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Formulation, characterization and *in vitro* release studies of terbinafine hydrochloride loaded buccal films

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ABSTRACT: Buccal administration of different active ingredients as an alternative to oral or dermal routes has been widely studied. The films are one of the most investigated dosage forms regarding the buccal formulations developed using bioadhesive polymers. Having thin and flexible structures, the films remain in the mouth for their duration of action without causing any discomfort. There are many studies conducted to develop buccal films for local treatment of oral fungal infections. In this research, buccal films were prepared using terbinafine hydrochloride, which is frequently used orally and systemically in the treatment of fungal infections and has low water solubility. The films were prepared by solvent casting method using hydroxypropyl methyl cellulose (HPMC) and polyvinyl pyrrolidone K30 (PVP) as the bioadhesive polymers and glycerin (2-3%) as the plasticizer. Characterization properties including thickness, weight uniformity, flexibility, tensile strength, swelling capacity were examined and the bioadhesive characteristics were determined by Texture Analyzer device using bovine buccal tissue. According to the findings of bioadhesion studies, the highest bioadhesive properties were detected in F1 and F7 formulations, which contained 5% HPMC. *In vitro* release studies exhibited that F1 and F7 film formulations with 5% HPMC represented slower and more controlled release compared to F2 and F8 film formulations having 4% HPMC + 1% PVP. The results revealed that the developed buccal formulations loaded with terbinafine hydrochloride might be convenient for the local treatment of oral fungal infections.

KEYWORDS: Terbinafine hydrochloride; hydroxypropyl methylcellulose; polyvinylpyrrolidone; buccal drug delivery; buccal film.

1. INTRODUCTION

Buccal drug delivery is of great importance owing to their multiple advantages [1,2]. The buccal mucosa covering the inner surface of the cheek and occupying the area between the gums and lips with an average surface area of 100 cm^2 is an extremely convenient area for the administration of dosage forms [3,4]. Additionally, easy application of the drug and its ability to be removed at any time offer high patient compliance [5]. Traditional liquid and semisolid dosage forms are unable to provide the desired therapeutic effect since they rapidly move away from the site of application due to mechanical stress in the oral cavity and the dilution effects of the saliva. In order to obtain the necessary therapeutic drug levels in mucosa; the contact between the dosage form and the mucosa should be increased and improved [6]. Thus, adhesive dosage forms, which allow the drug to remain in the mucosa for a longer time by sticking to the buccal mucosa, have been developed [7]. Bioadhesive tablets, films, wafers, lozenges, discs, gels and sprays are among the various dosage forms that are administered buccally [8,9]. Recently, development of bioadhesive and biocompatible films for buccal drug delivery has been widely studied [10]. Innovative and patient-friendly buccal films represent resistance against the mechanical stress in the mouth with their thin and flexible structures [10,11]. Buccal films are usually produced by traditional methods including solvent casting and hot-melt extrusion, yet innovative methods such as 3D printing technologies might be used to produce them as well [12].

Various bioadhesive polymers are used to add adhesive properties to buccal films [2]. Bioadhesive polymers might elevate the absorption and efficiency of the drug owing to its suitable adhesion capability and the time on the buccal mucosa [13]. Hydroxypropyl methylcellulose (HPMC), known for its mucoadhesive properties, is a non-ionic and neutral cellulose derivative polymer [14,15]. It is widely used

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due to high biocompatibility, biodegradability, flexibility and good film-forming features. It has the ability to swell rapidly through absorbing water since it is hydrophilic [13]. It is thought that the mucoadhesive behaviors of HPMC, which is one of the first generation mucoadhesive polymers, is due to chain entanglement between the polymer and the mucus; though not through the chemical bonds [14,16]. Moreover, in the literature, the first generation mucoadhesive polymers are suggested to bind to the mucosal membrane by non-specific interactions [17]. As a synthetic polymer, polyvinylpyrrolidone (PVP) is non-ionic, inert, pH-stable, nontoxic, biocompatible and heat resistant [18]. Having a great swelling property, PVP is water soluble and has an affinity for both hydrophilic and hydrophobic drugs [18,19]. Even though the mucoadhesive property of PVP is relatively controversial, it has been shown to have good film-forming properties [16,19]. The retention time was shown to increase when it is used together with the other polymers [16]. Therefore, it is utilized as a co-adjuvant to escalate the bioadhesion [19].

Terbinafine hydrochloride (THCl), which belongs to the class of synthetic allylamines, is used topically and orally for the treatment of various fungal infections, especially the *Candida* strains [20–22]. The water solubility of THCl, which is hydrophobic, is extremely low [22]. In the literature, numerous formulation studies of THCl including hydrogels, ethosomes, liposomal films and nanoemulgels that are applied through transdermal, topical and vaginal routes have been listed [20,21,23,24]. Yet, limited number of studies have concentrated on the buccal application of THCl [22,25].

In this study, it was aimed to develop and characterize buccal bioadhesive films containing THCl for the local treatment of oral fungal infections using HPMC and PVP K30 bioadhesive polymers. The bioadhesive properties of the developed formulations were examined using bovine buccal tissue and *in vitro* drug release studies were performed.

2. RESULTS AND DISCUSSION

2.1. Solubility studies

The solubility of THCl in ethanol and distilled water, which were used in the preparation of film formulations were detected. Its solubility was investigated in pH 6.8 phosphate buffer solution (PBS) since the buccal tissue has similar pH values. Additionally, the solubilities of THCl was tested in methanol and acetonitrile since they are used as the mobile phases of HPLC analysis (Table 1). THCl has low water solubility due to hydrophobicity [26,27]. The solubilities of THCl in PBS and distilled water were detected as 2.39 ± 0.061 and 1.21 ± 0.042 µg/mL, respectively, whereas the solubilities in ethanol and methanol were found relatively higher. The primary reason of using ethanol as a solvent in the preparation of formulations was the low solubility of THCl in water.

Table 1. The solubilities of terbinafine hydrochloride in different solvents.

Solvent	Solubility (mg/mL ±SD)
Methanol	335.00 ± 3.102
Ethanol	112.47 ± 1.105
Acetonitrile	10.14 ± 0.113
Distilled water	1.21 ± 0.042
Phosphate buffer (pH 6.8)	2.39 ± 0.061

2.2. Preformulation studies and preparation of buccal film formulations

Owing to their thickness and size, buccal films are the formulations with high patient compliance as they do not cause discomfort in the mouth [28]. Various polymers such as HPMC, chitosan, PVA, PVP were used alone or in combination during the preformulation process of buccal films prepared by solvent casting method. The ideal formulations containing THCl were determined to be prepared using HPMC and PVP. As the solutions ended up having gel textures in formulations with HPMC higher than 5%, the polymer concentration did not exceed this level. Since the aqueous solutions of PVP and HPMC are slightly acidic [29], sodium hydroxide has been added to the formulations to ensure that the films are compatible with the buccal pH.

When distilled water alone was used as a solvent, non-homogeneous and irregularly dried films were obtained due to the hydrophobic nature of THCl. In addition, the active ingredient was not dispersed

properly and settled to the bottom. Therefore, homogenous, decent and smooth films could not be prepared. For this reason, ethanol was used together with the distilled water. THCl was dissolved in ethanol and proper films were obtained. Additionally, the drying time of the THCl containing films was detected to be longer than the ones without THCl. During the preparation of buccal films through solvent casting method, the drying process is usually carried out at room temperature or at 40-50°C oven. The drying time and temperature vary based on the characteristics and quantities of the active ingredient, polymer and solvent used. They are significant factors in determining the homogeneity and flexibility of films [30–32]. Films loaded with THCl containing distilled water and ethanol as solvents were dried in the oven. However, homogenous films could not be obtained following the drying process at 50°C due to rapid evaporation of ethanol. Similarly, THCl crystallization was observed in some formulations. Thus, after discarding the air bubbles, the solutions were dried in the oven for 10-12 hours at 40°C.

The physicochemical analysis of the formulations containing 4% or more PVP (F5, F6, F11 and F12) could not be performed due to their gritty, fragmented or non-homogeneous structures after drying. Besides, as the amount of PVP and the ratio of glycerin increase in the formulations, the drying process delayed. For this reason, F4 and F10 took longer to dry than the other films. Substances such as glycerin, propylene glycol and polyethylene glycol are plasticizers that are added to formulations to make films more flexible [33–35]. It has been observed that the use of glycerin at a high rate while preparing the films significantly prolonged the drying time, which yielded very dry-hard or non-homogeneous films. For this reason, the proportion of glycerin used as a plasticizer in our study was determined to be 2-3%.

As a result, buccal film formulations containing HPMC and PVP were prepared by the solvent casting method and after the dried films were removed from glass petri, they were wrapped with an aluminum foil and kept in a desiccator. The films were cut to 1.5x1.5 cm^2 size prior to the physicochemical analysis and the studies were carried out accordingly.

2.3. Characterization of buccal films

2.3.1. Weight uniformity and thickness

The films were weighed in an analytical balance and the weights were found between 95-132 mg (Table 2). The lightest and the heaviest formulations were F3 and F10, respectively. The thickness analysis using micrometer revealed that F10 formulation had the greatest thickness with 0.571 ± 0.056 mm, whereas F3 formulation had the lowest thickness with 0.375 ± 0.019 mm (Table 2). According to the data obtained, among the films containing the same polymer with the same ratio, the ones with 3% glycerin represented elevated weight and thickness values compared to the ones with 2% glycerin. The difference in the weights of the films were statistically significant. However, only the thickness difference between F3-F9 represented significance ($p<0.05$).

2.3.2. pH of the buccal films

Formulations administered through the buccal route must be compatible with the buccal pH in order to avoid irritation in the mouth. In many studies, PBS (pH 6.8) was used to test the pH of the formulations prepared since the buccal pH was ascertained as 6.8 [36,37]. Following the measurements performed using a pH meter probe, the pH values of the films were detected as 6.415-6.692 (Table 2). The results demonstrated that the films were in compliance with the buccal area.

2.3.3. Percentage of moisture loss

The amount of solvent in the drying films is directly related to the moisture content. Freshly prepared films were immediately cut in appropriate size and the moisture controls were conducted. The moisture loss range of the films was detected as 9.21-24.66% (Table 2). The formulation with the highest moisture loss was F7, which included 5% HPMC. Moisture losses of formulations containing 2% glycerin were found to be very close to each other ($p>0.05$). In addition, the films containing 3% glycerin represented greater moisture loss.

Table 2. The characterization of buccal film formulations.

Formulations	Weight uniformity (mg±SD)	Thickness (mm±SD)	Moisture loss (%±SD)	Swelling index (%±SD)	pH (±SD)	Elongation (%±SD)	Tensile strength (N.cm²±SD)	Content uniformity (%±SD)
F1	104±6	0.403±0.029	9.72±1.32	184.76±12.92	6.686±0.020	36.15±2.33	0.169±0.013	98.76±7.48
F2	100±6	0.405±0.021	9.21±0.24	174.37±12.00	6.433±0.083	58.7913.48	0.042±0.002	99.01±7.19
F3	95±7	0.375±0.019	10.83±0.74	151.14±9.51	6.611±0.070	37.63±7.86	0.051±0.009	104.15±5.18
F4	113±8	0.522±0.031	10.34±0.68	255.82±14.26	6.451±0.096	29.81±2.38	0.097±0.005	113.90±6.14
F7	116±11	0.411±0.031	24.66±2.10	147.48±7.90	6.692±0.035	51.46±8.17	0.127±0.013	95.61±4.20
F8	113±7	0.428±0.027	9.92±0.75	155.23±13.54	6.581±0.102	79.37±5.33	0.052±0.008	99.85±4.61
F9	125±9	0.497±0.040	13.92±1.34	169.17±17.49	6.497±0.097	58.95±6.40	0.037±0.003	107.07±1.73
F10	132±13	0.571±0.056	10.62±2.25	221.57±13.21	6.415±0.031	35.35±2.38	0.088±0.005	124.81±8.80

2.3.4. Swelling studies

The swelling capacity of bioadhesive polymers is a crucial factor for bioadhesion. Since lacking sufficient swelling characteristics do not yield good bioadhesion in the mouth, the residence time and thus the efficiency decrease [38,39]. The swelling studies of buccal films were performed in petri dishes containing PBS (pH 6.8) at 37°C in order to provide compliance with the buccal area. Since the swollen films acquire a gel-like structure after a certain period of time, fragmentation or dispersion may occur in the second weighing process. For this reason, wire meshes were used to retain the integrity of the films while removing the wetness and weighing them without deterioration. In approximately half an hour of the swelling studies, the films started having jelly-like structures. Therefore, the studies were completed in this time period. The films had swollen through getting water that was 1.5 to 2 times of their own weights (Table 2), which caused a rapid dissociation. Even though there is a continuous flow of saliva in the buccal tissue, less amount of fluid exists at the same time, which in turn allows the films to swell slowly and remain in the buccal area longer. Among the films having the same polymer with the same amount, the ones containing 2% glycerin demonstrated higher swelling. In addition, F4 and F10 formulations had swollen greatly compared to the other formulations ($p<0.05$), yet they would disperse rapidly in the buccal environment.

2.3.5. Tensile strength and elongation

In order to detect the mechanical characteristics of buccal film formulations, studies were conducted using the Texture Analyzer device. The films were cut in 3x1 cm^2 dimensions and taped in both ends to avoid any damage that might have occurred while clamping. According to the findings of the study, the formulations with the highest tensile strength were F1 and F7, while the formulations with the greatest flexibility were F8, F9 and F2 (Table 2; Figure 1). The tensile strength of F1 and F7 were the highest compared to other formulations ($p<0.01$).

Figure 2 demonstrates that as the tensile strength increased, the flexibility decreased accordingly. However, high plasticizer content caused increased flexibility (Figure 2). The increasing amount of glycerin expanded the distance between the polymer chains, which in turn reduced the tensile strengths of the films and elevated their flexibilities [40–42]. Usually, in formulations containing the same amount of same polymer, the ones with 3% glycerin yielded higher flexibility and lower tensile strength. Statistically, the flexibilities of F2 and F8 formulations were significantly different compared to those having the same ratio of plasticizer ($p<0.01$). Both the qualitative evaluations and the data obtained as a result of this study revealed that the flexibilities of F3, F4 and F10 formulations were relatively low. When the results of the study were analyzed, it was determined that HPMC (low molecular weight) yielded higher flexibility than PVP K30 (F3>F4; F9>F10; $p<0.05$).

2.3.6. Drug content uniformity

In the study conducted to determine the homogeneity of THCl in films, the results were found between 95.61-124.81% (Table 2). The drug content uniformity findings of F4 and F10 formulations were 113.90 ± 6.14% and 124.81 ± 8.80%, respectively. One of the main reasons of observing 10-20% high drug content uniformity in these two formulations was the sliding of the film from the edge to the center of the petri by the end of the drying period. Therefore, the film was not homogeneous although the drying process was performed smoothly. In formulations prepared using petri dishes with a radius of 4.5 cm, approximately 4 - 4.25 cm radius films were obtained. Hence, since the area that the active ingredient dispersed and dissolved became smaller, the drug content uniformity results of these two formulations were higher. The content uniformity results of other formulations were mostly in accordance.

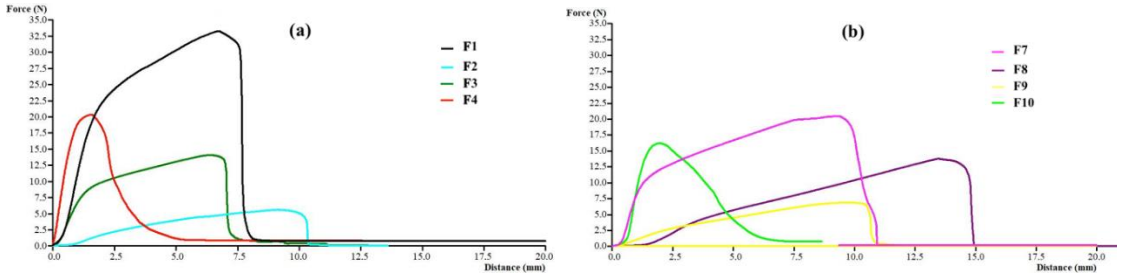

Figure 1. Mechanical characteristic profiles of buccal film formulations detected using Texture Analyzer (a- films with 2% glycerin; b- films with 3% glycerin).

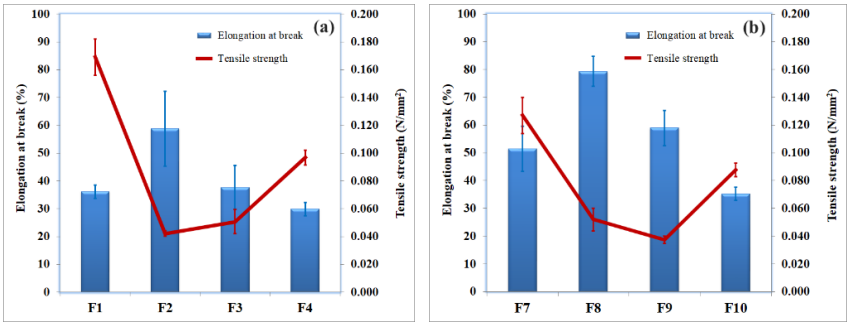

Figure 2. Graphs representing the relationship between flexibility and tensile strength (a- films with 2% glycerin; b- films with 3% glycerin).

2.3.7. FT-IR analysis

In the prepared buccal formulations, the interactions between hydroxyl groups of HPMC at 3300-3700 cm^{-1} and the hydrogen bonds in carboxyl groups of PVP at 1600-1750 cm^{-1} were highly expected [43]. Figure 3a represents the HPMC spectrum, in which C-O stretching vibrations are detected at 1060 cm^{-1}. The peak at 3447 cm^{-1} belonged to out-of-plane bending vibration of hydroxyl group. The characteristic peaks of the PVP spectrum observed in Figure 3b are C = O and N-C stretching vibration peaks of the carbonyl group that are located at 1648 and 1285 cm^{-1}, respectively [43,44]. In the spectrum of THCl seen in Figure 3c, C = C stretch vibrations were detected at 1515 cm^{-1}, whereas aromatic C-H stretching vibrations were at 3040 cm^{-1} and C = C-H peak with aromatic alkenyl stretch were at 2967 cm^{-1}. The peak of out-of-plane vibration that belonged to the benzene ring was observed at 777 cm^{-1} [21]. In the formulation lacking active ingredient (F8 free), the shift of HPMC peaks at 3447 cm^{-1} to 3360 cm^{-1} demonstrated the existence of hydrogen bonds (Figure 3d). The peaks in the fingerprint area that belong to C-O groups moved towards the lower wavelength values. When the peaks of the THCl containing formulation (F8) visible in Figure 3e were correlated to the spectrum of the formulation lacking active substance, it was observed that the characteristic peaks of THCl are highly preserved.

These facts confirmed the hypothesis that during solvent evaporation, THCl molecules found in the solution while preparing the buccal films were dispersed in the polymer structure without forming a chemical bond. Additionally, these finding verified that the polymer structure in the mouth began swelling and disintegrating by saliva and was released from the structure in a controlled manner.

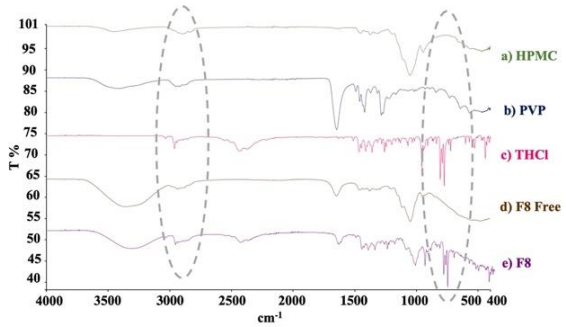

Figure 3. FT-IR Spectrums of a) HPMC, b) PVP, c) THCl, d) F8 Free, e) F8.

2.4. Bioadhesion studies

Bioadhesion is one of the most important criteria for evaluating the effectiveness of buccal formulations. The formulation lacking sufficient bioadhesive characteristics does not adhere to the site of application for the required time period and provide necessary effect. In the bioadhesion studies performed using the Texture Analyzer device, the films were cut in 1 cm diameter circles, which were in accordance with the probe and the buccal tissue was wetted using PBS (pH 6.8). Table 3 demonstrates that the formulations with the greatest work of bioadhesion values were F1 and F7 ($p<0.01$). Besides, the bioadhesion findings of F1 and F7 did not represent a statistically significant difference ($p>0.05$). Mostly, among the formulations containing the same amount of same polymer, the ones with escalated glycerin content yielded higher bioadhesive features. There are multiple studies showing that the increase in plasticizer ratio affects bioadhesion. In one study where buccal films of triamcinolone acetonide were prepared using pectin and gellan gum, 0.5-1% glycerin was used as a plasticizer. The bioadhesion studies of the formulations exhibited that the films containing high amounts of glycerin had greater bioadhesive strength [40]. In 2018, Paolicelli et al. investigated the effect of increasing glycerin concentration on bioadhesive strength [42]. In the study where gellan gum was used as a film forming agent, the glycerin concentration varied between 0.5-6. As the ratio of glycerin elevates, the bioadhesive strength gradually diminishes in formulations with constant amount of polymer. The film containing 3% glycerin only (OTF1.5) exhibited elevated bioadhesive strength compared to the film with 2% glycerin (OTF1) [42].

Table 3. Findings of bioadhesion studies using bovine buccal tissue.

Formulations	Bioadhesive force ($N/cm^2 \pm SD$)	Work of bioadhesion ($mJ/cm^2 \pm SD$)
F1	0.550 ± 0.040	0.381 ± 0.122
F2	0.195 ± 0.048	0.060 ± 0.017
F3	0.167 ± 0.056	0.075 ± 0.014
F4	0.147 ± 0.016	0.060 ± 0.005
F7	0.604 ± 0.055	0.407 ± 0.022
F8	0.303 ± 0.013	0.103 ± 0.029
F9	0.143 ± 0.029	0.045 ± 0.004
F10	0.169 ± 0.033	0.069 ± 0.017

These findings represent consistency with the data of our study. Figure 4a and 4b compare the bioadhesive characteristics of the films having 2% and 3% glycerin. As seen in Figure 4, bioadhesion diminished as the rate of HPMC decreased. Although both polymers had nonionic characteristics, HPMC demonstrated higher bioadhesive features compared to PVP [45,46]. The lowest bioadhesive property was detected in F9 formulation. Additionally, bioadhesion data of F2, F3, F4, and F10 formulations were quite similar ($p>0.05$). However, both bioadhesive strength and bioadhesion work data of F8 were greater than the other four formulations and the findings were statistically significant ($p<0.05$). As a result, the bioadhesive characteristics of F1, F7 and F8 formulations were better than other formulations.

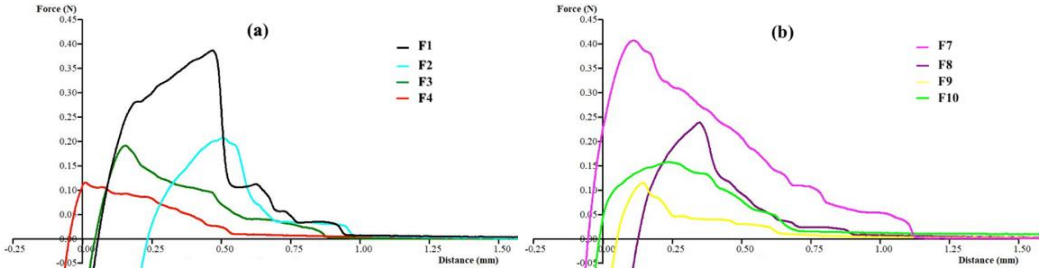

Figure 4. Bioadhesive profiles of buccal film formulations (a- films with 2% glycerin; b- films with 3% glycerin).

2.5. *In vitro* release studies

Formulations that are more suitable for buccal application were determined as a result of the characterization studies. Since the findings of these studies are relatively close to each other, especially the

mechanical properties and bioadhesion data have been considered as the criteria in evaluating the formulations. One of the most significant characteristics of film formulations for buccal application is good bioadhesiveness. Therefore, F1, F7 and F8 formulations were chosen due to their elevated bioadhesive features. F4 and F10 formulations were not found applicable, especially due to homogeneity and content uniformity issues. In addition, these formulations yielded weaker bioadhesive properties than the others. Although F2 formulation was not significantly different than F3 and F9 formulations in terms of bioadhesive property, it was chosen for release studies due to better flexibility. F1, F2, F7 and F8 formulations were more suitable for buccal administration and *in vitro* release study of these formulations was performed in 200 mL pH 6.8 PBS:Ethanol (1:1). Since the film formulations mostly dispersed in the release medium approximately within two hours, the release studies were conducted during this time period. By the end of two hours, the beakers containing the release medium were mixed at high speed and a sample was collected for the last time. The concentration of this sample was considered as 100%. The data obtained by the end of the study revealed that the release percentages of THCl were between 83.43% and 97.42% (Figure 5).

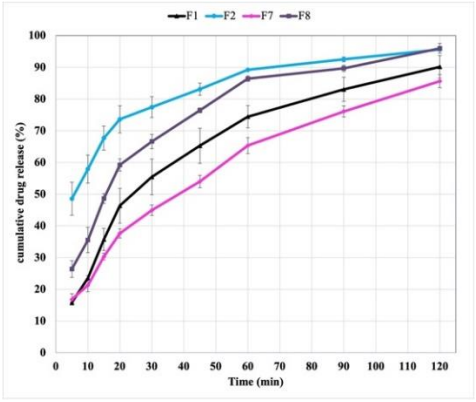

Figure 5. *In vitro* drug release profiles of THCl loaded buccal films.

F2 formulation yielded 48.57 ± 5.20% release at the 5th minute. Compared to other formulations, F2 exhibited a more rapid release in the first twenty minutes and released approximately 75% of THCl ($p<0.05$). However, a very rapid release poses a disadvantage in buccal administration. Due to the rapid release of the formulation in the mucosa or the active ingredient from the formulation, the majority of the active ingredient will be removed through the gastrointestinal tract by saliva flow. Nevertheless, when the release profile was examined, the F8 formulation showed a faster release compared to the F1 and F7 formulations. Briefly, the release profiles of F1 and F7 film formulations containing HPMC polymer only were more favorable compared to other formulations. The release profiles of F1 and F7 were comparable. Hence upon buccal administration, these two formulations will exhibit slower release during two-hour time period rather than the other formulations. As the PVP content escalates, gel texture of the films becomes denser, which cause faster dissociation. This is observed alongside the rapid release of F2 and F8 formulations.

The similarity factors (f2) of the formulations were calculated to evaluate the similarity between two dissolution profiles. The similarity factor of F1-F7 formulations was 56.2, which represented similarity between these two formulations (f2>50.0). On the other hand, the similarity factors of F1-F2, F1-F8, F2-F7, F2-F8 and F7-F8 were lower than 50.0, which indicated that these formulations were dissimilar.

In order to investigate the release data of buccal film formulations in terms of kinetics; zero-order, first-order, Higuchi and Hixson-Crowel kinetic models were applied. The obtained equations were evaluated based on r^2 values. According to this, the release values of the film formulations exhibited the highest determination coefficients in Higuchi and Hixson Crowel kinetic models. Additionally, Korsmeyer-Peppas kinetic model was applied to analyze release kinetics as well. In this model, the release kinetics are evaluated by "n" parameter. n=0.5 represents Fick's diffusion mechanism (Case I), whereas $0.5 < n < 1.0$ interval shows diffusion and non-Fickian (anomalous transport) release, n=1 is zero-order (Case II) and finally $n > 1$ is Supercase II release mechanism [47]. F1 and F7 yielded n values higher than 0.5, which represents that the drug release is time dependent and consistent with non-Fickian release kinetics (Table 4). n value higher than 0.5 points out the diffusion and erosion mechanism pair, in other words anomalous diffusion. This represents that the release of terbinafine hydrochloride from the buccal patches occurs by more than one process [48].

Table 4. Kinetic model analysis of THCl loaded buccal film formulations.

Formulations / Kinetic models	0		1st		Higuchi		Hixson-Crowel		Korsmeyer Peppas	
	(r^2)	k	(r^2)	k	(r^2)	k	(r^2)	k	(r^2)	n
F1	0.8574	0.614	0.8618	0.315	0.9776	11.624	0.9809	0.719	0.9578	0.5548
F2	0.7748	0.356	0.7964	0.155	0.9958	11.416	0.9509	0.298	0.9632	0.2118
F7	0.9363	0.592	0.9359	0.284	0.9916	8.897	0.9951	0.709	0.9888	0.5339
F8	0.8039	0.804	0.8044	0.240	0.9755	13.198	0.9551	0.541	0.9536	0.4131

3. CONCLUSION

Within the scope of this study, THCl loaded buccal film formulations were prepared using HPMC and PVP polymer. The characterization studies of the formulations were performed and their bioadhesive properties were investigated. *In vitro* release studies were conducted using the formulations that are selected in accordance with the findings and the release kinetics were analyzed. HPMC polymer has been detected to increase the bioadhesive characteristics more than PVP and provided more durable formulations. F1 and F7 formulations that were prepared using HPMC polymer only had suitable characterization properties for buccal application, along with elevated bioadhesive features and controlled release. In conclusion, THCl loaded buccal film formulations were shown to be effective in the local treatment of oral infections such as oropharyngeal candidiasis. The findings of this study are very promising since buccal formulation of THCl might be a significant alternative to conventional dosage forms such as creams or gels and used against oral fungal infections.

4. MATERIALS AND METHODS

4.1. Materials

Terbinafin hydrochloride (THCl) has been generously donated by Amino Chemicals Limited, (Malta). PVP K30 has been obtained from Fluka (USA). HPMC (low molecular weight; viscosity of ~15 cP, 2% in H_2O), ethanol, sodium hydroxide, methanol and glycerin were purchased from Sigma (USA). Acetonitrile and calcium chloride (anhydrous) were bought from Merck (Germany). All chemicals used in this study are analytical grade.

4.2. Solubility studies

The solubilities of THCl were detected in solvents used in formulation preparation, along with the mobile phase solvents used in HPLC. To do this, 1 mL of solvent was put into 1.5 mL eppendorf tubes and a large amount of THCl was added. Tightly capped and secured eppendorf tubes were shaken for 24 hours in a shaker (Stuart SSL2 reciprocating shaker, United Kingdom). If the active ingredient dissolved completely, more THCl was added, and the same procedure was repeated. Then, the eppendorf tubes were centrifuged in a centrifuge device (Sigma 3-18 KS, Germany) at 14,000 rpm for 15 minutes. A clear solution formed in the upper layer. 0.5 mL of this was collected and analyzed using HPLC (Agilent 1100, USA). The experiments were performed in triplicates.

4.3. Preformulation studies

Various polymers including HPMC, PVP K30, polyvinyl alcohol (PVA) and chitosan have been analyzed during the preformulation studies. Since THCl is not soluble in water, the suitability of the formulations was evaluated by adding different quantities of ethanol as a solvent in addition to distilled water. During solution preparation, optimal conditions were detected by changing drying time and temperature. Film preparation steps were optimized.

4.4. Preparation of buccal film formulations

Buccal film formulations were prepared by solvent casting method as previously described [49–51]. PVP and HPMC were used as bioadhesive polymers while preparing films to make total polymer concentration 5%. Glycerin (2-3%) was used as plasticizer (Table 5). Sodium hydroxide was dissolved in

distilled water, whereas THCl, having 10 mg of active ingredient on a 1.5x1.5 cm² film section, was dissolved in ethanol. Ethanol and distilled water were used in 3:1 ratio. These two phases were merged and dissolved after adding PVP. Then, HPMC was added, and the solution was mixed until it became completely homogenous. Finally, glycerin was added, and the formulation was finalized through mixing. All mixing steps were performed on a magnetic heating stirrer (Heidolph MR Hei-Standart, Germany). In order to remove the air bubbles in the homogeneous solution, the cap was tightly secured, and the solution was incubated overnight. Degasified homogeneous solution was poured into 9 cm diameter glass petri dish and left to dry at 40°C oven for 10-12 hours. Film formulations containing HPMC polymer only (F1, F7) were prepared following the same procedure. After dried films were removed from the petri dish, they were wrapped with an aluminum foil and stored in the desiccator.

Table 5. The contents of buccal film formulations.

Formulations	Terbinafine HCl (g)	PVP K30 (%)	HPMC (%)	Glycerin (%)	Sodium hydroxide (g)	Ethanol : Distilled water (3 : 1) (q.s.)
F1	0.282	-	5	2	0.040	31,50
F2	0.282	1	4	2	0.040	31,50
F3	0.282	2	3	2	0.040	31,50
F4	0.282	3	2	2	0.040	31,50
F5	0.282	4	1	2	0.040	31,50
F6	0.282	5	-	2	0.040	31,50
F7	0.282	-	5	3	0.040	31,50
F8	0.282	1	4	3	0.040	31,50
F9	0.282	2	3	3	0.040	31,50
F10	0.282	3	2	3	0.040	31,50
F11	0.282	4	1	3	0.040	31,50
F12	0.282	5	-	3	0.040	31,50

q.s. : quantum sufficient

4.5. Characterization of buccal film formulations

Dried films were cut in 1.5x1.5 cm² dimensions for physicochemical analysis including weight uniformity, thickness, pH, moisture loss, swelling percentage.

4.5.1. Weight uniformity and thickness

The films cut in abovementioned size were weighed on an analytical balance (Shimadzu, TW423L, Japan). The thicknesses of the films were measured using a digital micrometer (Insize, 0.001 mm, China). These steps were repeated for 5 times.

4.5.2. pH of the buccal films

The pH values of the prepared film formulations were measured to determine whether they were in compliance with the buccal area. For this purpose, 5 mL of PBS (pH 6.8) was added to the buccal films in a plastic petri dish with a diameter of 3.5 cm and then the petri dishes were closed. After 30 minutes, pH values were checked using a pH meter (WTW Inolab, Germany) [52,53]. This procedure was performed in triplicate.

4.5.3. Percentage of moisture loss

The films were weighed to detect the moisture loss of buccal film formulations (W_1). The films were put in a petri dish, which were then placed in a desiccator having anhydrous calcium chloride. After 72 hours, the films were weighed again (W_2). Percentage of moisture loss was calculated using Equation 1 [54]. This procedure was performed in triplicate.

$$\text{Moisture loss (\%)} = [(W_1 - W_2)/W_1] \times 100 \qquad \text{(Eq. 1)}$$

4.5.4. Swelling studies

Buccal film formulations with 1.5x1.5 cm^2 dimensions were weighed in an analytical balance (W_1) as previously described [55]. Then, the films were placed in a previously tared metal mesh and placed in a plastic petri dish with a diameter of 3.5 cm. pH 6.8 phosphate buffer (5 mL) heated to 37°C was added to the film. The petri was kept at a 37°C oven throughout the swelling studies (JSR, JSON 100, South Korea). The metal mesh was taken from the petri dish. The film was dried using a filter paper and weighed again. The swollen film was weighed on an analytical balance (W_2). The swelling index was calculated by Equation 2. Swelling studies were performed in triplicate.

$$\text{Swelling index (\%)} = [(W_2 - W_1)/W_1] \times 100 \qquad \text{(Eq. 2)}$$

4.5.5. Tensile strength and percentage elongation

In order to determine the mechanical properties of the prepared buccal film formulations, Texture Analyzer (TA.XT.Plus C Stable Micro Systems, Haslemere, Surry, UK) device with 5 kg load cell was used. The two ends of the 3x1 cm^2 film were taped to avoid breaking and the clamps were gently secured. Pre-test speed and test speed were adjusted to 0.5 mm/s. As the device initiated functioning, the upper clamp started moving above and stretched the film. The force and elongation at the break were determined using a software [56,57]. Tensile strength and elongation at break of buccal films were calculated using the following equations (Equation 3 and Equation 4). This experimental procedure was carried out for 4 times.

$$\text{Tensile strength } \left(\frac{N}{cm^2}\right) = \frac{\text{force at failure (N)}}{\text{cross-sectional area of the film } (cm^2)} \qquad \text{(Eq. 3)}$$

$$\text{Elongation at break (\%)} = \frac{\text{increase in length at breaking point (mm)}}{\text{initial length (mm)}} \times 100 \qquad \text{(Eq. 4)}$$

4.5.6. Drug content uniformity

In order to determine content uniformity, 1.5x1.5 cm^2 film was mixed with methanol (100 mL) until it completely disintegrated and dispersed. After adding methanol up to 200 mL and it was mixed again. 1 mL sample was collected and filtered through a membrane with 0.2 µm pore diameter (Isolab, cellulose acetate, 0.2 µm, Germany). This process was carried out in triplicate for each formulation and the samples were analyzed in HPLC.

4.5.7. HPLC studies

High performance liquid chromatography (HPLC) method was developed and validated for quantitation, which was required for active ingredient content uniformity, solubility and release studies. HPLC system (Agilent 1100) with thermostable column department, gradient pump and a UV detector, along with C18 column (GL Sciences, InertSustain C18, 150x4.6mm, 5µm) was used. In order to detect maximum absorption wavelength of THCl, Shimadzu UV 1800 double beam spectrophotometer (Japan) system was operated. The flow rate was set to 0.5 mL/min; the injection volume was 10 µL and the column temperature was 25°C. Methanol, acetonitrile and water (v/v/v 70:25:5) were used as the mobile phase, which was isocratic. Maximum absorption wavelength was detected as 224 nm using UV-VIS absorption spectrophotometer. Linearity of the method was analyzed in the range of 1-60 µg/mL THCl standard solution. The method was fully validated according to the International Conference on Harmonization guidelines with determination of linearity, precision, accuracy, limit of detection (LOD) and limit of quantification (LOQ) [58].

4.5.8. FT-IR analysis

FTIR spectra were obtained in order to verify the molecular interactions between HPMC, PVP, THCl and the optimized formulations that constitute the content of the buccal films prepared. Spectrum Two FT-IR Spectrometer (Perkin Elmer Inc., Waltham MA, USA) and UATR accessory with diamond crystal insert were used. All measurements were performed at 4000-400 cm^{-1} wavelength range and 4 cm^{-1} spectral

resolution in 8 repetitions. The mean values of the measurements were converted into wave number-transmittance graphs using SpectrumTM software (Perkin Elmer Inc., Waltham MA, USA).

4.6. Bioadhesion studies

In order to determine the bioadhesive characteristics of the buccal films, Texture Analyzer (TA.XT.Plus C Stable Micro Systems, Haslemere, Surry, UK) device was used. Freshly dissected bovine buccal tissue was obtained from a slaughterhouse. The underlaying fat and connective tissues were removed from the buccal tissue and it was stored at -30°C until further usage. Before initiating the bioadhesion studies, the buccal tissues were thawed at room temperature. Approximately 1 mm thick buccal tissue was fixed in between the plexiglass apparatus. 10 mm diameter cylindrical probe (SNSP/10, θ: 10 mm) was used for bioadhesion studies performed in a Texture Analyzer device with 5 kg load cell. Pre-test speed, test speed and post-test speed were set to 0.5 mm/s. 1 cm diameter film was sticked to the probe using double sided tape and buccal tissue was wetted using 50 µL PBS (pH 6.8). When the process started, the probe moved downwards to apply 1 N force to the buccal tissue for 120 seconds and then moved upwards. Equation 5 was used to calculate the work of adhesion (mJ.cm^{-2}) and the peak of adhesive force (N.cm^{-2}) from force-distance profile [56,59,60].

$$\text{Work of adhesion (mJ.cm}^{-2}) = AUC_{1\text{-}2} / \pi r^2 \qquad \text{(Eq. 5)}$$

$AUC_{1\text{-}2}$: Area under a curve of the force-distance profile
πr^2 : The surface area of the buccal film

4.7. *In vitro* release and release kinetic studies

According to the result of physicochemical and bioadhesion studies, the ideal formulations were determined, and *in vitro* release studies were carried out. The release studies were performed at 37°C and 100 rpm in a 250 mL beaker containing 200 mL pH 6.8 PBS: Ethanol (1: 1). The 1.5x1.5 cm^2 film was adhered to a thin glass disk using a small amount of cyanoacrylate adhesive and placed on the side surface of the beaker at a perpendicular angle [61]. 1 mL samples were collected at previously determined time points (5, 10, 15, 20, 30, 45, 60, 90 and 120 min) while stirring on the magnetic heating stirrer (Heidolph, Mr Hei-Standart, Germany). The samples were then filtered through a membrane with 0.2 µm pore diameter (Isolab, Germany). 1 mL of fresh medium was added to the release medium after each sample collection. The studies were performed for 3 times for each formulation and the samples were analyzed using HPLC. The similarity factors (f2) between the formulations were calculated, in which the f2 value less than 50.0 represented dissimilarity.

Data obtained as a result of *in vitro* release studies were analyzed in order to understand the release mechanism of films. For this purpose, zero order, first order, Higuchi, Hixson Crowell release mechanism and Korsmeyer-Peppas equation models were used to evaluate the release kinetics of the films.

4.8. Statistical analysis

Student's t-test was used to statistically evaluate the studies. $p<0.05$ was considered as statistically significant.

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