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Evaluation of Antimicrobial and Structural Properties of Thyme Essential Oil-Loaded Chitosan-Capric Acid and Chitosan-Stearic Acid Nanogels

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HIGHLIGHTS

- The use of chitosan nanogels to encapsulate Thyme Essential Oil is more efficient for antimicrobial applications compared to the ionic method.
- Use of long-chain acids such as stearic acid in the structure of chitosan than short-chain acids such as capric acid cause better formation of nanoparticles.
- The antimicrobial activity of the developed encapsulation systems significantly improved in the presence of Thyme Essential Oil.

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Acronyms and abbreviations
EDC=1-Ethyl-3-Dimethylaminopropyl Carbodiimide
FTIR=Fourier-Transform Infrared Spectroscopy
MTT=3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide
SEM=Scanning Electron Microscope
TEO=Thyme Essential Oil

ABSTRACT

Background: This study aimed to investigate the physicochemical properties, antimicrobial activity, and cytotoxicity of Thyme Essential Oil (TEO) encapsulated by chitosan nanogels.
Methods: In this study, chitosan-stearic acid and chitosan-capric acid nanogels were developed in two ratios of chitosan to fatty acid (10: 1 and 10: 3).
Results: The results of Fourier-Transform Infrared Spectroscopy analysis showed a successful binding of chitosan to capric and stearic acids. Scanning Electron Microscope images revealed that particle formation improved with increase of the ratio of fatty acid to chitosan. The antimicrobial capacity of both encapsulation systems on three species of microorganisms (*Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*) was studied. A sustained release of curcumin was observed in Simulated Intestine Fluid. The developed nanogels did not have any toxicity on different cell lines. The results also showed that the antimicrobial capacity of TEO encapsulated with chitosan nanogels was higher (p<0.05) than the ionic method (use of sodium triphosphate incorporating chitosan).
Conclusion: The results have shown that encapsulating TEO in chitosan nanogels is a suitable alternative for synthetic antibiotics in different products.

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Introduction

The increase of drug resistance of microorganisms and ever-increasing awareness of consumers about the harmful effects of chemical preservatives and synthetic nutrients have led to searching for natural compounds with wide range of biological activities. According to the literature, some of the essential oils have strong antimicrobial and antioxidant properties. Antimicrobial activity of the plant extracts is due to their high hydrophobicity, which enables them to cross the bacterial membrane and reduce the membrane potential (Hammer et al., 1999).

Essential oils have antibacterial, antifungal, antioxidant, and anti-cancer effects. Various studies have been shown the biological effects of essential oils (Lotfinia et al., 2013). Among the various essential oils, Thyme Essential Oil (TEO) is one of the most important essential oils with excellent antimicrobial activity. Essential oils are degraded when exposed to light, oxygen, and high-temperature. One of the ways to protect essential oils against external factors is encapsulation of these compounds into biopolymer-based encapsulation systems (Mousavian et al., 2021).

Nanocarriers can be divided into three categories: lipid, polymer, and lipid-based nanocarriers. Nanogels are a part of the category of polymer nanocarriers that have many applications in various fields, including tissue engineering, biotechnology, and medicine. Various biocompatible and biodegradable polymers such as chitosan, hyaluronate, dextran, cellulose, pullulan, alginate, etc. are commonly used to produce nanogels (Chacko et al., 2012). Chitosan is a polyunsaturated polymer composed of glucosamine and N-acetyl glucosamine units (with beta 1 and 4) obtained from the deacetylation of chitin. Cellulose is the most important polysaccharide in nature, and this cationic polymer is the second most significant.. Chitosan is a non-toxic, biodegradable, and biocompatible substance with antimicrobial and antifungal effects. The antimicrobial properties of chitosan are due to the positively charged amino groups. These groups react with the cell membrane of microorganisms, and as a results leads to leakage of protein components and other intracellular components of microorganisms (Mwangi et al., 2016).

This study aimed to encapsulate TEO in chitosan nanogels and to investigate the effect of nanogels (chitosan-capric acid and chitosan-stearic acid) as well as particle size on its antimicrobial properties for introducing one or more suitable formulations for replacement with synthetic antibiotics in various products.

Materials and methods

Materials

Chitosan (medium molecular weight with 75 to 85% degree of deacetylation), capric acid, stearic acid, acetic acid, methanol, ethanol, sodium hydroxide, 1-Ethyl-3-Dimethylaminopropyl Carbodiimide (EDC), Müeller Hinton agar, and Müeller Hinton broth were purchased from Sigma-Aldrich (Steinheim, Germany) and TEO was obtained from Barij Essential Oil Company (Iran).

Preparation of chitosan-stearic acid and chitosan-capric acid nanogels

Nanogels were prepared by sonication and binding stearic acid and capric acid to chitosan as described by Atarian et al. (2019). First, 1 gr of chitosan was dissolved in 100 ml of aqueous acetic acid (1% v/v). Afterward, 85 ml of methanol was added to the dispersion and the resulting mixture was ultrasonicated (Hielscher, UP200St-T Ultrasonic Transducer, Germany). Then, 40 mg of EDC that was mixed with 5 ml of methanol was added to 100 and 300 mg of stearic acid and capric acid. Then, the prepared samples were placed in the dark for 40 min to react, and after this time, the chitosan solution was added dropwise to the sample on a stirrer (Hotplate Magnetic Stirrer, MS-H380-Pro, Spain) at 500 rpm. The prepared solution was sealed with aluminum foil and put it in a shaker (Digital orbital laboratory shaker, 300×400mm, United Kingdom) at 190 rpm and at temperature of 20 °C for 24 h. After this time, for the preparation of the nanogel, the pH was set to 8.5-9 using sodium hydroxide (1 M). The mixture was then centrifuged (MPW-260 Laboratory Centrifuge, Germany) to separate the prepared nanogels. The obtained nanogels were washed three times with sodium hydroxide (1 M), acetic acid (2%), and distilled water to remove unreacted materials. The clear nanogel solution (dissolved in 1% acetic acid solution) was finally filtered by a Whatman filter with a 0.2 μm mesh. Figure 1 shows the reaction steps.

Evaluation of chitosan nanogels

-Fourier-Transform Infrared Spectroscopy (FTIR)

In this study, to confirm the formation of nanogels, infrared spectroscopy (AVATAR 370, USA) was performed in the wavenumber range of 500 to 4,000 1/cm at a temperature of 20 °C. To prepare the samples, dried samples were mixed with potassium bromide powder, and then compressed.

-Scanning Electron Microscope (SEM)

Morphology and particle size of TEO loaded chitosan-capric acid and chitosan-stearic acid nanogels were examined using a SEM. For this purpose, three drops of freshly diluted nanogels were poured on the slide and dried at ambient temperature, followed by covering with gold. Afterward, the morphological characteristics of the samples were evaluated by SEM made by KYKY Company (China),

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model EM 3,200 and the maximum voltage of 30 kV.

-Particle size and distribution

Particle size distribution and average diameter of nanogels were measured by a particle size measuring device (SHIMADZU, model SALD 2,101, Japan, model Wing Sald) based on static laser beam diffraction method.

TEO encapsulation in chitosan nanogels

For this purpose, first TEO was dissolved in ethanol in a ratio of 1:1 and then mixed with nanogels. The amount of 10,000 mg/L nanogel and 5,000 mg/L of essential oil was used to prepare the encapsulated essential oil. The resulting mixture was subjected to an ultrasound probe (70 KHZ) for 5 min (Beyki et al., 2014).

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

To evaluate the antimicrobial activity of different samples, a Gram-positive bacterial species (*Staphylococcus aureus* PTCC: 1112), a Gram-negative bacterial species (*Escherichia coli* PTCC: 1330), and a yeast species (*Candida albicans* PTCC: 5027) were used. First, a fresh culture of each microorganism was prepared by 16 h of incubation at 37 °C on Müeller Hinton agar, followed by fresh dilution of half-McFarland cultures with sterile normal saline (0.9%). The samples were then dissolved separately in Müeller Hinton broth. Subsequently, 180 µl of each concentration was transferred to wells in a 96-well cell culture plate, and subsequently, 20 µl of bacterial suspension (10^6 Colony Forming Unit (CFU)/ml) was inoculated into the wells. For negative control, Müeller Hinton broth without any additives was used to indicate sterile conditions. The bacteria were also inoculated separately as a positive control in the culture medium. The plates were then incubated at 37 °C for bacterial species and 25 °C for yeast species for 18 h. Finally, bacterial growth was examined by adding 20 µl of 2, 3, 5-Triphenyl Tetrazolium Chloride (TTC) (5 mg/ml) and re-incubating at 37 °C for 1 h using a colorimetric method. MBC was also studied by cultivating 20 µl of no-color-change on the surface of plates containing Müeller Hinton agar (18 h and 37 °C) (Fathi et al., 2021).

Toxicity test

The 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay was used to evaluate cell viability based on the reduction of yellow tetrazole compound (MTT) and its conversion to purple formazan. To evaluate the toxicity of the samples, several cell lines, including MCF-7, PC3, DU-145, HepG2, C26, HTC, Hella, PCL12, and A2, 780 were used. A specified number of cells were placed in each well containing 100 µl of RPMI medium. After 24 h of waiting for adhesion, various nanogels dilutions prepared in Dimethyl Sulfoxide (DMSO) and were added to the wells. After 48 h, 10 µl of MTT (5 mg/ml) was added to the wells and the plates were incubated at 37 °C for 4 h. Finally, the culture medium was discarded and the formazin blue dye was dissolved in 200 µl of DMSO. Toxicity was expressed as the concentration that inhibited cell growth by up to 50% (IC_{50}). IC_{50} values were calculated using CalcuSyn software version 2 (BIOSOFT, UK) (Fathi et al., 2021).

Study of TEO release

The release properties of TEO from the developed nanogels were investigated as reported by Sotelo-Boyás et al. (2017). Briefly, the nanoparticles were added to a dialysis bag and then placed into water under mechanical agitation. The release of TEO was determined by High Performance Liquid Chromatography (HPLC) (BIOSAN_21,001, Germany).

Statistical analysis

An analysis of variance (ANOVA) was carried out to examine the significant differences (p<0.05) between the treatments (seven treatments). All the tests were done considering triplicates. The statistical evaluation was carried out using SPSS software (version 26).

Results

Physical and microbial properties of nanogels

-FTIR analyze

As the initial step of this investigation, the nanogels were prepared by self-aggregation mechanism, using modified chitosan. To modify chitosan, a part of the free amino groups of chitosan was attached to stearic and capric acid groups using EDC intermediate. To confirm the formed bond between the chitosan amine groups and carboxylic acid groups of stearic and capric acid, the obtained spectra from FTIR were analyzed (Figure 2).

-SEM

SEM images of chitosan-capric acid (1:10), chitosan-capric acid (3:10), chitosan-stearic acid (1:10), and chitosan-stearic acid nanogels (3:10) are shown in Figure 3.

-The effect of TEO on the shape and size of nanogels

The uniformity of particle size distribution in the nanogels incorporated with essential oil was less (p<0.05) than nanogels without essential oil (Figure 3).

-Antimicrobial activity of chitosan nanogels with TEO

The antibacterial activity of nanogels was investigated, and the results are given in Table 1. To evaluate the antimicrobial effect, a Gram-positive bacterium (*S. aureus*),

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a Gram-negative bacterium (*E. coli*), and a yeast species were used.

Toxicity test of nanogels

When a covalent bond is formed between two compounds, a new compound with new properties is obtained. In the present work, a covalent bond was established between chitosan, capric acid, and stearic acid with EDC intermediate. Although chitosan, capric acids, and stearic acid have been approved for safety and no toxicity has been reported, but when these compounds are bonded together, a new structure is produced and has to be examined so that they can be used in the field of medicine. For this purpose, a toxicity test was performed on two nanogels of chitosan-capric acid (3:10) and chitosan-stearic acid (3:10) on different cell lines, and the results are presented in Table 2. The release profile of curcumin from TEO-loaded chitosan-capric acid nanogels is shown in Figure 6.

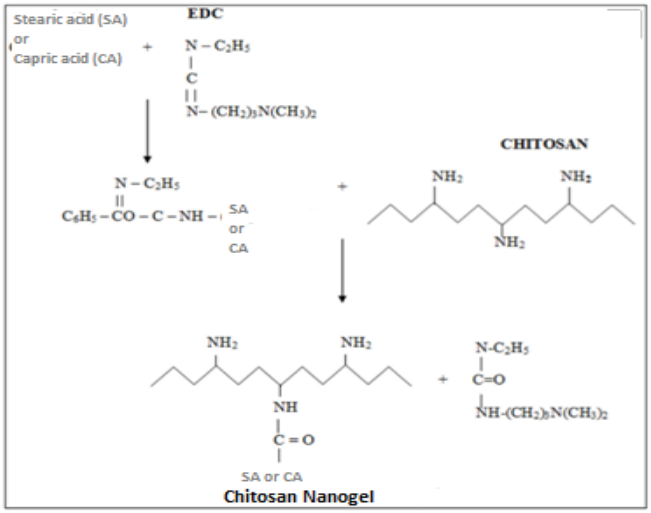


Figure 1: Schematic of the reaction between capric acid, stearic acid, and chitosan in the presence of 1-Ethyl-3-Dimethylaminopropyl Carbodiimide (EDC)

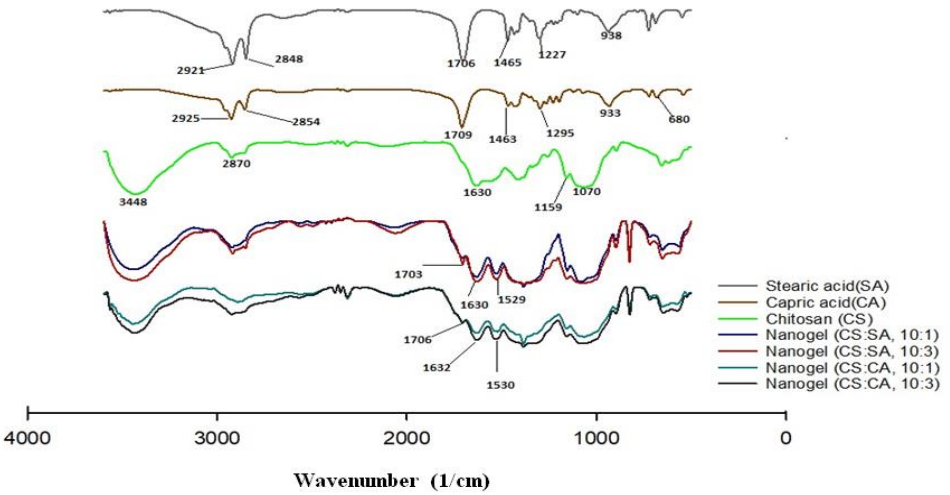


Figure 2: Fourier-Transform Infrared Spectroscopy (FTIR) spectra of chitosan, stearic acid, capric acid, chitosan-stearic acid nanogels, and chitosan-capric acid nanogels

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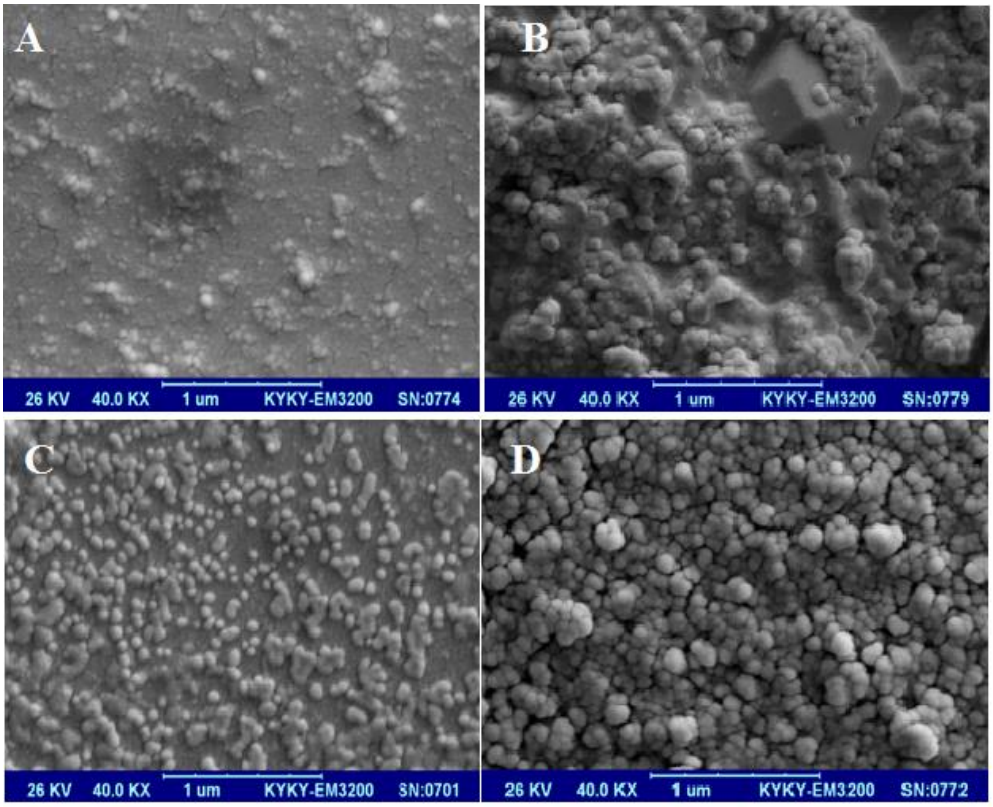


Figure 3: Scanning Electron Microscopy (SEM) of chitosan-capric acid nanogels (10:3) (A), chitosan-capric acid (10:3) (B), chitosan-stearic acid nanogels (10:3) (C), and chitosan nanogels-stearic acid (10:3) (D)

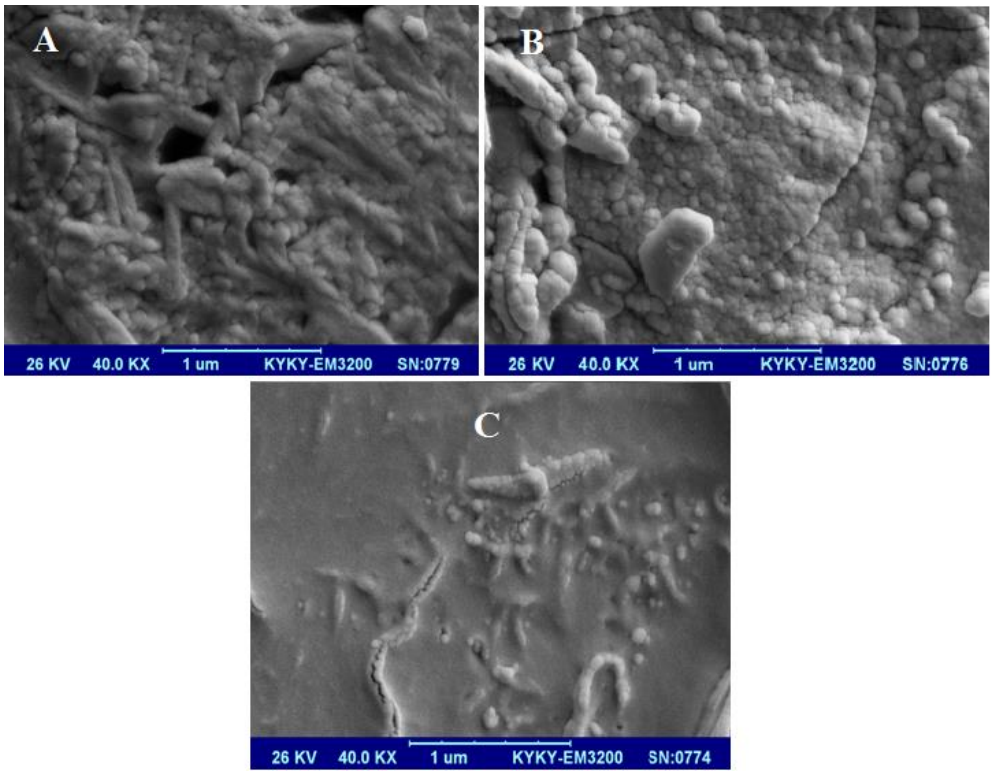


Figure 4: Scanning Electron Microscope (SEM) image of chitosan-capric acid nanogel (10:3) -Thyme Essential Oil (TEO)(A), chitosan nanogel-stearic acid (10:3) -TEO (B), and chitosan-triphosphate-thyme (C)

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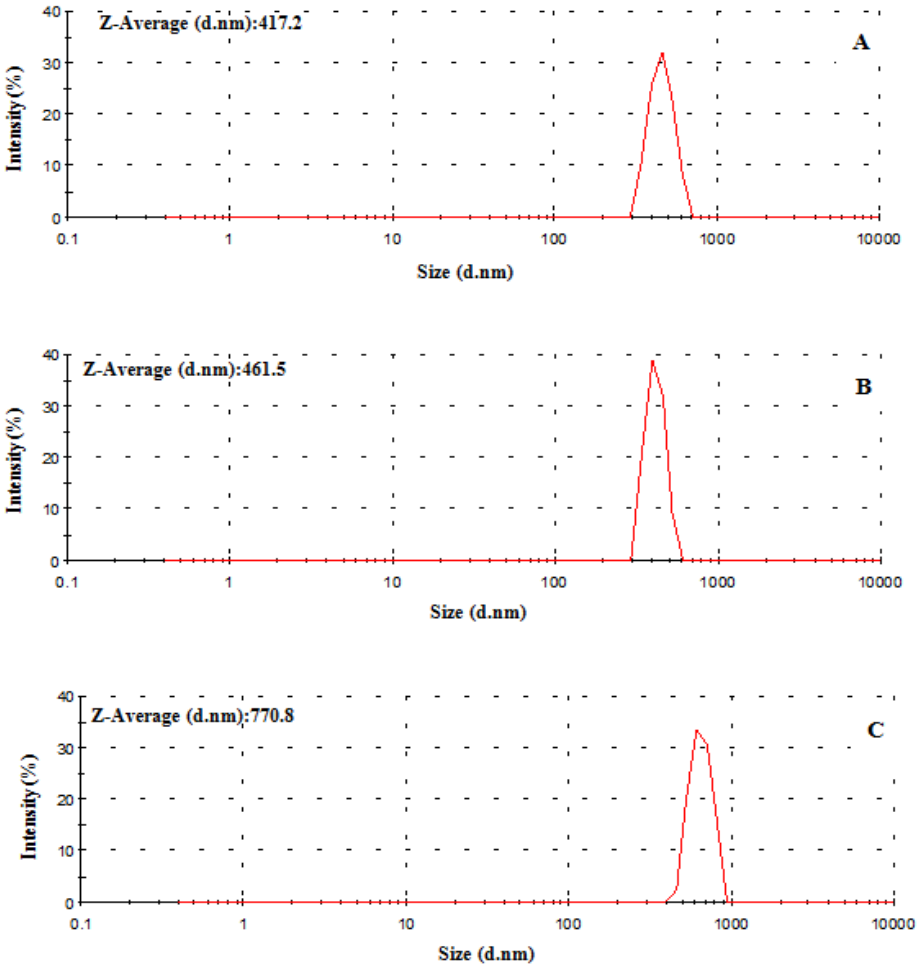


Figure 5: Particle size of chitosan-capric acid nanogel (10:3) -Thyme Essential Oil (TEO) (A), chitosan nanogel-stearic acid (10:3) -TEO (B), and chitosan-polyphosphate-TEO (C)

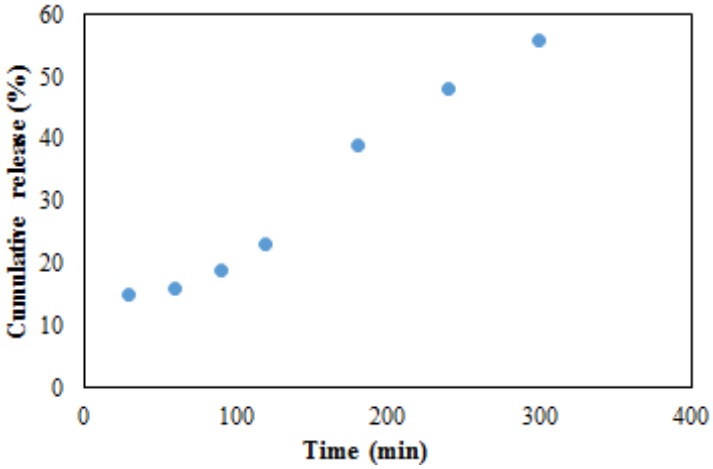


Figure 6: Release properties of Thyme Essential Oil (TEO) from chitosan-capric acid nanogels

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Table 1: Results of antimicrobial activity of chitosan-capric acid and chitosan-stearic acid nanogels with Thyme Essential Oil (TEO)

Sample	MIC/MBC (µg/ml)	*Staphylococcus aureus*	*Escherichia coli*	*Candida albicans*
Chitosan	MIC	78±5	156±3	156±6
	MBC	312±11	312±9	312±9
Chitosan-capric acid nanogel (10:1)	MIC	312±7	156±6	625±15
	MBC	312±6	312±7	625±14
Chitosan-stearic acid nanogel (10:1)	MIC	156±10	156±12	156±16
	MBC	156±14	156±14	156±21
Chitosan-capric acid nanogel (10:3)	MIC	156±12	78±12	78.1±14
	MBC	156±9	78±11	156±15
Chitosan-stearic acid nanogel (10:3)	MIC	156±8	156±8	156±10
	MBC	156±14	156±10	156±9
Chitosan-polyphosphate-TEO	MIC	78±10	156±12	156±18
	MBC	156±15	312±15	312±22
Chitosan nanogel-capric acid (10:1)-TEO	MIC	78±7	78±14	78±15
	MBC	78±10	156±12	156±19
Chitosan nanogel-stearic acid (10:1)-TEO	MIC	78±7	78±12	156±21
	MBC	78±9	78±7	156±17

MIC=Minimum Inhibitory Concentration
MBC=Minimum Bactericidal Concentration

Table 2: The results of toxicity test of chitosan-capric acid and chitosan-stearic acid nanogels

Cell line	IC_{50} (µg/ml)	
	Chitosan-stearic acid nanogel	Chitosan-capric acid nanogel
PC3	>100	>100
DU-145	>100	>100
HepG2	>100	>100
C26	>100	>100
HTC	>100	>100
Hella	>100	>100
PCL12	>100	>100
A2780	>100	>100

Discussion

In the FTIR spectrum of chitosan the peak observed at the wavenumber range of 3,400-3,500 1/cm is related to the partial overlap of the flexural vibrations of the alcohol and amine bonds. The peak at 2,870 1/cm is attributed to the stretching vibration of C-H in the methyl and methylene groups. The diagnostic peak at 1,163 1/cm is associated with the stretching vibrations of the C=O group in amides. The bands observed at 1,159 1/cm (C-O-C asymmetric stretching vibration) and 1,070 1/cm (C-O stretching vibration) are common structures in chitosan (Yang et al., 2010). In the FTIR spectrum of stearic acid, the peaks at 2,921 and 2,848 1/cm are related to C-H stretching vibration, and the main one at 1,706 1/cm is due to C=O tensile vibration. The detected peaks at 1,465, 1,227, and 938 1/cm are related to C-H flexural vibration, C-O tensile vibration, and the flexural vibration of O-H (Nikolić, 2011).

According to the FTIR spectrum of capric acid, the identified peaks at 2,925 and 2,849 1/cm are correspond to the C-H tensile vibration of the CH_2 groups, and the peak at 1,709 1/cm is attributed to the C=O tensile vibration of the fatty acid carboxylic group. The peaks at 1,463, 1,295, and 933 1/cm are related to the C-H flexural vibration of the CH_2 groups, tensile vibration of C-O carboxylic group of fatty acid, and flexural vibration of O-H carboxylic acid.

In the FTIR spectra of chitosan-stearic acid nanogels, a peak is observed at 1,630 1/cm, which is attributed to the vibrations of amide groups (Rao et al., 2012) as well as new amide bonds related to the reaction between chitosan and stearic acid. Furthermore, in the nanogel spectra, a peak is observable at 1,529 1/cm, which is due to the NH vibration of the second type of amide group (Wang and Chang, 2003). It confirms the reaction between chitosan and stearic acid. Besides, by comparing the nanogel spectra with each other, it can be seen that by increasing the ratio of stearic acid to chitosan from 1 to 3%, the intensity of the peak detected at 1,630 1/cm, which is related to tensile vibrations of C=O amide group increased, which may be due to the increase of carboxylic acid groups on chitosan. In each of the nanogel specimens, a peak was detected at 1,703 1/cm, and its intensity increased when the proportion of stearic acid to chitosan was raised. This peak is also observed in the stearic acid spectrum, which is related to the tensile vibration of the C=O group of the carboxylic acid group. Therefore, it can be inferred that some of the bindings of stearic acid to chitosan is through electrostatic interactions between carboxylic acid groups and chitosan amine groups, which with increasing the ratio of stearic acid to chitosan, the share of this type of binding has increased.

Based on Figure 2, it can be observed that the spectra of

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chitosan-capric acid nanogels closely resemble those of chitosan-stearic acid nanogels. Similarly, the interpretations made for chitosan-capric acid nanogels hold true. The results suggest that chitosan strands have successfully bonded with capric acid and stearic acid fatty acids. Also, the results of the present work are in agreement with those reported in previous studies (Atarian et al., 2019; Hosseini et al., 2020; Rajaei et al., 2017).

Based on Figure 3, the formation of nanoparticles improves when the ratio of capric acid and stearic acid to chitosan is increased. This is because it increases the hydrophobic portions of the chitosan filaments. These hydrophobic parts were better able to accumulate in the center of the particles and therefore better particles are formed. These results are consistent with the findings of other researchers (Atarian et al., 2019). Also, according to Figure 3, it can be seen that in general, stearic acid has caused better particle formation compared to capric acid. The reason for that is probably due to the longer chain of stearic acid (18 carbons) than capric acid (10 carbons). Stearic acid has a longer carbon chain compared to capric acid, which results in a higher level of hydrophobicity. Therefore, the nanogels containing stearic acid filaments are more hydrophobic in a similar proportion than capric acid nanogels. The presence of more hydrophobic parts in the structure of chitosan-stearic acid nanogels causes better accumulation of hydrophobic regions in aqueous media, resulting in better particle formation (Zhaveh et al., 2015). The microscopic images clearly showed that the nanoparticles were well-formed. In 2014, Beyki et al. examined the morphology of chitosan-cinnamic acid nanogels and found that the nanogels were formed with a spherical and uniform structure and the particle size of the nanogels measured by SEM was less than 100 nm.

The SEM method does not seem to be a suitable method for examining particles that contain volatile compounds such as essential oils. According to the results, the particles have become more interconnected. Due to its volatile nature, TEO likely causes the degradation and interconnection of particle structures when removed during the drying process in the SEM method. As an alternative method, dynamic laser beam scattering is used to investigate the size nanogel particles containing TEO. Since in this method the sample is used as a solution and no drying operation is performed, therefore this method does not change the TEO in nanogels and the results are more acceptable. Figure 4 shows the particle size distribution of chitosan-capric acid (3:10), chitosan-stearic acid (3:10), and chitosan-sodium triphosphate nanogels containing TEO.

According to Figure 5A, the particle size of chitosan-capric acid nanogels containing TEO was between 300 and 600 nm. In the case of chitosan-stearic acid nanogels containing TEO, the particle range was about 300 to 700 nm (Figure 5B). In the case of TEO encapsulated with chitosan-triphosphate, the particle range was between 400 and 1,000 nm. These findings indicate that, overall, the utilization of chitosan-capric acid and chitosan-stearic acid nanogels led to the production of smaller particles in comparison to chitosan-triphosphate. Besides, the encapsulating essential oil in the structure of chitosan-stearic acid nanogels caused larger particles than the encapsulating in the structure of chitosan-capric acid. This result is probably due to the longer stearic acid fatty acid chain (18 carbons) compared to capric acid. In addition, by comparing the results of Figure 3 i.e., nanogels without essential oils and the results of Figure 5 i.e., nanogels containing essential oils, it can be concluded that the placement of TEO in the structure of nanogels has increased the particle size.

According to the results of Table 1, the chitosan solution had the lowest inhibitory concentration against *S. aureus* compared to *E. coli*, and *C. albicans*. No significant difference was observed between the three species with regard to minimum lethal concentration. These results indicate that chitosan has greater inhibitory activity against Gram-positive bacteria. By comparing the results of chitosan with chitosan-polyphosphate-thyme solution, there was no difference between Gram-negative and yeast species. However, for Gram-positive species, the minimum lethal concentration of chitosan-polyphosphate-TEO has decreased. . The anticipation was that the inclusion of TEO would greatly enhance the antimicrobial effectiveness of chitosan, however, contrary to expectations, this did not occur. The reason for this result is probably related to the mechanism of antimicrobial behavior of chitosan. Regarding the antimicrobial effect of chitosan, various mechanisms have been reported; one of the most probable mechanisms being the positive charge of chitosan that can adhere to the wall of microbes and disrupt the entry and exit of cell compounds (Ashrafi et al., 2018; Fathi et al., 2021; Nejati Hafdani and Sadeghinia, 2011). Therefore, the positive charge of chitosan filaments is an important factor that the higher the positive charge of chitosan filaments, the better the attachment of the filaments to the wall and consequently the greater the antimicrobial effect (Goy et al., 2009). In the case of the chitosan-polyphosphate-TEO sample, because the sodium tri-polyphosphate compound is a compound with several negative charges, it interact with the chitosan and neutralize the positive charges of the chitosan (Gan et al., 2005). According to Table 1, the results of antimicrobial activity of chitosan-capric acid nanogels (1:10) show that compared to chitosan, antimicrobial activity has decreased, which is probably due to the decrease in the positive charge of chitosan filaments due to capric acid binds to the chitosan chain. In the case of

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chitosan-stearic acid nanogels (1:10), it can be seen that its antimicrobial activity has improved compared to chitosan as well as chitosan-capric acid nanogels (1:10). These results show that stearic acid has a better effect on increasing the antimicrobial properties of nanogels compared to capric acid.

The results of Table 1 also show that with the addition of TEO in the structure of nanogels (chitosan-capric acid nanogel (1:10) -TEO and chitosan nanogel-stearic acid (1:10) -TEO) the antibacterial activity increased significantly although this effect was less in the case of yeast species compared to bacterial species. The outcomes align with the discoveries of previous researchers who have affirmed that incorporating plant essential oils into nanogels enhances the antimicrobial impact of nanogels. (Beyki et al., 2014; Hadian et al., 2017; Nazem et al., 2016). The remarkable point about these results was that unlike the sample of chitosan-polysaphate-TEO which showed that the addition of essential oil to chitosan did not have much effect on increasing antimicrobial activity; in the case of nanogels the addition of essential oil to the structure of nanogels had a positive synergistic effect. These results clearly show that to encapsulate the destructive sensitive compounds such as plant essential oils as antimicrobial applications, it is more appropriate to use chitosan nanogel structures instead of compounds such as sodium triphosphate. In addition, chitosan nanogel structures have a permanent structure because the modifying agent (currently capric acid and stearic acid) is attached to the chitosan structure by covalent bonding, and unlike the chitosan-polyphosphate combination, which is a bond between chitosan and sodium triphosphate, is electrostatic, and it is not subject to environmental conditions such as pressure.

According to Table 2, the developed nanogels did not have a toxic effect on any of the cell lines and therefore the desired nanogels as their constituent performance, namely chitosan, capric acid, and stearic acid fatty acids can be used for pharmaceutical purposes.

During the initial release period (0 to 30 min), a rapid release was observed, which was subsequently followed by a controlled release. The observed fast release is related to the TEO molecules that are adsorbed to the surface of particles, leading to fast release. Comparatively, the release rate of TEO from the particles to simulate was considerably greater than Simulated Intestine Fluid (SIF), which may be due to the decrease of ionization degree and solubility of chitosan in the media with pH values greater than 6.5. In such media, chitosan macromolecules may be detached from nanogels, and then lead to the bridging between neighboring particles (Avadi et al., 2010). The same results have been reported by Fathi et al. (2021), who observed that the release rate of curcumin from chitosan NPs in SIF was more than of the intestine medium.

Conclusion

The results of FTIR analysis revealed a successful binding of chitosan to capric acid and stearic acid fatty acids. Furthermore, SEM results showed that particle formation has improved by increasing the ratio of fatty acid to chitosan. Stearic acid caused better nanoparticle formation compared to capric acid, which was due to the greater hydrophobicity of stearic acid filaments compared to capric acid. The antimicrobial activity of the developed encapsulation systems significantly improved in the presence of TEO. Furthermore, the nanogels generated exhibited no signs of toxicity. The findings of this study demonstrate that the utilization of chitosan nanogels for the encapsulation of TEO is significantly more effective for antimicrobial purposes in comparison to the ionic method. (using sodium tripolyphosphate in combination with chitosan). The results of this study also showed that the use of long-chain acids such as stearic acid in the structure of chitosan than short-chain acids such as capric acid cause better formation of nanoparticles.

Author contributions

A.R. investigated, analyzed data, and conducted the experimental work; D.S. supervised, investigated, and wrote the manuscript; M.T. and E.F. reviewed and edited the manuscript; Z.S. analyzed data and conducted the experimental work; F.S wrote the manuscript; S.R. and H.K. analyzed data. All authors read and approved the final manuscript.

Conflicts of interest

We declare that there is no conflict of interest.

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