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Effect of Tween 20 as Plasticizer on Cinnamyl Chitosan Membranes: Preparation, Characterization and Antimicrobial Evaluation

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IN recent decades, biopolymers have received considerable attention due to their renewability, broad availability and biodegradability. Scientists concern with antimicrobial biopolymers as an alternative to petroleum-derived polymers for implementing in several substantial applications such as food packaging, seeds preservations, wound dressing, cell culture and tissue engineering.

In the current study, cinnamyl chitosan Schiff base membranes were prepared using different amounts of tween 20 (T_{20}) as a plasticizer. A comparative study was carried out to explore the effect of T_{20} on the prepared membranes, including their physical, surface morphologies, optical and mechanical properties. In addition, the antibacterial activities were also investigated against five bacterial strains (two Gram-positive: *Streptococcus pyogenes* and *Staphylococcus aureus* & three Gram-negative: *Proteus vulgaris*, *Pseudomonas aeruginosa*, and *Shigella* sp.). The gained results revealed higher antibacterial activities for cinnamyl chitosan membrane compared to native chitosan membrane. Moreover, treatment of cinnamyl chitosan with different concentrations of T_{20} significantly enhanced the antibacterial activities of the membranes. Almost various concentrations of T_{20} showed identical trends of antibacterial activities towards the entire bacterial strains.

Keywords: Plasticization, Cinnamyl chitosan, Membranes, Tween 20, Antibacterial.

Introduction

Recently, much attention has been awarded to substitute petroleum-based plastics with biodegradable biomaterials. Biopolymers have been recognized as the most promising materials for this purpose. Although they usually exhibit limit mechanical properties regarding formability and end-use application, they display a fragility and brittleness during thermo-formation that restrict their potential for use. To overcome this problem, plasticizers are used to enhance the characteristic of biopolymers.

Plasticizers are group materials with low molecular weight and non-volatile compounds that are broadly applied in polymer industries as additives [1]. The primary function of such materials is to improve the elasticity and processability of polymers by lowering the second-order transition temperature, the glass transition temperature (Tg). These compounds overcome hardness, the tension deformation, density, viscosity and electrostatic charge of polymers, also, increasing the polymer flexibility, dielectric constant and resistance to fracture [2]. Properties such as the degree

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of crystallinity, electric conductivity, optical clarity, fire behaviour and resistance to biological degradation and other physical properties were also affected [3].

Chitosan is a biodegradable polysaccharide obtained naturally by some fungi [4, 5] and commercially by chemical deacetylation of chitin [6, 7]. The unique properties of chitosan can be attributed to its basic character. Chitosan was implemented in diverse applications such as water treatments [8], pharmaceutical applications [9, 11], antioxidant [12-14] antimicrobial [15-20] and biomedical applications [21-25]. Chemically, chitosan has different functional groups such as hydroxyl and amine groups regularly distributed along its backbone. Furthermore, the presence of various functional groups simplifies its chemical transformation and preparation of several chitosan derivatives. One of the simple reactions is a coupling of its amine groups with an aldehyde to form the corresponding Schiff base [16, 17]. Consumption of amine groups in Schiff base formation influence the mechanical and physical properties of chitosan.

In the current research, $T_{20}$was used as a plasticizer for the fabrication of cinnamyl chitosan Schiff base membranes. Impacts of T_{20} on Physical, mechanical and antibacterial properties of membranes were studied.

Experimental

Materials

Chitosan was prepared and purified in polymer department, ATNMRI, SRTA City, Alexandria (Egypt). Cinnamaldehyde (Purity 98%, M.W.132) was purchased from Scharlau, Spain. Tween 20 was supplied by Sigma Aldrich, Germany (Purity 98%, M.W.1228).

Bacterial strains

Five bacterial strains were utilized for evaluating the antibacterial activities of chitosan and modified chitosan. These strains were obtained from Genetic engineering and biotechnology research institute, Alexandria, Egypt. The tested bacterial strains included three Gram-negative strains (*Pseudomonas aeruginosa, Proteus vulgaris, Shigella*sp.) and two Gram-positive (*Staphylococcus aureus, Streptococcus pyogenes*). The strains were revived via growing into Luria Britani (LB) medium (1% peptone, 0.5% yeast extract, and 1% NaCl) and incubated overnight at 37°C and 200 rpm on a rotary shaker.

Methods

Preparation of membranes

Schiff base membrane was prepared as follow: 1 g of chitosan was dissolved in 50 mL of 2% acetic acid. 0.5 mL of cinnamaldehyde was added, and the solution was stirred well. Then, the different amount (0.25, 0.5, 0.75 and 1 mL) of T_{20} was added with continuous stirring. The solution was cast into a clean Petri dish and allowed to dry at room temperature for 48 h. Once the dried membrane was separated from the Petri dish, it was rinsed with 50 mL of 0.1 M NaOH. The rinsing of the membrane with a caustic solution gives the films water-resistance by neutralizing and removing the residual acetic acid anions in the membrane. The membrane was then washed with distilled water to remove the traces of alkali and neutralize it. The wet membranes were spread out and attached to the clean glass supported by clamps and allowed to dry for 24 h at room temperature.

Characterization of membranes

Water uptake

Water uptake (%) estimation was performed by placing a weighted sample previously dried in distilled water. After 6 h, the samples were filtered off, carefully eliminate the water over the surface with a filter paper and weighed. The water uptake was calculated using the following equation:

$$\textbf{Water uptake (\%)} = (M - Mo) / Mo \times 100$$

where M is the weight of the swelled sample, and M_0 is the weight of the dry sample

Moisture content

Films were placed in the humidity chamber with humidity ratio in the range of 70-80% overnight, and then weighed before and after drying in an oven at 105 °C for 3 h. Water content was calculated as follow:

$$\textbf{Moisture content (\%)} = (M - Mo) / Mo \times 100$$

where M is the weight of the sample before drying, and M_0 is the weight of the dry sample.

Contact angle measurements

Static water contact angle measurements for the prepared membranes were performed at room temperature by using advanced Ramêhart Instrument Co. Model 500-F1, UK. At least ten droplet images were obtained for each film.

Surface roughness

The average roughness of the surface of the prepared membrane was measured using surface

roughness tester SJ- 201P, Japan. Samples were mounted on a glass slide with double-sided tape. Minimum sample dimensions were 25 mm X 25 mm. All results are the average of triplicate measurements.

Optical properties

Film colour was measured by X-Rite (Model Sp64, USA). The colourimeter was calibrated with white and black plates. A white standard colour plate for the instrument calibration was applied as a background for colour measurements of the films. The system provides the values of three colour components; L* (black-white component, luminosity), and the chromaticness coordinates, a* (+ red to − green component) and b* (+ yellow to − blue component). Colour differences ΔE* were also calculated by the following equation:

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

where $\Delta \mathbf{L}^* = \mathbf{L}^* - \mathbf{L}_0^*$, $\Delta \mathbf{a}^* = \mathbf{a}^* - \mathbf{a}_0^*$, $\Delta \mathbf{L}^* = \mathbf{b}^* - \mathbf{b}_0^*$

where L^*_0; a^*_0; b^*_0; are the colour parameter values of the standard and L*; a*; b* the colour parameter values of the sample.

Mechanical properties

A method for testing the tensile properties of the prepared membranes was conducted according to ASTM D-882 standards for paper and paper broad using a constant-rate of elongation apparatus. The tensile properties were determined using a tensile instrument (Model AG-1S Shimadzu, Japan). Membrane thickness was obtained using an electronic digital micrometre.

UV-Vis Spectroscopic analysis

The electronic absorbance of chitosan and chitosan derivative membranes were analyzed using spectrophotometer scanned from 190-600 nm.

Scanning electron microscope (SEM)

Scanning of chitosan and chitosan derivative membranes was performed using a scanning electron microscope (Model Joel Jsm 6360LA, Japan).

Antibacterial activities

Antibacterial activities of the chitosan and chitosan derivatives were measured following the previous protocol [26]. The bacteria were inoculated into LB medium and incubated at 37 °C and 200 rpm for 24 h [27]. The bacterial suspension was diluted with the same medium solution 10 times. 0.1 mL of diluted bacteria suspension was cultured into 10 mL of LB medium, which contains 0.1 mL of chitosan solution that sterilized at 121 °C for 20 min. The inoculated medium was maintained at 37 °C for 18 h with shaking. Bacterial turbidity was measured using the UV-visible spectrophotometer at 620 nm. Growth Inhibition Percent (%) was calculated according to the following equation:

$$\textbf{Growth inhibition percent} = (1 - (A / A_0)) * 100$$

where A and A_0 is absorbance of bacterial growth in the presence and absence of tested polymer.

Results and Discussion

Physico-chemical characterization

wettability test

Water uptake of the chitosan, cinnamyl chitosan and plasticized membranes with Tween 20 was measured and recorded as shown in Table 1. There is a definite decrease in water uptake of cinnamyl chitosan membrane in comparison with chitosan films that can be attributed to the coupling of amine groups with highly hydrophobic cinnamyl groups. By adding the smallest concentration of T_{20} (Cin. $Cs.T_{20}$–0.25), water uptake of the membrane decreased that can be explained by the role of T_{20} in the emulsification of cinnamaldehyde in the casting solution. This emulsification process ameliorated the interaction between chitosan amine groups and its aldehyde groups. The continuous increase of T_{20} content to the membranes accumulated in the generated membranes and boost its hydrophilicity.

The same behaviour was monitored by analyzing the results of moisture content. Presence of hydrophilic groups such as hydroxyl and amine groups along polymer backbone give membranes the ability to trap moisture molecules from the surrounding atmosphere. Conversely, the existence of hydrophobic groups like cinnamyl or hydrophilic molecules like T_{20} can alter the moisture content.

Wettability of membranes was examined by determining the surface contact angle of water with the membrane. Obtained results

TABLE 1. Water uptake, Moisture content and contact angle measurements of chitosan and cinnamyl chitosan plasticized with different contents of T_{20}.

Sample	Water uptake (%)	Moisture content (%)	Contact angle (degree)
Cs	42.33 ± 1.945	8.66 ± 0.134	77.39 ± 0.957
Cin.Cs	35.12 ± 0.941	7.88 ± 0.235	89.77 ± 0.510
Cin.Cs.T_{20}_0.25	27.70 ± 1.498	5.95 ± 0.06	95.08 ± 0.706
Cin.Cs.T_{20}_0.5	34.51 ± 0.618	7.86 ± 0.055	92.01 ± 0.512
Cin.Cs.T_{20}_0.75	40.32 ± 0.935	8.04 ± 0.007	88.05 ± 0.87
Cin.Cs.T_{20}_1.0	42.08 ± 0.551	8.21 ± 0.096	80.4 ± 0.791

confirm the influence of T_{20} molecules on the hydrophilicity of the prepared membranes.

Surface roughness

Table 2 demonstrates the roughness of chitosan membranes, cinnamyl chitosan membranes and the membranes plasticized with different levels of T_{20}. The Interaction of chitosan with cinnamaldehyde to form Schiff base showed a significant increase in surface roughness. The action may be attributed to distortion of the internal structure of membranes by coupling with hydrophobic molecules. Moreover, the membranes exhibited smoother surface after supplementing with T_{20}. A small amount of T_{20} can emulsify the oily drops of cinnamaldehyde with chitosan and modify it to be homogenously distributed. At high concentration of T_{20}, an excess of $T_{20}$was removed from the membranes via washing that formed narrow bores.

Optical properties

The colour of the film is an essential index regarding general appearance. Table 2 presents the rectangular coordinates (L, a, and b) and the total colour difference (ΔE) of chitosan and cinnamyl chitosan Schiff base membranes plasticized with different quantities of T_{20}. Coupling of chitosan with cinnamaldehyde demonstrated a decrease in membrane brightness (ΔL) with an increase of red colour direction (Δa), yellow colour direction (Δb), and total colour difference (ΔE). Addition of T_{20} exhibited an increase of brightness and moderated improvement in (Δa), (Δb) and (ΔE) values that can elucidate the effect of T_{20} as a plasticizer. Furthermore, these findings show the potentiality of T_{20} to control the interaction between chitosan amine groups with cinnamaldehyde to have a homogenous distribution of immobilized groups.

Figure 1 exhibits the UV-vis spectra of membranes. Chitosan membranes illustrate a single peak ranged from 290-315 nm corresponding to σ-σ* transition [17,18]. on the other hand, cinnamyl, chitosan membranes showed broadening of this peak with redshift in addition to a new peak at 412 nm that corresponding of π-π* corresponding to the new bond formed –N=C of Schiff base [17,18].

SEM

Morphological structures of the prepared membranes were investigated using SEM at two magnifications (1000, and 5000X) as presented in Fig. 2. The modifications of chitosan structure by combining cinnamaldehyde make the surface of newly developed membranes rougher than chitosan. Additionally, T_{20} enahnced the membranes to possess a smoother surface. This can be indicated to the role of T_{20} in giving homogenous interaction of chitosan amine groups with cinnamaldehyde. By increase T_{20} concentration, the surface becomes rougher as a result ofT_{20}excess.

Mechanical properties

The mechanical properties of chitosan and cinnamyl chitosan plasticized with different contents of T_{20}were estimated from a critical breaking point of stretching pieces. The maximum stress σ_{max} (Nm^{-2}) was evaluated as the ratio of the stretching force divided by the cross-sectional area of broken membrane piece. The maximum strain $\lambda_{max}$was estimated as the elongation ratio of the initial length of the test piece. The results obtained are summarized in Table 3. Coupling of chitosan with cinnamaldehyde showed a decline in mechanical properties of membranes as a result of consumption of amine groups in Schiff base formation with cinnamaldehyde. The addition of T_{20} increased the efficiency of interaction between

TABLE 2. Surface roughness and color values of chitosan and cinnamyl chitosan plasticized with different contents of T_{20}.

Sample	Roughness (μm)	ΔL	Δa	Δb	ΔE
Cs	0.32 ± 0.101	23.51	6.26	7.58	26.06
Cin.Cs	0.612 ± 0.303	21.58	10.42	37.72	44.69
Cin.Cs.T20 _0.25	0.12 ± 0.152	30.31	0.93	13.93	29.72
Cin.Cs.T20 _0.5	0.164 ± 0.046	30.52	0.37	12.89	33.16
Cin.Cs.T20 _0.75	0.17 ± 0.087	30.66	0.45	10.98	32.58
Cin.Cs.T20 _1.0	0.182 ± 0.088	29.63	0.60	10.89	31.67

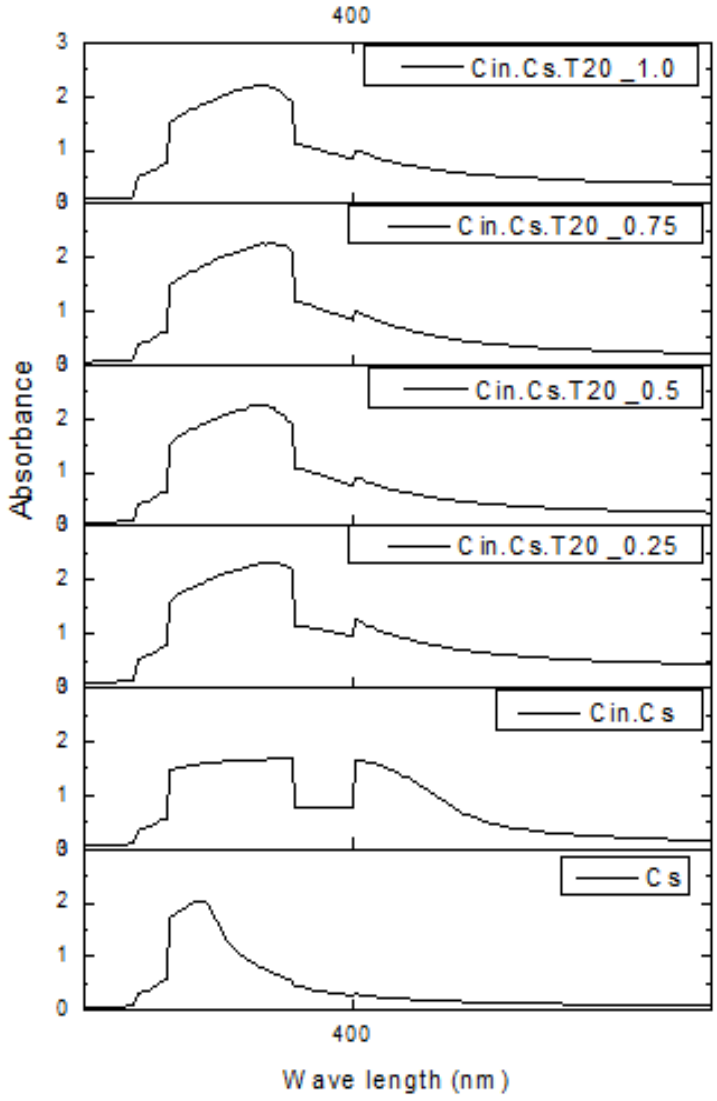

Fig. 1. UV-vis spectrum of chitosan and cinnamyl chitosan plasticized with different contents of T_{20}.

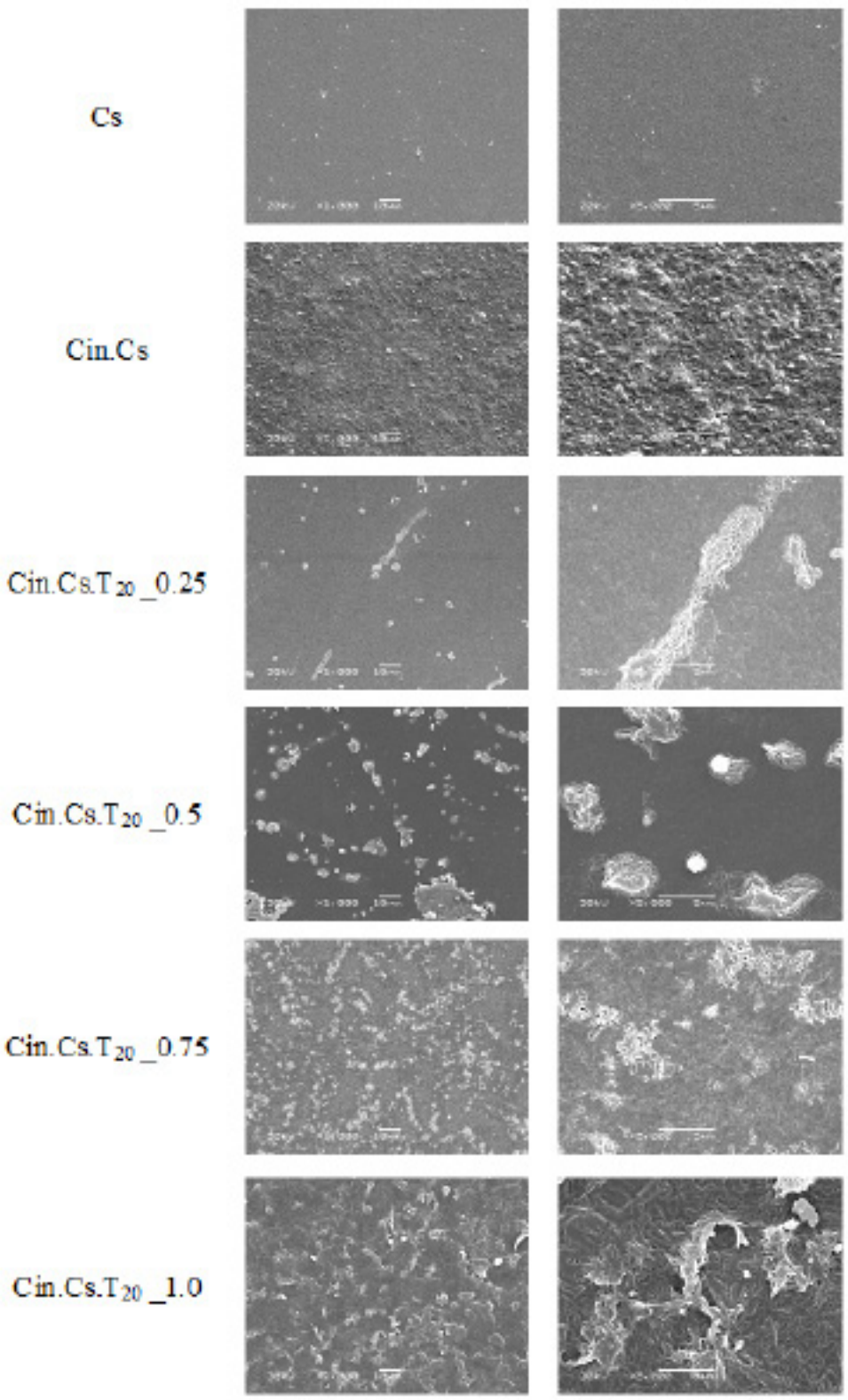

Fig. 2. SEM of chitosan and cinnamyl chitosan plasticized with different contents of T_{20}.

TABLE 3. Mechanical parameters of chitosan and cinnamyl chitosan plasticized with different contents of T_{20}.

Sample	Maximum Stress	Maximum Strain
Cs	64.08	20.53
Cin.Cs	38.28	2.18
Cin.Cs.T20 _0.25	17.36	2.388
Cin.Cs.T20 _0.5	14.51	2.496
Cin.Cs.T20 _0.75	19.25	8.27
Cin.Cs.T20 _1.0	16.43	10.307

chitosan and cinnamaldehyde via emulsification process. Control distribution of immobilization of cinnamyl group along the backbone of polymers revealed a rise in strain percent.

Antibacterial assessment of the fabricated membranes

Antibacterial activities of the fabricated films were investigated against five bacterial strains (*S. aureus*, *S. pyogenes*, *P. aeruginosa*, *P. vulgaris*, and *Shigella* sp.). Scientists concern with these pathogenic bacteria because they are responsible for many serious diseases and infections in hospitals. Most of these pathogenic bacteria can form a biofilm to escape the fatal action of antimicrobials via altering their physiological conditions for suppressing the effect of antimicrobials compounds [28, 29]. Fig. 3 shows an improvement of antibacterial activities of chitosan by boosting by cinnamaldehyde. This result is in agreement with that previously reported [30, 31]. Moreover, the antibacterial activities of cinnamyl chitosan membranes raised with the increase in T_{20}. T_{20} acts as an emulsifier for cinnamaldehyde into chitosan aqueous solution that would enhance the interaction of chitosan and cinnamaldehyde. Additionally, T_{20} not only increase the amine/

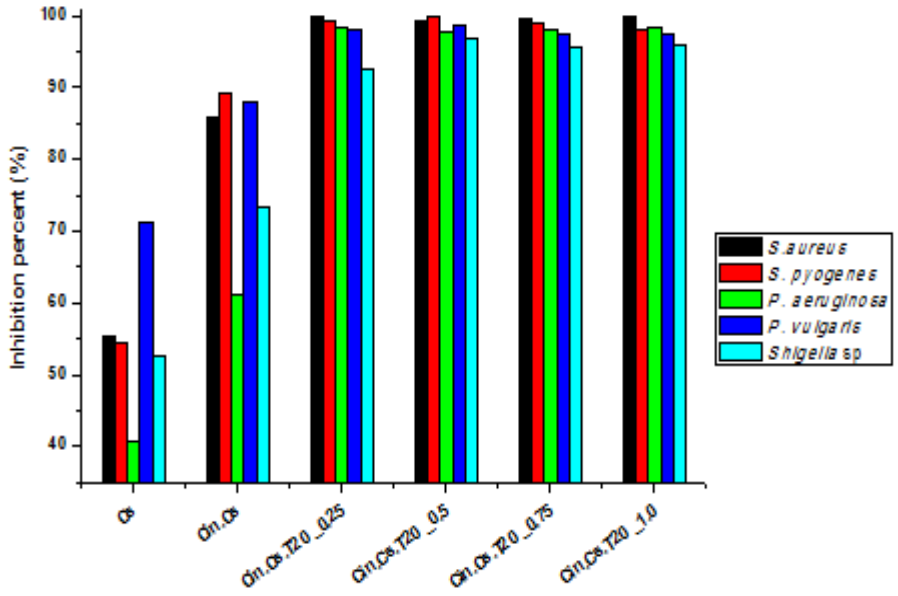

Fig. 3. Antibacterial activities of chitosan and cinnamyl chitosan plasticized with various contents of T_{20} against *S. aureus*, *S. pyogenes*, *P. aeruginosa*, *P. vulgaris*, and *Shigella* sp.

aldehyde interaction but also it may be generated homogenous distribution along with the polymer chain.The prepared membranes could be applied further as supportive materials in cell growth and proliferation [32].

Conclusion

Antibacterial cinnamyl chitosan membranes were successfully prepared using T_{20} as a plasticizer. The supplementation of cinnamyl chitosan membranes with T_{20} improved the interaction between chitosan and cinnamaldehyde that reflected on produced membranes properties. Physical and optical properties of the prepared membranes were enhanced. They can be summarized as follows:

- Hydrophilicity of membranes was evaluated through several parameters such as wettability, water uptake, and moisture content. Hydrophilicity was promoted with the increase in T_{20} contents.
- The tensile analysis showed that tensile strength enhanced by addition T_{20} portion in membranes, while all mechanical parameters were reduced by adding T_{20}.
- Roughness analysis revealed that the membranes exhibited some roughness of surface with the incorporation of cinnamaldehyde; on the other hand, it becomes smoother by adding T_{20}.
- Antibacterial activities of cinnamyl chitosan membranes supported by T_{20} were significantly augmented against the high resistant pathogenic bacteria.

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تأثيراستخدام التوين ٢٠ كملدنات على أغشية السينامبل كيتوزان: تحضير وتوصيف وتقييم التضاد الميكروبي

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في العقود الأخيرة، حظيت البوليمرات الحيوية باهتمام كبير بسبب قابليتها للتجديد وتوافرها على نطاق واسع وقابليتها للتحلل البيولوجي. اهتم العلماء بالبوليمرات الحيوية المضادة للميكروبات كبديل للبوليمرات الصناعية لاستخدامها في عدة تطبيقات حيوية مثل تغليف المواد الغذائية، والحفاظ على البذور، وضمادات الجروح، وزراعة الخلايا وهندسة الأنسجة.

في الدراسة الحالية، تم إعداد أغشية من سينامبل الكيتوزان واستخدام كميات مختلفة من توين ٢٠ (T_{20}) كملدنات. وأجريت دراسة مقارنة لتوضيح تأثير T_{20} على الأغشية المعدة، بما في ذلك الشكل البنائي و الخواص الفيزيائية والسطحية والبصرية والميكانيكية. وبالإضافة إلى ذلك، تم التحقيق في الأنشطة المضادة للبكتيريا أيضا ضد خمس سلالات بكتيرية (اثنين من إيجابية الصبغة: *Staphylococcus aureus , Streptococcus pyogene* وثلاثة سالبة الصبغة: بروتيوس الشائع، *Proteus vulgaris, Pseudomonas aeruginosa, Shigella* sp.).

وكشفت النتائج المكتسبة ارتفاع كفاءة التضاد البكتيري لاغشية السينامبل كيتوزان مقارنة مع أغشية الكيتوزان الأصلية. وعلاوة على ذلك، فإن علاج السينامبل كيتوزان مع تركيزات مختلفة من T_{20} عززت بشكل ملحوظ الأنشطة المضادة للبكتيريا لهذه الأغشية. وأظهرت زيادة تركيزات ال T_{20} ازدياد في التضاد البكتيري بنسب مختلفة بناء علي نوع السلالة البكتيرية.