CHITOSAN FILMS INCORPORATED WITH MASTIC OIL FOR POTENTIAL APPLICATION IN BIOMATERIALS: PHYSICOCHEMICAL CHARACTERIZATION

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Abstract

Chitosan is applied for novel and multifunctional biomaterials development. To enhance physicochemical and biological properties, as well as to improve the functionality of chitosan-based materials, multiple natural products are incorporated, such as essential oils. Essential oils with therapeutic potential can also be extracted from natural resins. Mastic gum, a soft resin collected mainly from *Pistacia lentiscus*, is a source of mastic oil, which is characterized by antimicrobial, antioxidant, anticancer activities, and wound healing properties. The purpose of the study was to prepare and physicochemically characterize chitosan films enriched with mastic oil and polysorbate 80 as an emulsifier. FTIR spectroscopy was used to evaluate chemical structure. Mechanical properties, swelling degree, and contact angle were also investigated. The effect of mastic oil and emulsifier on the properties of chitosan films was observed. Modified films incorporated with various concentrations of mastic oil were characterized by the hydrophilic surface, increased tensile strength, and elongation at break, thus greater flexibility as well as swelling capacity and stability in phosphate-buffered saline. These features might be an advantage in terms of material fabrication intended for biomedical applications, for example, wound healing. Chitosan and mastic oil might be a promising combination for use in biomaterials.

Keywords: *chitosan, mastic oil, polysorbate 80, biopolymer, biomaterial, film*

Introduction

Chitosan, a polycationic biopolymer derived from chitin by partial deacetylation, is a biodegradable, non-toxic, and biocompatible macromolecule. This versatile polysaccharide, consisting of D-glucosamine and N-acetyl-D-glucosamine units, is found in the exoskeleton of insects and crustaceans, but also in the cell walls of certain groups of fungi. Due to its antimicrobial activity, low immunogenicity, and susceptibility to physicochemical and biological properties modifications, chitosan can be used in the development of novel and multifunctional biomaterials in the form of films, membranes, scaffolds, particles, fibers, or gels. Chitosan has found application in generating wound healing materials, scaffolds for tissue engineering, drug delivery systems, and in cancer treatment [1-4].

To enhance the functional properties of chitosan-based materials, multiple natural products are applied. Numerous literature reports concern the enrichment of chitosan matrix with essential oils for biomedical purposes, namely wound treatment and drug delivery systems, or for the development of preservative coatings, food packaging materials, antibacterial agents, or metal corrosion inhibitors [5]. Due to the unique properties of essential oils, they have been eagerly combined with polymer-based biomaterials [6]. Essential oils can increase the antimicrobial, antioxidant, anti-inflammatory, or anticancer activities of chitosan-based systems [7-10], as well as modify their mechanical and physical properties [8,10,11]. Multiple types of essential oils were introduced to the chitosan-based materials, among others: oregano, cinnamon, thyme, lemon, clove, rosemary, and tea tree essential oils [8,9,10,12,13,14]. Some were also encapsulated into chitosan particles, e.g., clove, basil, mandarin, green tea, lemongrass, nettle, cumin, cardamon, or eucalyptus essential oils [15-17].

Essential oils with therapeutic properties can also be extracted from natural resins. Mastic gum, a soft resin collected as a liquid paste after the incision of bark and trunks mainly of *Pistacia lentiscus L*. or *Pistacia atlantica*, is rich in essential oils [18-22]. *Pistacia lentiscus* var. *Chia* is cultivated on the Greek island of Chios; however, various *Pistacia lentiscus L*. species are found in other parts of the Mediterranean area and the Middle East [23]. Mastic gum possesses anti-inflammatory, wound healing, antioxidant, antimicrobial, cardioprotective, and hepatoprotective properties confirmed by numerous studies [24, 25]. Mastic oil, extracted from mastic gum by steam or water distillation and recently by supercritical fluid extraction, has been used as a food additive, flavoring agent, and in the treatment of gastrointestinal disorders [18,19,21]. Resins collected from plants usually consist of terpenes and their derivatives [22]. Mastic oil consists of volatile terpenes, mainly aromatic monoterpenes such as α-pinene or myrcene, and terpenoids [23]. Furthermore, this essential oil also contains verbenone, linalool, α-terpineol, and camphene [26,27]. Since 2015, mastic oil has been described in the European Medicines Agency (EMA) monograph as a traditional herbal remedy for mild dyspeptic disorders and for minor wound healing and skin inflammation [19]. Mastic oil is characterized by antioxidant, anticancer, and antimicrobial activity against Gram-positive and Gram-negative bacteria [26, 28, 29]. Xanthis et al. [27] reported that mastic oil indicated indirect antioxidant activity, cytoprotective properties, and beneficial effects on cell migration and wound closure. Moreover, Maxia et al. [30] confirmed the anti-inflammatory activity of mastic oil, which contributed to the reduction of leukocyte migration to the damaged tissue. As it was reported by Spyridopoulou et al. [28], the anti-inflammatory properties of mastic oil could be ascribed to one of its constituents, i.e., β-pinene [28]. Mastic oil encapsulated into nanoparticles consisting of poly(lactic acid), poly(vinyl alcohol), and lecithin was applied for minor skin inflammation treatment [31]. Furthermore, this essential oil was investigated for functional dyspepsia treatment [32] and as an anticancer agent [19, 33, 34, 35]. Mastic gum and mastic oil can also be applied as aromatic agents and as ingredients for skin care, anti-aging, or oral cavity formulations. Besides mastic oil obtained from the resin, *Pistacia lentiscus L*. is a source of oils extracted from leaves and fruits [20,34].

The preparation of materials based on chitosan and essential oils requires the application of an emulsifier. Polysorbate 80 is a nonionic surfactant used as an emulsifier,

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solubilizer, or stabilizer in cosmetics, drugs, and food [36]. Polysorbate 80 is commonly used to emulsify essential oils due to its low cost, low toxicity, and biocompatibility. It has a high emulsification capacity and a hydrophilic-lipophilic balance (HLB) of 15. This compound improves the miscibility of essential oils with the medium, leading to their uniform distribution. Moreover, at pH values below 7 and in the presence of salt, it may provide steric stabilization, causing emulsion stability and preventing phase separation [37]. Nanoemulsion produced with essential oils and polysorbate 80 presented suitable physical characteristics and stability over time, mainly due to steric repulsion [38]. However, the effect of this surfactant on the antimicrobial activity of essential oils has not been fully recognized [39,40].

In the present study, we prepared and physicochemically characterized chitosan films enriched with various amounts of mastic oil with the addition of polysorbate 80 as an emulsifier. The purpose of the investigation was to observe the effect of mastic oil and polysorbate 80 on chitosan films' properties and determine the mastic oil concentration, which can have a beneficial effect on the properties of chitosan films in terms of their potential application in biomaterials. To the best of our knowledge, chitosan films incorporated with mastic oil have not been reported yet. The chemical structure of the obtained films was examined by FTIR spectroscopy. To investigate the mechanical properties, the following parameters: Young's Modulus, tensile strength, and elongation at break were determined. Swelling analysis was conducted in phosphate-buffered saline (PBS) with pH 7.4 for three days to assess the swelling degree of chitosan-based films. Contact angle measurements were performed for hydrophilic liquids such as glycerol to observe alterations of film surface properties after modification with selected essential oil.

Materials and Methods

Materials

To prepare and investigate biopolymeric films, following reagents were used: low molecular weight chitosan (Sigma Aldrich, St. Louis, MO, USA), mastic oil extracted from *Pistacia lentiscus* (Mustafa Enterprises, Karachi, Pakistan), polysorbate 80 (Greenaction, Kielce, Poland), acetic acid (Stanlab, Lublin, Poland), phosphate-buffered saline (PBS; Life Technologies Limited, Paisley, Scotland, UK), glycerol (Avantor Performance Materials Poland S.A., Gliwice, Poland).

Methods

Film preparation

Biopolymeric films were fabricated by the solution casting method. A 2% solution of chitosan was obtained with 0.1 Mol/L acetic acid solution. Each sample contained 25 grams of chitosan solution (CS), to which 5% of polysorbate 80 (P80) was added (by weight to biopolymer), and then various amounts of mastic oil (MO). Five samples of films were investigated, namely chitosan, chitosan with polysorbate 80 addition, and chitosan enriched with polysorbate 80 together with 1%, 2.5%, and 5% of mastic oil (by weight to biopolymer), respectively. Specimens were marked as follows: CS, CS_P80, CS_P80_MO 1%, CS_P80_MO 2.5%, CS_P80_MO 5%. Individual components of each film are summarized in Table 1. Samples were stirred on a magnetic stirrer for two hours at room temperature. The solutions (about 25 g) were poured onto the polystyrene plates (10 cm x 10 cm), and samples were placed in the drying oven. Solvent evaporation took place at a temperature of 37°C. The thickness of the films, measured by a thickness gauge, was about 0.03 mm. Films with the addition of mastic oil were smooth and uniform but slightly less transparent than pure chitosan film. Chitosan-based films are depicted in FIG. 1.

TABLE 1. Composition of chitosan-based films.

Specimen	2% chitosan solution in 0.1M acetic acid solution [g]	Polysorbate 80 [g]	Mastic oil [g]
CS	25	-	-
CS_P80	25	0.025	-
CS_P80_MO 1%	25	0.025	0.005
CS_P80_MO 2.5%	25	0.025	0.0125
CS_P80_MO 5%	25	0.025	0.025

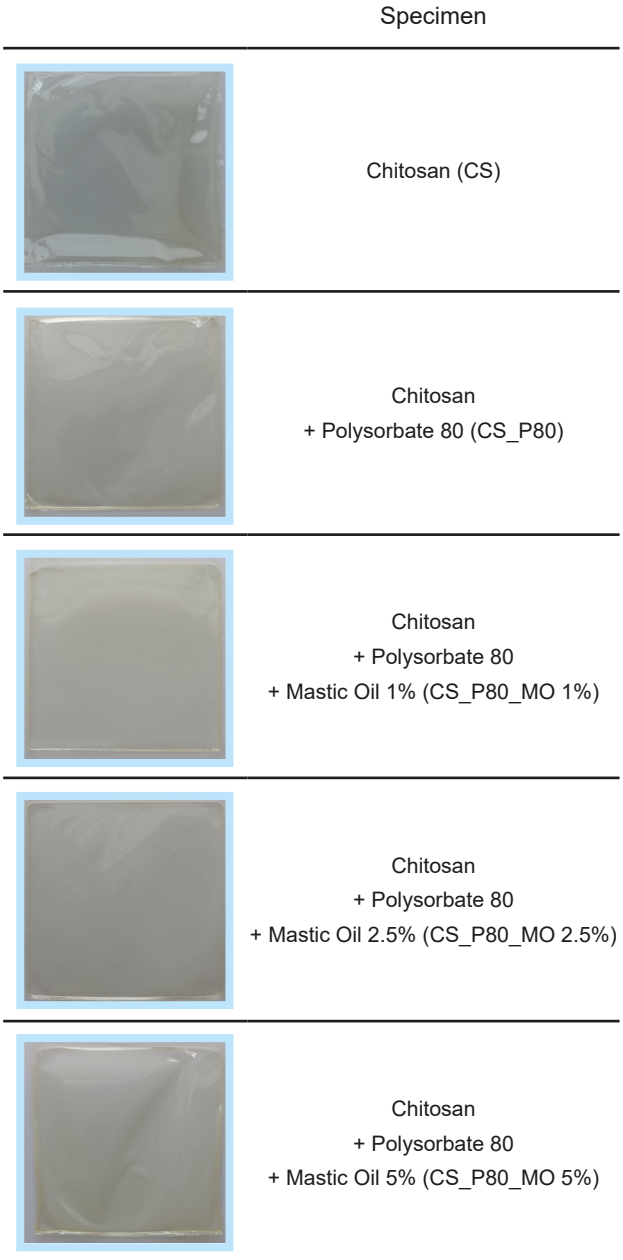

FIG. 1. Photos of chitosan-based film enriched with mastic oil and polysorbate 80.

FTIR spectroscopy

The chemical structure of chitosan-based films was assessed by ATR-FTIR spectroscopy. Spectra were registered with the following parameters: wavenumber range from 400 to 4000 cm^{-1}, 64 scans, resolution 4 cm^{-1} by Nicolet iS10 spectrometer equipped with diamond ATR accessory (Thermo Fisher Scientific, Waltham, MA, USA).

Mechanical testing

Young's Modulus (GPa), elongation at break (%), and tensile strength (MPa) of chitosan-based films were investigated by a mechanical testing machine (Z.05, Zwick and Roell, Ulm, Germany). For each film, about 10 measurements were performed. The total specimen length was 7.5 cm, and the total width was 1.25 cm, while the length and width of the analysed part of the sample were 2.5 cm and 0.4 cm, respectively. The parameters were as follows: the speed starting position 50 mm/min, the speed of the initial force 5 mm/min, and the initial force 0.1 MPa. Data were collected with the TestXpert II 2017 program. To indicate statistically significant differences between film samples, a one-way ANOVA test was used.

Contact angle measurements

A goniometer with a drop shape analyzer system (DSA 10, Krüss, Germany) was used for contact angle determination. Measurements were performed with glycerol at room temperature. For each film, about 10 measurements were done. The volume of the glycerol droplet used in the contact angle measurements was 3 µl. To indicate statistically significant differences between film samples, a one-way ANOVA test was used.

Swelling analysis

The swelling degree was examined in phosphate-buffered saline (PBS) at pH 7.4. Five samples in the shape of a square with a weight of about 0.0050g were cut for each film. Before the analysis, the samples were dried in a vacuum dryer at a temperature of 45°C. Subsequently, samples of each film were weighed and immersed in 50 mL of PBS. Polystyrene plates were placed in the laboratory incubator at a temperature of 37°C. Samples were gently dried and weighed after 1h, 2h, 8h, 24h, 48h, and 72h. To calculate the swelling degree, the following equation was used:

$$swelling = \frac{(m_t - m_0)}{m_0} \; x \; 100\% \qquad [\%]$$

m_t – weight of the material after immersion in PBS [g]
m_0 – the initial weight of the material [g]

Results

FTIR spectroscopy

To evaluate the chemical structure of prepared chitosan/polysorbate80/mastic oil films, FTIR spectroscopy was applied. Spectra of unmodified and modified chitosan films are presented in FIG. 2. Characteristic bands for chitosan were identified in the spectra, and are gathered in TABLE 2. The peak at about 1376 cm^{-1} was related to C-N stretching vibrations from acetamide groups and confirmed partial deacetylation of chitosan [14]. Similar FTIR spectra of chitosan films were registered by Shen et al. [14] and Altiok et al. [41]. For samples incorporated with additives bands assigned to -OH and -NH stretching vibrations, amide I, amide II, and C-O stretching vibrations were shifted to higher wavenumbers, while bands assigned to -CH stretching vibrations were altered to lower wavenumbers. In the spectra of mastic oil-enriched specimens, peaks at wavenumbers equal to about 1450 cm^{-1} and 2920 cm^{-1} occurred, which are ascribed to bending vibrations of $-CH_2/CH_3$ groups, and C-H stretching vibrations from $-CH_2/CH_3$ groups, respectively. These peaks are characteristic of mastic resin [22].

Mechanical testing

Young's Modulus, tensile strength, and elongation at break parameters were determined to evaluate the effect of mastic oil and polysorbate 80 on chitosan films. Results are presented in FIGs 3-5. As can be seen, both additives modified the mechanical properties of chitosan films. For

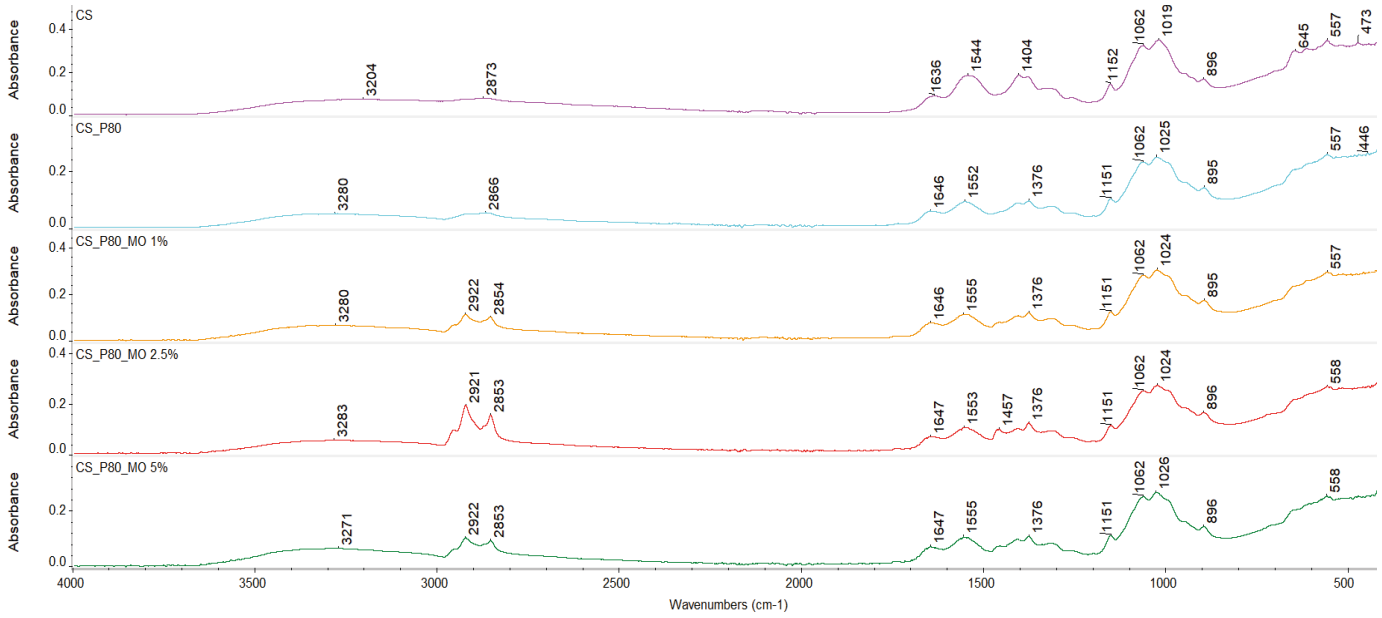

FIG. 2. FTIR spectra for chitosan-based films.

TABLE 2. Wavenumbers of characteristic bands for unmodified and modified chitosan films.

Specimen	Characteristic bands [cm^{-1}]									
	-OH -NH stretch	C-H stretch	Amide I	Amide II	CH_2 bend	C-N stretch	Amide III	C-O-C bridge asymmetric stretch	C-O stretch/ C-O-C asymmetric stretch	C-O stretch/ C-O-C symmetric stretch
CS	3204	2873	1636	1544	1404	1378	1258	1152	1062	1019
CS_P80	3280	2866	1646	1552	1405	1376	1258	1151	1062	1025
CS_P80_MO 1%	3280	2922/2854	1646	1555	1405	1376	1258	1151	1062	1024
CS_P80_MO 2.5%	3283	2921/2853	1647	1553	1405	1376	1258	1151	1062	1024
CS_P80_MO 5%	3271	2922/2853	1647	1555	1405	1376	1258	1151	1062	1026

samples with only polysorbate 80 addition and for samples enriched with emulsifier and mastic oil, increased tensile strength, elongation at break, and Young's Modulus (except the sample with 5% mastic oil addition) were observed in comparison to the pure chitosan specimen. The sample with the addition of emulsifier and the sample incorporated with 1% of mastic oil presented similar values of mechanical parameters. However, the addition of 2.5% of mastic oil significantly increased elongation at break, and the highest tensile strength among all specimens was noticed. A 5% addition of mastic oil led to the lowest Young's Modulus among all samples, the lowest tensile strength among all modified samples, while this sample was characterized by the highest elongation at break and flexibility.

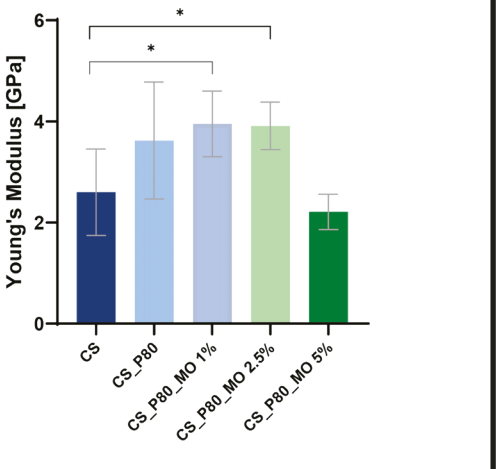

FIG. 3. Young's Modulus for chitosan-based films (statistically significant differences vs. CS sample are indicated as * p < 0.05).

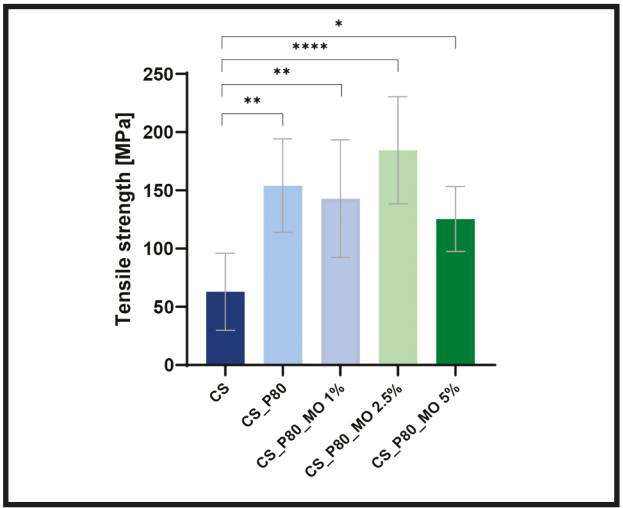

FIG. 4. Tensile strength for chitosan-based films (statistically significant differences vs. CS sample are indicated as * p < 0.05, ** p < 0.01, **** p < 0.0001).

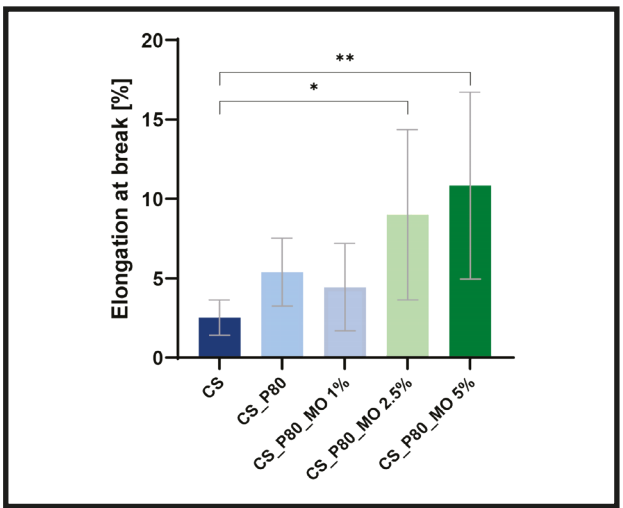

FIG. 5. Elongation at break for chitosan-based films (statistically significant differences vs. CS sample are indicated as * p < 0.05, ** p < 0.01).

Contact angle

Contact angle determination enables the evaluation of a sample's surface properties, its superficial hydrophilicity/hydrophobicity, and wettability [42]. Contact angle measurements were performed with glycerol, and results are presented in TABLE 3. Unmodified chitosan film was characterized by a hydrophilic nature, while the addition of polysorbate 80 caused an increase in contact angle, which was higher than 90°, thus the surface was hydrophobic. However, the addition of mastic oil to chitosan/polysorbate 80 films resulted in a decrease in contact angle; these films possessed hydrophilic surfaces. Furthermore, samples incorporated with emulsifier and mastic oil in concentrations of 2.5% and 5% displayed the lowest values of this parameter among all specimens.

TABLE 3. Contact angle results for chitosan-based films performed with glycerol (statistically significant differences vs. CS sample are indicated as 'a' $p < 0.01$).

Specimen	Θ^G
CS	83.37±5.60
CS_P80	93.10±3.26[a]
CS_P80_MO 1%	88.29±2.87
CS_P80_MO 2.5%	80.81±3.60
CS_P80_MO 5%	78.83±1.43

Swelling analysis

The results of the swelling degree for chitosan-based films are presented in FIG. 6. Analysis was conducted in phosphate-buffered saline (PBS) at pH 7.4 for three days, during which all modified samples were stable and no disintegration occurred. Unmodified chitosan materials indicated the highest swelling degree among all samples, which, after one hour of soaking, was about 1400%. A decrease in swelling degree was observed for all modified samples. However, mastic oil addition caused an even greater reduction of swelling degree in comparison to samples enriched only with polysorbate 80. A slight influence of the amount of mastic oil addition on the swelling degree of chitosan films was noticed. Among films incorporated with mastic oil, the lowest swelling degree was noticed for 2.5% addition, while the highest was for 5% addition.

Discussion

According to scientific reports, a combination of polymers and essential oils results in the production of materials with a wide range of potential applications, such as biomaterials, food systems, or packaging. Due to the properties of chitosan and mastic oil, the leading direction can be the development of wound dressings or other materials intended for skin regeneration. Our findings fit into the needs of further development of materials based on natural resources.

Based on the FTIR analysis, alterations of characteristic band positions of chitosan in modified samples, such as bands related to -OH and -NH stretching vibrations, might reflect newly created interactions, for example, possible hydrogen bonds between functional groups of components of mastic gum essential oil, polysorbate 80, and biopolymer. Chitosan molecules have numerous –OH groups and hydrogen bonds between –OH or $–NH_2$ groups from chitosan and additives might occur. As in our research, Song et al. [43] also observed increased intensities of bands at the 2800-2900 cm^{-1} region after the introduction of essential oil (pine needle) to chitosan/shellac blend films, which might be related to vibrations coming from functional groups present in the components of essential oil, in our study from mastic oil. Hosseini et al. [44] also reported newly created bonds between chitosan and cinnamon essential oil components in their films.

Alterations in the mechanical parameters of modified films might also be related to interactions between individual components and network microstructure [10]. In our study, both additives, i.e., emulsifier and essential oil, synergistically affected the mechanical properties of chitosan-based films. The type of essential oil, the complexity

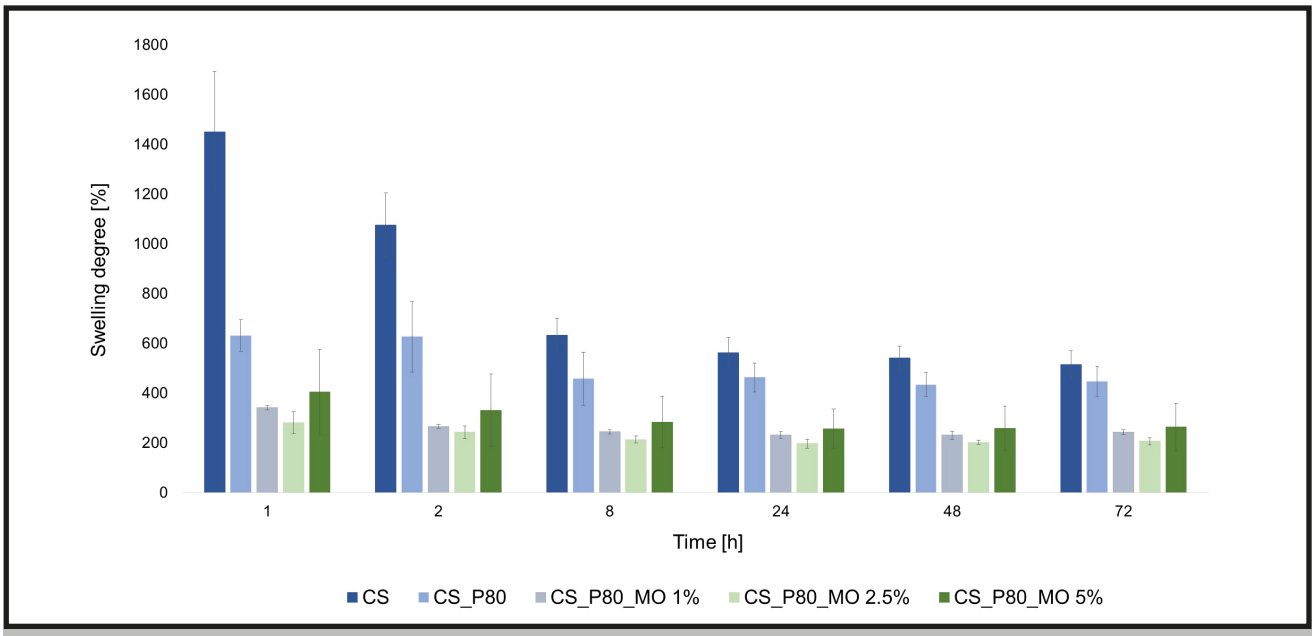

FIG. 6. Swelling degree for unmodified and modified chitosan films.

of its composition, the created interactions, and the type of applied emulsifier, not including the characteristics of chitosan itself, are just a few factors that can influence films' mechanical properties. Evaluation of the mechanical properties of polymeric materials is required in terms of their potential topical application, for example, in the treatment of skin lesions. Films should be characterized by appropriate tensile strength, mechanical resistance and flexibility to effectively adhere to the skin. As in our research, increased tensile strength after the incorporation of selected essential oils, such as cinnamon essential oil or marjoram essential oil, to chitosan films was also reported [10,44,45,46]. It might be related to the cross-linking effect in the chitosan/essential oil films, leading to a decrease in molecular mobility and compact structure; disruption of the chitosan network would be rather responsible for the opposite effect. However, several studies reported weaker mechanical properties of chitosan/essential oils films [14]. Decreased tensile strength after introducing essential oil to chitosan film was also observed, which might result from discontinuous microstructure, resulting in the lower mechanical strength of materials [8,12,47]. Increased elongation at break, thus greater flexibility of chitosan films after incorporation of essential oil, was also reported by Zivanovic et al. [8], similar to our research. The highest elongation at break was observed for the sample enriched with 5% of mastic oil, which may be caused by increased moisture content. Water molecules, inserted between polymer chains, can act as a plasticizer in the film, disrupting the intermolecular forces between polymer chains, leading to reduced chain rigidity and increased flexibility of the sample. For this film, the highest swelling degree among all chitosan/mastic oil samples and the most hydrophilic surface among all samples were also observed. Pereira dos Santos [48] also reported increased flexibility of chitosan/ essential oil films, but it might also be attributed to lactic acid application in film preparation. Film porosity and pore size may also adversely influence chitosan materials, leading to reduced tensile strength, Young's Modulus, and elongation at break, which was noticed by Altiok et al. [41] for chitosan/thyme essential oil films with increased concentration of additives. These comparisons confirmed that incorporation of the essential oil, the molecular weight of chitosan, the type of acid used to dissolve biopolymer, and the molecular weight of the counter ion influence films' elasticity and strength [8].

In our investigation, the addition of an emulsifier resulted in a hydrophobic film surface, whereas increasing concentrations of mastic oil introduced to chitosan films caused a reduction of contact angle. Samples with polysorbate 80 addition and samples with emulsifier and 1% of mastic oil addition were less hydrophilic than pure chitosan film. However, an increased amount of mastic oil in specimens caused decreased contact angle and greater surface hydrophilicity in comparison to chitosan film, which suggests that biopolymer chains were arranged with hydrophilic groups present on the film surface. According to results obtained by Wang et al. [12], the addition of clove bud oil also increased the chitosan film's hydrophilicity, while cinnamon oil caused an opposite effect. Essential oils are hydrophobic raw materials, and their addition to chitosan films in many studies led to more hydrophobic surfaces [45]. However, contact angle measurements performed with distilled water and phosphate-buffered saline (PBS) by Pereira dos Santos et al. [48] confirmed the hydrophilic nature of the chitosan film surface and reported that, independently of the content and the type of essential oil (clove or melaleuca) added to the chitosan matrix, film surface remained hydrophilic and decreased contact angle values were observed. Reduction in contact angle values determined for water and the hydrophilic surface of chitosan films enriched with terpene alcohols-rich essential oils was also reported by Mouhoub et al. [46]. The discrepancy of contact angle values of essential oils-incorporated chitosan films might result from the complex composition of these kinds of natural additives. The hydrophilic surface can be an advantage of materials intended for wound healing applications, due to ensuring the effective management of wound exudate. The type and amount of essential oil used should be considered during the fabrication of multifunctional materials for specific applications, for instance, wound dressing or engineered biomaterials.

The behavior of the biopolymer system in phosphate-buffered saline is a fundamental factor for designing biomaterials such as wound dressings. Decreased absorption of PBS solution occurred in modified samples, and our observations were similar to Zivanovic et al. findings [8]. Hafsa et al. [17] also reported that pure chitosan film was hydrophilic, absorbed water, and swelled. The reduction in the availability of groups able to create interactions with water molecules in modified films might result in an observed decrease in swelling degree and PBS absorption. A decrease in swelling degree in water for chitosan films enriched with essential oil was reported in other scientific reports [10,44,45]. Similar conclusions to our findings were presented for chitosan films (prepared from acetic acid) incorporated with terpene alcohols-rich essential oils (marjoram or tea tree), polysorbate 80, and glycerol [46] and for low molecular weight chitosan/PVA/essential oil films [49].

As essential oils might influence the physicochemical-properties and biologicalactivity of chitosan-based films further research is required to determine antimicrobial properties, antioxidant activity, and cytotoxicity of prepared materials intended for biomedical applications.

Conclusions

Physicochemical characterization of polymer-based materials, such as determination of chemical structure, mechanical properties, swelling degree, and contact angle, is an important preliminary step in the development of multifunctional materials for biomedical, cosmetic, or food applications. Investigation of the mechanical properties of polymeric materials is essential for their potential cutaneous application, for example, as wound dressings. Adequate tensile strength, mechanical resistance, and flexibility of polymeric films are required for proper skin adhesion. The evaluation of surface properties and the behavior of the polymer-based materials in phosphate-buffered saline are fundamental in biomaterial production. The selection of components will influence the physicochemical properties and biological activity of fabricated materials for specific applications; thus, the type and amount of biopolymers and additives, such as essential oils, are important factors.

Prepared chitosan films incorporated with various concentrations of mastic oil and polysorbate 80 as an emulsifier were smooth and uniform; however, essential oil addition caused slightly lower transparency of films. The effect of selected additives on the properties of chitosan-based films was observed. Modification of chitosan films resulted in a decrease in swelling degree, and the lowest swelling degree was shown by the sample with 2.5% of mastic oil addition. Changes in films' mechanical properties after the introduction of essential oil and emulsifier were observed. Modified films were characterized by increased tensile strength (the highest was noticed for the sample with 2.5% addition of

mastic oil) and elongation at break, thus they were more flexible (the highest was observed for the sample with 5% addition of mastic oil). The incorporation of an emulsifier into chitosan films caused an increase in contact angle, and the film surface was hydrophobic, whereas increasing concentrations of mastic oil resulted in a reduction of contact angle and a hydrophilic nature of the surface. Alterations of characteristic bands position of chitosan in modified specimens observed in the FTIR spectra might be associated with newly created interactions between functional groups of components of mastic gum essential oil, polysorbate 80, and biopolymer. The hydrophilic surface, increased tensile strength and flexibility, as well as swelling capacity and stability in PBS solution of prepared films, can be an advantage in terms of material fabrication intended for biomedical applications, for example, wound healing. Chitosan and mastic oil might be a promising combination for use in biomaterials.

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