Original Article

Formulation And Evaluation Of Chlorhexidine Oral Fast-Dissolving Films

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Abstract

Chlorhexidine is well known to be an effective antiseptic in managing wound care and oral diseases. Currently, there are a limited number of chlorhexidine formulations available, with the majority being conventional mouth rinses, gels, pocket chips and varnishes, and the minority being oral disintegrating films. Therefore, this study aims to formulate and evaluate oral fast-dissolving films of chlorhexidine with an additional objective to improve patient compliance and convenience while maintaining its efficacy as its standard oral rinses. Six formulations of chlorhexidine oral fast-dissolving films were prepared by solvent casting method. The films were then evaluated for their weight, dimensions, thickness, folding endurance, moisture content, in vitro disintegration time, surface pH, antimicrobial activity, and stability studies. Fourier-Transform Infrared spectroscopy (FTIR) of the films was also analysed. The films showed acceptable physicochemical and stability properties with a weight range of 33.23 to 85.90 mg, a thickness range of 0.32 to 0.79 mm, a folding endurance range of 21 to 437, a moisture content range of 9.16 to 26.22%, a disintegration time range of 4.91 to 7.09 mins and a surface pH range of 7.24 to 8.12. FTIR spectra displayed the incorporation of the film composition without any interactions. The antimicrobial activity of the chlorhexidine films was unable to produce satisfactory results despite using a potent antiseptic. It was concluded that despite not having fast-dissolving properties, the chlorhexidine films were still able to provide promising results that can be of great importance in managing oral diseases such as caries and gingival inflammation.

Keywords: chlorhexidine, fast-dissolving films, oral delivery, antimicrobial activity.

INTRODUCTION

With expanding urbanisation and alterations in living conditions, oral diseases are increasingly common and are expected to rise. Although they are usually preventable, dental treatments can impose large economic burdens on families and healthcare systems. Apart from the economic burden, oral disorders can also have an impact on people's quality of life, affecting roughly 3.5 billion people worldwide throughout the course of their lifespans [1]. Dental caries (tooth decay), periodontal disease, tooth loss, and cancer are some of the most common prevalent oral health conditions [2]. Untreated dental caries in permanent teeth and periodontal disorders, which respectively affected 35.5% and 10.8% of people worldwide in 2010, were two of the most prevalent health issues [3]. In the US, untreated caries were diagnosed in 25.3% of adults aged 45 and older, and in 20.2% of adults aged 65 years and older [4]. On the contrary, it is estimated that 47.2% of individuals aged 30 years and older, and 70.1% of individuals 65 years and older have periodontal disease [5].

Chlorhexidine is a cationic bisbiguanide with three forms that are utilised broadly in the pharmaceutical industry: diacetate, digluconate and dihydrochloride [6]. Particularly, chlorhexidine digluconate is one of the most widely used chlorhexidine salt forms in healthcare or commercial settings because of its ability to dissolve in water and efficiently

deliver the molecule [7]. Chlorhexidine is a broad-spectrum antimicrobial agent with rapid bactericidal activity that causes damage to the outer cell layers and cytoplasmic membrane, compromising its integrity to allow cellular components to leak out [6]. Depending on its concentration, it can also cause cytoplasmic coagulation and precipitation [6,8]. Currently, it has been often used as a disinfecting agent for catheters and non-living clinical surfaces [9]. Because it is also biocompatible, chlorhexidine can be used as an antiseptic mouthwash in order to prevent the build-up of plaque and oral biofilm [9]. The pathogenesis of oral disorders mentioned previously has been closely linked to the oral biofilm and its associated bacteria [5]. Even though toothbrushing is the primary method for preventing and reducing plaque and gingivitis, patients who are mentally or physically disabled, and in postsurgical situations where oral hygiene becomes nearly impossible, may find it difficult to use a toothbrush because of the time, willingness and dexterity-related skills required [5].

Chlorhexidine mouthwash is currently the gold standard in reducing oral biofilm due to its potency [5]. Apart from that, other chlorhexidine formulations such as gels, biodegradable chips, sprays, toothpastes, and varnishes are also available by prescription [5,9]. However, there are very few, if any, chlorhexidine oral films that are being commercialised in the pharmaceutical and dental industries.

Oral films apply the local drug delivery systems to provide high and sustained local drug concentrations without using large doses that could result in systemic toxicity. They are designed as an alternate formulation to prevent dysphagia and improve patient compliance by dissolving in the mouth without the aid of water and leaving a pleasant aftertaste [10]. The large surface area of the films allows rapid disintegration and absorption into the systemic circulation directly without undergoing first-pass hepatic metabolism, enhancing the bioavailability [11,12]. In particular, the term “fast-dissolving films” aims to achieve complete disintegration and dissolution of films within seconds. Compared to the conventional chlorhexidine formulations such as mouthwash and gels, the tiny size of the films also allows them to be packaged in a small container for the convenience of the individual to use at any time and place [11,12]. The small and thin films can simply be placed directly on the affected oral mucosal tissue, where they can be rapidly hydrated by saliva and adhered to the affected site, enabling targeted drug delivery.

Previous studies on the development and characterisation of chlorhexidine gluconate oral films were reported to have been successfully created with decent physicochemical characteristics. Chitosan-based films and hydrogens containing chlorhexidine gluconate were fabricated and observed that gel preparations provided a quicker drug release profile than films [13]. Chlorhexidine digluconate orodispersible films prepared by Brambati et al. (2021) reported that they had good and favourable stability properties. Yet, there has been limited evidence on the fabrication of chlorhexidine fast-dissolving films. Nirmala et al. (2016) successfully produced Zolpidem oral fast-dissolving films using the solvent casting method and produced satisfactory results on its physical characteristics and drug release. Moreover, another research conducted by Basu et al. (2022) found that Methylphenidate fast-dissolving films with higher amounts of hydroxypropyl methylcellulose (HPMC) E5 as the polymer demonstrated more than 95% drug release within 2 minutes. Oral fast-dissolving films of the antihypertensive drug, Losartan Potassium, also showed good physicochemical characteristics with a favourable drug release that ranged from 78 to 96% within 5 minutes [17]. The formulation of oral fast-dissolving films containing HPMC, polyethylene glycol (PEG) and Lisinopril as the active pharmaceutical ingredient provided satisfactory results on its physical and stability properties [18]. Furthermore, Wasim Hussain et al. (2017) successfully developed Chlorpromazine fast-dissolving films using the solvent casting technique for drug delivery in the buccal cavity while also adhering to the standard range of film-specific parameters.

The purpose of this study is to develop and evaluate antimicrobial oral fast-dissolving films of chlorhexidine digluconate, a biguanide compound, for the treatment of oral diseases.

The objectives of this research are to prepare the oral fast-dissolving films formulation, analyse its physicochemical properties and Fourier-Transform Infrared Spectroscopy (FTIR) characterisation according to its formulation, evaluate the in-vitro antimicrobial and stability properties, and to statistically analyse the results.

MATERIALS AND METHODS

Materials

Hydroxypropyl methylcellulose (HPMC) polymer, plasticisers such as glycerol and triethyl citrate, sodium starch glycolate (SSG) super disintegrant and chlorhexidine digluconate (CD) were obtained from the Sigma chemicals, USA. Other chemicals were of analytical grade.

Preparation of oral films

The preparation of the films was adapted from a study by David et al. (2018) that also followed the solvent casting method. A precisely weighed quantity of polymer was added to 10 mL of double distilled water and mixed using a magnetic stirrer until dissolved. Following that, sodium starch glycolate was added and stirred. A plasticiser was then added and stirred continuously until all ingredients were completely dissolved. CD was added with continuous stirring until a homogenous mixture was formed. The mixture was left to rest and ensured there are no bubbles for the next step. The mixture was then poured into the centre of stainless-steel rings with an aluminium foil as a backing layer, then dried in a hot air oven at 60°C for five days. The resulting oral fast-dissolving film was peeled from the glass petri dish and cut into films with a size of 1 x 1 cm^2, each film containing 25mg of CD. They were wrapped using airtight aluminium foil for storage [21].

Average weight

The weights of each film were measured with an electronic balance (A&D Company, Tokyo, Japan). The test was repeated three times, and the mean and standard deviation (SD) of the readings were recorded [22].

Film size and thickness

The lengths and heights of the oral films were measured using Fisherbrand™ Traceable™ Digital Calipers (Thermo Fisher Scientific, Waltham, USA). In addition to that, the thickness of the films was measured at three different points, i.e., left-end, centre, and right-end [23]. The mean and SD of the readings were calculated and recorded.

Folding endurance

The folding endurance of the films is determined by counting the number of times the films could be folded without breaking. Folding the films at the same place repeatedly could either break the films or develop visible curves [23–25]. The mean and SD values of triplicate readings will be calculated and recorded.

Surface pH

The films were allowed to dissolve in a beaker filled with distilled water [23,25]. The surface pH was determined using a Fisherbrand™ accumet™ AE150 pH meter (Thermo Fisher Scientific, Waltham, USA) and allowing it to equilibrate for at least 1 minute. The mean of triplicate readings for each film was recorded [22].

Moisture content

The moisture content was analysed by using an electronic moisture content analyser MOC63u (Shimadzu Corporation, Kyoto, Japan). The films were initially weighed and then dried for up to 105°C [26]. The percentage of moisture content was calculated as:

$$\% \text{ Moisture content} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

The mean and SD of triplicate readings for each formulation were reported.

Fourier transform infrared spectroscopy (FTIR) analysis

FTIR spectra of the formulations (F1, F2, F3, F4, F5 and F6), the pure drug (CD) and its excipients (HPMC, SSG, triethyl citrate and glycerol) were analysed. The spectra were obtained by using an FTIR spectrophotometer IRSpirit (Shimadzu Corporation, Kyoto, Japan). According to Bharti et al. (2019), the samples were scanned between 4000 and 400 cm^{-1} with a resolution of 4 cm^{-1} for 30 scans. The transmittance value for each reading was recorded [27].

In vitro disintegration time

In accordance with Bharti et al. (2019), three random films were placed in a beaker containing 20 mL of distilled water with a pH of 7.0 and continuously stirred with a magnetic stirrer (Cole-Parmer, Chicago, USA). The time when the film started to disintegrate into small fragments was recorded and the mean was reported [28,29].

Preparation of bacterial culture

Staphylococcus aureus bacteria were chosen as per the literature review over its prevalence of presence in the oral cavity as well as its occurrence in wounds. The ATCC 25923 strain was chosen to perform this experiment.

The assay performed was according to a protocol prepared by Wiegand et al. (2008). Mannitol salt agar was used to grow the ATCC 25923 S. aureus. A sterilised inoculating loop was used to inoculate thawed ATCC 25923 S. aureus onto the mannitol salt agar. Once inoculated, the petri dish was placed in an incubator at 37°C for 24 hours. The bacteria were confirmed to be Staphylococcus aureus through light microscopy and via a positive catalase test. The bacterial colonies were added to a Luria-Bertani (LB) broth solution with glycerol to create bacterial suspension and stored at -8°C [31].

Antimicrobial Disk diffusion test zone of inhibition (ZOI) test

The test was conducted following the standard guidelines set by the Clinical and Laboratory Standards Institute and the British Society of Antimicrobial Chemotherapy (BSAC). Mueller-Hinton Agar (MHA) was used to analyse the Zone of Inhibition (ZOI in mm) formed of the formulations containing CD with the ATCC 25923 S. aureus. 100µL of 0.5 McFarland Standard adjusted bacteria suspension was added onto each petri dish of MHA and spread using a plastic Biologix cell spreader (Cat65-1001) (Biologix, Jinan, China). Each formulation was shaped into 6mm diameter circles and then placed on the agar. A gentamicin 10µg disk and a UV-sterilised filter paper soaked in distilled water were used as positive and negative controls respectively. Then, the Petri dishes were kept in the incubator at 37°C for 16-18 hours, where the diameters of the zones of inhibition were measured for each formulation.

Stability study

An optimised batch of the oral dissolving films was left at three different conditions: 6°C, 25°C (room temperature) and 40°C. The films were kept for 3 weeks in a stability chamber and wrapped in aluminium foil. The samples were then inspected at the first-week interval, then the second week, and lastly the third week. The films were evaluated for their folding endurance, surface pH, moisture content and disintegration time [32].

Statistical analysis

Each characterisation test of the oral films was performed in triplicates according to the methods described previously. The mean values of the triplicate readings with standard deviations were calculated. The statistical difference was analysed by conducting a one-way ANOVA with a posthoc test according to Bonferroni's procedure to determine the mean differences between the films prepared using the RStudio computer program (Posit Software, Boston, USA). Statistical difference was set at $P < 0.05$.

RESULTS

Preparation of the oral films

The solvent casting approach was used to successfully create the chlorhexidine oral films. Table 1 shows the composition of the prepared films with differing amounts of excipients. The films appeared generally clear, opaque and smooth (Figure 1). Overall, they were thin, not brittle, no scent and had acceptable mechanical properties. They were then assessed for a variety of physicochemical tests including size, weight, folding endurance, moisture content, disintegration time and surface pH.

Table 1: Composition of oral films prepared

Formulation	HPMC (mg)	SSG (mg)	Triethyl citrate (%)	Glycerol (ml)	CD (ml)
F1	1000	300	5	-	0.5
F2		300	10		
F3		200	-	5	
F4		300			
F5		200		10	
F6		300			

Note: HPMC indicates hydroxypropyl methyl cellulose; SSG indicates sodium starch glycolate; CD indicates chlorhexidine digluconate

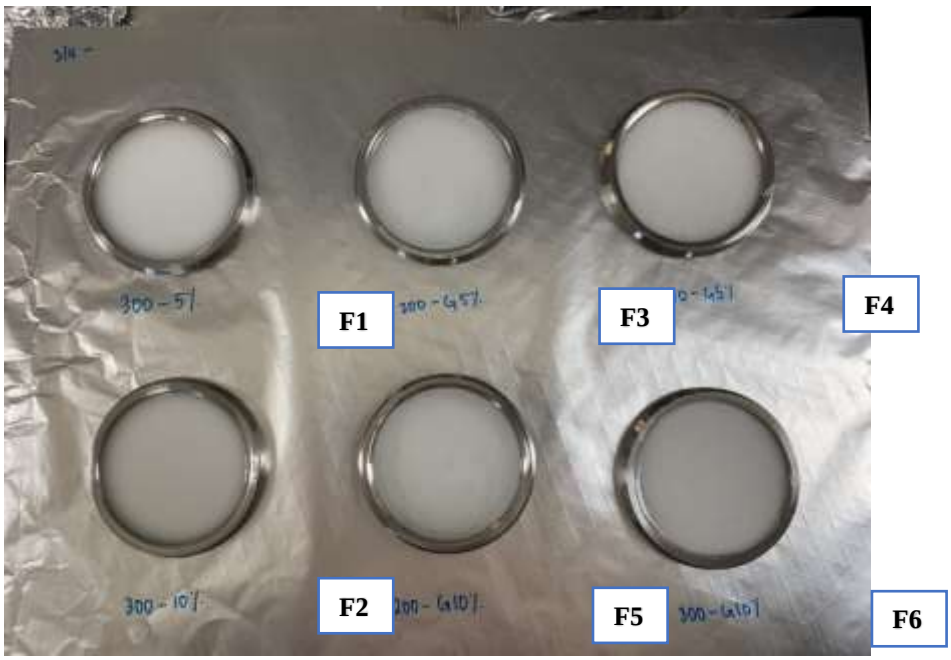

Figure 1: Chlorhexidine film preparations

Physical characteristics

Table 2 summarises the physical measurements of the six prepared formulations. The average weight and thickness of the films ranged from 33.23 to 85.90mg and 0.32 to 0.79mm respectively. These variations may be caused by the different amounts of excipients used in each formulation. The film dimensions were found to be quite uniform and near to their projected size of 1 cm x 1 cm. It is also important to note that an increasing concentration of plasticiser leads to an increase in both weight and thickness of the films.

Table 2: Evaluation of physical characteristics of film preparations

Formulation	n	Weight/mg	Length/mm	Width/mm	Thickness/mm
F1	3	33.23 (2.53) [c]	9.91 (0.11) [a]	9.94 (0.24)	0.32 (0.01) [c]
F2		56.43 (5.22) [c]	10.35 (0.24) [b]	10.53 (0.09) [a]	0.41 (0.04) [c]
F3		46.20 (2.16) [c]	9.82 (0.14) [a]	9.80 (0.49)	0.35 (0.02) [c]
F4		61.80 (4.56) [b]	9.74 (0.25)	9.86 (0.49)	0.49 (0.02) [c]

F5	87.83 (8.46) [c]	9.20 (0.32) [b]	8.99 (0.66) [a]	0.77 (0.03) [c]
F6	85.90 (5.00) [c]	8.96 (0.35) [b]	9.18 (0.50)	0.79 (0.04) [c]

Note: Values are represented as mean (SD); one-way ANOVA test (equal variance assumed), $p<0.05$; post-hoc test (Bonferroni's procedure), [a] for $p<0.05$, [b] for $p<0.01$, [c] for $p<0.001$ when compared to all formulations

Folding endurance test

The folding endurance of the films were measured manually, and results were tabulated in Table 3. The number of folds a film could withstand before breaking ranged from 21 to 437. A greater folding endurance suggests a lower possibility of the film easily rupturing. When the concentration of SSG and glycerol increased, the folding endurance remained low except for F4. Despite the increased amount of SSG from 300 to 400mg, F4 showed a greater folding endurance at 437 compared to that of F3 at 321.

Moisture content analysis

A low moisture content benefits film stability over the long term, brittleness reduction and protection from microbial contamination. All the prepared films had an average moisture content that ranged from 9.16 to 26.22% (Table 3). The results showed that the films had a slightly high moisture content that may be caused by the properties of the excipients or varying weight and size. The moisture content of F6 with the highest ratio of SSG to plasticiser (glycerol) was the highest at 26.22%, while the moisture content of F2 with the same ratio of SSG to plasticizer (triethyl citrate) was the lowest at 9.16%.

Disintegration test

The disintegration time for oral films indicate the onset of drug action. A low value would allow a quicker release and absorption of the drug through the oromucosal tissue. The range of the mean disintegration time was recorded to be 4.91 to 7.09 minutes (Table 3). Statistical analysis found that there were no significant differences of the disintegration times between the formulations ($P>0.05$).

Surface pH

To prevent any possible irritation to the mucosal lining from the alkalinity or acidity, the surface pH of the films should be close to the pH of the oral cavity (neutral pH). The films had a pH range of 7.24 to 8.12 on average (Table 3). Generally, films made with glycerol (F3, F4, F5 and F6) were significantly more basic compared to films made with triethyl citrate (F1 and F2).

Table 3: Evaluation of pharmacokinetic profiles of film preparations

Formulation	n	Folding endurance	Moisture content/%	Disintegration time/min	pH
F1		417 (162) [b]	12.59 (3.22) [c]	4.91 (1.64)	7.34 (0.14) [c]
F2		157 (38) [a]	9.16 (1.62) [c]	6.48 (1.44)	7.24 (0.07) [c]
F3	3	321 (30) [a]	21.69 (1.18) [c]	5.92 (0.18)	7.77 (0.22) [a]
F4		437 (101) [a]	18.02 (2.51) [a]	6.19 (0.90)	8.01 (0.13) [c]
F5		58 (29) [b]	18.02 (1.60) [a]	7.09 (0.76)	7.72 (0.11) [a]
F6		21 (15) [b]	26.22 (1.42) [c]	5.72 (0.87)	8.12 (0.05) [c]

Note: Values are represented as mean (SD); one-way ANOVA test (equal variance assumed), $p<0.05$; post-hoc test (Bonferroni's procedure), [a] for $p<0.05$, [b] for $p<0.01$, [c] for $p<0.001$ when compared to all formulations

Fourier transform infrared spectroscopy (FTIR) analysis

Figure 2 shows the spectra graph of all six formulations containing CD, the pure drug CD itself and its excipients. As shown in Figure 2, all six formulations were observed to have common peaks and no interactions. The absorption band at around 999 to 992 cm^{-1} were observed as the first common peak indicated the C-O stretching from C-O-C in the glycosidic ring of starch found in HPMC and SSG. In addition to that, there is also an absorption band at around 800 to 700 cm^{-1} that indicated a C-Cl stretch from CD in all formulations. The absorption peak at around 1750-1725

cm^{-1} observed in F1 and F2 suggested the ester bond presented in triethyl citrate. In films F3, F4, F5 and F6, the spectra demonstrated an absorption band at around 3645 to 3206 cm^{-1} that indicated a O-H stretching frequency and at 2935-2845 cm^{-1} that suggested a C-H_2 and C-H stretching found in glycerol. Moreover, the COO^- group in gluconic acid can be demonstrated in the IR peak at around 1640 to 1620 cm^{-1} in the films F3, F4, F5 and F6.

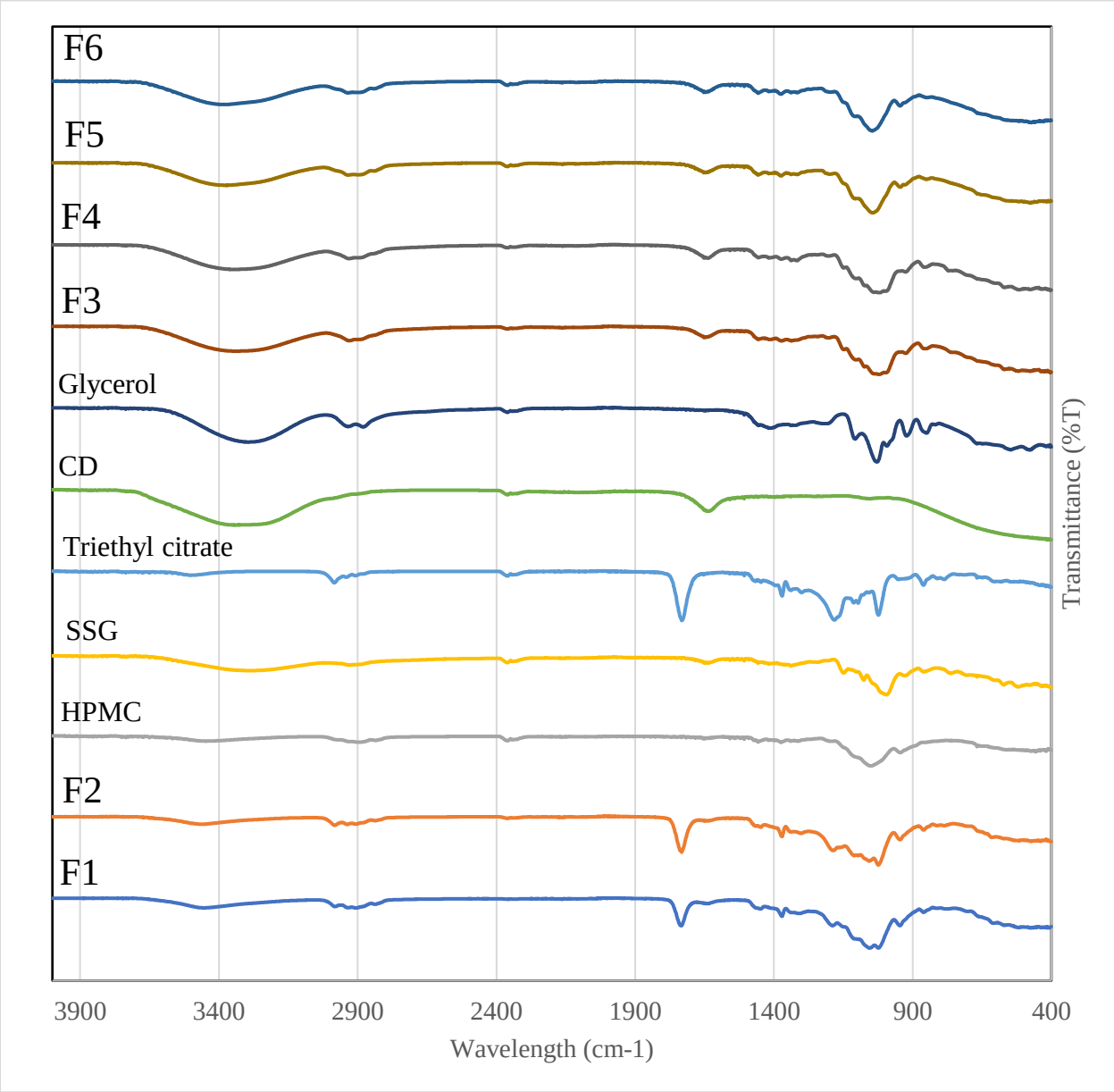

Figure 2: FTIR spectra of all formulations, HPMC, SSG, triethyl citrate, CD and glycerol

Antimicrobial activity of the films against S. aureus

The ATCC 25923 S. aureus bacteria were successfully grown on a freshly made mannitol salt agar for at least 24 hours.

The results of disk diffusion are displayed in Figure 3 and 4. The failure of bacterial growth inhibition by the negative controls exhibited that the experimental setting was appropriate for testing the antibacterial activities. The positive controls, gentamicin 10µg disks, inhibited the growth of S. aureus by 70mm in both ZOI dishes, demonstrating that S.

aureus is susceptible to the positive control [33]. None of the chlorhexidine film formulations could demonstrate that the S. aureus bacteria were susceptible to the formulation because there was no clear and distinct inhibition zone surrounding the film.

Figure 3: First zone of inhibition for positive control, negative control and all 6 film formulations

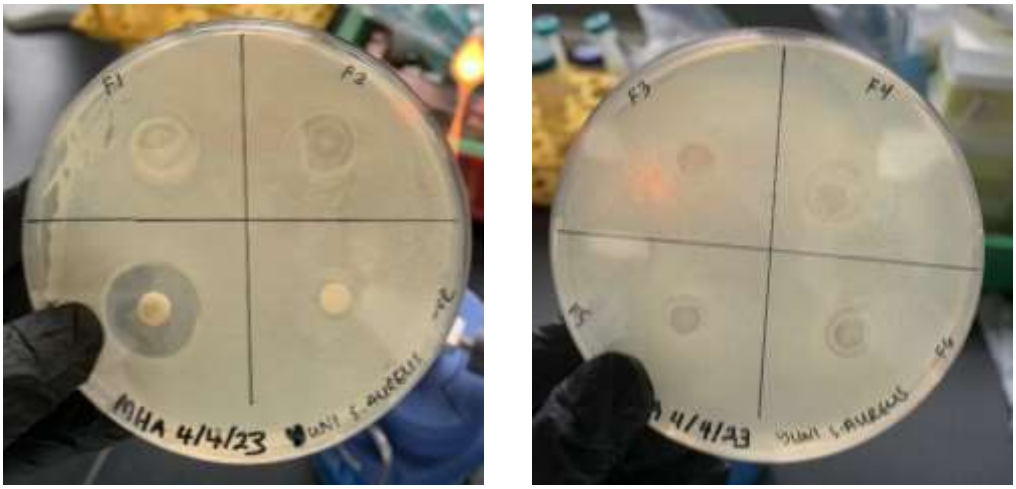

Figure 4: Second zone of inhibition for positive control, negative control and all 6 film formulations

Stability studies

Over the course of three weeks, the appearance of all films remained the same when stored at normal room temperature of 25°C and at 40°C. But the films F5 and F6 became more stiff and yellow in colour when kept at 6°C during the period.

The folding endurance of the films over the 3-week period are shown in Table 4. All the films generally had a decreased folding endurance when kept at a lower temperature throughout the 3-week period. In contrast, at higher temperatures, the films overall had the highest folding endurance over 3 weeks. The only formulations with a folding endurance below 100 folds during the period were F5 and F6, with F5 having the lowest folding endurance of just six.

It is also worth noting that as the weeks pass, the folding endurance of the films decreases, except for F2 stored at 40°C after 3 weeks, F3 stored at 6°C after 3 weeks, F4 stored at 6°C after a week and 40°C after 3 weeks.

The moisture content of the films over the 3-week period were more stable with low values at 6°C (Table 5). At room temperature, the moisture content of the films F4, F5 and F6 reduced as the three weeks passed. Storing F4 at 25°C throughout the period caused the moisture content to rise instead. However, the moisture content of F1 and F2 was significantly lower at the second week interval than it was at the first and third when kept at room temperature. During the 3-week period, there were no discernible trends in the moisture content of films at higher temperature. In comparison to the first and third weeks, the moisture content of the films F2, F4, F5 and F6 significantly increased at the second-week interval. On the contrary, F1 exhibited a decrease in moisture content throughout the period whereas F4 showed an increase. All films, apart from F2, still showed a high moisture content that ranged from 10.52 to 31.86% when stored at 25°C and 40°C.

Table 6 shows a distinct trend in the disintegration time of the films F1 and F2 over the course of three weeks. The disintegration times of F1 and F2 were slower as the weeks went on when stored at 6°C and 40°C. However, as the weeks went by, the time it took for the films F1 and F2 to disintegrate was faster at normal room temperature. In comparison to F1 and F2, there were no clear pattern in the disintegration time of the films F3, F4, F5 and F6. There were significant differences in the disintegration time of the films F3, F4 and F6 between the weeks. The disintegration time for F3 varied greatly from 5.15 to 8.00 mins at 6°C, 3.18 to 6.00 mins at 25°C and 4.56 to 7.92 mins at 40°C. On the other hand, the disintegration time for F6 ranged from 5.75 to 9.53 mins at 6°C, 4.61 to 7.04 mins at 25°C and 7.82 to 8.54 mins at 40°C. The range of disintegration time for film F4 was 5.80 to 6.61 mins at 6°C, 4.77 to 6.58 mins at 25°C, 5.80 to 8.36 mins at 40°C. However, the disintegration time of F5 was not significantly different between the weeks. The time it took for film F5 to disintegrate ranged from 7.38 to 8.07 mins at 6°C, 7.11 to 7.95 mins at 25°C and 7.05 to 8.44 mins at 40°C.

The pH of the films was found to be the most stable at lower temperatures over the course of three weeks (Table 7). On the other hand, the pH of all films was significantly different throughout the period when kept at room temperature. At the second week interval, all the films at 25°C recorded a pH of at least 8.05 compared to the rest of the week.

Table 4: Stability of folding endurance of formulations stored at 6°C, 25°C and 40°C over 3 weeks

Formulation	Folding endurance								
	Week 1			Week 2			Week 3		
	6±2°C	25±2°C	40±2°C	6±2°C	25±2°C	40±2°C	6±2°C	25±2°C	40±2°C
F1	102 [b]	479 [b]	626 [b]	161	206	70	74	91	246
F2	101 [b]	90 [b]	104 [b]	76	233	87	43	86	220
F3	70	130	365	89	99	347	112	70	120
F4	240	120	208	97 [b]	130 [b]	160 [b]	79	127	494
F5	6	11	29	19 [a]	20 [a]	14 [a]	16	10	63
F6	15 [a]	26 [a]	40 [a]	7	18	56	15	11	75

Note: one-way ANOVA test (equal variance assumed), $p<0.05$; post-hoc test (Bonferroni's procedure), [a] for $p<0.05$, [b] for $p<0.01$, [c] for $p<0.001$

Table 5: Stability of moisture content (%) of formulations stored at 6°C, 25°C and 40°C over 3 weeks

Formulation	Moisture content (%)								
	Week 1			Week 2			Week 3		
	6±2°C	25±2°C	40±2°C	6±2°C	25±2°C	40±2°C	6±2°C	25±2°C	40±2°C
F1	7.69 [c]	15.15 [c]	14.28 [c]	6.98 [b]	11.63 [b]	13.5 [b]	3.90	19.35	12.19
F2	6.90 [a]	10.52 [a]	7.84 [a]	14.81	6.78	18.96	7.27	12.07	7.55
F3	16.98 [a]	12.76 [a]	17.39 [a]	14.00 [a]	24.48 [a]	16.88 [a]	15.09 [b]	22.22 [b]	24.19 [b]

F4	13.79 [c]	30.30 [c]	25.42 [c]	13.56 [b]	24.38 [b]	27.63 [b]	18.52	15.87	25.80
F5	12.63 [b]	25.26 [b]	31.18 [b]	14.47 [a]	21.68 [a]	32.72 [a]	9.90 [a]	20.00 [a]	29.00 [a]
F6	14.94 [b]	34.51 [b]	30.43 [b]	15.38 [a]	25.35 [a]	35.59 [a]	15.79 [a]	23.40 [a]	31.86 [a]

Note: one-way ANOVA test (equal variance assumed), p<0.05; post-hoc test (Bonferroni's procedure), [a] for p<0.05, [b] for p<0.01, [c] for p<0.001

Table 6: Stability of disintegration test (mins) of formulations stored at 6°C, 25°C and 40°C over 3 weeks

Formulation	Disintegration time (mins)								
	Week 1			Week 2			Week 3		
	6±2°C	25±2°C	40±2°C	6±2°C	25±2°C	40±2°C	6±2°C	25±2°C	40±2°C
F1	3.66 [a]	6.06 [a]	4.40 [a]	7.89	8.98	5.95	8.58	3.93	6.69
F2	4.85	9.15	5.36	7.49	8.28	5.65	8.34 [a]	5.64 [a]	6.68 [a]
F3	5.15 [c]	5.12 [c]	5.14 [c]	8.00 [a]	6.00 [a]	7.92 [a]	7.67	3.84	4.56
F4	6.30 [a]	6.58 [a]	8.36 [a]	6.61 [a]	4.77 [a]	6.13 [a]	5.80 [b]	5.74 [b]	6.14 [b]
F5	7.38 [b]	7.11 [b]	8.44 [b]	8.07	4.83	7.05	7.51 [c]	7.95 [c]	8.28 [c]
F6	9.53 [b]	6.98 [b]	7.82 [b]	6.57	4.61	8.54	5.75 [a]	7.04 [a]	8.39 [a]

Note: one-way ANOVA test (equal variance assumed), p<0.05; post-hoc test (Bonferroni's procedure), [a] for p<0.05, [b] for p<0.01, [c] for p<0.001

Table 7: Stability of surface pH of formulations stored at 6°C, 25°C and 40°C over 3 weeks

Formulation	pH								
	Week 1			Week 2			Week 3		
	6±2°C	25±2°C	40±2°C	6±2°C	25±2°C	40±2°C	6±2°C	25±2°C	40±2°C
F1	7.22 [c]	7.15 [c]	7.48 [c]	7.28 [b]	8.14 [b]	7.25 [b]	7.27 [c]	7.14 [c]	7.34 [c]
F2	7.27 [c]	7.29 [c]	7.30 [c]	7.19 [b]	8.15 [b]	7.23 [b]	7.39 [c]	7.16 [c]	7.17 [c]
F3	7.90 [c]	7.70 [c]	7.01 [c]	7.60 [c]	8.12 [c]	7.51 [c]	7.63 [c]	7.54 [c]	7.39 [b]
F4	7.89 [c]	7.89 [c]	7.80 [c]	7.69 [c]	8.09 [c]	7.69 [c]	7.94 [c]	7.64 [c]	7.37 [c]
F5	8.09 [c]	7.83 [c]	7.90 [c]	7.98 [b]	8.05 [b]	7.51 [b]	8.15 [b]	7.94 [b]	7.50 [b]
F6	8.00 [c]	7.92 [c]	7.93 [c]	7.98 [b]	8.15 [b]	7.60 [b]	8.13 [c]	7.85 [c]	7.50 [c]

Note: one-way ANOVA test (equal variance assumed), p<0.05; post-hoc test (Bonferroni's procedure), [a] for p<0.05, [b] for p<0.01, [c] for p<0.001

DISCUSSION

Preparation of the chlorhexidine oral films by solvent casting technique was easy and six different formulations of oral films were created. Prior to drying, films F1 and F2 containing triethyl citrate as plasticiser appeared to be more homogeneous with less bubbles formed while films F3, F4, F5 and F6 with glycerol as a plasticiser were more prone to bubbles and more viscous; this made films made with glycerol slightly difficult to be poured into dishes and required more solvent than films made with triethyl citrate. Moreover, this may cause less content uniformity of the film formulations with glycerol which may explain the inconsistency in physicochemical results of F3, F4, F5 and F6. Particularly, the weight and thickness of the films had significant differences ($P < 0.001$) between the films made with triethyl citrate and those made with glycerol. None of the films achieved the recommended range for oral films, which is between 10 and 100 µm [34]. However, some researches that exceeded the recommended ranges were carried out in an effort to improve the mechanical properties of the films while maintaining rapid disintegration times [34]. Teixeira et al. (2021) clarified that glycerol is a hydrophilic substance and acts as a water container agent, leading to an increase in thickness from the swelling process.

Additionally, films with triethyl citrate exhibited more flexibility than those made with glycerol. Generally, plasticisers have been reported to improve mechanical properties such as tensile strength and flexibility. Teixeira et

al. (2021) explained that the increased in flexibility resulted from the insertion of the plasticiser into the polymeric matrix of the films which increased the distance between the polymer chains and provide them freedom to move and be less rigid. One of the factors that contributed to the difference in flexibility between plasticisers is the difference in molecular weight. Triethyl citrate has a greater molecular weight of 276 g/mol than that of glycerol at 92 g/mol. As a result, the distance between the polymer chains was further increased by the size of each triethyl citrate molecule, which enhanced the flexibility of the films [35].

There are multiple opinions from several researchers on the recommended range of moisture content in oral films. A study by Takeuchi et al. (2021) on the effects of moisture content in orally disintegrating films suggested that the moisture content of pharmaceutical orodispersible films should be limited to approximately 10% or less. On the contrary, Janigová et al. (2022) compiled the recommendations of some studies and found that the moisture content should be lower than 6%, specifically between 3 and 6%. In this study, none of the formulations were able to achieve the recommended moisture content of lower than 6%; however, only F2 was able to follow the recommendation from Takeuchi et al. (2021) and obtained a moisture content of less than 10% at 9.12%. The ability to create films with good moisture barriers is limited by the hydrophilic of the film-forming material [37]. It can be hypothesised that the high amount of HPMC added as the film-forming agent may contribute to the high moisture content. The addition of plasticizer provides more active sites available from its hydrophilic hydroxyl groups for the water molecules to bind to [37]. Furthermore, the presence of superdisintegrants such as SSG could also affect the moisture content as they tend to absorb water.

At present, there are still no official references available to determine an optimum disintegration time of orally disintegrating films. However, according to the European Pharmacopoeia and British Pharmacopoeia, the disintegration time of orally disintegrating tablets, which may be applied to films, should be limited to 3 minutes and below. Films should generally disintegrate or dissolve more quickly than conventional tablets due to their large surface area. Moreover, this present study focused more on developing films that dissolve within 1 minute. In spite of that, results in this study showed that the overall time it took for a film to disintegrate was too long (> 1 min) to qualify as a fast-dissolving film for all six formulations. Takeuchi et al. (2021) reported that an increase in moisture content resulted in a drop in the disintegration time. The film structure is influenced by moisture content because water molecules bounded by hydrogen bonds can produce pores with bigger sizes. As a result, the solvent molecules can easily penetrate the film through the pores at a higher moisture content, speeding up the disintegration time. However, there is no significant relationship between moisture content and disintegration time ($P>0.92$) in this study, rejecting the hypothesis. Theoretically, the superdisintegrant SSG should demonstrate a good disintegration time of the films. However, the high amount of HPMC added and the increased thickness of the films played a major role in increasing the disintegration time although it provided a strong and tough film with good mechanical properties.

Saliva has a normal pH range of 6.2 to 7.6, where it maintains the pH of the oral cavity near neutrality around 6.7 to 7.3 [38]. Keeping the pH near neutrality generally demonstrates a low incidence of dental decay; hence, it is recommended that the films should be in this neutral pH range to avoid oral mucosa irritation. There is a significant increase in pH values between films plasticised with triethyl citrate and those with glycerol. Films made with triethyl citrate showed pH ranges of 7.24 to 7.34 that are suitable and are likely to cause irritation. In contrast, those plasticised with glycerol showed mild basic pH ranges of 7.72 to 8.12. Nonetheless, there is a low possibility of oromucosal tissue irritation due to the low weight of the films.

In general, chlorhexidine has been proven to be an effective and potent antiseptic against a wide spectrum of microbes. However, unlike some polymers with ionic charges such as chitosan, HPMC polymers do not show antimicrobial activity because it acts as a neutral support to release the active ingredient to the surface; and so, the antimicrobial activity depends entirely on chlorhexidine. Several researches conducted on the study of HPMC films incorporated with antimicrobial substances have shown promising results on its antimicrobial activity on species such as S. aureus, E. coli and L. monocytogenes [39,40]. Another factor that could attribute to the antimicrobial activity of the films is the drying conditions of the formulations. Sun et al. (2020) studied on the effects of spray-drying temperature on

antimicrobial properties and reported that the increase in inlet air temperature used to dry resulted in a weaker antimicrobial activity. A research conducted by Shaaban et al. (2017) also found that the antimicrobial activity of films were affected by the drying methods and conditions, where the maximum antibacterial activity was achieved by films that were dried using ambient air and low-temperature hot air. In another study conducted, air dried formulations at 24°C exhibited higher antibacterial activity compared to those dried under the sun at 33°C and in the oven at 50°C [43]. Additionally, the films used for the disk diffusion ZOI test may not have suitable biological conditions, such as temperature, moisture, and pH, that can release the active pharmaceutical ingredient, chlorhexidine, to produce any antibacterial effects.

Results from stability studies showed that storing the formulations at a lower temperature were generally more stable as compared to higher room temperatures. Films containing a higher amount of glycerol observed a decrease in folding endurance, possibly due to the decomposition of glycerol during storage. On the contrary, films containing triethyl citrate demonstrated more constant results with appropriate physicochemical properties when stored at different conditions. Therefore, storage conditions for both triethyl citrate films and glycerol films can be suggested to be kept at normal room temperature.

Study limitations and future research

The study was limited to a number of experiments due to time constraints. Prior in commencing the research, small batches of formulations with different types and amounts of excipients were done. The formulations with the best appearance, texture and toughness were chosen to be evaluated for its properties. The limited time hindered the opportunity of creating a formulation with the better excipient and drug ratio for an enhanced drug delivery and antimicrobial activity. Other experiments that could be performed for further evaluations of the films prepared include microscopical analysis, drug content uniformity, swelling properties, dissolution studies, in vitro drug release kinetics, permeation studies, histopathology investigations, animal and human clinical studies. Further studies can re-evaluate on the polymer and plasticiser ratio in chlorhexidine oral film formulations for improved physicochemical properties or focus on the antimicrobial activities and efficacy of chlorhexidine oral films in reducing oral biofilms and plaque accumulation.

CONCLUSION

Oral films of chlorhexidine were successfully formulated by the solvent casting method using HPMC as the polymer, SSG as the superdisintegrant and triethyl citrate and glycerol as the plasticisers. In vitro evaluation of the films illustrated a satisfactory observation of the chlorhexidine films, especially those prepared with triethyl citrate and low amounts of glycerol. Due to the long disintegration times of all films, it was not qualified to be referred as an oral fast-dissolving film. The formulations were also overall stable of its physicochemical properties when stored at normal room temperature. Films plasticised with triethyl citrate are expected to be more stable under cool conditions for a longer period. Unfortunately, however, the study did not produce satisfactory results in its antimicrobial activity despite chlorhexidine being a potent antiseptic agent. Formulating chlorhexidine oral fast-dissolving films can be extremely beneficial for local drug delivery in the oromucosal tissue and convenience of individuals with oral disorders. Further research can be done to further improve the disintegration times and antimicrobial properties of chlorhexidine oral fast-dissolving films for a possible alternative that is just as effective as the conventional chlorhexidine mouthwash in the pharmaceutical and dental industries.

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CONFLICT OF INTEREST

There is no conflict of interest reported.

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Appendices

Appendix A

Statistical analysis of differences for each evaluation parameters are displayed in the tables below.

Table 8: Comparing mean weight (mg) of formulations

Formulation	n	Weight Mean (SD)	F stat (df)[a]	P value[a]
F1	3	33.23 (2.53)	36.16 (5)	<0.001[b]
F2		56.43 (5.22)		
F3		46.20 (2.16)		
F4		61.80 (4.56)		
F5		87.83 (8.46)		
F6		85.90 (5.00)		

[a] One-way ANOVA test (equal variance assumed)

[b] All pairs except F1 and F2 with F3, F2 and F3 with F4, F5 with F6 were significant different by post-hoc test (Bonferroni's procedure).

Table 9: Comparing mean length (mm) of formulations

Formulation	n	Length Mean (SD)	F stat (df)[a]	P value[a]
F1	3	9.91 (0.11)	8.0969 (5)	0.002[b]
F2		10.35 (0.24)		
F3		9.82 (0.14)		
F4		9.74 (0.25)		
F5		9.20 (0.32)		
F6		8.96 (0.35)		

[a] One-way ANOVA test (equal variance assumed)

[b] All pairs except F5 and F6 with F3 were not significantly different by post-hoc test (Bonferroni's procedure).

Table 10: Comparing mean width (mm) of formulations

Formulation	n	Width Mean (SD)	F stat (df)[a]	P value[a]
F1	3	9.94 (0.24)	3.0521 (5)	0.05[b]
F2		10.53 (0.09)		
F3		9.80 (0.49)		
F4		9.86 (0.49)		
F5		8.99 (0.66)		
F6		9.18 (0.50)		

[a] One-way ANOVA test (equal variance assumed)

[b] All pairs except F2 with F5 were not significantly different by posthoc test (Bonferroni's procedure).

Table 11: Comparing mean thickness (mm) of formulations

Formulation	n	Thickness Mean (SD)	F stat (df)[a]	P value[a]

F1		0.32 (0.01)		
F2		0.41 (0.04)		
F3	3	0.35 (0.02)	390.31 (5)	<0.001[b]
F4		0.49 (0.02)		
F5		0.77 (0.03)		
F6		0.79 (0.04)		

[a] One-way ANOVA test (equal variance assumed)

[b] All pairs except F3 with F1 and F5 with F6 were significantly different by posthoc test (Bonferroni's procedure).

Table 12: Comparing mean folding endurance of formulations

Formulation	n	Folding endurance Mean (SD)	F stat (df)[a]	P value[a]
F1		417 (162)		
F2		157 (38)		
F3	3	321 (30)	9.8957 (5)	<0.001[b]
F4		437 (101)		
F5		58 (29)		
F6		21 (15)		

[a] One-way ANOVA test (equal variance assumed)

[b] All pairs except F1 and F4 with F5 and F1, F3 and F4 with F6 were not significantly different by posthoc test (Bonferroni's procedure).

Table 13: Comparing mean moisture content (%) of formulations

Formulation	n	Moisture content Mean (SD)	F stat (df)[a]	P value[a]
F1		12.59 (3.22)		
F2		9.16 (1.62)		
F3	3	21.69 (1.18)	17.823 (5)	<0.001[b]
F4		18.02 (2.51)		
F5		18.02 (1.60)		
F6		26.22 (1.42)		

[a] One-way ANOVA test (equal variance assumed)

[b] All pairs except F2, F4 and F5 with F1, F3 and F4 with F5 and F3 with F6 were significantly different by posthoc test (Bonferroni's procedure).

Table 14: Comparing mean disintegration time (mins) of formulations

Formulation	n	Disintegration time Mean (SD)	F stat (df)[a]	P value[a]
F1		4.91 (1.64)		
F2		6.48 (1.44)		
F3	3	5.92 (0.18)	0.941 (5)	0.49[b]
F4		6.19 (0.90)		
F5		7.09 (0.76)		
F6		5.72 (0.87)		

[a] One-way ANOVA test (equal variance assumed)

[b] All pairs were not significantly different by posthoc test (Bonferroni's procedure).

Table 15: Comparing mean pH of formulations

Formulation	n	pH Mean (SD)	F stat (df)[a]	P value[a]
F1	3	7.34 (0.14)	14.477 (5)	<0.001[b]
F2		7.24 (0.07)		
F3		7.77 (0.22)		
F4		8.01 (0.13)		
F5		7.72 (0.11)		
F6		8.12 (0.05)		

[a] One-way ANOVA test (equal variance assumed)

[b] All pairs except F2, F4 and F5 with F1, F3 and F4 with F5 and F3 with F6 were significantly different by posthoc test (Bonferroni's procedure).

ABBREVIATIONS

HPMC – hydroxypropyl methylcellulose;

SSG – sodium starch glycolate;

CD – chlorhexidine digluconate;

FTIR – Fourier Transform Infrared spectroscopy;

ZOI – zone of inhibition.