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Preparation and Physicochemical Evaluation of Nystatine Mucoadhesive Buccal Film

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A B S T R A C T

A new type of drug delivery system known as mucoadhesive film has been developed for oral thrush treatment. Due to comfortable use of this drug delivery system, this would be encouragingly utilized, especially in babies and patients under chemotherapy. Nystatinmucoadhesive film (NMF) is a polymeric film which is adhered to front upper gum and back to the upper lip and releases the drug within 5-6 hours in desired concentration. Because of sustained-release nature and permanent contact of drug with infectious agent of *Candida albicans*, the disease healing process would happen more rapidly, and in the meantime the patients can go on with their usual activities such as eating, drinking and talking without feeling the film in their mouth cavity. NMF was prepared using Eudragit RL100 and HPMC as film-forming constituents, and glycerin, propylene glycol and poly ethylene glycol 400 as plasticizers, according to casting method. Finally, physical appearance, weight, *in vitro* drug release rate and adhesion were assessed. with respect to considered parameters, prepared films with HPMC did not show uniform appearance; however, prepared films with Eudragit RL100 showed more desirable results and later showed longer drug release time with approximate time of 3.5 hours. Prepared NMF with dimensions of 2×1 Cm containing 10 mg nystatin using Eudragit RL100 polymer with mixture of glycerin, propylene glycol as plasticizer, not only showed desirable pharmaceutics characteristics but also exponential value (n) of 0.779 showed non-Fickian behavior of drug release.

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Introduction

One of the fungal infections in mouth that is caused by *Candida Albicans* is oral thrush.The most common symptom of oral thrush is white patches and the presence of creamy white, slightly raised lesions in mouth and usually on tongue or inner cheeks. Thrush can cause an uncomfortable burning feeling in the mouth and throat.This is a very common fungal infection in cancer and transplant patients and because of the severe ensuing pain of this infection the patients are not able to eat solid foods and this leads to their weakness [1].

Oral candidiasis can be treated with topical anti-fungal drugs, such as nystatin, miconazole, gentian violet or amphotericin. B. Nystatin is a macrolide antifungal antibiotic that is used to treat vaginal candida infections, skin, mouth and esophagus.Nystatin is both fungistatic and fungicidal. It binds to sterols in the fungal cell membrane, altering its permeability and allowing leakage of intracellular components such as $k^{+,}$ thus cells will be killed[2]. Various dosage forms of nystatin including oral suspension, vagina- ltablets, topical ointments, suppositories and lozeng form are available in the market. Since oral suspension has short residence times at the site of adminis- trationand is not easy and comfortable for children to use, so a new form of mucoadhesive films was considered for this study. Mucoadhesive drug delivery systems (MDDS) are novel systems in pharmacy and because of their ability to adhere and remain on mucosal surfaces and their ability to release the drug slowly and for longer times have caught great attention.. Some advantages of mucoadhesive films are the simple and comfortable use special in infants, suitable for slow drug release on oral cavity without causing problem for eating or speaking.Mucoadhesive dosage forms are available in tablets, batch, and film and gel forms [3]. So far, different drugs have been studied in these mucoad hesive drug delivery systems such as carbamazepine mucoadhesive tablets[4], diltiazem[5] morphine sulfate[6] miconazolenitrate[7] ibuprofen[8] clotrim- azole mucoadhesivefilm[9] and etc. There are various methods for the preparation of mucoadhesive films such as solvent casting, semisolid casting, hot melt extrusion, rolling spray and solid dispersion extrusion [10].

The aim of this study was to prepare a mucoadhesive sustained release film that could control a sufficient amount of drug for a long time in oral cavity to cure the fungal infections, especially in children and patients treated with immunosuppressive drugs rapidly. In this study the pharmaceutical characteristics of film including the rate of drug release, *in vitro* adhesion, having appearance uniformity without bubbles; weight and thickness have been also evaluated.

Method and Material

The material used included nystatin powder (Jabereb- nhayan Pharmaceutical Co.), HPMC15000cps (Dow Co.),Eudragit RL100 powder (Evonik Industries), Glycerin, Propylene glycol , PEG 400, Acetone and Ethanol 96° (Merck) in pharmaceutical grade.

The instruments used in the study were UV / VIS double-beam spectrophotometer (UV-1650 PC, Shimadzu, Japan), Franz cell device attached bath Gallenkamp thermo stirrer 100 made in EEC, the bath's construction battalions KBLee2020 Daiki orbital water bath, the pH meter (pH Meter 632 METROHM company MetrohmHerisau, Switzerla-nd), the digital micrometer (Calper digital microme-ter 0-25mm GB/T14899-94 Chinese model) and IKA RH basic 2 Stirrer machine model built in Brazil.

Preparation of nystatin films

The first film was prepared by casting method with HPMC15000cps polymer using water (20 ml) as solvent. HPMC15000cps was dispersed in water at 50 °C and mixed with hitter stirrer (100rpm). Nystatin was dispersed in plasticizer and added to polymer at 45°C. The formulations were stored at room temperature for 12 h to remove all the contingency air bubbles entrapped and then the viscose solution poured into the petri dish and dried in oven at 50 °C.

Preparation of Eudragit films was different and the solvent was a mixture of acetone and alcohol with proportion 1 to 4. Then Plasticizer was added to mixture. Desired amount of nystatin was dissolved in plasticizer and was then slowly added to the solution of Eudragit to become completely uniform and transparent. The Eudragit solution was poured in petri dish and dried in oven 50^{o}C (table 1&2).

Table 1. Formulations of HPMC 15000films with different amounts of plastisizer

Formulation	HPMC15000(mg)	PEG400(ml)	PG(ml)	GLY(ml)	DRUG(mg)
F1	500	1	0	0	220
F2	500	0	1	0	220
F3	500	0	0	1	220
F4	500	0.5	0.5	0	220
F5	500	0.5	0	0.5	220
F6	500	0	0.5	0.5	220
F7	500	1.5	0	0	220
F8	500	0	1.5	0	220
F9	500	0	0	1.5	220
F10	500	1	0.5	0	220
F11	500	1	0	0.5	220
F12	500	0	1	0.5	220
F13	500	0	0.5	1	220
F14	500	0.5	1	0	220
F15	500	0.5	0	1	220

Table 2. Formulations of Eudragit RL100 films with different amounts of plastisizer

Formulation	Eudragite RL 100	PEG400(ml)	PG(ml)	GLY(ml)	Drug(mg)
M1	1000	1	0	0	220
M2	1000	0	1	0	220
M3	1000	0	0	1	220
M4	1000	0.5	0.5	0	220
M5	1000	0.5	0	0.5	220
M6	1000	0	0.5	0.5	220
M7	1000	1.5	0	0	220
M8	1000	0	1.5	0	220
M9	1000	0	0	1.5	220
M10	1000	1	0.5	0	220
M11	1000	1	0	0.5	220
M12	1000	0	1	0.5	220
M13	1000	0	0.5	1	220
M14	1000	0.5	1	0	220
M15	1000	0.5	0	1	220

Physicochemical examinations of films

Physical examinations

The films must have visually uniform surface without bubbles and must be flexible. To convert qualitative parameters to measurable quantitative ones, films were graded from not good to acceptable as +1 to +3. The results are shown in the Tables 3 and 4.

Determination of thickness and weight of films

The thickness of film in plate was determined by digital micrometers in five different points and the average was reported. (Tables 3&4).

Table 3. The pharmaceutical properties of film formulations "F" series

Formolation	Mucoadhesion Strength(N)	bubble	weight (g)	Uniformity	Clarity	Tension (N)	Thickness (µm)	Result
F1	-	+2	0.672	+1	+1	+1	472±17	fail
F2	-	+3	0.528	+1	+1	+3	341±9	fail
F3	-	+3	0.634	+1	+1	+3	521±18	fail
F4	-	+2	0.593	+1	+1	+3	432±15	fail
F5	-	+3	0.659	+1	+1	+3	336± 25	fail
F6	-	+3	0.635	+1	+1	+3	445±17	fail
F7	-	+2	0.743	+1	+1	+1	494±18	fail
F8	-	+2	0.711	+1	+1	+3	432±12	fail
F9	-	+2	0.821	+1	+1	+3	519±18	fail
F10	-	+3	0.783	+1	+1	+2	492±14	fail
F11	-	+3	0.803	+1	+1	+2	460±24	fail
F12	-	+2	0.723	+1	+1	+3	450±20	fail
F13	-	+3	0.793	+1	+1	+3	547±24	fail
F14	-	+3	0.843	+1	+1	+3	566±22	fail
F15	-	+1	0.819	+1	+1	+3	615± 28	fail

Table 4. The pharmaceutical properties of film formulations "M" series

Formulation	Muccoadhesion Strength(N)	bubble	Weight (g)	Uniformity	Clarity	Thickness (µm)	Result
M1	0.233±0.077	+1	1.276	+2	+1	194±10	Fail
M2	0.231±0.009	+3	1.021	+3	+3	181±3	Pass
M3	0.250±0.007	+3	1.312	+2	+1	190±07	Fail
M4	0.234±0.015	+2	1.091	+2	+1	184±8	Fail
M5	0.244±0.019	+2	1.295	+2	+1	203±8	Fail
M6	0.250±0.007	+3	1.212	+2	+2	193±6	Fail
M8	0.258±0.012	+3	1.385	+3	+3	206±3	Pass
M10	0.289±0.008	+1	1.231	+2	+1	202±10	Fail
M12	0.263±0.011	+3	1.305	+3	+3	205±3	Pass
M13	0.279±0.011	+3	1.211	+2	+1	194±9	Fail
M14	0.273±0.027	+2	0.987	+2	+1	203±7	Fail

Determination of inflation of films

The inflation of films was measured by putting the weighed film samples (1× 2 Cm) on the surface of plate containing 2% agar and incubated at 37°C. Periodically the swollen films were weighed until the maximum and stable weight. The inflation index was calculated by taking the following formula: [11].

$[(W_t - W_o) / W_o] \times 100$

W_t: weight of film at timeWo: weight of film at zero

The results were reported in Table 5.

Table 5. Inflation (%) after 30 -60min for "S" series of films

Time(minute)	Inflation (%) S1	Inflation (%) S2	Inflation (%) S3	Inflation (%) S4
30	30.72±3.04	34.24±2.83	31.01±1.98	28.46±3.56
60	60.54±2.89	53.38±3.34	49.03±2.28	42.63±4.43

The assay of nystatin in the film

The film was cut into 1×2 Cm and weighted. The film was immersed in100ml of phosphate buffer solution with pH6.8 at 37°C and was shaken until the film was completely dissolved(about 3 h).Then a certain amount of sample was taken and filtered

through 0.45micronfilter and one milliliter of sample diluted with 10 mlofphosph- atebuffer. The resultant solution measured with UV-Vis spectrophotometer in a wave length of 307.8 nm. (Table 6)

Table 6. The pharmaceutical properties of film formulations "S" series

Formulation	Muccoadhesion Strength(N)	The bubble	Weight(g)	Uniformity	Clarity	Thickness (µm)	Drug content (%)	Result
S1	0.282±0.008	+3	1.092	+3	+3	178±8	100.20±1.6	Pass
S2	0.279±0.008	+3	1305	+3	+3	194±9	96.84±3.47	Pass
S3	0.265±0.006	+3	1.486	+3	+3	228±5	96.51±6.17	Pass
S4	0.258±0.004	+3	1.729	+3	+3	278±8	98.02±1.93	Pass

Determination of in vitro drug release

To assess *In vitro* drug release a Franz cell with 1×2 Cm piece of film was placed on the 0.45micron filter located on the cell surface and phosphate buffer solution with pH6.8 at 37°C was performed. The 1ml of samples volume was removed after 15, 30, 45, 60, 90, 120, 180, 210, 240, 300, 330, 360, min and replaced with phosphate buffer. The samples were analyzed by UV-Vis spectrophotometer and profile of release is shown in curve No 1.

Drug release modeling

To investigate modeling of drug release from polymeric delivery systems Peppas-Korsmeyer equation was used.these parameters were calculated by taking the following formula:

$$\text{Log}\,(M_t / M_\infty) = \log k_m + n\text{Log}_t$$

30 formulations have been investigated and the results have shown that only 3 of 30 formulations have significant physical properties.After choosing a suitable plasticizer, the connection between polymer concentration and drug release rates was investigated and the results were shown in Table 6.
Results of the profile of drug release from the formulation film S1, S2, S3 and S4 are illustrated in figure1.

M_t:amount of drug released up to time t
K_m:constant drug release
n: exponential value[12, 13].
Results of this section ares hown in Table 3and 4.

Results

The results of drug assay, uniformity, flexibility, mucoadhesion strength, weight, thickness and percentage of inflation of nystatin film with different polymers in formulations are shown in the Table 5, 6 and 7.

Table 7. Diferent amount of Eudragit RL100 with Constance amount of Plasticizer and drug

Formulation	Eudragite RL 100(mg)	GLY(ml)	PG(ml)	NYSTATIN(mg)
S1	750	0.5	1	220
S2	1000	0.5	1	220
S3	1250	0.5	1	220
S4	1500	0.5	1	220

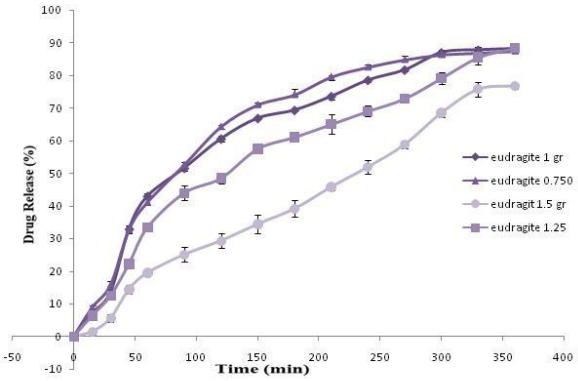

Fig. 1. Effect of Eudragit RL100 concentration on nystatin release in phosphate buffer 6.8

Discussion

Early solid oral drugs forms have been only comprised of tablet and capsule. The emergence of tablet and controlled-slow drug release capsules or drug delivery systems has been remarkable in short span of time and has surpassed conventional methods. Among the systems developed most recently for drug delivery, mucoadhesive systems owing to their mucoadhesion ability, remaining attached to mucosal surface and continuous controlled release of drug have already attracted a great deal of attention. There exist different forms of mucoadhesive systems including tablet, gel and film which are topically placed within the oral cavity and releas their contained drug orally. In this regard, these can offer novel possibilities for the effective treatment of oral diseases like oral thrush. Indeed, to start oral thrush treatment, mucoadhesive film containing nystatin is adhered to oral mucosal layer and then the drug is released into oral cavity. Thereby, in this case, prolonged and slow release of drug, direct and continuous contact with infectious agent (*C.Albicanse*) accelerate the treatment of candidiasis. Furthermore, mucoadhesive film containing nystatin is preferable over adhesive tablet in terms of flexibility and patient comfort[15].

Earlier studies have been prepared nystatin film using different types of polymers including chitosan, carbomer934 and eudragit RL100. Although the release of nystatin from films prepared using chitosan was about 4/5 hours however film formation procedure due to their PH dependence was time-consuming and difficult[16]. In comparison with prepared film using eudragit RL100, prepared films with carbomer934 which release nystatin in the time course of 3 hours, have shown shorter release time[17]. In the present study, in addition to the polymers as applied before, we further used Eudrgit RL100. In contrast to prepared films with HPMC polymer, prepared films using Eudragit RL100 shown higher transparency and more uniformity due to more dispersion of drug in the polymer and plasticizer. It should be noted that nystatin is insolube in water and alcohol though it is soluble in DMSO which is a harmful solvent [18]. Thus, dissolving of drug in a suitable solvent which also has a plasticizer role as well causes improvement of solubility and more dispersion of drug in the polymer.

Among plasticizers, glycerin owing to sweet taste formula and appropriate viscosity led to improvement of taste and the film adhesion whereas preparation of films with HPMC K15M polymer, because of its solubility in the water, and after addition of plasticizer resulted in nystatin sedimentation and its undispersion in the film. Moreover, morphological characteristics and other pharmaceutical parameters of film were ruined. The results obtained from assessment of drug release from the prepared films with Eudragit RL100 revealed that increasing in the polymer concentration of films prolongs the release time of nystatin as well as an increasing in the thickness and a dropping in the adhesion strength of films was observed. Among prepared formulations, S3 formula had an appropriate morphology and thickness and showed favorable adhesion strength. It also released 90% of drugs in the time course of 5-6 hours by a non-Fick-mechanism (exponential value (n) of 0.779) and thereby it was considered as a desirable formulation.

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Conflict of interest

Authors certify that no actual or potential conflict of interest in relation to this article exists.

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