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Development of orodispersible films for buccal antiseptic delivery of chlorhexidine: preparation, evaluation, and *in vitro* antimicrobial activity

Desenvolvimento de filmes orodispersíveis para administração antisséptica bucal de clorexidina: preparo, avaliação e atividade antimicrobiana *in vitro*

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This study focuses on formulating and evaluating orodispersible films (ODFs) for targeted chlorhexidine delivery as a buccal antiseptic. ODFs were prepared using blends of natural polymers, either alone or in combination with polyvinylpyrrolidone (PVP), employing the solvent-casting method. The ODFs were loaded with a chlorhexidine digluconate solution at concentrations of 0.625, 1.25, and 2.5% w/w, and subjected to characterization, encompassing parameters such as appearance, thickness, average weight, folding endurance, disintegration time, pH, and residual water content. These analyses revealed favorable attributes for buccal administration. *In vitro* dissolution studies revealed rapid disintegration and release, facilitating the achievement of therapeutic levels of chlorhexidine within a short timeframe. The antimicrobial activity of the released chlorhexidine against Gram-positive and Gram-negative bacterial strains, as well as a fungus, was evaluated via agar diffusion assays. The ODF containing 1.25% w/w chlorhexidine exhibited the highest antimicrobial activity, with chlorhexidine content of 3.76 mg, meeting content uniformity standards. The compounding process proved to be simple, fast, reproducible, and robust, using low-cost excipients and a film applicator device. These findings hold significant implications for improving oral hygiene practices and addressing oral infections. In conclusion, this personalized formulation shows promise for magistral pharmacies in the compounding of buccal antiseptics as ODFs.
Keywords: personalized medicine, buccal hygiene, antimicrobial activity.

Este estudo concentra-se na formulação e avaliação de filmes orodispersíveis (FODs) para a administração direcionada de clorexidina como antisséptico bucal. Os FODs foram preparados utilizando misturas de polímeros naturais, seja isoladamente ou em combinação com polivinilpirrolidona (PVP), empregando o método de moldagem e evaporação do solvente. A solução de digluconato de clorexidina foi incorporada nos FODs em concentrações de 0,625, 1,25 e 2,5% m/m e estes foram submetidos à caracterização, abrangendo parâmetros como aparência, espessura, peso médio, resistência à dobra, tempo de desintegração, pH e teor de água residual. As análises revelaram atributos favoráveis para a administração bucal dos FODs. Estudos de dissolução *in vitro* demonstraram rápida desintegração e liberação, favorecendo o alcance de níveis terapêuticos de clorexidina em um curto período de tempo. A atividade antimicrobiana da clorexidina liberada contra cepas bacterianas Gram-positivas, Gram-negativas e um fungo foi avaliada por meio de ensaios de difusão em ágar. O ODF contendo 1,25% m/m de clorexidina apresentou a maior atividade antimicrobiana, com teor de clorexidina de 3,76 mg, atendendo aos padrões de uniformidade de conteúdo. O processo de manipulação mostrou-se simples, rápido, reprodutível e robusto, utilizando excipientes e dispositivo aplicador de filme de baixos custos. Essas descobertas têm implicações significativas para a melhoria das práticas de higiene oral e o tratamento de infecções bucais. Em conclusão, esta formulação personalizada apresenta-se promissora para a manipulação de antissépticos bucais baseados em FODs pelas farmácias magistrais.
Palavras-chave: medicina personalizada, higiene bucal, atividade antimicrobiana.

1. INTRODUCTION

Oral hygiene is crucial for maintaining good dental health and preventing various dental diseases. Effective oral hygiene entails the removal of food debris, plaque, and other harmful substances that accumulate on the teeth and gums. Inadequate oral hygiene practices can lead to the onset of dental diseases, including tooth decay, gum disease, and halitosis. Pathogenic microorganisms, including bacteria, viruses, and fungi, can thrive in the oral cavity, thereby precipitating dental diseases [1]. The use of antiseptic agents such as chlorhexidine digluconate (CD) is a viable strategy for preventing dental diseases attributed to pathogenic microorganisms.

CD is an antiseptic that works against both Gram-positive and Gram-negative bacteria, as well as fungi. Its active intablegredient forms an electrostatic bond with the bacterial cell membrane, remaining adsorbed on the cell wall. At bacteriostatic concentrations, it disrupts the membrane, leading to the leakage of cytoplasmic components. At higher concentrations, it acts bactericidally by disrupting the cytoplasmic membrane, forming irreversible precipitates with intracellular adenosine triphosphate and nucleic acids [2-4] determined the minimum inhibitory concentrations (MIC) of CD against oral pathogens, including *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Candida albicans*, *Prevotella intermedia*, *Porphyromonas gingivalis*, *Porphyromonas endodontalis*, *Prevotella denticola*, and *Prevotella melaninogenica*. MIC values are lower for Gram-positive bacteria due to their greater affinity for the cell wall compared to Gram-negative bacteria, and the literature reports values ranging from 2.67 to 4 µg/mL [4].

CD is a broad-spectrum antimicrobial with important residual activity. The main advantages of using CD in oral care products lie in the fact that the active ingredient does not cause bacterial resistance; its ability to adhere to tissues, acting for a prolonged period; and its ability to retain an antimicrobial activity even in the presence of blood and other biological fluids [2-4]. In oral hygiene products, it binds to the surfaces of oral mucosa tissues through electrostatic interactions, inhibiting dental plaque formation and exerting bacteriostatic action for several hours [5]. However, CD is currently available in limited dosage forms, such as solutions, gums, gels, and toothpaste, which require removal after application by rinsing with water. The development of new personalized dosage forms of CD can improve patient compliance and provide a more convenient and effective mode of delivery. In this context, the development of personalized dosage forms, such as orodispersible films, can provide a tailored approach to oral hygiene.

Orodispersible films (ODFs) are a solid dosage form that rapidly dissolves when placed in the buccal cavity, allowing for targeted delivery of active pharmaceutical ingredients (APIs) to patients with swallowing difficulties, such as the elderly, children, and pets. ODFs are made of hydrophilic polymers and are designed to dissolve quickly upon contact with saliva, leaving no residue in the mouth. They are also known as polymeric thin-films or strips and have been shown to have great potential for personalized drug delivery [6-10].

ODFs offer several advantages over traditional solid dosage forms, including the ability to be administered without water or chewing, reduced risk of choking or suffocation due to their rapid disintegration and lack of residue, rapid release of API in the buccal cavity, potential for pleasant texture and flavor, ease of transport, flexibility in dosing, and suitability for both synthetic and bioactive APIs [9-11]. However, ODFs may not be appropriate for high-dose APIs or those with properties that can degrade in oral pH, cause irritation, or possess a bitter taste [6, 12]. Moisture is a critical factor that can cause instability, stickiness, and API hydrolysis in ODFs, necessitating special packaging and storage considerations [13].

Among the methods described for the preparation of polymeric films on a small scale, the solvent casting technique using a film applicator is the most often used because of its best reproducibility, ease, and cost-effectiveness compared with other methods [14]. In this context, the objective of this study was to develop an ODF formulation containing CD e for use as a personalized dosage form in oral hygiene, based on natural polymers isolated or associated with polyvinylpyrrolidone (PVP) (0.5% w/w), using the solvent casting technique. This research was carried out to provide a high-quality formulation that can be used as a reference for ODF production in compounding pharmacies for personalized oral hygiene dosing.

2. MATERIALS AND METHODS

Carrageenan gum (Kappa type; viscosity of 5 to 25 cP, 0.3% in H2O at 25° C), polyvinylpyrrolidone (PVP40; average molar mass 40,000), porcine skin gelatin (pharmaceutical grade), polysorbate 80 (molar weight 79,000) and chlorhexidine digluconate (secondary standard, content of 20.1%) were purchased from Sigma-Aldrich (USA). Chlorhexidine digluconate (20% w/v; Neobrax; lot number 747FE) and pullulan (from Aureobasidium pullulans; Kumar Organic; lot number K05018) were acquired from Embrafarma and Vepakum (Brazil), respectively. The others inactive ingredients (xanthan gum, maltodextrin, mannitol, simethicone, propylene glycol, polyethylene glycol 400, sorbitol, and potassium sorbate), presented pharmaceutical-grade and were purchased from national suppliers (Brazil).

2.1 Preparation of polymer blends and obtainment of ODFs

The preparation of polymer blends was carried out by weighing all components (Shimadzu analytical balance, model AUY220, Japan) and following a standardized protocol. Firstly, the soluble components were dissolved in purified water in a beaker and heated in a water bath (Solab, model SL-150/10, Brazil), at a temperature between 60 and 70 (± 5° C). Subsequently, the polymers were sequentially dispersed into the solution under continuous mechanical stirring (Fisatom, model 713D, Brazil), while maintaining the heating and stirring conditions until the complete absence of clumps. The plasticizers were added next, followed by surfactant and silicone, when present in the formulations. The final weight of each formulation was adjusted with purified water and the solutions were brought back to slow stirring without heat. After complete homogenization, the formulations were subjected to cycles of 20 to 40 min in an ultrasonic bath (Soniclean, model 6, Brazil) to eliminate any bubbles. The pure base formulations were named ODFP1, ODFP2, and ODFP3. The qualitative and quantitative composition of the pure blends is presented in Table 1. All formulations were prepared by weighing the ingredients (w/w).

Table 1: Qualitative and quantitative composition of oral dispersible film polymer (ODFP) formulations.

	ODFP1*	**ODFP2***	**ODFP3***
Composition	**Quantity (% w/w)**		
Pullulan	16.0	13.0	16.0
Xanthan gum	0.06	0.15	0.06
Carrageenan gum	0.37	0.30	–
Gelatin	–	–	0.37
Acacia gum	–	0.15	–
Polyvinylpyrrolidone	–	–	0.50
Maltodextrin	2.00	2.00	5.00
Mannitol	2.00	–	–
Propylene glycol	1.75	–	1.75
Polyethylene glycol	2.00	2.00	2.00
Sorbitol	–	4.20	4.20
Polysorbate 80	1.00	1.00	–
Simethicone	0.50	–	–
Potassium sorbate	0.20	0.20	0.20
Flavor (bacon)	0.01	0.01	0.01
Purified water	73.62	75.00	69.92

*ODFP1, ODFP2, ODFP3: refer to the distinct pure base formulations developed in this study.

After preparing the polymeric blends, the commercial CD solution was incorporated at proportions of 0.625, 1.25, and 2.5% w/w, after applying the correction factor. The concentrations were defined based on the consulted literature, which will be discussed later. The CD was carried out by magnetic stirrer for 45 min. The obtained solutions were used to prepare ODFs by the solvent casting method. Each blend was deposited on a glass plate (30 x 9.5 cm) using an adapted applicator film (Figure 1) to achieve a thickness of 0.75 mm, established experimentally. The plates were then left exposed to air for up to 48 h for drying, and the continuous films were cut into ODFs with dimensions of 2 x 2 cm, according to the standard dimensions given in the literature [15]. The ODFs were wrapped in waxed paper and individually packed in airtight plastic bags containing silica gel to maintain their quality parameters. Nine formulations containing CD were presented, namely ODFC1 to ODFC9.

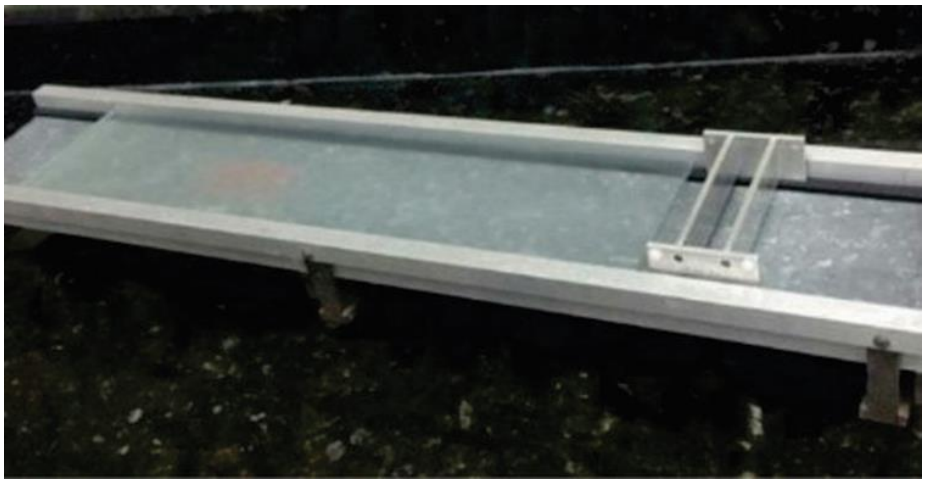

Figure 1: Applicator film device adapted for spreading blends and obtaining ODFs.

2.2 Evaluation of quality parameters of the films

2.2.1 Macroscopic characterization

The aspect of the films was determined according to visual analysis, observing the transparency, color, brightness, presence of clumps (insoluble solids) and bubbles.

2.2.2 Weight determination and thickness

The average weight and weight variation of the twenty (20) films of each formulation were determined using an analytical balance (Shimadzu, model AUY220, Japan), following the methodology recommended by European Pharmacopeia (2018) [8].

The study considered the recommended average weight of formulations for oral mucosa use, with a value of less than 80 mg and a variation of ± 10%. Additionally, two dosage units with a deviation less than or greater than 10% were allowed, and only two units with double this deviation were acceptable.

The thickness of the ten (10) films of each formulation was measured, using a digital external micrometer (Mitutoyo, model MDC-25PX, USA), with 10 random, central, and peripheral points measured in each film.

2.2.3 Disintegration time

Disintegration time was evaluated according to the method adapted from Desai et al. (2011) [16]. Two media, water and simulated salivary fluid (pH = 6.8), were used for the evaluation, both

maintained at 37 (± 2° C). Six (6) films of each formulation were placed in glass Petri dishes with a diameter of 6.5 cm, and 25 mL of the respective medium was added to each dish. The plates were then stirred in circular motions (three turns every 10 s) and the onset time of disintegration was recorded.

2.2.4 Folding endurance

Folding endurance was determined as described by Mukherjee and Bharath (2013) [17]. Ten (10) films of each formulation were repeatedly folded at a 180° angle until the film cracked or broke. The number of times the film could be folded without breaking was considered as the folding endurance value.

2.2.5 pH measurement

The pH of the films was determined by potentiometry using a pH meter (Hanna, model HI2221, USA). The electrode was immersed in a beaker containing a 2x2-cm film that had been previously solubilized in CO_2-free water at room temperature.

2.2.6 Residual water content

The residual water content was determined using a moisture analyzer (Shimadzu, MOC63u, Japan) by measuring the water loss through infrared radiation. Three (3) films of each formulation were weighed and heated up to 105° C until the mass balance was achieved. The percentage of mass loss and the time required to reach the mass balance were determined by the device.

2.3 In vitro antimicrobial activity

The *in vitro* antimicrobial activity was assessed using the agar-well diffusion method in accordance with the Clinical & Laboratory Standards Institute with modifications [18]. In this method, wells of 6-mm diameter were made in the culture medium placed in a Petri dish using a sterile mold. The Gram-negative bacterium *Escherichia coli* (ATCC 25.922), the Gram-positive *Staphylococcus aureus* (ATCC 25.923) and *Staphylococcus epidermidis* (ATCC 12.228) bacteria, and the yeast strain *Candida albicans* (ATCC 24.433) were used as standard strains. For this experiment, microorganisms were previously cultivated in brain heart infusion broth at 35.5° C for 24 h, and the concentration of the microbial suspension was adjusted to 1.5 x 10^8 CFU.mL/mL in sterile saline solution (0.9% NaCl) based on the 0.5 McFarland standard. Freshly prepared Mueller Hinton agar medium was then transferred to the plates and, and the strains were seeded using the swabbing technique.

In the wells made in the agar plates, were placed 175 mg of each blend prepared with CD in the same concentrations presented in the ODF studied. Blends without the drug were used in the same amount as a negative control. Disks containing tetracycline (30 µg/disk) and amphotericin B (10 µg/disk) were used as positive controls for comparison between replicates and with CLSI (2019) [18]. Plates were incubated at 36° C for 24 - 48 h. The diameter of the zones of inhibition was measured in millimeters (mm).

2.4 Determination of chlorhexidine digluconate content

The determination of the CD content was performed on the ODF formulation that exhibited the best physicochemical attributes and highest antimicrobial activity, using ultraviolet spectrophotometry (Rigol spectrophotometer, model Ultra 3660, China) with purified water and ethanol as solvents. Initially, a stock solution of CD (secondary standard) at a concentration of 200 µg/mL was prepared in each solvent and scanned using the spectrophotometer (400 – 200 nm)

in the absorption mode to confirm the wavelength. To assess the presence of interference from other components in the blend at the defined reading wavelength, spectra were also collected from pure ODF dissolved in each solvent. After confirming the wavelength and selecting the best solvent, a calibration curve was prepared by preparing successive dilutions of the stock solution (200 µg/mL) in purified water, with concentrations ranging from 4 to 20 µg/mL. The absorbance at 262 nm was measured for these solutions using purified water as the blank. The mean absorbances were plotted against the concentration to generate graphs, and the equation of the line and correlation coefficient were determined using linear regression.

In order to determine the CD content, 10 ODFs were minced and homogenized. An amount of ODF equivalent to the average weight of one was dissolved in purified water in a 1000 mL volumetric flask, and the absorbance of each solution was measured at 262 nm. The uniformity of dosage units was evaluated using the content uniformity method, repeating the process for 10 units of ODFs individually. The acceptance value (VA) was calculated according to the Brazilian Pharmacopoeia 6th ed. [19], using Equation 1, where: VA = acceptance value; $\bar{X}$ = average of individual contents; M = 98.5% (for T < 101.5% and $\bar{X}$ < 98.5%); k = 2.4 (for n = 10); and, s = standard deviation.

$$VA = |M - \bar{X}| + ks \qquad \text{Eq. (1)}$$

2.5 Statistical analyses

All analyses were performed in triplicate, and the results were expressed as mean ± standard deviation. For weight variation, the mean weight, standard deviation, relative standard deviation (coefficient of variation), variation, and lower and upper weight limits of the ODFs were calculated. In order to assess thickness, folding endurance, and pH, calculations were performed to determine the standard deviation and relative standard deviation. The data obtained for antimicrobial activity were subjected to analysis of variance (ANOVA), and the average halo measurements were compared by the Tukey test, both at a significance level of 5%, using the PAST program (v. 2.17). To construct the calibration curve, each concentration of the standard solution was prepared in triplicate, and three readings were taken for each. For the determination of content and uniformity of dosage units, each analysis was repeated two times.

3. RESULTS

3.1 Physicochemical characterization of orodispersible films with digluconate chlorhexidine

The study focused on the preparation and characterization of various ODFs formulations. ODFP1, formulated using natural polymers, served as the baseline. ODFP2 and ODFP3 formulations introduced modifications, including alterations in the proportions of natural polymers and the addition of gelatin and PVP to enhance film-forming capabilities. The appearance, weight, thickness, disintegration time, water residual content, folding endurance, and pH of the ODFs were evaluated.

Incorporation of CD into polymeric blends resulted in changes in the appearance and quality of the films compared to pure films. ODFP1 films and their CD derivatives had similar characteristics - white, opaque, with one side smooth and the other rough, and palpable solids.

The films derived from ODFP2, initially smooth, transparent, and shiny, became less transparency with the presence of insoluble solids, especially at higher CD percentages, as did ODFP3-derived films. All ODFs showed good handling resistance. Notably, the ODFP2 formulation with the lowest CD concentration (0.625% w/w; ODFC4) produced a rigid and brittle film, which was discarded. Figure 2 shows images of both pure ODFs and those containing CD after drying.

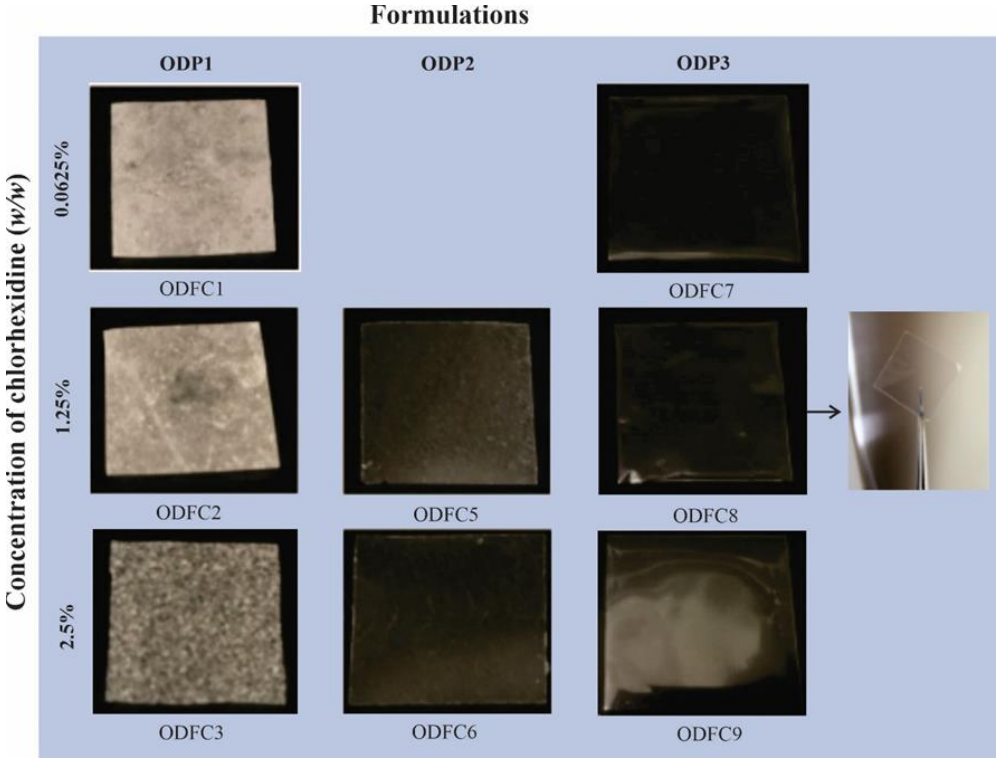

Figure 2: Representative image of ODFs containing CD at concentrations of 0.625% w/w (ODFC1 and ODFC7); 1.25% w/w (ODFC2, ODFC5, and ODFC8); and 2.50% w/w (ODFC3, ODFC6, and ODFC9).

The weight uniformity among dosage units was evaluated, and the results are presented in Table 2. None of the dosage units deviated from the weight by more than ± 20%. However, formulations ODFC3 and ODFC7, containing CD, exhibited variations exceeding 10% in more than two units, indicating a test failure.

Table 2: Determination of weight, standard deviation, weight variation tolerance and lower and higher weight values of ODFs.

	Average weight (mg)	Variation (± 10%) (mg)	IL (mg)	UL (mg)	LV (mg)	HV (mg)	Nonconforming units
ODFP1	52.80 (±4.92)	5.28	47.52	58.08	44.00	60.00	2 below e 1 above
ODFC1	22.50 (±2.22)	2.25	20.24	24.74	1750	25.40	1 below e 1 above
ODFC2	21.96 (±1.29)	2.20	19.76	24.16	20.20	23.80	None
ODFC3	19.57 (±2.31)	1.96	17.61	21.53	16.90	23.80	3 below e 2 above
ODFP2	24.48 (±2.46)	2.45	22.03	26.93	20.70	27.20	1 below e 1 above
ODFC5	25.20 (±1.69)	2.52	22.68	27.72	23.10	28.80	1 above
ODFC6	26.58 (±1.44)	2.66	23.92	29.24	23.30	28.00	1 below
ODFP3	33.81 (±2.21)	3.38	30.43	37.19	29.80	37.50	1 below e 1 above
ODFC7	32.32 (±8.36)	3.23	29.09	35.55	15.00	44.50	2 below e 1 above
ODFC8	33.91 (±3.39)	3.39	30.52	37.50	31.40	32.60	1 above
ODFC9	35.38 (±1.65)	3.54	31.84	38.92	33.50	39.00	1 above

IL = inferior limit; UL = upper limit; LV = lower value; HV = highest value.

Standard deviation values were generally less than 5%, indicating good weight uniformity among most tested dosage units. Similarly, thickness measurements were conducted (Table 3), with all CD-containing formulations classified as ultra-thin, ranging from 55.25 to 96.76 μm.

Table 3: Quality parameters analysis of pure and CD-containing ODFs: thickness, disintegration time, folding endurance, pH e water residual content.

	Thickness (μm) (n = 100) (±SD; RSD)	Disintegration time (s) (n = 3) (±SD)		WRC (%)	FE (n = 10) (±SD; RSD)	pH (n = 9) (±SD; RSD)
		Water	SSB			
ODFP1	0.173 (±0.017; 9.825%)	109 (± 0.02)	117 (±0.01)	9.14	208 (±74; 46%)	6.23 (±0.23)
ODFC1	0.063 (±0.016; 24.543%)	57 (±0.01)	74 (±0.00)	12.07	31 (±16; 53%)	8.78 (±0.13)
ODFC2	0.088 (±0.004; 5.024%)	29 (±0.00)	37 (±0.00)	11.47	4 (±2; 53%)	8.76 (±0.42)
ODFC3	0.097 (±0.010; 10.025%)	27 (±0.00)	48 (±0.00)	18.33	5 (±3; 59%)	9.40 (±0.44)
ODFP2	0.050 (±0.028) (5.623%)	> 180 (±0.00)	> 180 -	7.14	24 (±23; 98%)	7.97 (±0.22)
ODFC5	0.072 (±0.008; 11.69%)	44 (±0.01)	47 (±0.01)	5.26	2 (±1; 53%)	8.27 (±0.45)
ODFC6	0.083 (±0.011; 12.99%)	38 (±0.02)	41 (±0.01)	7.50	2 (±1; 32%)	7.91 (±0.32)
ODFP3	0.066 (±0.008; 12.672%)	24 (±0.00)	29 (±0.00)	7.22	162 (±45; 40%)	8.40 (±0.72)
ODFC7	0.055 (±0.015; 27.149%)	27 (±0.01)	34 (±0.00)	6.87	90 (±43; 48%)	8.51 (±0.39)
ODFC8	0.064 (±0.002; 3.455%)	23 (±0.00)	34 (±0.00)	5.04	105 (±50; 48%)	8.42 (±0.30)
ODFC9	0.069 (±0.005; 7.920%)	23 (±0.00)	38 (±0.00)	6.86	56 (±15; 26%)	8.66 (±0.17)

SD = standard deviation; RSD = coefficient of variation; WRC = water residual content; FE = folding endurance; SSB = simulated salivary buffer.

The disintegration time of the ODFs was evaluated in both water and simulated salivary fluid. All CD-containing formulations disintegrated in less than 180 s in both media. However, the disintegration time was longer in simulated salivary fluid than in water for all formulations. Water residual content values ranged from 5.04 to 18.33%. Formulations containing PVP and CD at 1.25% w/w exhibited water residual content values below 6%. The folding endurance test revealed that most formulations were brittle, particularly those derived from ODFP1 and ODFP2. Only ODFC8 exhibited a folding endurance value greater than 100. The pH values of all ODFs ranged from 7.9 to 9.4, with higher values observed in formulations derived from ODFP1.

3.2 *In vitro* antimicrobial activity

The antimicrobial activity of the ODFs was evaluated using blends incorporating CD derived from ODFP3, which exhibited superior physicochemical characteristics. Blends were formulated to include CD in quantities equivalent to the average weights of their corresponding films. The values found from the determination of the inhibition halo measurements are given in Figure 3.

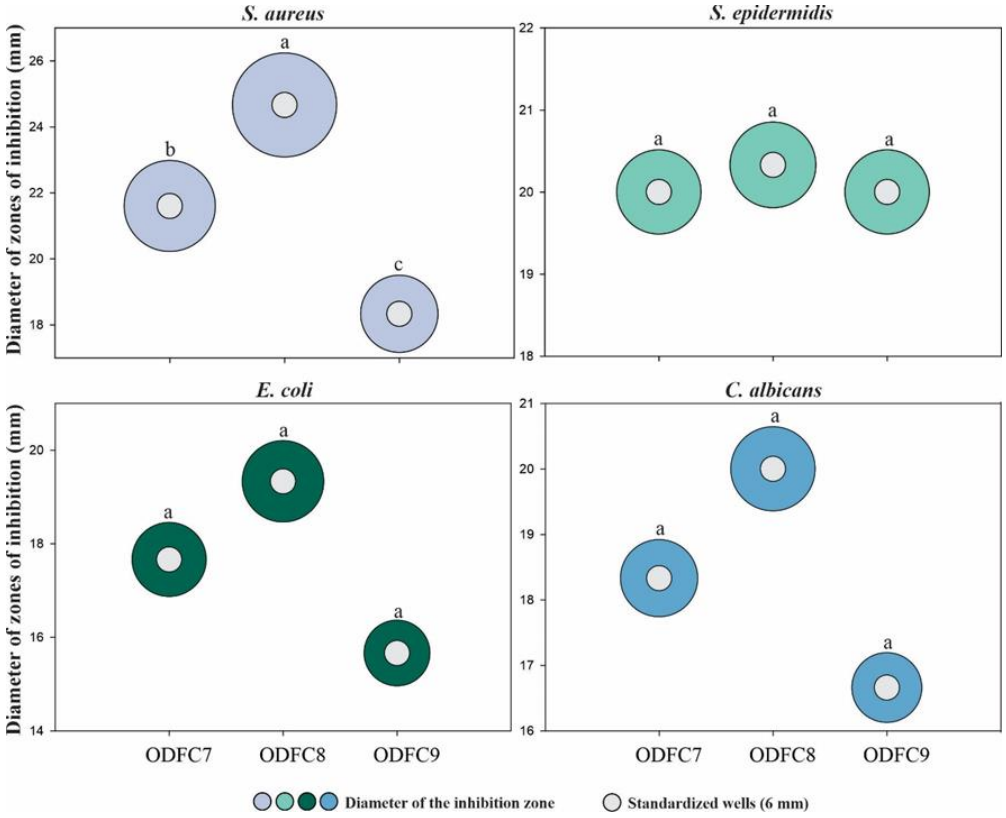

Figure 3: Mean and standard deviation of zones of inhibition (mm) observed for formulations derived from ODFP3.

In this test, the inhibition zones observed for tetracycline and amphotericin B agreed with the recommended standard for the test [18]. There was no growth inhibition observed for the pure blends, indicating no interference from the blends without CD in the antimicrobial activity. All blends containing the active ingredient showed zones of inhibition, demonstrating the ability of ODFs with CD to inhibit the growth of all evaluated microorganisms, even at the lowest concentration used. ODFC9 exhibited the lowest values for the zone of inhibition for all microorganisms. Regarding the statistical analysis to determine the most effective concentration, there was no significant variation ($p<0.05$) between the inhibition zones determined for the studied microorganisms in ODFC7. However, for *S. aureus*, ODFC8 containing the active ingredient at 1.25% w/w resulted in a significantly higher inhibition zone diameter ($p<0.05$) than the other concentrations, suggesting its superior effectiveness in inhibiting the growth of this strain and better performance of this formulation.

3.3 Determination of CD content in ODFC8 film

Considering that the ODFC8 formulation exhibited the best performance in physicochemical tests and the antimicrobial activity assay, the content of CD in this formulation was determined using a spectrophotometric method at 262 nm, following the methodology described by Márquez et al. (2017) [20].

A mathematical equation was derived from the least-squares regression line, resulting in $y = 0.285 x + 0.1155$, with an estimated correlation coefficient (r) of 0.9998. The high correlation coefficient value close to 1 indicates a strong relationship between the studied concentrations and the absorbances, which ranged from 0.230 to 0.698, in accordance with the Lambert-Beer Law (Figure 4).

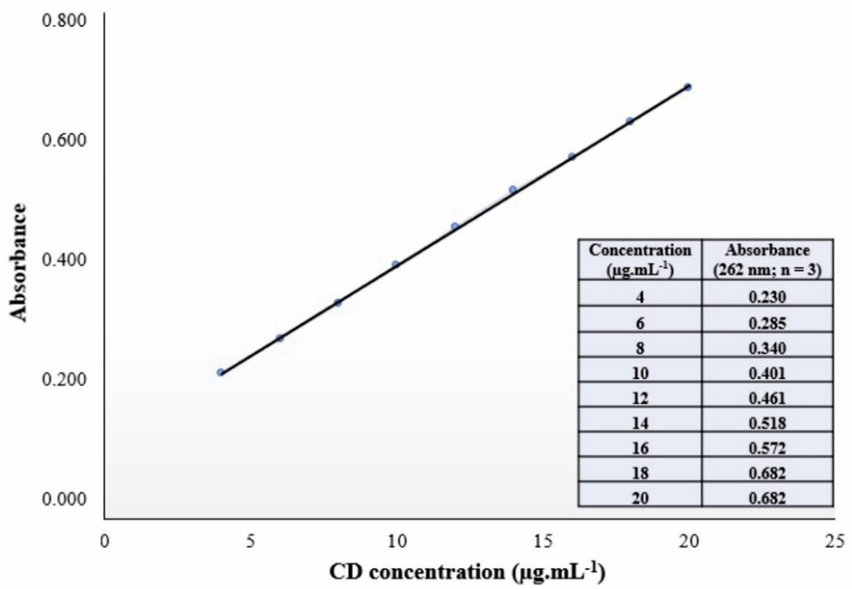

Figure 4: Curve calibration obtained for CD secondary standard solutions in purified water at 262 nm.

The CD content in the ODF samples was determined using the equation of the straight line, with the average value obtained from two separate analyses. Three absorbance readings were taken from each analysis. The mean CD content was found to be 3.76 mg, with a standard deviation of ± 0.36 mg and a relative standard deviation of ± 9.5%. The determined content represents 90.97% of the expected value, calculated based on the average weight.

The content uniformity test was conducted to ensure accurate doses in each dosage unit. The values obtained from the content uniformity determination, including the acceptance value, are presented in Table 4. The dosage units of ODFC8 successfully passed the content uniformity test as the acceptance value was below 15, which is the maximum allowed value (L1) when the first 10 units are tested. For set 1, the content ranged from 88.2% to 101.47%, while for set 2, it ranged from 79.92% to 100.45%. The theoretical value considered was based on the average weight of the 20 tested dosage units (33.25 mg; SD ± 1.73; RSD = 5.25).

Table 4: Results of content uniformity test for ODFC8 dosage units.

	Set 1		Set 2		Mean content
ODF	**Content (mg)**	**Content (%)**	**Content (mg)**	**Content (%)**	**(%)**
1	3.50	91.73	4.63	97.49	-
2	3.88	90.09	4.88	93.21	-
3	4.25	88.20	4.25	100.03	-
4	4.00	89.91	4.50	93.70	-
5	3.88	90.09	4.75	95.91	-
6	3.50	93.07	4.50	100.45	-
7	3.63	101.47	4.88	92.97	-
8	4.25	95.36	4.63	91.16	-
9	3.88	93.72	4.28	93.86	-
10	4.13	90.92	4.50	79.92	-
Mean	-	**92.63**	-	**93.87**	**93.25**
SD	-	**3.81**	-	**5.79**	**4.80**
RSD	-	-	-	-	**5.14**
AV	**7.13**		**8.80**		-

SD = standard deviation; RSD = coefficient of variation; AV = acceptance value.

4. DISCUSSION

In this study, the ODFP1 formulation based on natural polymers was prepared as according to the method described by de Santana et al. (2020) [21]. The ODFP2 formulation was prepared with modified proportions of natural polymers other components, while the carrageenan gum was replaced with gelatin and PVP was added to the ODFP3 formulation due to its excellent film-forming capacity. ODFs are prepared using natural or modified natural polymers, such as cellulose, cellulose-derived, and polysaccharides, due to their quick dissolution and swelling properties in water. Synthetic polymers like PVP are often combined to enhance their film-forming abilities and regulate the release of the API. Others excipients commonly used in ODF formulations include plasticizers, diluents, thickeners, superdisintegrants, sweeteners, flavorings, preservatives, antioxidants, surfactants, and salivation-promoting agents. The main solvents used in ODF preparation are water, ethanol, and hydroalcoholic solutions [6, 12, 15]. Several studies have investigated the preparation of oral antiseptic films with varying disintegration rates using different chlorhexidine salts. Senel et al. (2000) [22] formulated two gel and film-based chitosan preparations containing CD at concentrations of 0.1 and 0.2% w/w, which continued to release the active ingredient for up to 4 hours. Soares Neto et al. (2018) [23] developed ODFs using starch or starch associated with chondroitin sulfate, sorbitol as a plasticizer, and CD at 0.12% w/w, targeting oral hygiene of geriatric patients. Ricardo et al. (2017) [24] used gelatin to prepare a CD-containing film as an oral antiseptic. The commercial product PerioChip®, which utilizes a gelatin matrix, was developed to provide sustained release of chlorhexidine in the oral cavity [25].

According to Visser et al. (2015) [7], Visser et al. (2015) [13] and Borges et al. (2015) [6], achieving uniform weight and thickness of ODFs requires a polymer dispersion with adequate viscosity for spreading. Carrageenan gum and gelatin, which solubilize when heated, are commonly used but need to be prepared under heating to form thermoreversible gels, as done in this study. The formation of bubbles can also compromise film homogeneity, which was reduced by subjecting the blends to ultrasonic cycles prior to spreading, as described by Irfan et al. (2016) [26]. Additionally, a device was used in this study to spread silica gel on chromatography plates as a film applicator, which improved the spreading process's efficiency and reproducibility.

The determination of weight and weight variation among dosage units in a batch is essential to ensure consistent mass and indirectly, a consistent amount of active ingredient in each unit [13, 27]. Preis et al. (2013) [9] prepared ODFs of dimenhydrinate alone or in combination with cyclodextrins and maltodextrin using a starch derivative as a film-forming polymer and obtained mean weight values considered acceptable ranging from 50.50 to 71.90 mg. Chaithanya et al. (2018) [28] prepared films containing ebastine dispersions in blends of polyethylene glycol 6000 and propylene glycol with different proportions of hydroxypropyl methylcellulose (HPMC), pullulan, and PVP, obtaining films with average weights ranging from 30 to 36 mg, similar to the present study.

With regard to thickness measurements, literature reports indicate that oral films should have thickness values between 50 and 1000 μm, with the possibility of ultra-thin films ranging from 50 and 150 μm [10, 12, 27] For all formulations, except ODFC7 and ODFC8, the calculated values for standard deviation and relative standard deviation were high. For ODFC7 and ODFC8, these values were less than 5%, indicating uniformity between measurements. Juliano et al. (2008) [29] prepared films for the treatment of oral candidiasis containing chlorhexidine diacetate (5 and 10 mg) using different polymers, such as sodium alginate, HPMC, chitosan, PVP, and pullulan, alone or associated, in mono- or bilayers. The thickness of the films prepared with HPMC was above 0.33 mm, whereas those with PVP had a thickness of 0.23 to 0.26 mm. The smallest thickness values were found for pullulan films, ranging from 0.07 to 0.12 mm. According to the authors, the thickness of the films was homogeneous and close to 19.8 μm, a value lower than those obtained in the present study.

In addition to their relationship with the uniformity of doses, the average weight and thickness of oral films can provide indications of the reproducibility of the manufacturing process, and highlight the need for possible adjustments to ensure consistency [6]. The present study also found that the method of preparation and the use of a spreading accessory influenced the results

obtained. AnjiReddy and Karpagam (2020) [30] developed ODFs using cellulose derivatives and donepezil hydrochloride, which had average weight and thickness values similar to those observed in this study. The authors considered the quality attributes of their ODFs to be adequate.

The disintegration time of films is regarded as a critical quality attribute for ODFs. Conventional-release films should be designed to disintegrate rapidly and release the APIs within a period that can range from few seconds to 180 s [10]

Water residual content is a crucial quality parameter for ODFs as it strongly affects their mechanical strength, physical, chemical, and microbiological stability. However, there is no uniform specification or quantification method in the literature for this parameter. Excessive water content can result in problems such as the formation of tack films that adhere to the packaging and API hydrolysis, while low residual water content or moisture loss can make ODFs brittle [6]. According to Nair et al. (2013) [27], the moisture content in oral films should be less than 5%, while in other studies, the limit was experimentally determined to be in the range of 3-6%. Borges et al. (2017) [31] analyzed the water content in commercial ODFs and found that all had residual water content below 10%, and the majority had values below 5%.

The folding endurance test measures the flexibility, elasticity, and durability of the film during handling and storage [12, 17]. Zahid et al. (2020) [32] prepared ODFs containing risperidone and monitored folding endurance values for 60 days, which ranged between 104 and 109.

The pH value of a formulation is decisive in maintaining the quality and efficacy of the final product [21]. According to Wiegand et al. (2015) [33], the antimicrobial activity of CD is independent of pH within a range of 5-9, and the active ingredient may precipitate at pH levels above 8 without affecting its antimicrobial activity. This phenomenon could potentially explain the presence of visible particles in ODFs containing CD.

The evaluation of antimicrobial activity was conducted using polymer blends, as the ODFs rapidly dispersed upon deposition on the agar medium and incubation, rendering it difficult to measure the diameters of the inhibition zones. Therefore, blends incorporating CD derived from ODFP3, which exhibited superior physicochemical characteristics, were prepared in equivalent amounts as found in the average weights of these films. The results obtained for the films containing the CD concentrations studied are similar to the data found in the literature, which port antimicrobial activity for CD at concentrations ranging from 0.5 to 2% w/w [34-36]. Rodrigues et al. (2019) [34] evaluated the antibacterial activity of mucoadhesive films containing CD at concentrations of 0.5, 1, and 2% w/w against buccal microbiota from salivary samples seeded on blood agar medium. The authors reported the formation of inhibition zones around all discs, indicating the films' ability to inhibit bacterial growth even at the lowest concentration of CD tested. Barragan et al. (2020) [35] developed mucoadhesive films composed of a blend of chitosan:acacia gum (1:1) and polycarbophil, incorporating chlorhexidine diacetate (2% w/w), which effectively inhibited the growth of *C. albicans* (ATCC 90.028), *S. aureus* (ATCC 25.923), *E. coli* (ATCC 25.922), *Enterococcus faecalis* (ATCC 29212), and *Pseudomonas aeruginosa* (ATCC 29.853). Kloster et al. (2018) [36] investigated the antimicrobial efficacy of buccal bioadhesive films based on chitosan and containing chlorhexidine at different concentrations (0.2, 0.6, 1, and 2% w/w) against *C. albicans* and *Streptococcus mutans*. The authors observed the greatest inhibition zones for films containing 2% w/w of the active ingredient.

The comprehensive antimicrobial action of chlorhexidine helps reduce the overall bacterial load and modulate the oral microbiome, creating an environment less conducive to periodontitis development. Additionally, extensive clinical and preclinical studies have consistently demonstrated the efficacy of chlorhexidine in managing plaque and periodontal diseases [2-4]. The demonstrated efficacy against the strains studied in this research suggests that chlorhexidine is effective against a variety of oral pathogens.

The determined CD content in ODFC8 indicates successful incorporation of the active ingredient. The CD spectra in purified water and ethanol showed three peaks, consistent with the literature. However, the pure ODFs did not dissolve in ethanol, hence purified water was chosen as the solvent. By comparing the spectra of pure ODFs and ODFs with CD in water, it was confirmed that the film components did not interfere with the reading at the selected wavelength. It is worth noting that the amount of active ingredient presents in ODFC8 exceeded the MIC values reported in the literature for CD against microorganisms commonly found in endodontic

diseases, as reported by Amorim et al. (2004) [4]. Juliano et al. (2008) [29] obtained films containing CD with concentrations ranging from 72 to 95.1% of the theoretical value and attributed the significant variation to the preparation process.

In order to minimize errors and ensure quality control in finished products, compounding pharmacies in Brazil are regulated by legislation that requires the monitoring of the compounding process for solid pharmaceutical forms. This includes the implementation of a standard operating procedure for monitoring and analysis of content and uniformity of content, particularly for products with dosage units containing APIs equal to or less than 25 mg, with priority given to those with a content equal to or less than 5 mg [37]. Consequently, the content uniformity test was conducted to ensure the administration of accurate doses in each dosage unit, with the API content being close to the declared amount. The compliance of the product with the unit dose uniformity test is determined by the acceptance value not exceeding the specified limit according to pharmacopoeial standards [19].

5. CONCLUSION

In conclusion, our study successfully developed ODFs incorporating CD using the solvent casting method. Through rigorous quality-control tests and antimicrobial activity assays, we identified the ODFC8 formulation as the standout performer. This formulation surpassed all quality standards, exhibited enhanced antimicrobial activity against *S. aureus*, and demonstrated desirable characteristics such as appropriate weight (33.91 mg), thickness (62.40 μm), rapid disintegration, low moisture content (5.04%), adequate pH (8.42), excellent handling durability, accurate CD content (3.76 mg), and uniform dosage units. Notably, incorporating 1.25% w/w of CD into the gelatin-based formulation with the addition of PVP proved to be a promising approach for delivering chlorhexidine to the buccal cavity as a personalized treatment option. These findings highlight the potential of compounding pharmacies in providing high-quality ODFs that cater to individual patient needs, ensuring both safety and efficacy.

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