Original Research Article

Hydroxypropyl methylcellulose-based films embedded with garlic oil-loaded chitosan microparticles as a potential oral mucosal drug delivery system

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Abstract

In the present study, hydroxypropyl methylcellulose (HPMC)-based films embedded with garlic oil-loaded chitosan microparticles were characterized and evaluated for their suitability as a potential oral mucosal drug delivery system concerning bacterial infections. Chitosan was used to encapsulate garlic oil and form microparticles using different weight ratios by ionic gelation technique. The microparticles were then incorporated into HPMC solution and films were fabricated using the film casting technique. A blank film without microparticles was also prepared. The microparticles were examined for their particle size, uniformity, shape, zeta potential and antimicrobial activities. The results suggest that the microparticles exhibit a spherical shape, are electrically unstable and tend to agglomerate due to having low zeta potential (-7.06 to -1.24 mV). Nevertheless, they showed antimicrobial activities against both *Escherichia coli* and *Staphylococcus aureus*. Additionally, the films were characterized by weight, thickness, wettability and antimicrobial activities. All films produced were generally light (0.20 to 0.25 g), thin (0.02 to 0.12 mm) and had good wettability based on their low contact angles (below 90°). In comparison to the microparticles, the films exhibited antimicrobial activity against *S. aureus* whereas no zone of inhibition was observed when the films were tested against *E. coli*. HPMC-based films embedded with encapsulated garlic oil have the potential to act as a new drug treatment to treat oral mucosal bacterial infections. Additional studies should be performed to evaluate the effectiveness of this treatment, such as extensive physicochemical characterization, mucoadhesion studies and evaluation of drug release and drug permeation across the oral mucosa.

Keywords: HPMC; garlic oil; chitosan; oral mucosal drug delivery; film

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1.0 Introduction

More than 700 different microorganisms can be found in the oral cavity (1), which is regulated by the microbial-ecological homeostasis (2). The critical determinant in the early phase of an infection is the bacterial adhesion mechanisms. Oral resident flora and the host live harmoniously as long as the microbial load is low and the microorganisms do not invade the oral tissues. On the other hand, transient microorganisms are unable to compete with the resident microflora that has already been established. Furthermore, these transient microorganisms have a weak capacity to adhere to the host's tissue or other microorganisms. However, some pathogenic bacteria can compete, grow and finally manage to infect the host when there is an impairment of the microbial homeostasis (3); this is usually seen in immune-compromised patients. Opportunistic bacteria are then able to take over either as a result of successful competition with the stationary organisms or by filling a vacancy caused by a decline in the normal flora (2). *Staphylococcus aureus*, beta-hemolytic *streptococci*, *Haemophilus* spp, and gram-negative enteric rods, such as *Escherichia coli* and *Pseudomonas*, are the types of bacteria that can cause infections of the oral mucosa (2).

Oral mucosal infections can be treated systematically or locally. However, these treatments are not as successful when immunosuppressive factors are at play. Even though antibiotics show good activity against the causative bacteria in *in vitro* antimicrobial susceptibility tests, their use often fails in immunocompromised patients. This is because, antibiotics themselves can induce changes in the oral microbial homeostasis (2). Moreover, when antibiotics are given systematically, they do not achieve high concentrations in the oral mucosa required to fight off infections. As for local treatment, oral chlorhexidine rinsing can exert little effect on aerobic gram-negative bacilli and may contribute to worsening of infections (4). A study by Wahlin (5) showed that, in leukaemia patients, there is a tendency for opportunistic bacteria, such as *Pseudomonas*, enterococci and enteric rods, to increase in numbers after the use of chlorhexidine. Acidified sodium chlorite in mouth rinses is reported to have the ability to reduce the number of *S. aureus* in oral mucosal infections (6). However, little is known about the mouth rinses' antimicrobial effect in mucosal lesions. Additionally, many conventional mouth rinses contain alcohol and astringents that cause the formulations to have an unpleasant taste. Meanwhile, food consumption and saliva's flushing action in the mouth cause reductions in drug concentration, resulting in these drugs being less effective at fighting infections (7). Additionally, the

development of drug resistance poses another difficulty in managing infections with current treatments (8).

The buccal mucosal membrane lining of the oral cavity has become an attractive target for drug administration. Absorption through the buccal region can avoid gastrointestinal tract conditions and the first pass effect. Mucoadhesive buccal films have better patient compliance compared to mouthwashes. They generally have good adaptation to the mucosal surface and do not cause irritation to the patient while mouthwashes can cause tooth discoloration, taste alteration and also oral mucosa irritation (9,10). Furthermore, the films can be formulated to provide either local or systemic action, as mucoadhesion infers attachment to the buccal mucosa (11). This can lead to an improvement of intraoral administration of drug delivery. The films can also help reduce pain, protect the surface of the wound and treat the disease more effectively when they are used to treat oral disease locally (12).

Hydroxypropyl methylcellulose (HPMC), also known as hypromellose, is a hydrophilic cellulose derivative used as a pharmaceutical excipient in oral sustained drug release matrix systems. HPMC is a non-ionic and water-soluble polymer. HPMC is stable between pH values of 3.0 to 11.0, is enzyme resistant (13) and can form a gel layer due to its swelling ability. Liquid molecules can penetrate the spaces of the polymer and cause hydration as the water content increases. The pores of the high-viscosity HPMC block further water uptake, leading to a turbid gel formation that resists erosion and dilution, resulting in slower and sustained drug diffusion and release rates (14). HPMC is commonly employed in the preparation of buccal films due to its excellent mucoadhesive properties that are provided by the hydrogen interpenetration of HPMC molecules with the mucin chains (15). HPMC has also been used in the development of antimicrobial edible films for food packaging. Lee *et al.* (16) and R. Moghimi *et al.* (17) have both used HPMC, combined with either oregano essential oil nanoemulsion or *Thymus daenensis* essential oil nanoemulsion, respectively, to produce edible films; both of these films exhibited good antimicrobial properties.

Natural products are currently being investigated as an alternative strategy for treating bacterial infections as there is an increase in microbial resistance to conventional antibiotics. Garlic, or *Allium sativum*, is a conventional spice from the Alliaceae family (18). Garlic is not only used as a flavour enhancer; since ancient times, it has been utilized as a medicine (19,20). Garlic is known to have antibacterial properties due to an oxygenated sulphur compound, thio-2-propene-1-sulfinic acid S-allyl ester, which is also known as allicin (20). The antimicrobial activities of garlic, garlic-derived organosulfur compounds and garlic essential oil have been widely investigated for use against food spoilage bacteria and foodborne pathogens (21–23). Most of these studies have focused on garlic oil as edible films in food packaging systems (24–27). Esmaeili *et al.* (25) and Pranoto *et al.* (26) proved that incorporating garlic essential oil and garlic oil, respectively, into edible films can retard the growth of bacteria and enhance the film's antimicrobial efficacy. Garlic extracts were found to have the ability to prevent the growth of oral pathogens in a study done by Bakri and Douglas (20). However, garlic oil is prone to degradation by oxidation and must, therefore, be encapsulated to protect it from oxidation and control its antimicrobial agent release. Encapsulation can be done with chitosan. Chitosan has been used to encapsulate essential oils due to its status as generally recognized as safe

(GRAS) (28), as well as other characteristics, such as its biodegradability, biocompatibility and bioadhesiveness (29,30). Previous study by Amiri *et al.* (31) showed that nanoencapsulation of garlic essential oil with chitosan enhanced the antibacterial and antioxidant properties of broiler nutrition. Tavares *et al.* (32) had successfully encapsulated garlic essential oil using complex of soy protein isolate and chitosan as a wall material, in producing microparticles. The wall material was effective for protecting the garlic sensitive compounds by improving its thermal stability.

The present study aimed to evaluate the suitability of HPMC-based films embedded with garlic oil-loaded chitosan microparticles as a potential oral mucosal drug delivery system concerning bacterial infections. Various formulations were prepared and preliminary characterization of physical and morphological properties, as well as antimicrobial activity were performed.

2.0 Materials and Methods

2.1 Materials

Natural, food-grade garlic oil and sodium tripolyphosphate (TPP) was purchased from Sigma-Aldrich (United States). Chitosan powder (medium molecular weight, 86% degree of deacetylation) was obtained from Zulat Pharmacy (Malaysia). Acetic acid (glacial) 100% was supplied by EMD Millipore Corporation (Merch, Darmstadt, Germany). Hydroxypropyl methylcellulose, HPMC K100 (Methocel*K100 Premium LV), was purchased from Dow Chemical Company (Michigan, USA). All other chemicals used were of analytical grade.

2.2 Encapsulation of garlic oil

Garlic oil was encapsulated with chitosan via ionic gelation technique to form microparticles. Briefly, a chitosan solution (0.3% w/v, 100 ml, pH = 3.4) was prepared by dissolving the chitosan in 1% v/v acetic acid overnight (18 h) at ambient temperature (25 ± 2 °C). Then, garlic oil was dropped gradually into the stirring mixture of the chitosan solution. The garlic oil was used in various amounts to obtain different weight ratios of chitosan to garlic oil (Table 1). TPP solution (0.3% w/v, 40 ml, pH= 9.2) was added into the mixture, drop-wise, and was stirred continuously at room temperature for 60 minutes. This process results in the formation of garlic oil-loaded chitosan microparticles spontaneously. The prepared garlic oil-loaded chitosan microparticles were then collected by centrifugation at 10,000 rpm at 25 °C for 10 minutes. The supernatant was removed, and the precipitate was freeze-dried using Eyela FDU-1200 Bench Top Freeze Dryer (Tokyo Rikakikai Co. Ltd, Japan) at a temperature of -45 °C and vacuum pressure between 18 to 23 Pa for further analyses.

Table 1: Weight ratios of garlic oil-loaded chitosan microparticles.

Samples	M1	M2	M3	M4	M5
Weight ratios of chitosan to garlic oil	1: 0	1: 0.25	1: 0.50	1: 0.75	1: 1.00

2.3 Characterization of garlic oil-loaded chitosan microparticles

The microparticles' (freeze-dried precipitates) morphological characteristics were examined by taking transmission electron microscopy (TEM) images using a Tecnai G2 20 S-TWIN TEM (Fei Company, United States). To evaluate particle size and zeta potential of the garlic oil-loaded chitosan microparticles, they were first diluted with distilled water to a concentration of 1 mg/ml and then measured using a Zetasizer Nano-ZS (Malvern Instruments, UK.) at 25 ± 1 °C. The data were expressed as mean of three repeats with the corresponding standard deviation (SD).

2.4 Preparation of mucoadhesive HPMC films embedded with garlic oil-loaded chitosan microparticles

The HPMC films were prepared by film casting technique. Briefly, 1.25 g of HPMC powder was dispersed in 50 ml of distilled water to form a polymer solution with a concentration of 2.5% w/v. The mixture was stirred continuously for 1 hour until a clear solution was obtained. Subsequently, 0.5 g of glycerol was added into the HPMC solution and mixed for 15 minutes. Next, 15 mg of each garlic oil-loaded chitosan microparticle from Table 1 were added into 6.5 g of HPMC solution and mixed until becoming homogenous.

Additionally, an HPMC solution without the garlic oil-loaded chitosan microparticles was prepared. The 6.5 g solution was then poured onto a glass petri dish and allowed to evaporate for 24 hours at 40 °C in a hot air oven. The films were peeled off and kept in a vacuum desiccator until further use. Table 2 shows various formulations of the HPMC films.

2.5 Film thickness & weight measurement

The films were first cut into 3.5 cm × 0.6 cm rectangles. Then, each of the films was weighed three times using a digital balance. The film thickness was measured using a High-Accuracy Digimatic Micrometer (Mitutoyo Corporation, Japan) at three different points, and the results were averaged.

Table 2: Various formulation of HPMC films.

Ingredients	Formulation code					
	F1	F2	F3	F4	F5	F6
HPMC (g)	1.25	1.25	1.25	1.25	1.25	1.25
Glycerol (g)	0.5	0.5	0.5	0.5	0.5	0.5
Microparticles	-	M1	M2	M3	M4	M5

2.6 Wettability of the films

The films were cut into 3.5 cm × 0.6 cm rectangles. The wettability of the films was examined by measuring the contact angle of the films using distilled water. The contact angle was measured using the sessile drop technique using a contact angle instrument (Data Physics, OCA15ES). The reading was taken thrice for each film.

2.7 Antimicrobial activity of garlic oil-loaded chitosan microparticles and films

Both microparticles and films were dissolved in phosphate buffer solution (pH = 6.8) with a concentration of 75 mg/ml to mimic the mouth's natural condition. Disk diffusion method was employed using the Kirby-Bauer technique (33). Trypticase soy agar (TSA) plates were seeded with 0.1 ml of inoculums containing approximately 105 – 106 CFU/ml of *S. aureus* and *E. coli*, respectively. The plates were divided into three areas: positive control, negative control and microparticles or films. Chlorhexidine digluconate 2 mg/ml mouthwash solution was used as the positive control, while, for the negative control, the disk was not dipped into any solution. The plates were then incubated for 24 hours at 37 °C. The diameter of the inhibitory zone surrounding the film discs was measured and repeated three times. F1 was excluded from this experiment.

2.8 Statistical analysis

The results obtained in the study were expressed as means of three repeats with the corresponding standard deviation (SD). The statistical analysis was carried out using SPSS software version 26 and a statistically significant difference is denoted by $p < 0.05$. Student's t-test and analysis of variance (ANOVA) were used to analyse the data. Post hoc analysis by Tukey HSD test was used when necessary.

3.0 Results and Discussion

3.1 Characterization of garlic oil-loaded chitosan microparticles

Garlic oil-loaded chitosan microparticles were prepared using the ionic gelation technique. TPP, a nontoxic poly-anion, was employed in this technique. TPP can form microparticles via ionic interactions between the positively charged amino groups of the chitosan and the negatively charged counter ions of TPP (34). Microparticles are solid colloidal particles with a size generally between 1 to 1000 µm (35,36). Table 3 shows the physical properties of the various garlic oil-loaded chitosan microparticles. The results indicated that the particle size of the microparticles were in the range of 2.09 to 5.64 µm, with a uniformity of 0.30 to 0.91.The particle size uniformity is obtained from polydispersity index (PDI). PDI is defined as the standard deviation of the particle diameter distribution divided by the mean particle diameter (37). It is used to estimate the average uniformity of a particle solution. Large PDI values usually correlate to a large size distribution in the particle sample. Thus, the microparticles in this study are considered as polydisperse as the values of the particle uniformity are larger than 0.1 (37,38). Meanwhile, the zeta potential of the microparticles was in the range of -7.06 to -1.24 mV (Table 3). The particle size, uniformity of particle size and zeta potential were not significantly different between various garlic oil-loaded chitosan microparticles of M1, M2, M3, M4 or M5 (ANOVA: $p > 0.05$). Zeta potential is an important feature used to characterize the microparticle surface charge and evaluate the colloids' stability (39,40). Zeta potential also plays an essential role in the garlic oil's interaction at the target site and, consequently, the garlic oil's bioavailability (29). High zeta potential indicates that the

particles are highly charged, which can prevent particle aggregation due to electric repulsion. A minimum zeta potential of ± 30 mV is needed in order to obtain a physically stable microparticle suspension by electrostatic repulsion (29). However, the low zeta potential of M1, M2, M3, M4 and M5 indicates that the microparticles formed are electrically unstable and tend to agglomerate (Table 3); this is proven through the TEM images. The TEM images (Fig. 1) suggest that the microparticles are spherical in shape with most of them being aggregated, especially as seen in Fig. 1(e). Apparently, the particle sizes showed by TEM images are generally smaller than the sizes captured by Zetasizer. The particle sizes from the Zetasizer were in micron range with slightly negative zeta potential, whereas the sizes from TEM were in nano-range. This could be due to the measurement in the Zetasizer included the unformed materials inside the formulation, while the TEM only showed the formed microparticles. The future study should improve the preparation method by using low centrifugation speed (3,000 – 4,000 rpm) to remove the unformed materials first, followed by the centrifugation at 10,000 rpm to collect the microparticles.

3.2 Film weight and thickness

The weight of the films ranged from 0.20 to 0.25 g, while the thickness ranged from 0.02 to 0.12 mm, as shown in Table 4. The prepared films were thin and light, making them suitable for buccal application as these properties can minimize discomfort to the patient. In this study, it was found that there was a significant difference in film weight and thickness between F1 and the other films (F2, F3, F4, F5 and F6) (ANOVA: $p < 0.05$). Meanwhile, F2, F3, F4, F5 and F6 had uniform thickness and weight, demonstrating no significant differences between the various formulations (ANOVA: $p > 0.05$). The incorporation of garlic oil-loaded chitosan microparticles increased the film weight and thickness. This is beneficial, as the films must have a proper thickness to avoid dissolving too quickly in the oral cavity (41).

3.3 Wettability of films

The wettability of the film surface was examined using contact angle measurement. A small contact angle, lower than 90°, indicates that the surface has high wettability, while a large contact angle, specifically larger than 90°, indicates low wettability. Generally, a mucoadhesive film that is meant for buccal administration must have a good wetting ability, as the wetting ability allows intimate contact with mucin in the mucus layer, which is the crucial step for mucoadhesion (42).

Table 3: Effect of different weight ratios of chitosan to garlic oil on particle size, particle size uniformity and zeta potential of the microparticles (mean ± SD, n=3).

Sample	Particle size (µm)	Particle size uniformity	Zeta-potential (mV)
M1	2.09 ± 0.14	0.68 ± 0.37	-4.23 ± 1.31
M2	3.36 ± 2.92	0.75 ± 0.12	-7.06 ± 3.88
M3	2.76 ± 1.02	0.91 ± 0.11	-3.49 ± 2.31
M4	5.64 ± 1.25	0.64 ± 0.31	-1.24 ± 0.76
M5	2.61 ± 0.86	0.30 ± 0.31	-4.41 ± 5.27

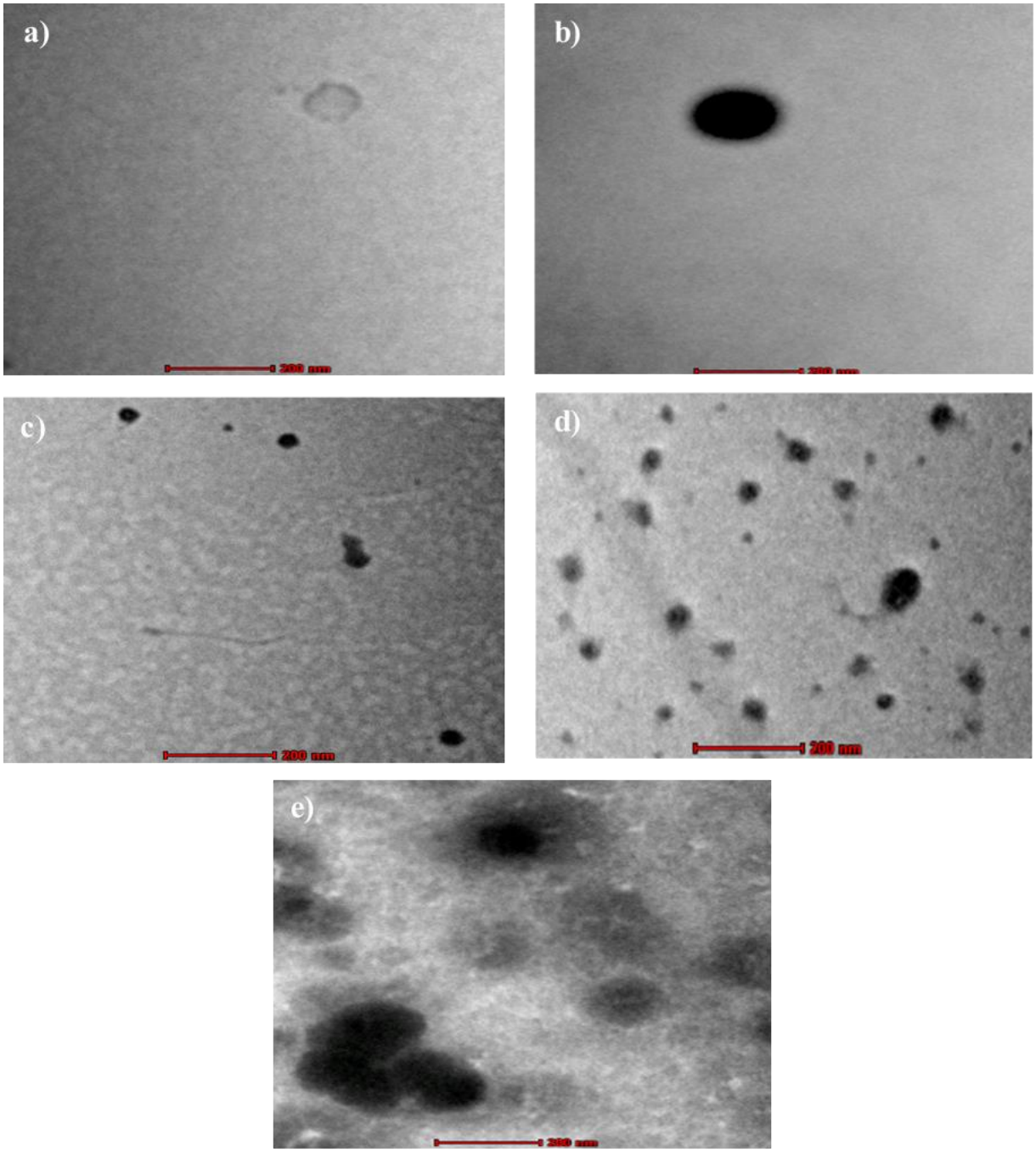

Figure 1: TEM images of garlic oil loaded chitosan microparticles at 43 K magnification; a) M1, b) M2, c) M3, d) M4 and e) M5.

The results showed that all films had contact angles of less than 90°, indicating good wettability (Table 4). The contact angle of F2 was found to be significantly lower than the rest of the films (ANOVA: $p < 0.05$). There was no garlic oil in the chitosan microparticles incorporated in the F2 film. When chitosan is diluted in an acidic medium it forms a cationic polymer that appears to have protonated amine groups (NH^{3+}) disposed at the chitosan structure (43). These positive charges are scattered over and possibly repel themselves by electrostatic repulsion, migrating to the surface of the films and weakening its adhesion with water (43). However, the addition of glycerol as a plasticizer in the formulation can enhance the wettability of the films, owing to the high affinity between molecules of water and glycerol, as both of them are polar (43). Generally, HPMC films embedded with garlic oil-loaded chitosan microparticles possessed higher contact angles compared to blank films and films containing chitosan microparticles. This is expected, since garlic oil increases the hydrophobicity of the films (33). Nevertheless, the wettability between the HPMC films embedded with garlic oil-loaded chitosan microparticles (F3, F4, F5 and F6) were not significantly different, as shown by their contact angles (ANOVA: $p > 0.05$).

3.4 Antimicrobial properties of garlic oil-loaded chitosan microparticles and films embedded with garlic oil-loaded chitosan microparticles

According to Piletti *et al.* (33), garlic oil has good antimicrobial effects against both *E. coli* and *S. aureus*. This proves that garlic oil has excellent antimicrobial activity and can be used to treat oral mucosal infections. However, garlic oil has not been studied for this purpose due to its strong odour and thermal instability (24,33,44). From Table 5, regarding *E. coli*, the microparticles alone showed a zone of inhibition in the range of 7.00 to 9.00 mm, while films that were embedded with the same sample of microparticles did not exhibit any zone of inhibition. Concerning *S. aureus*, the microparticles showed a zone of inhibition in the range of 7.00 to 9.00 mm, while the films incorporated with the same sample of microparticles also exhibited zones of inhibition in the range of 7.00 to 8.00 mm. The results suggest that garlic oil-loaded chitosan microparticles showed better antimicrobial activities against *S. aureus* than *E. coli*, as evidenced by the significant difference for films between *E. coli* and *S. aureus* (Student's t-test: $p < 0.05$).

Table 4: Film weight, thickness, and contact angle measurement (mean ± SD, n=3).

Film	Weight (g)	Thickness (mm)	Contact angle, Θ (°)
F1	0.20 ± 0.01	0.02 ± 0.00	50.93 ± 3.53
F2	0.25 ± 0.02	0.12 ± 0.01	47.70 ± 0.53
F3	0.24 ± 0.01	0.11 ± 0.01	54.77 ± 0.57
F4	0.22 ± 0.03	0.12 ± 0.02	57.20 ± 1.47
F5	0.23 ± 0.02	0.11 ± 0.00	56.07 ± 4.65
F6	0.25 ± 0.01	0.12 ± 0.00	56.80 ± 1.41

The positive control also displayed a significant difference between these two bacteria for both microparticles and films embedded with the same microparticles (Student's t-test: $p < 0.05$). This is due to S. aureus being a Gram-positive bacteria, with the major constituent of its cell wall being peptidoglycan with very little protein. *Escherichia coli*, on the other hand, is a Gram-negative bacteria and, although its cell wall is thinner, it is more complex and contains various polysaccharides, proteins and lipids. The cell wall of *E. coli* also has an outer membrane that constitutes the wall's outer surface (26). HPMC films, in contrast to chitosan, do not show antimicrobial activities and only act as a neutral support in releasing the antimicrobial properties of the active compounds (45). This may be the cause of why there was no zone of inhibition for *E. coli* when using HPMC films embedded with garlic oil-loaded chitosan microparticles, as *E. coli* has several layers that need to be penetrated. These findings are similar to those of Klangmuang & Sothornuit (46), who noted that HPMC-based nanocomposite films incorporated with essential oil were more effective in inhibiting *S. aureus* than *E. coli*.

Nevertheless, the effects regarding garlic oil-loaded chitosan microparticles and *E. coli* contradict the findings of Piletti *et al.* (33). In their study, garlic oil encapsulated with beta-cyclodextrin did not show any inhibition halos for *E. coli*. The difference could be due to the use of chitosan as an encapsulating agent in the current study, as chitosan possesses antimicrobial properties of its own. Chitosan has antimicrobial ability due to its positively charged amino group, which can interact with the negative charge of bacterial cell membranes, leading to a decrease in the bacterial cell membranes' permeability (26). This is supported by Sánchez-González *et al.* (45) where blank chitosan films without any essential oil exhibited a significant antimicrobial activity against *E. coli*. Nonetheless, the blank chitosan films did not show antimicrobial activity against *S.aureus* and only with the incorporation of essential oil into the chitosan films, they managed to reduce the pathogen growth. Garlic oil is known to have antimicrobial ability. The antimicrobial activity of garlic oil is mediated by its lipophilic properties, which can perforate the bacterial membrane and alter its chemical composition (31,47), resulting in the release of membrane components from bacterial cells to the external environment (31,48). Thus, combination of garlic oil and chitosan together may synergistically increase antimicrobial activity.

Based on Table 5, for *S. aureus*, M3 was found to have a significantly larger zone of inhibition compared to the rest of the microparticles (M1, M2, M4 and M5; ANOVA: $p < 0.05$). However, this was not the case for F4, even though F4 contains M3 microparticles, as F6 was found to have a significantly larger inhibition zone than F2, F3 and F4 (ANOVA: $p < 0.05$). The results suggest that, the higher the amount of garlic oil in the chitosan microparticles, the more effective the HPMC films embedded with garlic oil-loaded chitosan microparticles are when developed as a treatment for bacterial infections in the mouth.

4.0 Conclusion

In this study, the suitability of HPMC-based films embedded with garlic oil-loaded chitosan microparticles was evaluated as a potential oral mucosal drug delivery system concerning bacterial infections due to increasing bacterial resistance to conventional antibiotics. Garlic oil has great importance due to its antimicrobial properties; however, it needs to be encapsulated with chitosan to preserve its stability. Garlic oil-loaded

chitosan microparticles (M1-M5) were successfully prepared using ionic gelation method. The microparticles were spherical in shape, sized between 2.09 to 5.63 µm and had a uniformity of 0.30 to 0.91. However, the microparticles were electrically unstable and tended to agglomerate due to having a low zeta potential. The microparticles were added into HPMC solution to form films (F2-F6); a blank film was also prepared (F1). All the fabricated films were thin and light, suitable for buccal use. Additionally, the films had good wettability and the films that were embedded with garlic oil-loaded chitosan microparticles (F3-F6) had higher contact angles compared to the blank film (F1) and films that contained only chitosan microparticles (F2); this is due to the presence of garlic oil increasing the films' hydrophobicity. The films also demonstrated in vitro antimicrobial activities, especially against Gram-positive bacteria, namely *S. aureus*. HPMC-based films embedded with encapsulated garlic oil have the potential to act as a new drug treatment against oral mucosal bacterial infections. More studies should be performed to evaluate the effectiveness of this treatment, such as extensive physicochemical characterization, mucoadhesion studies, and studies evaluating drug release and drug permeation across the oral mucosa. Additionally, the particle preparation should be improved by using low centrifugation speed (3,000 – 4,000 rpm) to remove the unformed materials first, followed by the centrifugation at 10,000 rpm to collect the microparticles.

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Conflict of interest

The authors declare that there are no conflicts of interest.

Table 5: Zone of inhibition for positive control, garlic oil-loaded chitosan microparticles and films embedded with garlic oil-loaded chitosan microparticles for *E.coli* and *S.aureus* (mean ± SD, n=3).

Bacteria	Zone of inhibition (mm)				
	Positive control	Garlic oil-loaded chitosan microparticles		Films embedded with garlic oil-loaded chitosan microparticles	
E.coli	14.13 ± 0.43	M1	7.00 ± 0.00	F2	0.00 ± 0.00
		M2	7.00 ± 0.00	F3	0.00 ± 0.00
		M3	7.00 ± 0.00	F4	0.00 ± 0.00
		M4	8.00 ± 0.00	F5	0.00 ± 0.00
		M5	9.00 ± 0.00	F6	0.00 ± 0.00
S.aureus	19.00 ± 2.27	M1	7.00 ± 0.00	F2	7.00 ± 0.00
		M2	7.00 ± 0.00	F3	7.00 ± 0.00
		M3	9.00 ± 0.00	F4	7.00 ± 0.00
		M4	8.00 ± 0.00	F5	7.67 ± 0.58
		M5	7.67± 0.58	F6	8.00 ± 0.00

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