

Design and Development of Nanocomposite of Antifungal Miconazole Drug

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Abstract: In this work, zein/casein composite nanoparticles loaded with Miconazole (Miconazole-Z/C NPs) were prepared and then embedded into a gelatin matrix to enhance the film's physicochemical performance and antifungal activity. Dynamic light scattering (DLS) analysis showed that the nanoparticles had an average diameter of 69.6 ± 1.3 nm and a zeta potential of -34.2 ± 0.9 mV. Once incorporated, the nanoparticles dispersed uniformly throughout the gelatin film and significantly strengthened its mechanical behavior—raising the tensile strength from 31.6 ± 3.3 MPa to 52.5 ± 3.1 MPa, and increasing the elongation at break from 0.226 ± 0.088 to 0.686 ± 0.104 . Serving as hydrophobic fillers, the nanoparticles also enhanced the film's water-resistance, reducing water vapor permeability to 0.336×10^{-9} g·m/m²·Pa·s and increasing the water contact angle to $123.56 \pm 1.55^\circ$. Differential scanning calorimetry (DSC), Fourier-transform infrared spectroscopy (FTIR), and scanning electron microscopy (SEM) were used to explore the interactions between the nanoparticles and the gelatin matrix. Encapsulation enabled a controlled, sustained release of Miconazole, and the release profile fit well with the Avrami kinetic model. Additionally, the resulting nanocomposite films demonstrated strong antifungal activity against *Aspergillus niger*, *Botrytis cinerea*, and *Penicillium citrinum*. Overall, the findings indicate that Miconazole-Z/C nanoparticles can act both as reinforcing nanofillers and as functional bioactive agents, enhancing the structural properties and antimicrobial performance of gelatin-based films.

Keywords: Miconazole, Antifungal, Gelatin, Nanocomposite, Zein, Casein, etc.

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Introduction

Interest in antimicrobial biopolymer films has grown substantially as the use of disposable plastics declines and consumers seek products that are safer and free from synthetic preservatives. These functional packaging materials are typically produced by incorporating antimicrobial compounds into biopolymer matrices. Various active agents—including potassium sorbate, carvacrol, citral, melanin, rosmarinic acid, thymol, and cinnamon oil—have been successfully embedded in different biopolymer films to inhibit bacteria and fungi associated with food spoilage. Despite this progress, reports on antifungal films remain limited, and most existing examples rely on polymer blends infused with essential oils ^[1-3].

Miconazole (MZ) is a broad-spectrum antifungal compound from the azole group that acts through two primary pathways in *Candida albicans*: it interferes with ergosterol biosynthesis and suppresses peroxidase activity, ultimately causing fungal cell death. Despite its effectiveness, its therapeutic application is limited by issues such as cytotoxicity, the development of resistance, and very low water solubility, which leads to inadequate dissolution. These drawbacks underscore the importance of developing advanced formulation approaches to improve the drug's physicochemical behavior, enable

more precise delivery, and minimize side effects, including hepatic toxicity and gastrointestinal discomfort ^[4, 5].

Advances in biomass-derived nanomaterials have opened new avenues for improving both the mechanical strength and functional performance of biopolymer films. Among these materials, zein-based nanoparticles stand out as promising nanofillers and delivery vehicles due to their biocompatibility, hydrophobic nature, low cost, and straightforward preparation. Incorporating such nanoparticles into films can significantly enhance physicochemical attributes. For instance, poly (ethylene oxide) films fortified with thymol- and carvacrol-loaded zein nanoparticles have shown improved structural integrity and antioxidant performance ^[6, 7].

A major drawback, however, is that unmodified zein nanoparticles tend to be unstable and prone to aggregation in aqueous environments. To address this, our research team recently developed robust composite nanoparticles derived from zein proteins for the encapsulation of Miconazole, resulting in markedly improved dispersion and stability in water. These highly stable composites show potential not only as nanocarriers for Miconazole but also as hydrophobic reinforcing agents within biopolymer matrices. To date, there appear to be no documented

studies on the use of Miconazole-loaded nanoparticles for producing antifungal biopolymer films^[8, 9].

This research investigated how incorporating Miconazole-loaded zein/casein colloidal nanoparticles (Miconazole-Z/C NPs) influences the physicochemical characteristics and antifungal performance of gelatin-based films. The films were assessed for their mechanical behavior, water vapor transmission, optical attributes, surface wettability, and structural morphology. The release profile of Miconazole from the films was also examined. In addition, the antifungal efficacy of the resulting nanocomposite films was evaluated against *Aspergillus Niger*, *Penicillium citrinum*, and *Botrytis cinerea*.

Material and Method

Materials

Miconazole was kindly supplied by Glenmark (Washi, Mumbai, India). Zein (97% purity, AR grade) was sourced from Macklin Co. Ltd. (Shanghai, China). Casein (protein content > 90%), phosphoric acid (GR grade), and sodium hydroxide (AR grade) were obtained from Aladdin (Zhejiang, China). Glycerol (AR grade) and gelatin (Bloom 100) were purchased from Xilong Chemical (Zhejiang, China). Potato dextrose agar (PDA) was acquired from TCI. The fungal strains *Aspergillus Niger* (ATCC 6275), *Penicillium citrinum* (ATCC 8506), and *Botrytis cinerea* (ATCC 12481) were supplied by Preservation Biotechnology Company (Shanghai, China).

Preparation of the Miconazole-Z/C NPs

Suspensions of Miconazole-loaded Z/C nanoparticles were produced using a modified pH-driven coprecipitation technique. Briefly, total proteins (4 mg/mL; zein and casein in a 1:1 mass ratio) and Miconazole (0.4 mg/mL) were accurately weighed and dissolved in 0.03 M NaOH under continuous magnetic stirring. The pH of this solution was then carefully brought to 7.00 ± 0.02 by adding 0.4 M phosphoric acid while maintaining stirring at 600 rpm. This process yielded a translucent, milky dispersion of the Miconazole-Z/C nanoparticles, which was subsequently stored at 4 °C for later use^[10].

Dynamic light scattering (DLS)

The mean particle diameter (MPD), zeta potential (ZP), and polydispersity index (PDI) of the Miconazole-Z/C nanoparticles were determined using a Nano-ZS90 Zetasizer (Malvern Instruments, UK). Measurements were conducted at a fixed scattering angle of 137°, with a detection wavelength of 658 nm, and at a controlled temperature of 25 °C. Prior to analysis, each sample was allowed to equilibrate for 2 minutes. All evaluations were completed in triplicate, and the reported values represent the corresponding averages^[11].

Film preparation

Gelatin was dissolved in distilled water at 60 °C to obtain a 6% (w/v) stock solution. The Miconazole-Z/C nanoparticle (NP) dispersion was prepared as previously described, containing 4 mg/mL of total protein and 0.4 mg/mL of Miconazole. To make the film-forming mixtures, predetermined proportions of the gelatin solution and the NP suspension were combined so that the final gelatin concentration remained constant at 3% (w/v). The resulting formulations contained nanoparticle (total protein) levels of 0, 0.1, 0.2, 0.6, 1.0, and 1.4 mg/mL, and the corresponding films were designated as GNP0, GNP1, GNP2, GNP3, GNP4, and GNP5. Glycerol, used as a plasticizer, was incorporated at 35% of the gelatin mass and the mixture was stirred for 1 hour. Entrapped air was removed through ultrasonication. Each film-forming mixture (35.00 ± 0.02 g) was poured into a 10 cm × 10 cm × 1 cm square Petri dish and dried in an oven at 30 °C until solidified. The finished films were conditioned for 24 hours at 25 °C and 50% relative humidity prior to characterization^[12, 13].

Film characterizations

Color parameters

The color of the films was assessed with a CR-400 colorimeter (Konica Minolta Co., Ltd., Japan) and reported in terms of L*, a*, and b* values. A calibrated white tile served as the reference standard. Each color parameter represents the average of five separate readings.

Light transparency

The transparency of the film was evaluated using a UV spectrophotometer (UV-2600, Shimadzu, Japan). A rectangular piece of film (4 cm × 0.5 cm) was fixed onto the side of a quartz cuvette, and the absorbance was recorded at 600 nm^[14]. The transparency value (TV) of the films was then determined using the following formula:

$$TV = \frac{-\log(T_{600})}{x}$$

Where x represents the film thickness in mm, and T600 represents the fractional transmittance at a wavelength of 600 nm.

Mechanical properties

The mechanical properties of the films, including tensile strength (TS) and elongation at break (EAB), were evaluated using a texture analyzer (TA.XT Plus, Stable Micro Systems, England). Film samples were cut into pieces measuring 15 × 70 mm for testing. Each sample was secured on the grips with an initial distance of 50 mm between them. Tests were performed at a crosshead speed of 200 mm/min, with five replicates conducted for each film. Film thickness was determined using a digital micrometer (Sanhe, China) with a precision of 0.001 mm. Measurements

were taken at five random points on each film, and the mean value was recorded ^[15, 16].

Water vapor permeability

The water vapor permeability (WVP) of the films was determined following the ASTM standard, with slight adjustments. Each film sample (7.5 cm × 7.5 cm) was placed over aluminum infiltration cups filled with 40 g of silica gel and sealed securely ^[17]. The cups were then stored in a desiccator containing distilled water at 25 °C, and their weight was recorded every hour for a duration of 8 hours. The WVP was subsequently calculated using the measured data.

$$WVP = \frac{wL}{At\Delta p}$$

Where w is the weight increase (g), L is the thickness of the film (m), A is the exposed area of the film (m²), t is the time (h), and Δp is the difference in partial pressure.

Water contact angle

The water contact angle (WCA) of the films was determined using a Theta Lite Optical Tensiometer (Attension Theta, Biolin Technologies, Sweden) following a slightly adapted method. Film samples measuring 15 mm × 40 mm were secured on a glass slide and placed on the instrument stage. A 7 μl droplet of distilled water was carefully applied to the film surface, and the contact angle was recorded. Images of the droplet were captured over 10 seconds at a rate of 10 frames per second, and the droplet profile was digitally analyzed to obtain measurements. Each film was tested at six randomly chosen points, and the mean value was calculated ^[18, 19].

Fourier-transform infrared spectroscopy

The films' infrared spectra were recorded using a Nicolet iS50 FTIR spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) fitted with an attenuated total reflection (ATR) crystal. Measurements were taken in the range of 600–4000 cm⁻¹ at a resolution of 4 cm⁻¹. The film samples were positioned directly on the ATR crystal and analyzed at room temperature. Each measurement was repeated three times, and the results were reported as the average spectra ^[20].

Differential scanning calorimeter

Calorimetric measurements were conducted using a differential scanning calorimeter (DSC 250, TA Instruments, and New Castle, DE, USA). Approximately 3 mg of each film was carefully weighed and sealed in aluminum pans under a dry nitrogen atmosphere. Following equilibration at 25 °C, the samples were heated from 25 to 280 °C at a rate of 10 °C per minute. Each experiment was performed in triplicate ^[21].

Scanning electron microscopy

A Hitachi GENIMI 300 scanning electron microscope (Hitachi Co., Ltd., Japan) was employed to examine the freeze-dried Miconazole-Z/C nanoparticles and the corresponding films at an accelerating voltage of 3 kV. The Miconazole-Z/C nanoparticle suspensions were subjected to freeze-drying for 48 hours. Representative images obtained from the scanning electron microscopy (SEM) analysis are presented ^[22].

Release properties of Miconazole from the films

The release of Miconazole from the films was evaluated using a full-immersion technique. Films were cut into 3 cm × 3 cm pieces and each piece was placed inside a dialysis bag, which was then immersed in 50 mL of PBS buffer (pH 7.4) contained in a glass bottle. The bottles were maintained on a shaker at 50 rpm and a temperature of 37 ± 1 °C. Aliquots of the medium were withdrawn at predetermined time intervals, and the Miconazole concentration in the samples was measured at 280 nm using a UV spectrophotometer (UV 2600, Shimadzu, Japan) based on a previously established standard curve. The cumulative percentage of Miconazole released (Q_t %) from the films was then calculated ^[23].

$$Q_t\% = \frac{M_t}{M_0} = \frac{C_t V + \sum C_{t-1} V_s}{M_0} \times 100$$

In the equations, M_t and M₀ denote the amount of Miconazole released and its initial quantity, respectively. C_t represents the concentration of Miconazole in the release medium at a given time t, while V is the volume of the release medium.

The release kinetics of Miconazole were analyzed using Avrami's model, which can be represented in a double logarithmic form as follows:

$$\ln(-\ln(1 - Q_t)) = n \ln(k) + n \ln(t)$$

Where k and n are the Avrami release rate constant and Avrami parameter, respectively. A linear plot of ln(−ln(1 − Q_t)) versus ln(t) was used to obtain the parameters k (intercept) and n (slope).

Antifungal activities

The antifungal properties of the film samples were evaluated against three fungi: *Aspergillus niger*, *Penicillium citrinum*, and *Botrytis cinerea*, using the agar diffusion technique. Petri dishes were prepared by combining potato dextrose agar (PDA) with the respective fungal suspensions. Once the agar had solidified, 9 mm strips of the films were placed on the surface. Pure gelatin films (CK) served as the negative control. The plates were kept in the dark at 4 °C for 2 hours before being incubated. Subsequently, they were incubated at 28 °C in an MJX-160B-Z incubator (Shanghai, China) for 72 hours. The resulting plates were photographed and examined, with antifungal activity determined by measuring the diameter of the clear inhibition zones ^[24, 25].

Statistical analysis

Results are presented as the average value with the corresponding standard deviation. Statistical analysis was performed using analysis of variance (ANOVA), and differences were considered significant at a 95% confidence interval.

Result and Discussion

Characterization of the Miconazole-Z/C NPs

The DLS analysis of the synthesized Miconazole-loaded Zein/Casein (Miconazole-Z/C) nanoparticles is presented in Fig. 1(A). The nanoparticles exhibited a small size and narrow size distribution, with an average particle diameter (MPD) of 69.54 ± 1.22 nm and a polydispersity index (PDI) of 0.18 ± 0.03 . SEM images revealed that the nanoparticles were dense and roughly spherical, with diameters around 60 nm, corroborating the DLS findings. The zeta potential (ZP) of the Miconazole-Z/C nanoparticles was measured as -34.08 ± 0.83 mV, exceeding the ± 30 mV threshold, indicating that electrostatic repulsion likely contributed to their stabilization.

Figures 1B and 1C display suspensions prepared by the pH-driven method without and with casein, respectively. Zein nanoparticles prepared without casein showed poor colloidal stability and tended to aggregate, with visible sediment forming at the bottom of the container. This aggregation occurred as the pH approached the isoelectric point of zein ($\sim$ pH 6.8), promoting precipitation. In contrast, Miconazole-Z/C nanoparticles remained evenly dispersed even after 7 days of storage at room temperature. Previous studies, including our own, have demonstrated that polyelectrolyte macromolecules such as casein can effectively stabilize zein nanoparticles and prevent aggregation under neutral conditions.

In the present study, the final optimized formulation contained total protein at 4 mg/mL with a zein-to-casein ratio of 1:1, and Miconazole at 0.4 mg/mL. This Miconazole concentration is over ten times its solubility at room temperature (0.02–0.04 mg/mL), potentially enhancing its antifungal activity in solution. The combination of small size, narrow size distribution, and high colloidal stability makes these zein-casein composite nanoparticles promising candidates for use as both nanocarriers and nanofillers in biopolymer films.

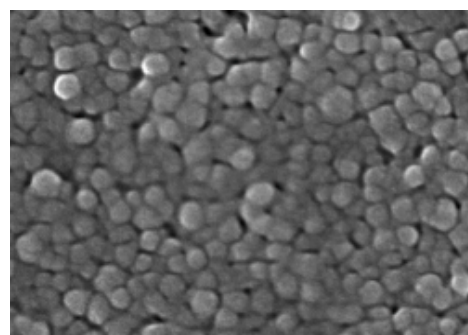
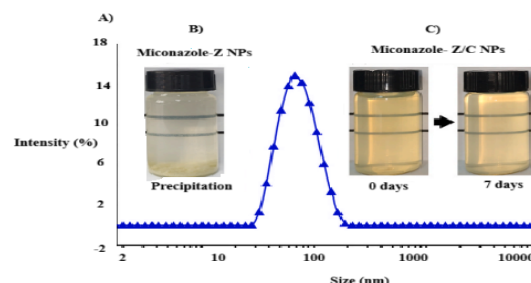


Fig. 1: Dynamic light scattering (DLS) analysis of Miconazole-loaded zein/casein nanoparticles (Miconazole-Z/C NPs) (A); images showing precipitation in freshly prepared Miconazole-loaded zein nanoparticles (Miconazole-Z NPs) colloidal solution (B) and the stable colloidal state of Miconazole-Z/C NPs after 0 and 7 days of storage (C); SEM micrograph of freeze-dried Miconazole-Z/C NPs (D)

Properties of the Miconazole-Z/C NP-incorporated gelatin films

Optical properties

The impact of nanoparticles (NPs) on the color characteristics of the films, including L^* (lightness), a^* (red-green), and b^* (yellow-blue), is summarized in Table 1. As the concentration of Miconazole-Z/C NPs increased, the L^* values decreased, indicating a reduction in film brightness. Both a^* and b^* values showed an upward trend with the addition of NPs, with b^* exhibiting a more pronounced change. Specifically, the b^* value rose from 0.89 ± 0.08 to 6.45 ± 0.08 , reflecting a gradual shift of the film color from nearly colorless to yellow. This change may be attributed to the natural yellow tint of zein and Miconazole. Incorporating natural additives into films often results in noticeable color variations compared to films without additives. Additionally, films became less transparent (higher transparency index) as NP content increased. Comparable alterations in color were reported for zein/chitosan blend films and chitosan films containing epigallocatechin gallate nanocapsules.

Table 1: The color characteristics and transparency indices of nanocomposite films were evaluated for samples containing varying

concentrations of Miconazole-loaded zein/casein nanoparticles. The samples—labeled GNP0, GNP1, GNP2, GNP3, GNP4, and GNP5—corresponded to total protein concentrations of 0, 0.1, 0.2, 0.6, 1.0, and 1.4 mg/mL, respectively

Sample s	L*	a*	b*	Transparenc y index
GNP0	88.5 9 ± 0.08a	— 0.06 ± 0.03a	0.89 ± 0.08f	0.50 ± 0.04f
GNP1	88.3 3 ± 0.08 b	— 0.14 ± 0.02 b	2.43 ± 0.03e	0.85 ± 0.08e
GNP2	88.2 2 ± 0.03c	— 0.19 ± 0.04c	3.56 ± 0.16 d	2.36 ± 0.06d
GNP3	87.8 7 ± 0.06 d	— 0.36 ± 0.06 d	4.81 ± 0.20c	3.47 ± 0.13c
GNP4	87.3 0 ± 0.08e	— 0.44 ± 0.03e	5.51 ± 0.03 b	5.52 ± 1.22b
GNP5	86.3 6 ± 0.03f	— 0.53 ± 0.04f	6.45 ± 0.08a	6.04 ± 0.66a

Values are means ± SD (n = 3). Different superscript letters in the same column indicate significant differences (p < 0.05).

Mechanical properties

Table 2 presents the thickness and mechanical characteristics, including tensile strength (TS) and elongation at break (EAB), of the nanocomposite films. Incorporating miconazole-Z/C nanoparticles (NPs) led to an increase in the gelatin film thickness,

likely due to the higher NP content. A comparable pattern has been reported in studies of chitosan/zein edible films. The TS of the films rose from 31.6 ± 3.3 MPa to 52.5 ± 3.1 MPa as the NP concentration increased from 0 to 0.6 mg/mL. Similarly, the EAB values showed a gradual rise with the addition of nanoparticles, with the highest EAB being about three times greater than that of the control. Other research has also shown that nanocomposite films generally exhibit improved TS and EAB compared with pure films. This enhancement is attributed to the formation of hydrogen bonds and/or ionic cross-linking between nanoparticles and the gelatin matrix. Proteins such as gelatin, zein, and casein contain numerous terminal carboxyl (COOH) and amino (NH₂) groups, which promote these interactions. However, when NP content is increased further (as in GNP4 and GNP5), the TS begins to decline, though it remains higher than that of pure gelatin. This reduction is thought to result from NP agglomeration and/or disruption of the gelatin's internal hydrogen bonding network. Comparable observations have been made in gelatin films incorporating tea polyphenol-loaded chitosan nanoparticles.

Table 2: The thickness, tensile strength (TS), and elongation at break (EAB) of nanocomposite films were evaluated for varying concentrations of Miconazole-loaded zein/casein nanoparticles. The samples—GNP0, GNP1, GNP2, GNP3, GNP4, and GNP5—contained 0, 0.1, 0.2, 0.6, 1.0, and 1.4 mg/mL of total protein, respectively.

Samples	TS (MPa)	EAB (%)	Thickness (mm)
GNP0	31.6 ± 3.3c	23.6 ± 8.8d	0.131 ± 0.008f
GNP1	43.9 ± 4.2b	43.2 ± 5.1c	0.152 ± 0.007e
GNP2	47.8 ± 3.4b	53.9 ± 8.8b	0.168 ± 0.005d
GNP3	52.5 ± 3.1a	59.7 ± 9.8b	0.181 ± 0.008c
GNP4	47.8 ± 4.8b	69.7 ± 7.5a	0.197 ± 0.007b
GNP5	39.6 ± 3.2bc	69.6 ± 11.4a	0.217 ± 0.005a

Values are means ± SD (n = 3). Different superscript letters in the same column indicate significant differences (p < 0.05).

Water vapor permeability (WVP)

The influence of nanoparticles (NPs) on the water vapor permeability (WVP) of gelatin films is presented in Fig. 2(a). The WVP of the control gelatin film was measured at $0.569 \times 10^{-9} \text{ g}\cdot\text{m}/\text{m}^2\cdot\text{Pa}\cdot\text{s}$. Incorporating NPs into the gelatin matrix resulted in a gradual decrease in WVP, suggesting an improvement in the film's resistance to water vapor transmission. This effect can be attributed to the presence of nanoparticles, which create a more convoluted and narrow pathway for water vapor, thereby limiting its diffusion. In addition, the hydrophobic nature of zein protein increases the barrier to water molecules, which are inherently hydrophilic. Since the inherent hydrophilicity of biopolymer films can restrict their practical applications, the results suggest that zein NPs can serve as effective hydrophobic fillers to enhance water barrier performance. Comparable improvements in moisture resistance and mechanical strength have been observed in films based on whey protein isolate when zein nanoparticles were incorporated.

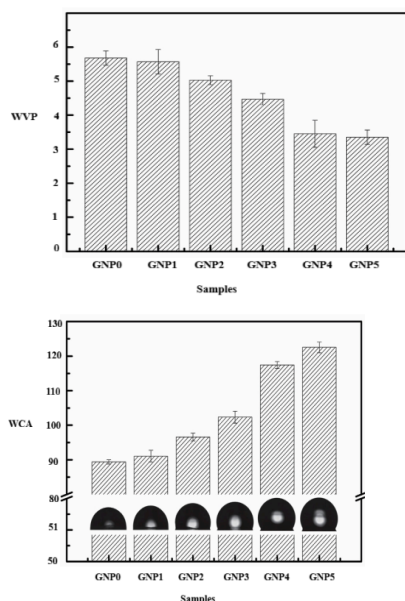


Fig. 2: The impact of incorporating varying concentrations of Miconazole-Z/C nanoparticles (GNP0–GNP5, representing 0, 0.1, 0.2, 0.6, 1.0, and 1.4 mg/mL of total protein) on the water vapor permeability (WVP) and water contact angle (WCA) of gelatin films was investigated.

Water contact angle (WCA)

Figure 2(b) illustrates the water contact angle (WCA) measurements for the various nanocomposite films. As anticipated, films incorporating Miconazole-Z/C nanoparticles exhibited higher contact angles than pure gelatin, which had a WCA of $90.05 \pm 0.68^\circ$. Increasing the concentration of Miconazole-Z/C nanoparticles led to a corresponding rise in the films' surface hydrophobicity. For instance, the GNP4 sample (1 mg/mL total protein) showed a WCA of $118 \pm 0.92^\circ$, representing an increase of nearly 32% compared to the unmodified gelatin film. Gelatin's abundance of hydrophilic groups contributes to its moisture-absorbing nature. The interaction between gelatin and zein nanoparticles limits the availability of gelatin's hydrophilic groups on the film surface. Moreover, at the air-water interface, proteins generally orient their hydrophobic regions outward, further replacing hydrophilic gelatin surface sites with hydrophobic zein groups and thereby increasing the water contact angle. Overall, the hydrophobicity of these nanocomposite films is influenced both by the interactions between the polymer and nanoparticles and by the intrinsic hydrophobic properties of the nanoparticles themselves.

Overall, the hydrophobicity of these nanocomposite films is influenced both by the interactions between the polymer and nanoparticles and by the intrinsic hydrophobic properties of the nanoparticles themselves.

Fourier-transform infrared spectroscopy (FTIR)

Figure S1 presents the ATR-FTIR spectra of pure gelatin, zein, casein, and Miconazole. Except for Miconazole, the spectra display the typical features of proteins. In gelatin, the band at 3282 cm^{-1} is associated with N-H and O-H stretching in amide A, while the signal at 2935 cm^{-1} corresponds to C-H stretching in amide B. The bands near 1634 , 1522 , and 1239 cm^{-1} are attributed to C=O stretching of amide I, N-H bending and C-N stretching of amide II, and in-plane vibrations of C-N and N-H in amide III, respectively. Similar absorption patterns were observed for zein and casein. In the case of Miconazole, distinctive peaks were identified, including 1003 and 1268 cm^{-1} for C-O-C stretching in ether groups, 1714 cm^{-1} for conjugated ester, and 1573 cm^{-1} for primary amine groups.

Figure S2 shows the ATR-FTIR spectra of gelatin films with and without nanoparticles (NPs). Because the protein bands dominate and the NP content is relatively low, the spectra of all films appear largely similar. For comparison, the spectra of gelatin powder, the film without NPs (GNP0), and the film with the highest NP loading (GNP5) are presented in Fig. 3. The amide I band shows negligible changes, suggesting that the secondary structure of gelatin remains largely unaltered during film formation. The amide II band shifts from 1522 cm^{-1} in the gelatin powder to around 1541 cm^{-1} in the films, a change more reflective of hydration effects than structural alterations. A peak at $\sim 1034 \text{ cm}^{-1}$ corresponds to glycerol, the plasticizer, while the 3282 cm^{-1} peak becomes stronger and broader due to water content in the films. These observations suggest that gelatin's amide groups engage in hydrogen bonding with glycerol and/or water, forming interconnected networks that enhance film flexibility. Overall, the findings indicate that the interactions involved in forming the composite films are predominantly physical.

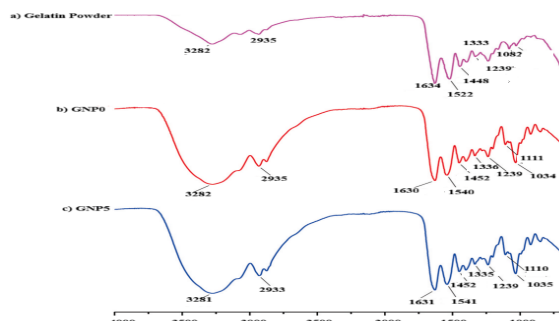


Fig. 3: FTIR spectra were recorded for (a) pure gelatin powder, (b) gelatin film without nanoparticles (GNP0), and (c) gelatin film containing the maximum concentration of nanoparticles (GNP5).

Differential scanning calorimeter (DSC)

Figure 4(a) displays the DSC thermo grams of the gelatin-based nanocomposite films. Two prominent endothermic peaks are evident in all gelatin films, appearing in the ranges of 116–126 °C and 231–251 °C. The first peak corresponds to the energy required for water evaporation from the films, consistent with observations in gelatin/chitosan nanofibre films. The second peak, observed between 230–250 °C, is mainly associated with gelatin melting, which aligns with previously reported melting peaks of 233–269 °C for gelatin-based films. Upon zooming in on the DSC profiles (Fig. 4(b)), a minor endothermic peak emerges between 40–70 °C, which can be attributed to the glass-to-rubber transition of gelatin. The glass transition temperature (T_g) increases when Miconazole-Z/C nanoparticles are incorporated, likely due to hydrogen bonding interactions between gelatin and the additives. These interactions create steric hindrance that restricts the mobility of the polymer chains, resulting in a higher T_g . Comparable findings have been reported for other gelatin-based blended films.

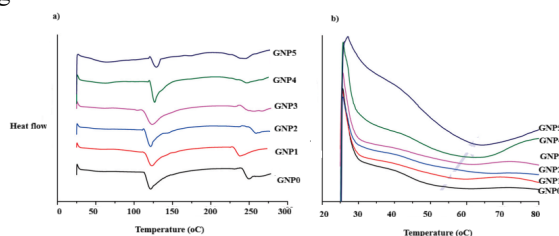


Fig. 4: (a) Differential scanning calorimetry (DSC) analysis was conducted on gelatin films containing varying amounts of Miconazole-loaded zein nanoparticles, labeled as GNP0, GNP1, GNP2, GNP3, GNP4, and GNP5, which corresponded to total protein concentrations of 0, 0.1, 0.2, 0.6, 1.0, and 1.4 mg/mL, respectively. (b) DSC profiles of the nanocomposite films were recorded within a temperature range of 20–80 °C.

Scanning electron microscopy (SEM)

The cross-sectional and surface morphologies of the nanocomposite films were analyzed using SEM, as presented in Fig. 5. The images revealed no formation of large particle clusters on either the surface or cross-section, suggesting that the zein/casein composite nanoparticles remained stable throughout the film fabrication process. The small nanoparticle size made it difficult to visualize them clearly at the standard magnification. Attempts to observe the films at higher magnification were unsuccessful, as the increased electron beam energy caused damage to the films. Overall, the Miconazole-loaded Z/C nanoparticles were evenly distributed within the film matrix and maintained a non-aggregated state during film preparation.

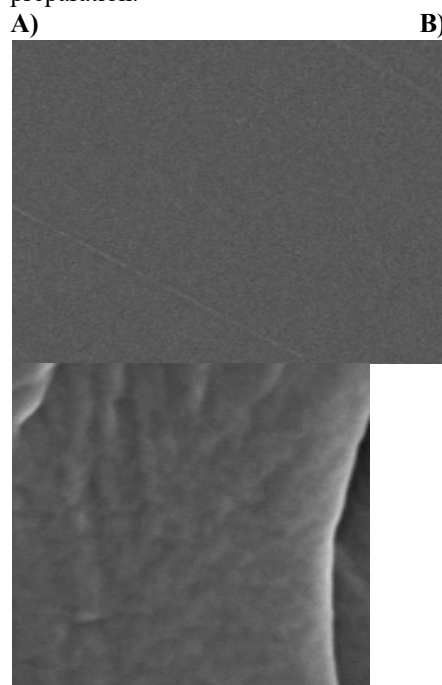


Fig. 5: The surface (A) and cross-section section (B) of the gelatin film incorporated with the Miconazole-Z/C NPs observed by SEM

Release properties of Miconazole from the films

The release behavior of Miconazole from the films is presented in Fig. 6. Compared to the film containing only Miconazole (GNATA), the nanocomposite films exhibited a slower drug release. This observation aligns with previous studies on the controlled release of hydrophobic compounds from polymeric films. Initially, we attempted to model the release data using a first-order kinetic approach, but the fit was unsatisfactory, particularly for the nanocomposite films (Table S1). The release rate was higher during the first 75 minutes and slowed down afterward. Consequently, the release profile was divided into two stages—region 1 (0–75 min) and region 2 (75–210 min)—and analyzed using the Avrami model. The

resulting parameters are summarized in Table S2. The model provided a good fit for both regions. In region 1, the Avrami exponent n was 1.07 for the GNATA film and averaged 1.56 for the nanocomposite films. An n value of 1 corresponds to first-order kinetics. These findings suggest that Miconazole release involves two mechanisms: a faster release from the surface or loosely bound drug, and a slower release from drug encapsulated deeper within the nanoparticles, which must diffuse through the swollen gelatin matrix. Additionally, strong interactions between the nanoparticles and gelatin further delayed Miconazole release. Overall, encapsulation in Z/C nanoparticles effectively enables sustained release of Miconazole from the films.

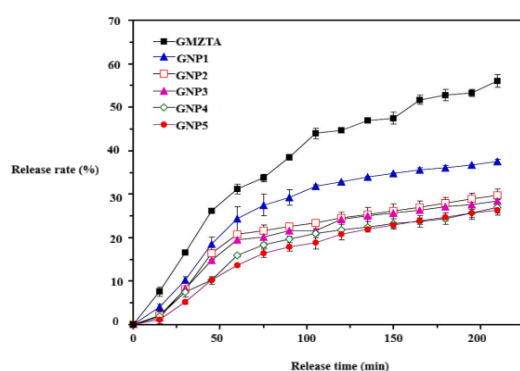


Fig. 6: The percentage of Miconazole released (Q_t %) over time (minutes) was measured for gelatin films containing pure Miconazole (GMZTA) and for films incorporating varying amounts of Miconazole-loaded zein nanoparticles. The samples—GNP0, GNP1, GNP2, GNP3, GNP4, and GNP5—correspond to nanoparticle concentrations of 0, 0.1, 0.2, 0.6, 1.0, and 1.4 mg/mL of total protein, respectively.

Antifungal activities

The antifungal effects of the plain gelatin film (CK), gelatin films containing pure Miconazole, and films with varying amounts of Miconazole-Z/C nanoparticles (GNP 3 and GNP 5) against fungi such as *Aspergillus niger*, *Botrytis cinerea*, and *Penicillium citrinum* are illustrated in Fig. 7. As expected, the plain gelatin film exhibited no antifungal properties. Films containing Miconazole demonstrated inhibitory effects against all the tested fungi. Increasing the concentration of Miconazole-Z/C nanoparticles in the films led to a corresponding increase in antifungal activity. Miconazole effectiveness is attributed to its polyene structure, which binds to ergosterol in fungal cell membranes, forming a complex that alters membrane permeability and inhibits fungal growth. Interestingly, the nanocomposite film at the highest concentration tested (GNP5) displayed antifungal activity comparable to that of the GNATA sample,

suggesting that encapsulating Miconazole within Z/C nanoparticles does not diminish its antifungal efficacy. Previous studies have also shown that incorporating Miconazole into films enhances their antimicrobial properties. For instance, polyethylene films containing Miconazole inhibited the growth of *Aspergillus niger*, while edible starch films loaded with Miconazole and nisin suppressed yeast and mold growth and improved the microbial stability of Salut cheese. In the present study, nanoparticle encapsulation not only ensures uniform distribution of Miconazole throughout the gelatin films but also improves their mechanical strength.

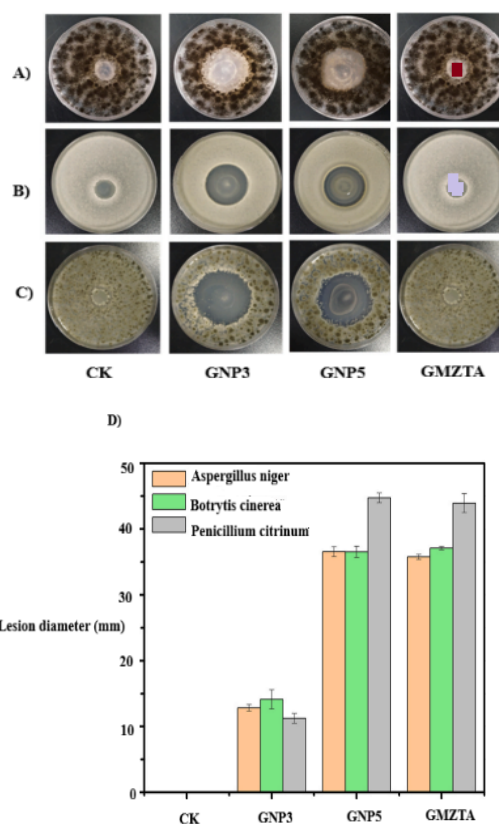


Fig. 7: Antifungal effects were evaluated for the control (CK) and various nanocomposite films, including GNP3 (containing 0.6 mg/mL total protein and 0.06 mg/mL Miconazole), GNP5 (with 1.4 mg/mL total protein and 0.14 mg/mL Miconazole), and GMZTA (a film supplemented with 0.14 mg/mL Miconazole suspension without nanoparticle encapsulation) against three fungal species: (A) *Aspergillus niger*, (B) *Botrytis cinerea*, and (C) *Penicillium citrinum*. The lesion diameters produced by each treatment on the fungi are shown in (D)

Conclusion

In this research, zein/casein nanoparticles loaded with Miconazole were successfully embedded into gelatin

films to enhance their physical, chemical, and antifungal characteristics. The zein/casein nanoparticles exhibited stability and improved the dispersion of Miconazole in water. Acting as effective hydrophobic nanofillers, the nanoparticles strengthened the gelatin films by increasing tensile strength, elongation at break, water resistance, and surface hydrophobicity. Encapsulation of Miconazole within the nanoparticles allowed for its controlled, sustained release from the films. Additionally, gelatin films containing the Miconazole-loaded nanoparticles demonstrated enhanced antifungal activity in vitro against *Aspergillus niger*, *Botrytis cinerea*, and *Penicillium citrinum*. These results suggest that gelatin-based nanocomposite films could serve as promising antifungal edible films for food packaging applications.

References

1. Ahammed, S., Liu, F., Khin, M. N., Yokoyama, W. H., & Zhong, F. Improvement of the water resistance and ductility of gelatin film by zein. *Food Hydrocolloids*. 2020; 105, Article 105804.
2. Alias, S. A., & Sarbon, N. M. Rheological, physical, and mechanical properties of chicken skin gelatin films incorporated with potato starch. *Npj Science of Food*. 2019; 3: 26.
3. Bernardi, A. O., Garcia, M. V., & Copetti, M. V. Food industry spoilage fungi control through facility sanitization. *Current Opinion in Food Science*. 2019; 29: 28–34.
4. Casanova, F., Mohammadifar, M. A., Jahromi, M., Petersen, H. O., Sloth, J. J., Eybye, K. L., et al. Physico-chemical, structural and techno-functional properties of gelatin from saithe (*Pollachius virens*) skin. *International Journal of Biological Macromolecules*. 2020; 156: 918–927.
5. Dai, L., Zhou, H., Wei, Y., Gao, Y., & Mc Clement, D. J. Curcumin encapsulation in zein-rhamnolipid composite nanoparticles using a pH-driven method. *Food Hydrocolloids*. 2019; 93:342–350.
6. Deng, L., Kang, X., Liu, Y., Feng, F., & Zhang, H. Characterization of gelatin/zein films fabricated by electrospinning vs solvent casting. *Food Hydrocolloids*. 2018; 74:899.
7. Eghbal, N., Dumas, E., Yarmand, M. S., Mousavi, M. E., Oulahal, N., & Gharsallaoui, A. Antimicrobial films based on pectin and sodium caseinate for the release of antifungal natamycin. *Journal of Food Processing and Preservation*. 2019; 43: 13953.
8. Fang, S., Zhao, X., Liu, Y., Liang, X., & Yang, Y. Fabricating multilayer emulsions by using OSA starch and chitosan suitable for spray drying: Application in the encapsulation of β -carotene. *Food Hydrocolloids*. 2019; 93: 102–110.
9. Galus, S., & Lenart, A.. Development and characterization of composite edible films based on sodium alginate and pectin. *Journal of Food Engineering*. 2013; 115: 459–465.
10. Grafia, A. L., Vazquez, ' M. B., Bianchinotti, M. V., & Barbosa, S. E. Development of an antifungal film by polyethylene surface modification with natamycin. *Food Packaging and Shelf Life*. 2018; 18: 191–200.
11. Jridi, M., Abdelhedi, O., Salem, A., Kechaou, H., Nasri, M., & Menchari, Y. Physicochemical, antioxidant and antibacterial properties of fish gelatin-based edible films enriched with orange peel pectin: Wrapping application. *Food Hydrocolloids*. 2020; 103: 105688.
12. Karim, M., Fathi, M., & Soleimani-Zad, S. Incorporation of zein nanofibers produced by needleless electrospinning within the casted gelatin film for improvement of its physical properties. *Food and Bioproducts Processing*. 2020; 122: 193–204.
13. Liang, J., Yan, H., Zhang, J., Dai, W., Gao, X., Zhou, Y., et al. Preparation and characterization of antioxidant edible chitosan films incorporated with epigallocatechin gallate nanocapsules. *Carbohydrate Polymers*. 2017; 171: 300–306.
14. Lin, M., Fang, S., Zhao, X., Liang, X., & Wu, D. Natamycin-loaded zein nanoparticles stabilized by carboxymethyl chitosan: Evaluation of colloidal/chemical performance and application in postharvest treatments. *Food Hydrocolloids*. 2020; 106: 105871.
15. Ma, W., Tang, C. H., Yin, S. W., Yang, X. Q., Wang, Q., Liu, F., et al. Characterization of gelatin-based edible films incorporated with olive oil. *Food Research International*. 2012; 49: 572–579.
16. Mazzarino, L., Borsali, R., & Lemos-Senna, E. Mucoadhesive films containing chitosan-coated nanoparticles: A new strategy for buccal curcumin release. *Journal of Pharmaceutical Sciences*. 2014; 103(11): 3764–3771.
17. Nieto-Suaza, L., Acevedo-Guevara, L., S' anchez, L. T., Pinzon, ' M. I., & Villa, C. C. Characterization of Aloe vera-banana starch composite films reinforced with curcumin-loaded starch nanoparticles. *Food Structure*. 2019; 22: 100131.
18. Oll'e Resa, C. P., Gerschenson, L. N., & Jagus, R. J. Starch edible film supporting natamycin and nisin for improving microbiological stability of refrigerated argentinian Port Salut cheese. *Food Control*. 2016; 59:737–742.
19. Pan, K., & Zhong, Q. Low energy, organic solvent-free co-assembly of zein and caseinate to prepare stable dispersions. *Food Hydrocolloids*. 2016; 52:600–606.
20. Roy, S., & Rhim, J. W. Preparation of carrageenan-based functional nanocomposite films incorporated with melanin nanoparticles. *Colloids and Surfaces B: Biointerfaces*. 2019; 176: 317–324.

21. Saez-Orviz, ' S., Marcet, I., Weng, S., Rendueles, M., & Díaz, M. PLA nanoparticles loaded with thymol to improve its incorporation into gelatine films. *Journal of Food Engineering*. 2020; 269: 109751.
22. Shankar, S., Wang, L. F., & Rhim, J. W. Effect of melanin nanoparticles on the mechanical, water vapor barrier, and antioxidant properties of gelatin-based films for food packaging application. *Food Packaging and Shelf Life*. 2019; 21: 100363.
23. Umaraw, P., Munekata, P. E. S., Verma, A. K., Barba, F. J., Singh, V. P., Kumar, P., et al. Edible films/coating with tailored properties for active packaging of meat, fish and derived products. *Trends in Food Science & Technology*. 2020; 98: 10–24.
24. Wang, W., Xiao, J., Chen, X., Luo, M., Liu, H., & Shao, P. Fabrication and characterization of multilayered kafirin/gelatin film with one-way water barrier property. *Food Hydrocolloids*. 2018; 81: 159–168.
25. Zhang, L., Liu, Z., Wang, X., Dong, S., Sun, Y., & Zhao, Z. The properties of chitosan/zein blend film and effect of film on quality of mushroom (*Agaricus bisporus*). *Postharvest Biology and Technology*. 2019; 155: 47–56.