Role of chitosan on controlling the characteristics and antifungal activity of bioadhesive fluconazole vaginal tablets

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Article history:
Received 14 October 2017
Accepted 19 December 2017
Available online 23 December 2017

Keywords:
Fluconazole
Chitosan
Bioadhesive polymers
Prolonged release
Swelling
Antifungal activity

Abstract
Vaginal fluconazole (FLZ) prolonged release tablets containing chitosan in physical blends with other bioadhesive polymers were designed. Chitosan was mixed with hydroxypropyl methylcellulose (HPMC), guar gum or sodium carboxymethyl cellulose (NaCMC) at different ratios and directly compressed into tablets. In-vitro release profiles of FLZ were monitored at pH 4.8. Compressing chitosan with HPMC at different ratios slowed FLZ release, however, time for 80% drug release (T80) did not exceed 4.3 h for the slowest formulation (F11). Adding of chitosan to guar gum at 1:2 ratio (F3) showed delayed release with T80 17.4 h while, in presence of PVP at 1:2:1 ratio (F5), T80 was 8.8 h. A blend of chitosan and NaCMC at 1:2 ratio (F15) showed prolonged drug release with T80 11.16 h. Formulations F5 and F15 showed fair physical characteristics for the powder and tablets and were subjected to further studies. Fast swelling was observed for F15 that reached 1160.53 ± 13.02% in 4 h with 2 h bioadhesion time to mouse peritoneum membrane compared with 458.83 ± 7.09% swelling with bioadhesion time exceeding 24 h for F5. Extensive swelling of F15 could indicate possible dehydration effect on vaginal mucosa. Meanwhile, antifungal activity against C. albicans was significantly high for F5.

1. Introduction
Vaginal route serves as a potential site for local and systemic absorption of a variety of therapeutic agents especially for female related conditions. Despite being a non-invasive route of drug administration, the vagina has not been extensively explored as compared to other routes. Intravaginal drug delivery has been traditionally restricted to the delivery of anti-infective to the local vaginal cavity, however, the vagina has great potential for systemic drug delivery (Benziger and Edelson, 1983). Vaginal route for local effect has several advantages such as, reducing hepatic side effects (Cedars and Judd, 1987), overcoming the inconvenience caused such as pain, tissue damage and probable infection by parental routs (Guyot and Fawaz, 1993), reduction in the incidence and severity of gastrointestinal side effects of oral doses (Vermesh et al., 1988), in addition to the possible self-insertion and removal of the dosage form (Calis et al., 1994).

Local vaginal drug delivery systems include creams, gels, foams, suspensions, solution and tablets (Knuth et al., 1993). Vaginal creams, gels and foams tend to be messy while, suspensions and solutions tend to spread unevenly in the vagina (Patel and Patel, 2012). Local vaginal tablets appear to be an appropriate therapeutic strategy aimed for successful eradication of infectious agents, achievement of high drug levels at the target site, reduction of repeated daily administration and side effect minimization (Ghelardi et al., 1998). Moreover, these particular formulations can offer easy portability, precise dosing, ease of storage, handling and administration, feasibility of large scale production and low cost. Recently, bioadhesive vaginal tablet formulations have been developed and studied as they are capable of delivering the active agent for an extended period at a predictable rate using polymers such as carbopol (Perioli et al., 2011), carboxymethyl cellulose (Karasulu et al., 2004), methylcellulose (Ameen, 2011), HPMC...
Chitosan is a hydrolyzed derivative of chitin, a biopolymer widely distributed in nature. Chitosan is non-toxic, biocompatible and biodegradable (Perioli et al., 2009; Muzzarelli et al., 1988). It has both antibacterial and antifungal activity (Calamari et al., 2011; Kumar, 2000). Its cationic character allows the establishment of hydrogen bonding with anionic mucin chains, resulting in a good mucoadhesive property (Dodane and Vinod, 1998; Sandri et al., 2005). Therefore, chitosan could be considered as an excellent candidate to prepare formulations applicable on vaginal surface.

Vaginal candidiasis is a common condition and up to 75% of women suffer at least one episode of this infection during their lifetime (Karasulu et al., 2004). Candida albicans is the main cause of vaginal candidiasis where most patients respond to local and oral treatments (Karasulu et al., 2004). The most commonly prescribed treatment for vulvovaginal candidiasis is imidazole. Imidazole antifungal agents, including fluconazole (FLZ), are available in various dosage forms such as vaginal creams, pessaries and oral tablets (Bachhav and Patravale, 2009). Fluconazole is known considered as the primary treatment option for almost all forms of susceptible Candida infections (Meis et al., 2000). Moreover, treatment of vaginal candidiasis with FLZ is even more effective than for other sites of infection (Moosa et al., 2004). The main adverse reactions related to the use of FLZ oral tablets are nausea, vomiting, headache, rash, abdominal pain, diarrhea, and alopecia in patients undergoing prolonged treatment with a dose of 400 mg/day (Bennett, 2003). Bisht and Ghosh have studied FLZ vaginal tablets that contain a single polymer including carbopol, HPMC and guar gum (Bisht, 2011) while, Mahours et al. used these polymers in combinations (Mahours, 2016). The tablets showed prolonged release of the drug in both reports in favor of carbopol as a single polymer and carboxypol with HPMC as combined polymers.

Chitosan was reported to be a carrier for antifungal drugs (including FLZ) alone as a single unmodified (Szymarska et al., 2014; Notario-Perez et al., 2017) and modified polymer (Perioli et al., 2009). Other reports compared chitosan with other bioadhesive polymers (Patel et al., 2011) or studies chitosan combined with other polymers. Chitosan in a blend with PVP tailored the release of FLZ as solid dispersion for oral administration (Papageorgiou et al., 2008) while, it was used as a film forming agent in presence of other bioadhesive polymers for formulation of FLZ mucoadhesive buccal films (Yehia et al., 2009). For vaginal treatment, chitosan of different molecular weights was evaluated as a vaginal mucoadhesive gel (Senyiqit et al., 2014). It was also combined with bioadhesive polymers including carboxymethylcellulose and alginate as complexes for local vaginal delivery of chlorhexidine (Bigucci et al., 2015; Abuzzo et al., 2001) or compressed into tablets with HPMC by wet granulation methods (Amish et al., 2011; Khan and Thakur, 2014). However, chitosan was not tested to be a key polymer to control the properties of tablets formulated with other mucoadhesive polymers using direct compression.

In this study, bioadhesive long acting vaginal tablets containing FLZ were formulated by direct compressing chitosan in physical blend with other mucoadhesive swellable polymers including, HPMC, Na CMC and guar gum. Due to the unique characteristic of chitosan specially in acidic environment of vagina, it was selected to modify the tablets’ physical characteristics including swelling and bioadhesion as well as drug release profiles to provide properly designed bioadhesive vaginal tablets with long term therapeutic effect at the site of infection with improved efficacy, reduced frequency of administration and minimized drug side effects.

2. Materials and methods

2.1. Materials

Fluconazole (FLZ) was received as a gift sample from Al-Jazeera Pharmaceutical Industries, Riyadh (Saudi Arabia). High molecular weight chitosan (fine powder) was purchased from Sigma Aldrich, Missouri (USA). Hydroxypropyl methylcellulose E 10 M premium CR (HPMC) was obtained from Dow Chemical, Michigan (USA) while, high viscosity carboxymethyl cellulose sodium salt (NaCMC) was purchased from BDH chemicals, Parkstone (England). Guar gum was purchased from Merck Specialties, Bumbai (India) and polyvinyl pyrrolidone K30 (PVP) from Loba Chemie, Mumbai (India). McIlvaine’s citrate buffer of pH 4.8 were prepared according to British Pharmacopoeia using citric acid which was purchased from Avon Chemicals, Cheshire (UK) and di-sodium hydrogen phosphate anhydrous from Winlab, Leicester (England). All other chemicals used were of analytical grades. Mouse peritoneum membrane was obtained from King Saud University animal house, Riyadh (Saudi Arabia).

2.2. Methods

2.2.1. Evaluation of physical properties of the selected polymers and their blends with chitosan

Each powder blend contains 1:3 wt ratio of FLZ to polymer or polymer blend was mixed by cube mixer (Cube mixer, Erweka, Germany) and passed through a sieve of mesh size #60. The composition of different FLZ polymer blends is shown in Table 1. Flowability of powder was evaluated by determining the angle of repose, Carr’s compressibility index and Hausner ratio.

Angle of repose was determined by the previously described conventional funnel method (Shah et al., 2008). Carr’s compressibility index was calculated based on the bulk and tapped volumes. The bulk volume (V_{bulk}) occupied by 10 g of sample was measured and corresponding tapped volume (V_{tapped}) when no change in volume was obtained after 15 repeated taps (Aguilar-de-Leyva et al., 2011). The test was performed in triplicate and the mean values of the V_{tapped} and V_{bulk} were used to calculate the compressibility index according to the following equation:

\[
\text{Carr’s Compressibility index (\%)} = \frac{100 \times (V_{bulk} - V_{tapped})}{V_{tapped}}
\]

The method of determining the Hausner ratio was applied using the relation:

\[
\text{Hausner Ratio} = \frac{V_{bulk}}{V_{tapped}}
\]

2.2.2. Preparation of FLZ vaginal tablets

Each tablet of total weight 200 mg was containing 50 mg FLZ and 150 mg single polymer or polymer blends (including chitosan) were prepared by direct compression of the powder blend using flat 9 mm punch (RoTab T, rotary tablet press, kg-Pharma, Berlin, Germany). No lubricant or glidant was needed during the compression process. The amount of 100 tablets was prepared for each tested formulation. The compression force of the tablet machine was adjusted to give tablet hardness ranging between 4 and 8 Kp.

2.2.3. Assay of FLZ in simulated vaginal secretion

Solution of FLZ (400 µg/ml) in McIlvaine’s citrate buffer of pH 4.8 to mimic vaginal secretion was spectrophotometrically assayed at λ_{max}261 nm (Corrêa and Salgado, 2011). The absorbance values of serial concentration of FLZ in range 50–400 µg/ml were measured at the denoted λ_{max}. The measured absorbances were plotted
against the corresponding concentrations and a mean calibration curve was constructed. The slope of the linear regression line representing the calibration curve was calculated (n = 5).

2.2.4. Physical characteristics of the formulated tablets

A total of 20 tablets were weighed and their average weight was compared with the claimed 200 mg and the deviation was measured.

Friability testing was applied according to USP method (UPS, 2016). Twenty tablets were de-dusted and weighed (W₀). The tablets were then placed in the friabilitator drum (Friability tester, Erweka, Germany). The apparatus was adjusted at 25 rpm for 4 min then the tablets were removed, de-dusted and weighed (W). Amount of loss was calculated by the following equation:

\[
\text{% loss} = \frac{W₀ - W}{W₀} \times 100
\]

The hardness test was performed on ten tablets selected randomly from each formulation and measured by Erweka hardness tester (Erweka, Germany) and the mean ± SD were recorded.

For drug content, one tablet of each formula were crushed and powdered in a mortar, an aliquot of 100 mg was accurately weighed and extracted with 100 ml McIlvaine’s citrate buffer pH 4.8. The solution was filtered, spectrophotometrically assayed at 261 nm and the amount of FLZ in each tablet was calculated. The test was performed in triplicate and mean drug content was reported as mean ± SD.

The thickness of twenty tablets from each formulation was also determined by micrometer (Fowler, Massachusetts, USA) and thickness mean ± SD were reported.

2.2.5. In-vitro FLZ release study

Drug release was determined using USP dissolution apparatus II (Model DT 70, PharmaTEST, Germany). Each tablet was glued using cyanoacrylate glue on the center of a 9 cm glass disc (Bhat and Shivakumar, 2010). Dissolution medium was 250 ml McIlvaine’s citrate buffer pH 4.8 (Cevher et al., 2014), maintained at 37 ± 0.5 °C and stirred at 50 rpm. Samples of 5 ml were withdrawn and filtered through 0.45 μm filter at predetermined time intervals and assayed spectrophotometrically at 261 nm for its FLZ content. Samples were replaced with fresh buffer. Cumulative percentage of drug release was determined and each study was done in triplicate. The drug release profile was characterized by initial percent-

age release at 5 min, time for 50% FLZ release (T₅₀), time for 80% FLZ release (T₈₀) and dissolution efficiency (% DE).

Dissolution efficiency was calculated by the following equation:

\[
\text{% DE} = \int_0^t \frac{y \cdot dt}{y_{100} \cdot t} \times 100
\]

where y is percentage of drug dissolved at time t; y₁₀₀ is maximum percentage of drug dissolved over the time period 0 – t. The time of 480 min (8 h) for drug release was chosen for the determination of % DE.

For formulations that have not released 50% or 80% in the 8 h dissolution time, Weibull mathematical model (Costa and Lobo, 2001) was employed for T₅₀ and T₈₀ prediction. Weibull model is described by the following equation:

\[
F = 100\left[1 - e^{-\left(\frac{t}{\tau}\right)^{\beta}}\right]
\]

where F is the fraction (%) of drug released in time t, α is the scale parameter which defines the time scale of the process; β is the shape parameter which characterizes the curve as either exponential (β = 1; case 1), Sigmoid, S-shaped, with upward curvature followed by a turning point (β > 1; case 2), or parabolic, with a higher initial slope and after that consistent with the exponential (β < 1; case 3).

2.2.6. Release kinetics

The release data were tested for release kinetic model to compare dissolution profiles of promising formulations using model-dependent approaches. DDSolver program was used to evaluate the goodness of fit of a model; the coefficient of determination (r²) was calculated in order to discriminate the most appropriate model.

The data were analyzed for zero and first-order as well as Higuchi release kinetic models.

Drug release data were also treated in a Korsmeyer–Peppas semi-empirical model to further confirm the kinetic model of drug release using the relation:

\[
\frac{M_t}{M_\infty} = k_α t^n
\]

where \(M_t/M_\infty\) is the fractional amount of drug released at time t, \(k_α\) is the release rate constant, and n is the diffusion exponent that characterizes the type of release mechanism used during the dissolution process (Peppas, 1985). The values n and \(k_α\) were estimated using a linear regression of \(\log(M_t/M_\infty)\) vs log t.

2.2.7. Swelling study

The swelling behavior of tablet, described as the water absorbing capacity, was determined by gravimetric methods (Cevher et al., 2014; Kast et al., 2002). In this test, each tablet was placed into a stainless steel basket and weighed. The basket was then placed in 100 ml McIlvaine’s citrate buffer pH 4.8, allowing the tablet to swell at 37 ± 0.5 °C for 6 h. The basket was periodically weighed after removing the excess water on the surface with a filter paper. The swelling % was calculated using the following equation:

\[
\text{Swelling (}% = \frac{W_t - W_0}{W_0} \times 100
\]

where: \(W_t\) is the weight of the basket containing the tablet at time t and \(W_0\) is the initial weight. The swelling study on each formulation was done in triplicate and mean ± SD was recorded.

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### Table 1

Composition of FLZ polymer blends in mg to be directly compressed into vaginal tablets.

| Formulation | FLZ  | Chitosan | HPMC | NaCMC | Guar gum | PVP |
|-------------|------|----------|------|-------|----------|-----|
| F1          | 50   | 100      | 50   |       |           |     |
| F2          | 50   | 75       |      | 100   |          |     |
| F3          | 50   | 50       | 50   | 50    |          |     |
| F4          | 50   | 37.5     | 75   |       | 37.5     |     |
| F5          | 50   | 150      |      |       |          |     |
| F6          | 50   | 150      |      |       |          |     |
| F7          | 50   |          | 150  |       |          |     |
| F8          | 50   | 100      | 50   |       |          |     |
| F9          | 50   | 75       | 75   |       |          |     |
| F10         | 50   | 50       | 100  |       |          |     |
| F11         | 50   | 50       | 0    | 50    |          |     |
| F12         | 50   |          | 150  |       |          |     |
| F13         | 50   | 100      |      | 50    |          |     |
| F14         | 50   | 75       | 75   |       |          |     |
| F15         | 50   | 50       | 100  |       |          |     |
| F16         | 50   | 50       | 50   | 50    |          |     |
| F17         | 50   |          | 150  |       |          |     |

* High molecular weight Chitosan.
wetted and placed in a petri dish containing one hole to ensure the effectiveness of FLZ on C. Albicans. All plates were incubated at 37 ± 0.5 °C for 24 h. Antifungal activity of the selected formulations and its control drug free tablets were compared. The test was performed in triplicate and the diameter of the inhibition zones were measured with a gauge and expressed in mm ± SD.

2.2.11. Statistical analysis

All the results were expressed as mean values ± standard deviation (SD). Statistical analysis was done using student t-test or one-way analysis of the variance (ANOVA) at a significant level p < 0.05. Instat® software was used for statistical analysis.

3. Results and discussion

3.1. Physical characteristics of FLZ-polymer blends

Angle of repose, Carr’s compressibility index and Hausner ratio have been widely used to describe powder flowability which has a great impact on many pharmaceutical process such as blending, compression and handling. Powder flowability was determined and classified according to USP classification. Table 2 shows that all tested drug polymer blends had either fair or passable flowability. Generally, blending of chitosan with other polymers showed to decrease flowability of these polymers. On the other hand, adding PVP to the polymer blend improved the powder flowability. This was shown when guar gum blend with chitosan (F2) was compared with F4, HPMC blend with chitosan (F9) was compared with F11 and NaCMC blend with chitosan (F14) was compared with F16.

3.2. Physical characteristics of formulated tablets

Chitosan alone or its blend with other polymers were used for the formulation of FLZ vaginal tablets. Polymers were chosen mainly for their bioadhesion properties and prolonged release effect in addition to their ability to enhance physical properties of powder blend of the formulated tablets. Therefore, attempts were made to investigate and assess the pharmaceutical quality of different tablet formulations.

According to BP (BP, 2017), ±7.5% deviation in tablet weights is allowed for uncoated tablet weighing 200 mg. Table 3 shows that deviation in tablet weights was within the acceptable range. Tablet thickness (Table 3) was mainly affected by the composition of each formulated tablet, which depends on cohesiveness, adhesiveness and bulk density of the ingredients in the formulae. Tablets thickness were generally decreased with increased ratio of chitosan due to its large bulk density, meanwhile, decreased thickness was also observed in presence of PVP due to the adhesive effect of the polymer. The resistance of the tablet to chipping, abrasion, or breakage during storage, transportation or handling depends on the tablet hardness (crushing strength). All formulations showed hardness of 4–8 kg, except formulations containing chitosan and guar gum at 1:1 (F2) and 1:2 (F3) ratios and guar gum alone (F7) which had values of 3.3 ± 0.15, 3.6 ± 0.23 and 3.5 ± 0.73 kg, respectively (Table 3). The presence of guar gum is possibly the reason for this low hardness, since it has been found that the commercially available guar gum does not provide tablets with sufficient hardness. Thus, additional polymer must be used to provide the desired properties (Gebert et al., 1995). Increasing amount of chitosan in the formulation by changing the ratio into 2:1 (F1) resulted in 4 ± 0.4 kg hardness.

Generally, high ratios of polymers such as HPMC, NaCMC and PVP in blends with chitosan would produce tablets with significantly higher hardness values especially with PVP due to their high binding effect compared with chitosan. However, this was not

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2.2.8. In-vitro determination of tablet bioadhesion time

In-vitro bioadhesion time was determined using mouse peritoneum membrane (Wang and Tang, 2008). The tissue was cut in 2 × 2 cm squared pieces and stored frozen at −20 °C. Before examination it was thawed in 0.9% sodium chloride solution and fixed in the internal side of a beaker with cyanoacrylate glue. Tablet of each formulation was wetted with 50 μl of McIlvaine’s citrate buffer pH 4.8 from one side and put in contact with the biological membrane surface applying a fingertip force for 20 s. The beaker was then put in contact with the biological membrane surface applying a fingertip force for 20 s. The beaker was then filled with 75 ml McIlvain’s citrate buffer pH 4.8 and kept at 37 ± 0.5 °C. Tablet behavior and bioadhesion time was monitored until complete detachment or dissolution occurs (Perioli et al., 2011). The test was done in triplicate and mean ± SD was recorded.

2.2.9. Determination of ex-vivo bioadhesion strength

Mouse peritoneum membrane frozen at −20 °C was thawed in 0.9% sodium chloride solution before examination. The test was performed using Instron® tensile tester. The tissue was glued in the middle of glass slide with cyanoacrylate glue, the slide was fixed to the lower plate of the tensile tester and the tablet was glued to the upper rod of the apparatus. The surface of the membrane was moistened with 50 μl of McIlvaine’s citrate buffer of pH 4.8. The tablet and the membrane were brought in contact. A 0.5 N force was applied after initial contact for one minute. Next, the tablet and the mucosa were pulled apart at a speed of 5 mm/min until a complete rupture between the tablet and the tissue is observed (Ameye et al., 2002). The test was done in triplicate and mean ± SD was recorded.

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### Table 2

| Formulation | Angle of repose (°) | Compressibility index (%) | Hausner ratio | Flow character |
|-------------|---------------------|---------------------------|---------------|---------------|
| F1          | 43.6                | 22.7                      | 1.22          | Passable      |
| F2          | 44.0                | 25.0                      | 1.25          | Passable      |
| F3          | 43.6                | 22.0                      | 1.22          | Passable      |
| F4          | 36.9                | 20.1                      | 1.20          | Fair          |
| F5          | 37.8                | 20.0                      | 1.20          | Fair          |
| F6          | 36.9                | 19.5                      | 1.19          | Fair          |
| F7          | 39.8                | 19.4                      | 1.19          | Fair          |
| F8          | 43.2                | 25.2                      | 1.33          | Passable      |
| F9          | 43.6                | 22.5                      | 1.29          | Passable      |
| F10         | 44.0                | 23.3                      | 1.30          | Passable      |
| F11         | 40.4                | 20.0                      | 1.25          | Fair          |
| F12         | 40.5                | 20.0                      | 1.25          | Fair          |
| F13         | 43.2                | 22.2                      | 1.28          | Passable      |
| F14         | 43.2                | 23.1                      | 1.30          | Passable      |
| F15         | 40.5                | 20.3                      | 1.25          | Fair          |
| F16         | 39.3                | 17.2                      | 1.20          | Fair          |
| F17         | 40.1                | 20.0                      | 1.20          | Fair          |

* Composition of each code is presented in Table 1.*
The designed tablet formulations depended on direct compressing bioadhesive polymer blends containing chitosan, as a key polymer, in absence of other non-bioadhesive excipients that are commonly added in formulation of direct compressed tablets. This could allow for formulation of prolonged release vaginal tablets with superior characteristics such as high bioadhesion strength and long bioadhesion time in addition to simple formulation processing that could be applied in large scale production.

### 3.3. In-vitro FLZ release study

Release of FLZ was monitored for tablets containing a single polymer, including chitosan, or combination of chitosan with other polymers in order to select the best formulation(s) with prolonged release properties.

The release characteristics of FLZ from tablet formulations of the selected single polymers chitosan (F6), guar gum (F7), HPMC (F12) and NaCMC (F17) are presented in Table 4 and their release profiles in Fig. 1. Tablet formulations containing only chitosan as a single bioadhesive polymer (F6) showed rapid initial FLZ release where 80% was released in less than 5 min with high DE% in 480 min. This could be due to observed high swelling rate of chitosan followed by predominance erosion of the tablet matrix (Shao et al., 2015). Meanwhile, HPMC tablet (F12) gave high initial FLZ release and high DE% with observed complete tablet disintegration within 5 min due to high hydrophilic property of the polymer where fast high expansion of the matrix along with increase of tablet size could be the reason (Ju et al., 1995). Unlike chitosan and HPMC, formulation F7 containing only guar gum showed very slow FLZ release with almost 90 min lag time for detected drug release and predicted >24 h to release 80% of the drug from the formulation. This is possibly because of the compact viscous gel layer formed on tablet surface (Malviya et al., 2010). Moreover, formulation F17 containing only NaCMC showed slow release profile, where, 80% of the drug was predicted to be released in 681.07 min. This could be due to observed high swelling rate of chitosan where fast high expansion of the matrix along with increase of tablet size might cause that slow release (Conti et al., 2007). The formation of high viscosity gel layer on the tablet surface might cause that slow release (Conti et al., 2007).

### Table 3

Weight variation, tablet thickness, hardness, friability and drug content of the formulated tablets.

| Formulation | Tablet weight (mg) | Tablet thickness (mm) | Hardness (kg) | Friability (%) | Drug content (%) |
|-------------|--------------------|-----------------------|---------------|---------------|-----------------|
| F1          | 200.55 ± 1.25      | 2.611 ± 0.033         | 4.0 ± 0.40    | 0.724         | 105.02 ± 3.66   |
| F2          | 199.60 ± 2.30      | 2.649 ± 0.022         | 3.3 ± 0.15    | 0.985         | 104.51 ± 2.76   |
| F3          | 201.05 ± 1.93      | 2.639 ± 0.021         | 3.6 ± 0.23    | 0.995         | 99.32 ± 0.99    |
| F4          | 200.75 ± 2.74      | 2.598 ± 0.019         | 4.9 ± 0.33    | 0.523         | 98.35 ± 2.78    |
| F5          | 203.05 ± 1.42      | 2.661 ± 0.053         | 4.8 ± 0.32    | 0.625         | 97.63 ± 4.77    |
| F6          | 200.65 ± 2.09      | 2.586 ± 0.015         | 4.6 ± 0.16    | 0.595         | 99.05 ± 3.61    |
| F7          | 202.45 ± 3.08      | 2.721 ± 0.070         | 3.5 ± 0.73    | 0.808         | 101.51 ± 1.45   |
| F8          | 203.55 ± 2.24      | 2.503 ± 0.042         | 4.1 ± 0.29    | 0.272         | 100.65 ± 0.92   |
| F9          | 200.02 ± 1.09      | 2.520 ± 0.052         | 4.2 ± 0.5     | 0.249         | 105.08 ± 1.88   |
| F10         | 202.08 ± 1.82      | 2.517 ± 0.044         | 5.8 ± 0.51    | 0.274         | 103.33 ± 3.51   |
| F11         | 198.50 ± 2.91      | 2.486 ± 0.012         | 65.5 ± 4.1    | 0.149         | 104.66 ± 4.41   |
| F12         | 204.50 ± 1.88      | 2.524 ± 0.045         | 4.4 ± 0.43    | 0.334         | 99.09 ± 0.75    |
| F13         | 204.05 ± 0.93      | 2.509 ± 0.019         | 5.3 ± 1.18    | 0.400         | 99.34 ± 1.65    |
| F14         | 203.05 ± 2.13      | 2.465 ± 0.022         | 5.7 ± 0.20    | 0.290         | 97.54 ± 1.42    |
| F15         | 203.80 ± 1.11      | 2.471 ± 0.047         | 5.7 ± 0.16    | 0.315         | 104.76 ± 0.99   |
| F16         | 200.15 ± 1.01      | 2.519 ± 0.048         | 63.3 ± 0.18   | 0.299         | 101.99 ± 1.23   |
| F17         | 199.65 ± 1.22      | 2.527 ± 0.055         | 4.5 ± 1.01    | 0.655         | 99.54 ± 1.55    |

Weight variation, tablets thickness and friability test n = 20, Hardness test n = 10 and drug content, n = 3 (claimed FLZ content = 50 mg).

### Table 4

Drug release properties from tablet formulations containing single polymer and polymer blends with chitosan.

| Formulation | Initial release (%) | DE (%) | T50 (min) | T80 (min) |
|-------------|---------------------|--------|-----------|-----------|
| F1          | 90.97               | 94.72  | 1.09      | 2.60      |
| F2          | 5.23                | 40.70  | 262.09    | 571.08    |
| F3          | 4.16                | 38.32  | 309.80    | 1048.49   |
| F4          | 13.18               | 82.17  | 45.25     | 105.21    |
| F5          | 6.41                | 60.77  | 144.08    | 526.31    |
| F6          | 56.65               | 94.33  | 3.18      | 28.71     |
| F7          | ND                  | 13.42  | >653.12   | >1440     |
| F8          | 15.76               | 87.48  | 25.43     | 90.60     |
| F9          | 10.45               | 80.37  | 45.71     | 150.56    |
| F10         | 10.99               | 76.52  | 56.92     | 188.50    |
| F11         | 12.76               | 71.04  | 78.03     | 258.98    |
| F12         | 55.72               | 98.96  | 4.47      | 7.97      |
| F13         | 19.67               | 92.23  | 23.52     | 61.50     |
| F14         | 15.48               | 84.38  | 42.15     | 134.31    |
| F15         | 6.67                | 44.22  | 380.69    | 670.20    |
| F16         | 9.74                | 79.72  | 75.95     | 161.67    |
| F17         | ND                  | 25.17  | 437.24    | 681.07    |

| a Initial release at 5 min. |
| b DE in 480 min (8 h). |
| c T50 = time for 50% release. |
| d T80 = time for 80% release. |
| e ≥1440 min = 24 h. |
| f ND = not detectable. |

observed with guar gum where high ratio of the polymer decreased the tablet hardness (Table 3).

A tablet property related to hardness is friability, which evaluates the ability of the tablet to withstand shock during manufacturing, packaging, and handling. All the tested formulations had friability values within the acceptable ±1% loss stated by USP (USP, 2016), as shown in Table 3. The formulations that showed lower hardness F2, F3 and F7 gave the highest% loss in the friability study but, were still within the limit.

Mixing of ingredients is a critical process in the production of tablets. Properly mixed powder blend should give uniform drug content. For studying the uniformity of drug content, randomly selected tablets from each formulation were tested. All tablets showed less than 6% deviation from the 50 mg claimed amount of FLZ in each tablet (Table 3).
presence of PVP are present in Table 4, while, Fig. 2 shows FLZ release profiles. Guar gum alone (F7) showed very slow drug release with DE 13.42% and $T_{90} > 1440$ min compared with 94.33% and 28.71 min respectively, for chitosan alone (F6). Adding chitosan to guar gum in 2:1 ratio (F1) had resulted in rapid and complete release of FLZ from the tablet in less than 5 min, due to observed tablet disintegration. While, chitosan and guar gum at 1:1 ratio (F2) showed a faster release compared with that of 1:2 ratio (F3) of higher guar gum content with $T_{90}$ 571.07 and 1048.49 min (9.5 and 17.4 h) and DE 40.70 and 38.32% respectively (Table 4). Thus in presence of chitosan, increase of guar gum ratio showed a more acceptable prolonged release profile of FLZ. This is probably due to gelling effect of guar gum on the tablet surface (4), which predominantly controlled the release of FLZ from the tablet matrix.

Addition of PVP to chitosan-guar gum blends at ratios of 1:1:1 (F4) and 1:2:1 (F5) for chitosan:guar gum:PVP, respectively, changed FLZ release profile. In presence of PVP, the release of FLZ was significantly accelerated compared to F2 and F3 as shown in Fig. 2 where PVP is a binder of moderate toughness property (Joneja et al., 1999). Formulation F5 of higher ratio of guar gum favors a slower release of FLZ compared with F4 where high ratio of guar gum is responsible for delayed drug release (Yousaf et al., 2017). Although, formulations F2, F3 and F5 exhibited prolonged release of FLZ from the tablet matrices; F5 was chosen to undergo further investigations, based on its more desirable powder and tablet physical properties compared with F2 and F3 as shown in Tables 2 and 3.

Release of FLZ from tablet formulations using chitosan and HPMC blends in different ratios with and without PVP are shown.
in Table 4. Compressing chitosan with HPMC in ratios 2:1 (F8), 1:1 (F9) and 1:2 (F10), respectively, slowed down FLZ release compared with both chitosan alone (F6) and HPMC alone (F12) as shown in Fig. 3. The higher chitosan ratio was accompanied with slower drug release. Adding PVP to chitosan-HPMC tablets slowed down FLZ release due to PVP binding effect. However, the release profile was not satisfactory to design prolonged release bioadhesive tablets, where, T80 did not exceed 259 min (4.3 h) for the slowest release formulation (F11).

Although, tablet formulation containing NaCMC alone (F17) showed slow initial FLZ release, adding chitosan to NaCMC at 2:1 (13) and 1:1 ratio (F14), respectively, highly accelerated the drug release (Fig. 4). Formulations containing chitosan and NaCMC in presence of PVP at 1:1:1 ratio (F16) were compared with F14. A slower release was observed in presence of PVP where T80 increased from 134.31 to 161.67 min and DE% significantly decreased from 84.38 to 79.72%. The presence of PVP could inhibit the polymers swelling owing to its binding property (Joneja et al., 1999). However, release profiles were still not satisfactory for prolonged release bioadhesive tablets, where, the n value for F15 was 0.857 which indicate anomalous non-Fickian diffusion rather than zero order (Yang and Fassihi, 1996). This deviation from zero order model could be due to fast swelling and observed erosion of tablet matrix during the course of drug release. While, the n value for F5 was 0.231 which is deviated from the value of 0.5. It could be considered as two phase release profiles, where, the first phase is applied during matrix swelling followed by steady release in a second phase. Fast release was observed for about 2 h and the data were found to obey Higuchi model with r2 equal to 0.967 and n value equal to 0.136 (not shown in table) which indicates quasi-Fickian diffusion during tablet swelling (Apu et al., 2009). The second phase of release (from 2 to 8 h) was found to obey zero order kinetic (steady state release) with r2 equal to 0.998 and n value equal to 0.978 which indicates a perfect super case II zero-order diffusion (Apu et al., 2009).

3.5. Swelling study

The chosen formulations have been subjected to hydration studies as this phenomenon is directly connected to its bioadhesive ability. Bioadhesive polymers should hydrate in presence of water and produce gel layer. If the hydration level is too high, the bioadhesion property is expected to be reduced due to the competition between water molecules and the active groups in the mucin chains of the biological membrane to bind to the polymer active groups. On the other hand, the hydration ability of formulation is important because it affects tablet size, drug retention and drug release kinetics (Tur and Ch'ng, 1998; Perioli et al., 2009). The vaginal pH of women in reproductive age is acidic with pH 4–5 (Vermani and Garg, 2000), therefore, swelling studies were carried out in McIlvaine’s citrate buffer of pH 4.8 and kept at 37 ± 0.5 °C.

3.4. Release kinetics

The release of FLZ from the selected tablet formulations were subjected for apparent release kinetic model. The release data were examined for zero order, first order and Higuchi diffusion kinetic models. The model with higher r2 values was judged to be the most appropriate model of drug release. Table 5 shows the values of r2 as well as the slope (K) for the tested models. It was clear that the apparent release kinetics is following Higuchi diffusion model for formulations F5 while F15 showed apparently release profile that obeyed zero order kinetic model. In order to further verify the release model, the simple power law expression of Korsmeyer-Peppas mathematical model was utilized. Table 5, also, shows the obtained n values for the tested formulations. The n value for F15 was 0.857 which indicate anomalous non-Fickian diffusion rather than zero order (Yang and Fassihi, 1996). This deviation from zero order model could be due to fast swelling and observed erosion of tablet matrix during the course of drug release. While, the n value for F5 was 0.231 which is deviated from the value of 0.5. It could be considered as two phase release profiles, where, the first phase is applied during matrix swelling followed by steady release in a second phase. Fast release was observed for about 2 h and the data were found to obey Higuchi model with r2 equal to 0.967 and n value equal to 0.136 (not shown in table) which indicates quasi-Fickian diffusion during tablet swelling (Apu et al., 2009). The second phase of release (from 2 to 8 h) was found to obey zero order kinetic (steady state release) with r2 equal to 0.998 and n value equal to 0.978 which indicates a perfect super case II zero-order diffusion (Apu et al., 2009).
Fig. 5 shows the collective swelling profile for the selected formulations (F5 and F15). It was clear that formulations containing chitosan have high swelling rates. Chitosan is a weak base due to the presence of large number of amino groups on its chain, hence, it exhibit pH-sensitive swelling. At the acidic swelling medium (pH 4.8) it undergoes protonation, which extends to the flexible conformation of the chitosan backbone (Sonia and Sharma, 2011). This allows more water penetration and swelling. Both tablet formulations showed high swelling percentage within 15 min after contact with buffer that was 174.50 ± 13.09% for F15 and 378.99% for F5. However, F15 showed continuation of fast swelling that reached 1160.53 ± 13.02% in 4 h due to presence of chitosan with NaCMC compared with only 458.83 ± 7.09% for F5 in presence of chitosan and guar gum. This might be due to the formation of a protective gel layer of the guar gum prior to entry of water into the matrix hindering fast hydration of the tablet inner core (Prasad et al., 1998). Generally, the initial swelling was followed by constant swelling rate (zero order) till it reached hydration equilibrium. It was also noticed that equilibrium time for F5 is about 4 h, while, it was only 2 h for F15. Formula F15 also showed a decrease in the calculated swelling percentage after 4 h, which may be due to tablet erosion.

3.6. In-vitro determination of tablets bioadhesion time

In-vitro bioadhesive time and behavior were performed in order to evaluate tablet residence time at the application site and to observe tablet behavior in contact to mucosal surface in presence of vaginal fluid. In general, the swelling state of the polymer could impact on its bioadhesive behavior (Bottenberg et al., 1991). Formulation F5 containing guar gum showed high adhesion times exceeding 24 h with slow erosion of the compact viscous gel layer surrounding the tablet which came in agreement with the swelling results. On the other hand, tablets of F15 containing chitosan with NaCMC showed a short adhesion time for about 2 h. This also came in agreement with the observed swelling behavior which showed the highest hydration capacity. Thus, the hydrated polymer produced on the tablet surface is exposed to erosion, which led to continuous fragment falling.

3.7. Determination of bioadhesion strength

The bioadhesive polymers are activated by the presence of moisture. Moisture plasticizes the system, allowing the bioadhesive molecules to break free and to link up with biological membrane (Hogerstrom et al., 2003). Several theories of bioadhesion have suggested that it might occur via physical entanglement (diffusion theory) and/or chemical interactions, such as electrostatic, hydrophobic, hydrogen bonding, and van der Waals interactions (Salamat-Miller et al., 2005). Both tablet formulations showed good mucosalhesive forces to mouse peritoneum membrane with values of 2.560 ± 0.636 and 2.530 ± 0.036 N for the mean break load for F5 and F15, respectively, with no significant difference in the adhesion strength between both formulations (p > .05). It could be suggested that the cationic chitosan creates the strong electrostatic bonding with negative charge of epithelium at the surface of the biological membrane (Roy et al., 2009).

3.8. In-vitro antifungal activity study

The antifungal activity of formulated tablets was tested using tablet diffusion method. As tablet formulations need to hydrate...
to become adhesive and able to release drug, 200 µl of McIlvaine’s citrate buffer was added after their introduction into the agar wells before incubation. Fig. 6 shows the inhibition zone after 24 h incubation produced by 50 mg pure FLZ disc compared with the two selected formulations as well as their negative control (drug free tablets).

All the formulations were gradually swelled and the released drug was able to diffuse in agar inhibiting *C. albicans* growth. Formulation F5 produced the largest microbial inhibition zones of 21.33 ± 1.15 mm, indicating that FLZ was able to diffuse through the polymeric network of swelled tablet and gradually released out of tablets to retard the growth of candida. However, drug free control tablets of formula F5 (denoted as F5c) showed inhibition zone of 13.66 ± 1.15 mm after 24 h incubation, due to the presence of free chitosan in the formulation, where chitosan has been proven to have an antifungal activity against *C. albicans* by the formation of electrostatic forces between the protonated NH+3 groups and the negative charge on microbial cell membrane (Seyfarth et al., 2008). Although control tablet of formulation F15 (F15c) contained chitosan, it did not show any sign of antifungal activity. This is probably due to the interaction between the cationic group of chitosan and anionic group of NaCMC in the formula at pH 4.8, which inhibit the chitosan antimicrobial activity. Meanwhile, it was observed that formulation F15 swelled rapidly and extensively in the agar plate. This behavior was responsible for cracking and dehydration of surrounding agar area as shown in Fig. 6. These results came in agreement with observed high swelling profile for F15 compared with F5. The presence of chitosan with NaCMC that have high swelling ability could be the reason for dehydration effect on the surrounding agar. It was concluded that these tablets are not suitable to be exposed to vaginal environment as they could harm the vaginal mucosa. On the other hand, guar gum in formulation F5 formed a gel layer that favorably controlled the swelling. Thus, formulation F5 is the most desirable with high antifungal activity.

### 4. Conclusions

The precise addition of chitosan, as a key polymer, in physical blends with other bioadhesive polymers including HPMC, guar gum, NaCMC as well as PVP was utilized in the design of prolonged...
release FLZ vaginal tablets. Chitosan significantly controlled the tablets’ physical characteristics as well as drug release profiles in acidic vaginal secretion (pH4.8). Out of 17 tested formulations at different chitosan:polymer ratios, two formulations were selected. The first has chitosan:guar gum:PVPP at 1:2:1 ratio (F5) while, the second was formulated with chitosan and NaCMC at 1:2 ratio (F15). Both formulation showed fair powder flowability and compressed tablets of acceptable hardness and friability. Drug release at pH 4.8 had essentially prolonged profiles for both formulations, with good bioadhesion characteristics. However, the first formulation could be the more desirable with longer bioadhesion time, high antifungal activity and no expected harm to vaginal mucosa.

Declaration of interest

Authors do not have personal and/or financial conflict of interest.

Acknowledgement

This research project was supported by a grant from the “Research Center of the Female Scientific and Medical Colleges”, Deanship of Scientific Research, King Saud University.

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