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Chitosan And Carboxymethylchitosan Mucoadhesive Oral Film Forming Spray For Local Delivery Of Fluconazole For Treatment Of Oral Candidiasis

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Abstract
This study aims to develop an efficient local oral mucoadhesive film-forming spray for the targeted delivery of fluconazole, intended for the treatment of oral candidiasis. Chitosan and its water-soluble derivative, carboxymethylchitosan, were employed as key polymers in the formulation. While chitosan was solubilized in 0.5% acetic acid aqueous solutions due to its insolubility in water, carboxymethylchitosan was readily soluble in water. Notably, chitosan formulations, characterized by an acetic aroma and low pH, were primarily included for comparative purposes, acknowledging their potential for oral mucosal irritation.

Ten formulations, denoted as C14-C24, were meticulously prepared for this investigation. Utilizing a quadratic user-defined mathematical optimization model within Design-Expert 13, the optimal concentrations of carboxymethylchitosan and PEG 400 for maximal mucoadhesion were determined. The resultant formulation, comprising 20.03% PEG and 1.5% CMCH, exhibited a high desirability score of 0.989 in the Design-Expert 13 analysis. This composition closely resembled formulation C18, consequently identified as the selected formulation due to its superior mucoadhesive properties and rapid drying time.

Subsequent analyses, including ex vivo permeation studies, in vivo irritation assessments, in vitro anti-candida effectiveness, and stability evaluations, were conducted on formulation C18. It can be concluded from the comprehensive findings from these advanced analyses collectively affirmed the optimal pharmaceutical attributes of formulation C18, underscoring its potential as a promising candidate for effective oral candidiasis treatment as spray forming film drug delivery system.

INTRODUCTION

Oral thrush, or oral candidiasis, arises from an overgrowth of Candida yeasts within the oral cavity (Karajacob et al., 2023). The primary cause of oral candidiasis is a breakdown in the protective mechanisms of the mucosal lining (Qasim, 2020), often evident in individuals with compromised immune systems such as those with human immunodeficiency virus/acquired immunodeficiency syndrome (Vila et al., 2020). Candidiasis stands as the most prevalent oral fungal infection in clinical and dental settings, predominantly attributable to Candida albicans. Notably, a consistent rise in oral candidiasis cases has been noted over the last decade (Malec et al., 2023).

Fluconazole, administered orally at 100 mg/day for one to two weeks, stands as an effective treatment for oral candidiasis. Compared to other antifungal medications, fluconazole demonstrates superior efficacy in reducing the risk of mycological cure rates in oral candidiasis (Fang, Huang and Ding, 2021). Nonetheless, systemic administration of fluconazole can lead to an array of adverse effects ranging from nausea and headaches to liver failure and dysfunction. Furthermore, the medication is associated with interactions with oral hypoglycemics, coumarin-type anticoagulants, cyclosporins, terfenadine, theophylline, phenytoin, rifampin, and astemizole (Rençber et al., 2016).

Given that the pathogenic yeasts responsible for oral candidiasis primarily inhabit the superficial layers of the oral mucosa and fluconazole achieves effective concentrations in oral secretions, the efficacy of systemic fluconazole may be partly attributed to its topical action (Dornelas-Figueira et al., 2023). While fluconazole demonstrates topical efficacy, its systemic adverse effects and potential drug interactions underscore the necessity for a local buccal formulation in clinical practice, devoid of the aforementioned risks. Such a topical administration route might impede the development of fluconazole-resistant strains and bolster antifungal potency by delivering the medication directly to the oral cavity (Malec et al., 2023).

Sprays offer numerous advantages over alternative topical forms, including ease of application, minimal irritation, sterility, uniform medicine distribution upon administration, and customizable dosages (Sneha et al., 2017). In recent years, significant progress has been made in crafting efficient and potent spray formulations, exemplified by the adoption of film-forming sprays (FFS) in pharmaceutical preparations (Zhong et al., 2019). FFS involves the application of a solution as a spray, which upon contact with the target area, forms a film using a polymer matrix for film formation (Zhuang, Zhong and Zhao, 2019).

By adjusting dosages based on the amount of solution per spray, practitioners can regulate both systemic and local effects. A Fixed-Film System ensures consistent medication dispersion and excellent spreadability, thereby enhancing ease of use and patient compliance(Umar et al., 2020). Chitosan, a biocompatible film-forming polymer, boasts positively charged amino groups that interact with the negatively charged sialic acid residues of mucin, a pivotal component of the oral mucosal surface. These interactions entail electrostatic attractions, hydrogen bonding, and van der Waals forces, endowing chitosan with mucoadhesive properties that promote adhesion to the buccal mucosa, prolonging drug residence time and augmenting drug absorption (Arpa et al., 2023).

The introduction of a carboxymethyl group to the chitosan parent structure facilitates the synthesis of carboxymethylchitosan. This alteration enhances chitosan's solubility in neutral and alkaline solutions while preserving other vital properties (Thanakkasaranee et al., 2021).

METHODS

MATERIALS

Fluconazole (Meryer, China), mucin powder Type II (Shanghai-Hai D and B Biological and Technology, China), Low molecular weight chitosan (Glentham Life Sciences, UK), low molecular weight Carboxymethylchitosan (Beijing Jin Ming Biotechnology Co., Ltd., China), Tween 80 (Sigma-Aldrich). Thomas Baker-Thailand provided the polyethylene glycol 400 and ethanol were purchased from the market.

PREPARATION OF MUCOADHESIVE BUCCAL FILM FORMING SPRAY OF FLUCONAZOLE

As shown in Table 1 formulations containing chitosan (CH) and carboxymethylchitosan (CMCH). The polymeric solutions were obtained by dissolving CH in a 0.5% acetic acid aqueous solution and the CMCH in deionized water using a magnetic stirrer for 60 minutes. Tween 80 was added to each solution, to reduce surface tension and increase wettability (Umar et al., 2021). Then Polyethylene glycol (PEG 400) was add gradually which is in addition to being a plasticizer, increases the solubility of polymers in polymeric solutions, stabilizes the solution. PEG400 also act as a solvent for fluconazole, stabilize the drug in polymeric solution (Umar et al., 2020). PEG400 also increase penetration of antifungal drugs as they solubilize and carry the drug (Mori et al., 2017). Fluconazole (FLC) was dissolved in ethanol which is used as a solvent for fluconazole and it is typically used as solvent for film forming spray dosage forms, it is the last was add gradually to each polymeric solution. The design of experiments involve study of effects of two factors percentages (PEG 400, and CMCH%) at three levels on the mucoadhesion of film forming spray formulations of Fluconazole.

Table 1. Compositions of mucoadhesive buccal film forming spray of Fluconazole formulations

Formulation	FLC (g)	CH (g)	CMCH (g)	Tween 80 (mL)	PEG 400 (mL)	Ethanol (mL)	Water up to mL
C14	1	1		2	10	40	100
C15	1	1.5		2	10	40	100
C16	1		1	2	10	40	100
C17	1		1.5	2	10	40	100
C18	1		1.5	2	20	40	100
C19	1		1.5	2	30	40	100
C20	1		0.5	2	10	40	100
C21	1		0.5	2	20	40	100
C22	1		1	2	20	40	100
C23	1		0.5	2	30	40	100
C24	1		1	2	30	40	100

CHARACTERIZATION OF THE PREPARED FLUCONAZOLE MUCOADHESIVE BUCCAL SPRAY FORMULATIONS

DRUG CONTENT ASSAY

A volume of 1 milliliter of prepared film forming solution, supposed to contain 10 milligrams of FLC, was mixed with 50 milliliters of ethanol to get the right volume for the UV absorbance reading. The prepared solution's absorbance was measured at 261 nm (Salian, 2023) using a UV-visible spectrophotometer.

PH MEASUREMENT

The pH values of the formulations were determined in triplicate using a pH meter (Hanna, Italy) (Ashoor et al., 2021) (Tamer et al., 2022).

SPRAY ANGLE MEASUREMENT

Each spray solution formulation was directed horizontally 15 cm onto a sheet of white paper and sprayed until a circle was observed. The radius of the circle was then determined, and the spray angle (θ) was calculated using the provided equation (Desai et al., 2022) (Al-Anbagi, Rajab and Khalil, 2018):

θ = tan^(-1)(l/r) (Eq. 1)

Here, θ represents the spray angle, l is the spacing between the nozzle and the sheet, and r is the mean radius of the circle.

TIME FOR DRYING AND FILM FORMATION

The film-forming solution was sprayed onto a glass petri dish, and the time required for solvent evaporation and film formation was recorded (Gohli and Shah, 2019).

VISCOSITY STUDIES

A Brookfield DV3T viscometer was utilized to measure viscosity. The prepared formulations were placed in a beaker for viscosity measurements using spindle LV (62) and LV. Viscosity was determined at various speeds (12, 30, 60, and 100 rpm) and conducted in triplicate (K. Ali Allah and N. Abd-Al Hammid, 2017).

MUCOADHESION MEASUREMENT BY TURBIDITY TEST

Turbidimetric measurements were employed to compare the absorbance of a mixture of mucin and each formulation with the absorbance of a mucin-only dispersion at a wavelength of 650 nm using an ultraviolet-visible

spectrophotometer. Five milliliters of each formulation were added to a 5 mL dispersion of 0.1% mucin in water and agitated at a speed of 200 rotations per minute at a temperature of 37 °C. The turbidities of the dispersions were recorded at 30-minute intervals and compared to the turbidity of the mucin dispersion. The increase in turbidity of the mucin-formulation dispersion indicated the mucoadhesive characteristic (Rençber et al., 2016).

MATHEMATICAL MODELING AND ANALYSIS OF MUCOADHESION

For optimization purpose, Response surface methodology (User defined) by Design expert13 was used for analysis. Formulations C16-C24 were enrolled in the 2^3 factorial design for optimizations in which two independent variables (X1) represents PEG 400%, and (X2) CMCH % at three levels while the response is the force of mucoadhesion, and the coded variables are shown in Table 2

Table 2. Coded variables X1 PEG400% and X2 CMCH% for formulations from C16 to C 24 and response variable mucoadhesion force

Formulation	Independent variables		Response
	(X1) PEG %	(X2) CMCH %	Mucoadhesion (%Transmitance)
C16	-1	0	
C17	-1	1	
C18	0	1	
C19	1	1	
C20	-1	-1	
C21	0	-1	
C22	0	0	
C23	1	-1	
C24	1	0	

DRUG RELEASE STUDY

An in vitro release study was conducted in the laboratory using a USP Type II dissolution test (Paddle Method). Five puffs of approximately 1 ml of solution were sprayed onto a petri plate (Ashoor et al., 2021). The solution was placed in 500 ml of dissolution media of simulated saliva fluid at 37°C (Raheema and Kassab, 2022) and stirred at 50 rpm. Samples were collected at specified intervals and analyzed for FLZ content using a UV spectrometer.

IN VITRO ANTIFUNGAL ACTIVITY AGAR WELL DIFFUSION METHOD

The antifungal activity of FLZ from the optimized formulation was assessed in vitro using the Muller Hinton agar diffusion disc method. After adjusting the turbidity of fresh C. albicans cultures to a McFarland 0.5, the formulations were aseptically placed into 6 mm diameter drilled wells on the inoculation medium, and the plates were incubated at 37°C. The sizes of the inhibitory zones were measured 24 hours later. The tests were conducted three times, and the averages of the measurements were calculated.

DRUG CONTENT PER EACH SPRAY ACTUATION

The volume of each actuation in the film-forming solution is determined using the equation:

$V = ((Wi - Wa))/LD$ (Eq. 2)

Where V represents the volume of spray produced with each actuation, Wi is the initial weight of the liquid before actuation, Wa is the weight after actuation, and LD is the liquid density.

The FLZ content is evaluated after five puffs by adjusting the volume to 50 ml with methanol and measuring the absorbance in UV spectroscopy. This test is essential for metered-dose sprays (Reem Wael Shahadha and Nidhal Khazaal Maraie, 2023).

EX VIVO FLUCONAZOLE PERMEABILITY STUDY

The chicken cyst was selected as a model membrane due to its histological similarities with human buccal mucosa. The cyst mucosa was obtained from freshly slaughtered chickens and prepared to form a thin membrane by washing with a 7.4 phosphate buffer solution (Hashem et al., 2022).

In this study, a modified Franz diffusion cell was used. Two milliliters of simulated saliva solution and two milliliters of the formulation were placed in the donor compartment, while fifty milliliters of 7.4 phosphate buffer solution filled the receptor compartment. The temperature was maintained at 37°C, and the solution was stirred at 100 rpm. Samples were collected at specific intervals from the receptor compartment and analyzed using UV spectroscopy.

IRRITANCY TEST FOR THE SELECTED SPRAY FORMULATION

An irritancy study was conducted for each of the two optimized formulations individually. Six male rats were selected for macroscopic observation and histological examination. Rats refrained from consuming food at least one hour before the study. Food intake was restricted throughout the research, although liquid consumption was allowed 60 minutes after the buccal liquid formulation was administered. The selected oral liquid formulation was applied to the buccal mucosa using an art brush for 30 seconds. Macroscopic observations were performed on all animals before the formulation was administered, as well as at 1 hour, 2 hours, and 24 hours post-administration, to detect any signs of sloughing, discoloration, ulceration, or bleeding. After 24 hours, the animals were euthanized, and their heads were preserved in a 10% neutrally buffered formalin solution. The buccal mucosa and tongue of each animal underwent decalcification, dehydration, dealcoholization, and embedding in paraffin. The paraffin-embedded tissue samples were thinly sliced, stained, and examined under a light microscope for histological analysis (Kimoto et al., 2016), in comparison to a group of untreated rats serving as the control.

STABILITY TEST OF SELECTED FORMULATION

The best FLC oral spray solution formulation, C18, was evaluated through a stability test by maintaining it at 25°C, 40°C, and 5°C conditions for three months in accordance with the International Council for Harmonization. The sample was reassessed for pH, drug content, and viscosity (International Council for Harmonisation, 2003). Before and after the stability study, C18 was centrifuged at 3000 rpm for 30 minutes to evaluate its physical appearance and identify any signs of separation or turbidity.

RESULTS AND DISCUSSION

PHYSICAL PROPERTIES OF PREPARED FILM FORMING SPRAY

The drug content of the formulations ranged from 88.1 to 110.5% as shown in table 3, falling within the acceptable range as specified by the Pharmacopoeia (Younus Alkwak and Rajab, 2022). This indicates that the prepared formulations exhibit appropriate levels of drug uniformity distribution in the preparations.

A pH range of 5.5–7.0 of the prepared formulations as shown in table 3 which is suitable because it matches saliva pH (Abdella et al., 2022). All manufactured films had an acceptable pH values (6.5-7.2), except C14 and C15 in which acetic acid was used to solubilize chitosan. C14 and C15 considered irritant to oral mucosa.

The spray angle measurement is a crucial parameter in evaluating the performance of spray formulations, particularly for oral sprays where the direction and coverage of the spray are essential for effective drug delivery. The spray angle directly impacts the area covered by the spray and the uniformity of drug distribution on the target surface.

From the results as shown in table 3, it can be observed that the spray angles vary slightly among the different formulations. Formulations C19, C23, C24 exhibited relatively lower spray angles, indicating a narrower

dispersion pattern. On the other hand, formulations C20 and C21 showed higher spray angles, suggesting a wider dispersion pattern.

Optimal spray angles are crucial for ensuring efficient and uniform drug delivery. A narrow spray angle may result in localized drug deposition, leading to uneven distribution and potential inefficacy. On the contrary, too wide a spray angle might result in drug wastage or reduced drug concentration at the intended site of action.

The time required for drying and film formation is a critical parameter in the development of spray formulations, especially for applications such as buccal sprays where rapid film formation is essential for patient convenience and adherence. The drying time directly impacts the usability and effectiveness of the product.

From the results as shown in table 3, it can be observed that formulations C17, C15, and C18 exhibited relatively shorter drying times, indicating quick evaporation of the solvent and rapid film formation specifically C18 shows the shortest time for film forming as shown in figure (1). On the other hand, formulations C21, C24, and C23 showed longer drying times, suggesting slower solvent evaporation and film formation.

Optimal drying and film formation times are crucial for ensuring patient compliance and comfort during product application. A rapid drying time can enhance patient convenience, while a longer drying time may affect the practicality and usability of the formulation.

Figure 1. Film formed after 48 sec of spraying formulation C18

Table 3. Physical properties of prepared film forming spray of Fluconazole formulations

Formulation	Drug content %	pH	Spray angle mm	Time for Drying and Film Formation Sec.
C14	88.1	5.2	45.74±0.625	52
C15	91.8	4.9	44.01±0.920	46
C16	99.3	7.1	48.85±0.531	55
C17	110.5	6.9	47.02±0.610	45
C18	98.6	6.5	43.05±0.520	48
C19	95.5	7.2	40.84±0.741	55
C20	98.6	6.9	55.31±0.436	75
C21	95.9	6.8	52.48±0.665	84
C22	96.6	7.2	45.94±0.509	70
C23	93.1	7.0	44.25±0.431	100
C24	89.4	6.6	43.84±0.658	85

Viscosity

Chitosan is insoluble in neutral and alkaline solutions. Carboxymethylation involves the chemical modification of chitosan's amine group (-NH2) to form the -NHCOCH3 group, resulting in the creation of CMCH. The introduction of carboxymethyl groups enhances the solubility of CMCH in aqueous environments, rendering it water-soluble. Importantly, the augmented solubility of CMCH coincided with a reduction in its viscosity relative to unmodified chitosan. Notably, as depicted in Figure 2 and table 4, C14 (1% CH) exhibited higher viscosity than C16 (1% CMC), while C15 (1.5% CH) displayed greater viscosity than C17 (1.5% CMC). Both CH and CMCH behave as non-Newtonian, shear-thinning polymers.

Across formulations C13 to C24, viscosity decreased with escalating shear rates. Additionally, an increase in PEG 400 concentration was found to elevate viscosity. This outcome can be ascribed to the enhanced interchain entanglement of polymer chains caused by the augmented PEG concentration. While PEG, an oligomer with a relatively low molecular weight and high chain flexibility, increases viscosity by impeding the free flow of the solution. This phenomenon mirrors observations made with clotrimazole transdermal spray formulations containing varying concentrations of PEG 400 (Paradkar et al., 2015).

Table 4. Viscosities of the film forming spray of Fluconazole formulations at different speeds

Formulation	12 rpm	30 rpm	60 rpm	100 rpm
C14	375±5.6	288±3.4	174±0.9	105±3.5
C15	462±5.9	347±7.5	235±4.1	168±2.7
C16	270±6.1	171±5.0	121±9.6	75±1.3
C17	312±3.8	199±7.7	159±5.5	110±3.8
C18	460±11.1	325±4.9	204±6.1	160±2.4
C19	613±3.9	498±7.2	411±8.5	294±4.2
C20	247±2.9	186±3.1	107±8.8	61±3.8
C21	324±7.1	243±1.9	166±2.9	89±5.6
C22	373±4.7	244±1.5	160±12.5	98±7.8
C23	312±8.4	275±2.6	184±6.3	104±2.5
C24	491±4.9	358±3.4	291±1.8	177±5.1

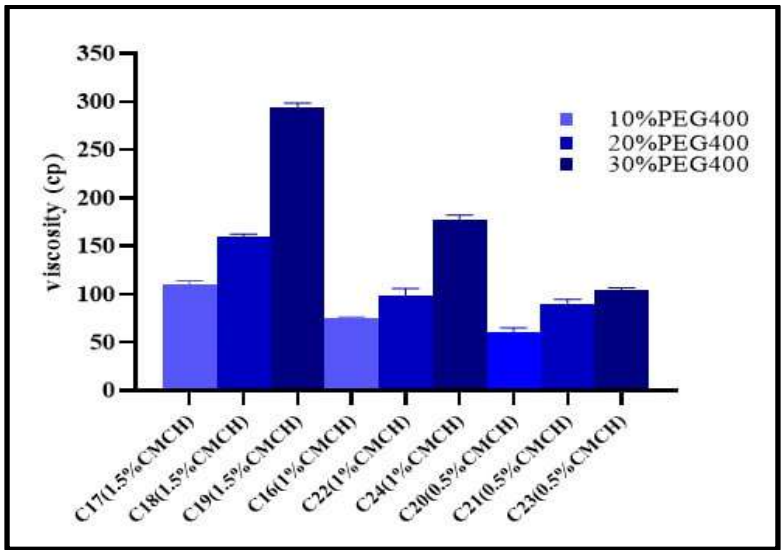

Figure 2. Effect of PEG 400 concentration and polymer concentration on viscosity of CMCH included spray forming formulations

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MUCOADHESIVE MEASUREMENT BY TURBIDITY TEST

For an effective buccal mucosal delivery system, maintaining continuous and close contact with the mucous layer above the epithelial tissue is essential. Mucus, primarily composed of mucin, a protein secreted by epithelial cells, plays a key role. The complex mucoadhesion mechanism involves the formation of various bonds (ionic, hydrogen, covalent, van der Waals) between mucin and the polymer (Alkufi and Kassab, 2019). In turbidity mucoadhesion tests, changes indicate interactions between the formulation and mucin rather than particle movement. Chitosan, with amine groups, can form hydrophobic and covalent bonds when solubilized at a low pH. The positive charge resulting from protonation of chitosan's amino groups facilitates strong electrostatic contact with sialic acid and epithelial surfaces. Additionally, chitosan's hydroxyl group can form hydrogen bonds with mucin, enhancing mucoadhesion. Substitution with carboxymethyl groups reduces the negative charge density of CMCH, which remains slightly positive and can form hydrogen bonds with mucin.

Consequently, formulations with CH exhibit higher mucoadhesion than those with CMCH. The presence of hydroxyl groups in PEG 400 facilitates intermolecular hydrogen bonding with CMCH, increasing hydration and mucoadhesion strength. Excessive hydration, as seen in C19 with 30% PEG400, may reduce mucoadhesion due to chain washing and steric hindrance. Higher polymer concentrations generally enhance mucoadhesion force by providing more interacting chains. C16(1%CMCH) and C17(1.5%CMCH) are less mucoadhesive than C14(1%CH) and C15(1.5%CH) as shown in Figure 3 . It can be concluded that PEG 400 increases the mucoadhesion strength of CMCH solutions, which result in an increase in mucoadhesion of C18 (20% PEG400), which is more mucoadhesive than C17 (10% PEG400); however, C19 (30% PEG400) is less mucoadhesive than C18 (20% PEG400), which might be due to excessive hydration of polymer chains, which leads to the washing away of polymer chains (Madhavi et al., 2010). Also, more PEG 400 might sterically hinder the interaction of CMCH with mucin. For all the prepared formulations, increasing polymer concentration increases mucoadhesion force. The concentration of the polymer influences the mucoadhesion force, and at a lower concentration, the number of polymer chains available to interact with mucin is less than at a higher concentration (Chikwado Okeke, 2017)

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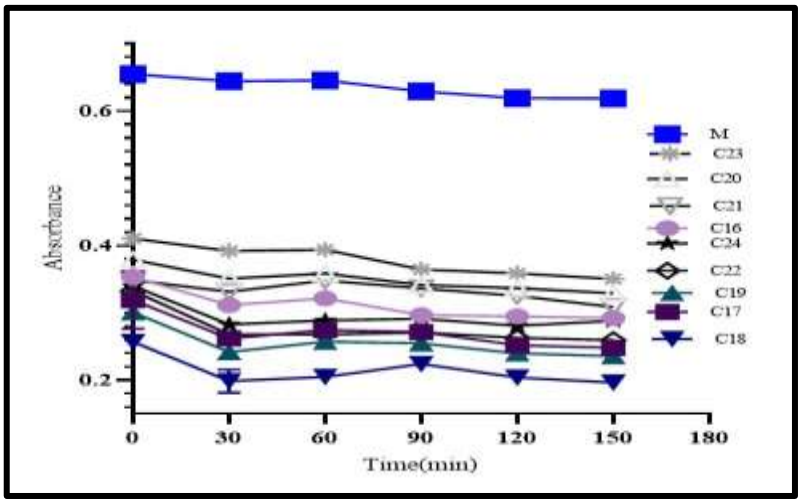

Figure 3. Estimation of interaction between the design formulations and mucin for the turbidity assay as index for mucoadhesion force at different time

Using design- expert13 to analyze the effect of percentage of two polymers in the formulations PEG and CMCH %) individually and combined on the mucoadhesion force represented by transmittance at 150 minute as shown in table 5. The analysis shows significant ($p<0.05$) effect of PEG and CMCH% on mucocadhesion force of film forming spray as shown in Figure 4.

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Table 5. Value of mucoadhesion force of prepared film forming spray of Fluconazole formulations

Formulation	Independent variables		Response
	(X1) PEG %	(X2) CMCH %	Mucoadhesion (%Transmitance) at 150 min
C16	-1	0	0.708
C17	-1	1	0.752
C18	0	1	0.804
C19	1	1	0.765
C20	-1	-1	0.670
C21	0	-1	0.690
C22	0	0	0.740
C23	1	-1	0.650
C24	1	0	0.710

A quadratic model of Adjusted R^2 equal 0.984 was selected and mathematical modeling was done by a design expert13 for calculated response and variables for two independent variables

$Y= 0.7430-0.0008*X1+0.0518*X2+0.0082*X1X2-0.0355X^21+ 0.0025X^22$

Where is Y is the response (mucoadhesion force), X1 is first independent variable (PEG400%), and X2 is second independent variable (CMCH %).

The analysis shows significant ($p<0.05$) effect of PEG and CMCH% on mucocadhesion force of film forming spray as shown in Figure 4.

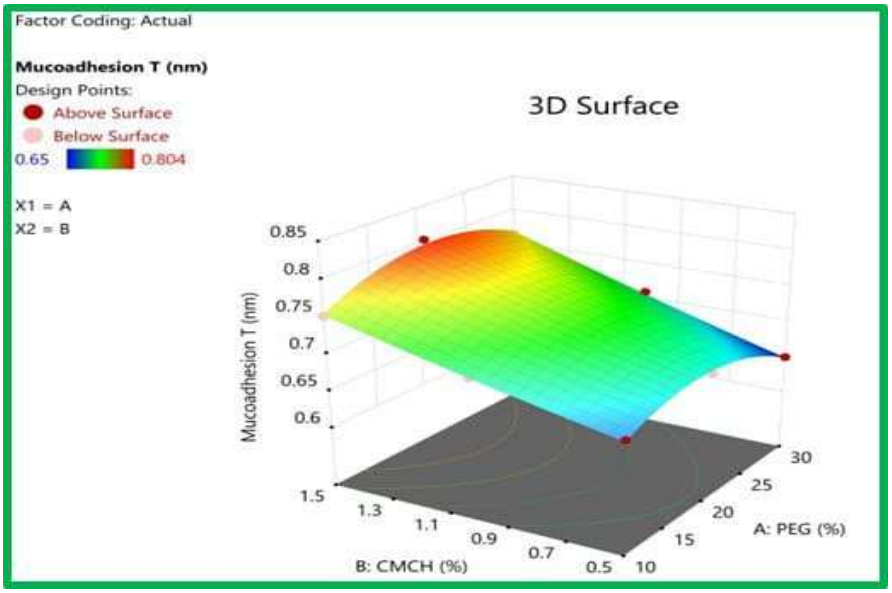

Figure 4. Response surface methodology (RSM) plots of Effect of PEG and CMCH on force of bioadhesion

The suggested formulation for optimum mucoadhesion is consist of 20.03% PEG and 1.5% CMCH with high desirability (0.989), and this formulation is close to formulation C18 which consider as the selected formulation.

FLUCONAZOLE RELEASE STUDY

In the fluconazole release study, it was observed that within formulations with the same polymer concentration, fluconazole release from CMCH was higher compared to that from CH, possibly due to CMCH's solubility in aqueous media. As depicted in Figure 5, the release of fluconazole from C19 (30% PEG) exceeded that from C18 (20% PEG) and both were higher than the release from C17 (10% PEG), attributed to fluconazole's high

solubility in PEG400. This trend aligns with findings in the release of fluconazole from fluconazole ointments containing varying quantities of PEG400 (Siddiqui et al., 2022).

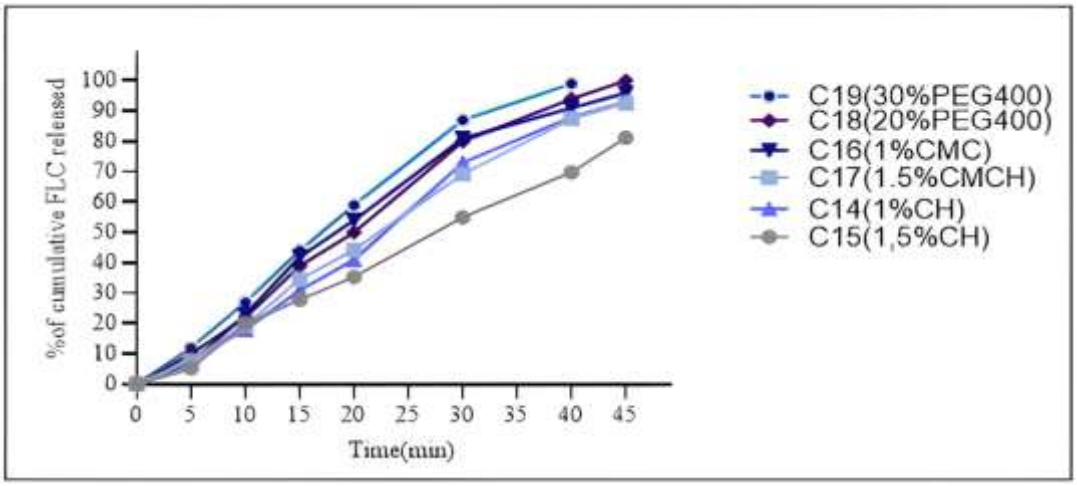

Figure 5. In- vitro dissolution of drug from CH and CMCH formulations in phosphate buffer (pH 6.8) at 37°C

EVALUATION OF THE OPTIMUM FORMULATION C18

IN VITRO ANTIFUNGAL ACTIVITY AGAR WELL DIFFUSION METHOD

In the in vitro antifungal activity agar well diffusion method, the optimal formulation C18 exhibited a sizable inhibition zone of 45 mm against Candida albicans, while its control group (with the same components but lacking fluconazole) displayed a smaller zone of inhibition measuring 15 mm, as illustrated in Figure 6. This enhanced effect can be attributed to the inherent anti-candida activity of carboxymethylchitosan, as demonstrated in previous studies such as the investigation of gauze coating with CMCH as an antifungal agent (Mahdy Samar et al., 2013). These findings underscore CMCH's superior antifungal properties compared to chitosan. Additionally, ethanol, known for its anti-candida activity, may contribute to the inhibition zone observed in the control group.

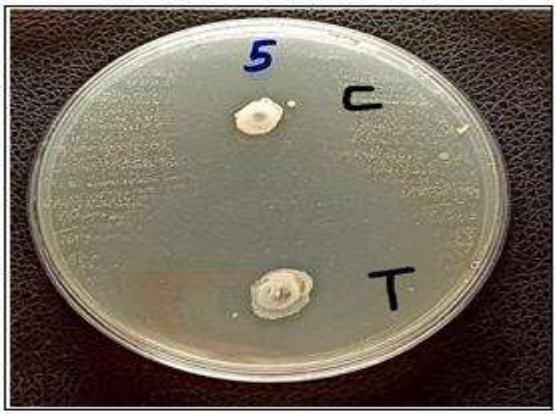

Figure6. Anti-Candida albicans Activity (Inhibition Zone) of C18 Oral Spray Formulation with Fluconazole (T) Compared to Control Formulation without Fluconazole (C)

DRUG CONTENT PER EACH SPRAY ACTUATION

The study found that the volume of actuation per puff was 0.21 ml for C18. This suggests that 5 puffs of formulation contain approximately 1 ml of formulation and 10 mg of fluconazole, Five puffs are needed to cover the entire buccal cavity, just like the nicotine buccal spray where 5 puffs distributed the drug evenly (Chikwado Okeke, 2017).

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EX VIVO FLUCONAZOLE PERMEABILITY STUDY

In Figure 7, it is evident that approximately 31% of fluconazole (FLC) in the optimal formulation C18 permeated through the membrane after 180 minutes. This permeation of the drug signifies its potential antifungal action against microorganisms that may penetrate host tissues. Effective treatment of oropharyngeal candidiasis often necessitates the release of fluconazole directly into the oral cavity. A similar approach is observed in the use of borneol-based oral spray containing clotrimazole for localized treatment of oropharyngeal candidiasis (Lertsuphotvanit et al., 2023).

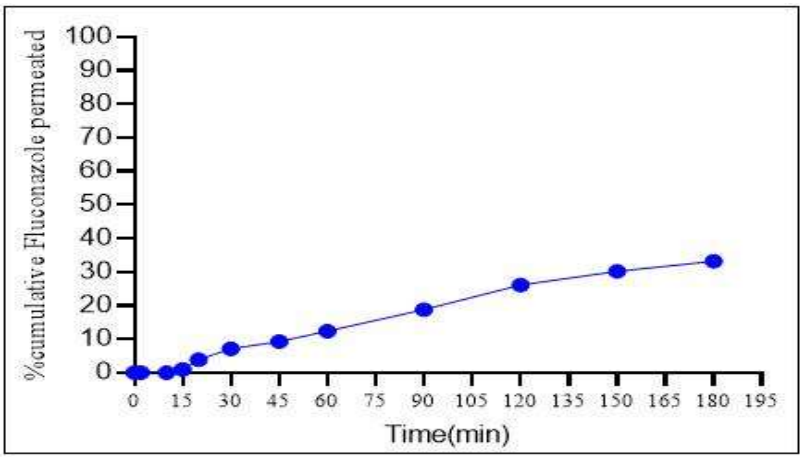

Figure 7. The percentage of cumulative fluconazole from formulation C18 that permeated a chicken cyst

IRRITANCY TEST FOR THE SELECTED SPRAY FORMULATIONS

MACROSCOPIC EXAMINATION

No adverse responses such as discoloration, ulceration, or any other forms of irritation were noticed on the examination sites of any tested animal after the administration of the buccal liquid formulation C18 for durations of one hour, two hours, and 24 hours. therefore, it is evaluated as nonirritant (Cavalcante et al., 2011) as shown in figure 8.

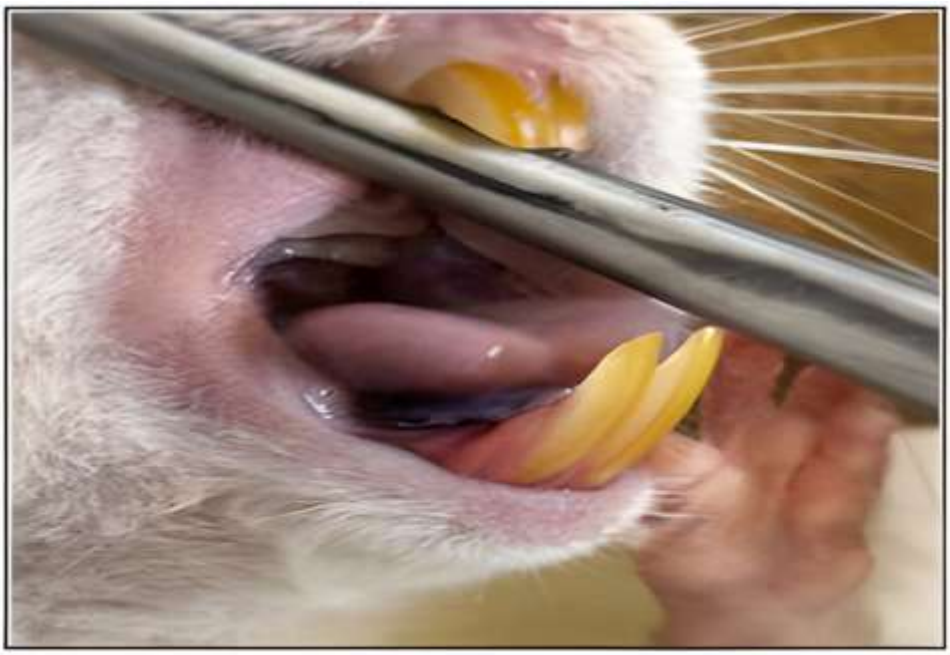

Figure 8. Macroscopic examination of rats after treated with film forming spray formulations C18 after 24 hours

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HISTOPATHOLOGICAL EXAMINATION

The histopathological examination of both control (untreated) and treated rats revealed a normal appearance of lingual structure and cytoarchitecture. It showed normal lingual papillae, epithelium, fibrous lamina propria, and normal intrinsic bundles of skeletal muscle in the rat's tongue. On the other hand, the histopathological findings of the oral mucosa in both control and treated rats also showed a normal structural appearance and cytoarchitecture, consisting of a normal thin keratinized stratified squamous epithelium supported by a normal thick fibrous lamina propria, adipose tissue, and normal bundles of masseter muscle (see Figure 9 and 10)

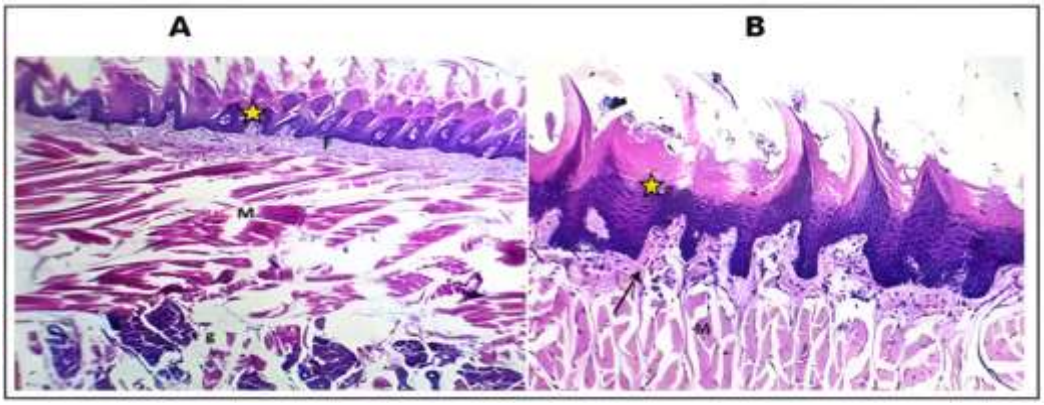

Figure 9. (A) Section of normal (control) lingual mucosa, (B) Section of lingual mucosa of a rat 24 hours after exposure to formulation C18

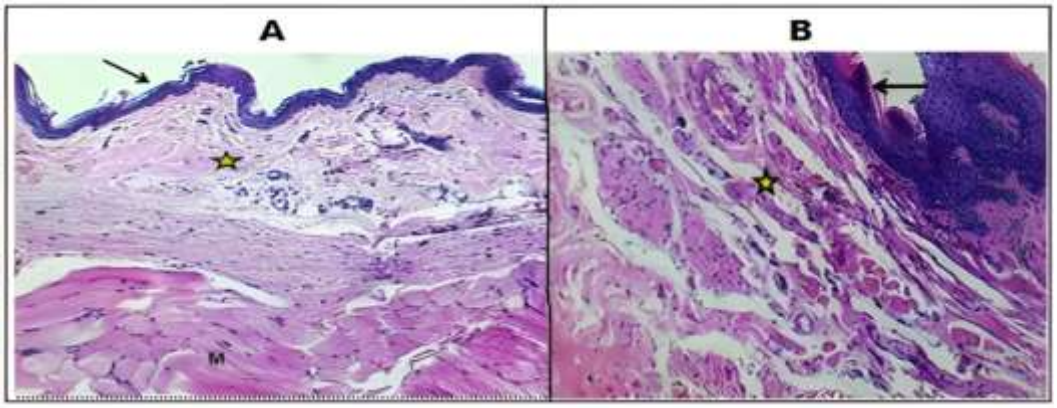

Figure 10. A. Section of normal buccal mucosa showing normal mucosa with normal lining epithelium (arrow). B. Section of buccal mucosa after 24 hrs of exposure to formulation C18

Stability test of selected formulation

The drug viscosity, pH, and drug content of the fluconazole selected formulation C18 were evaluated after three months of storage at 5°C, 25°C, and 40°C. Visually, the formulation remained clear with no signs of aggregation or degradation, suggesting that the prepared mucoadhesive film-forming oral spray formulation is stable for storage in refrigerated conditions and at various temperatures.

Table 6. Stability parameters of selected film forming spray formulation C18 upon different storage temperatures

Parameter	Temperature °C		
	5°C	25°C	40°C
pH	6.5	6.4	6.4
Viscosity (cp)	164	160	158
Drug content (%)	97.6	98.2	97.2

CONCLUSION

The study focused on evaluating the physical properties and performance of a mucoadhesive film-forming oral spray formulation containing fluconazole. The results indicated that the optimal formulation (C18) exhibited good pharmaceutical properties.

In the ex vivo permeability study, the optimal formulation (C18) showed significant permeation of fluconazole, highlighting its potential for localized antifungal action.

Irritancy tests confirmed that the selected formulation, C18, was non-irritant based on macroscopic examination.

The stability test results demonstrated that formulation C18 remained stable after three months of storage at different temperatures, indicating its suitability for storage in various conditions.

Overall, the study provides valuable insights into the formulation, performance, and stability of the mucoadhesive film-forming oral spray containing fluconazole, emphasizing its potential for effective drug delivery and clinical applications.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest related to this work.

AUTHOR CONTRIBUTION

Ilaf J. Attoosh: MSc Student, Mowafaq M. Ghareeb: Supervisor

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