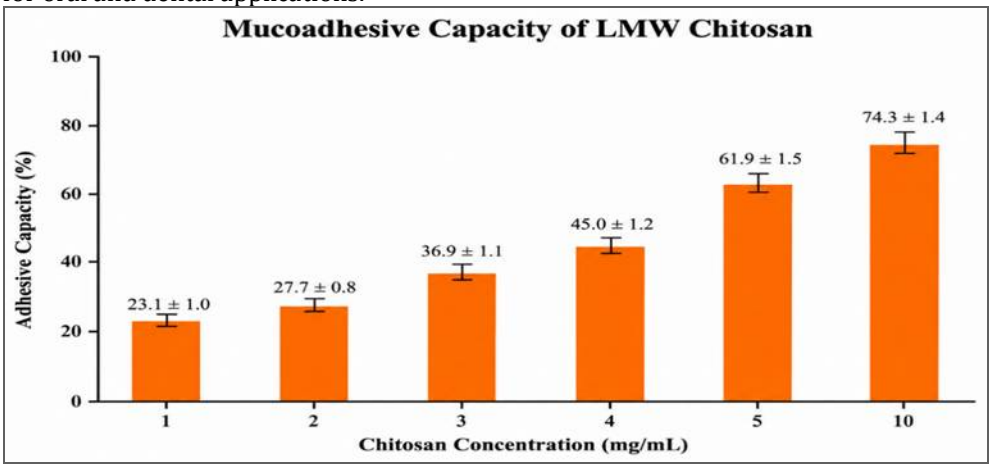

Figure 5. Bar graph representing the mucoadhesive capacity of LMW chitosan.

The concentration-dependent mucoadhesive behaviour of LMWC may be due to electrostatic and hydrogen bonding interactions between chitosan and mucin. Similar mucoadhesive behaviour was reported by Ways et al. [22] and Bernkop-Schnürch and Dünnhaupt [23] and are demonstrated mucosal adhesion and retention properties of chitosan for drug delivery applications. Increased mucoadhesion at higher concentrations may improve retention of dental formulations within the oral cavity and enhance local therapeutic effectiveness.

CONCLUSIONS

The present study demonstrated that low-molecular-weight chitosan undergoes gradual lysozyme-mediated biodegradation under simulated oral conditions. Weight loss analysis confirmed progressive degradation of the chitosan matrix over time, while FTIR analysis verified structural modification of the polymer following enzymatic treatment. LMWC also showed concentration-dependent mucoadhesive behaviour with good interaction towards mucin glycoproteins. These properties make low-molecular-weight chitosan suitable for dental applications because the material can adhere effectively to oral tissues and degrade gradually without long-term accumulation. Its biodegradability and good mucoadhesive nature support its potential use in oral drug delivery systems, periodontal therapy, wound healing materials and bioadhesive dental formulations.

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CONFLICT OF INTERESTS

Authors declared that there is no conflict of interest for this study.

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