

Development and Characterisation of Natamycin Mini-Matrices Prepared by Hot-Melt Extrusion for Vaginal Delivery

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Abstract

In this study, bioadhesive mini-matrices of natamycin were prepared for vaginal application by hot-melt extrusion. In addition, melt viscosity measurements, thermogravimetric analysis, *in vitro* drug release studies and *in vitro* mucoadhesion test were performed. High molecular weight grades of Klucel™ hydroxypropylcellulose were used as a thermoplastic polymer. TEC and PEG 400 were chosen as plasticizer. According to the obtained results of melt viscosity measurements, the maximum torque of extrudates prepared using PEG 400 increased with increasing drug loading. The thermogravimetric analyses showed that natamycin is stable up to 198°C and this result gives the opportunity to hot melt extrusion process at 90°C. *In vitro* drug release results showed that the release was extended up to 72 hours and drug release rate increased with increasing drug loading. In respect to the *in vitro* mucoadhesion test results, the values of work of mucoadhesion were found high as 771,977 mN·mm, 753,199 mN·mm, 686,356 mN·mm for the prepared hot melt extruded mini-matrices. Our results showed that the developed formulations were found worthy of further studies.

Keywords

Hot Melt Extrusion, Natamycin, Controlled Release, Bioadhesive, Vaginal Delivery

1. Introduction

Vaginal candidiasis (VC) often referred to as vulvovaginal candidiasis, is a common mucosal infection of vagina, mainly caused by *Candida* species. It is

also known as second most prevalent mucosal infection later than bacterial vaginosis [1]. Yeast colonization occurs relatively frequently, with up to 30% of healthy asymptomatic women having a positive culture for yeast at any single point of time and up to 70% if followed longitudinally over a 1-year period [2]. Frequent recurrences of symptomatic vulvovaginitis result in considerable suffering and cost and have a markedly negative effect on sexual relations [3] [4].

Natamycin (NT), the first antifungal compound was approved by the US Food and Drug Administration for topical and ocular use [5]. Natamycin is a polyene antifungal produced by *Streptomyces natalensis* and related species. It has low toxicity and prevents the growth of mould and yeast [6]. NT has been applied topically as a suspension or ointment for skin, ocular or oral candidiasis, as an ointment for dermatomycosis. NT is poorly water-soluble drug and it causes bioavailability problems.

Controlled release of a poorly water soluble drug is one of the most challenging issues for the pharmaceutical scientist. In recent years, hot-melt extrusion technology has gained popularity due to pharmaceutical applications of poorly water-soluble drugs. The drug release is primarily controlled by the type and concentration of thermoplastic polymer, other excipients (e.g., plasticizers) and the processing conditions (e.g., temperature) [7]. In melt extrusion processes, the drug/carrier powder blend is exposed to elevated temperatures for approximately two to three minutes. Due to the simplicity of the manufacturing process, the properties of solid dispersions are not expected to change during scale-up, and when solid solutions are formed, the influence of the physical properties of the drug including the particle size distribution on drug release is eliminated [8]. With hot melt extrusion technique, the solubility can be increased also excipients and processing steps can be decreased.

The vagina is often an ideal route for drug administration because it allows for the administration of lower doses, steady drug levels, and less frequent administration than the oral route. With vaginal drug administration, absorption is unaffected by gastrointestinal disturbances, there is no first-pass effect, and use is discreet [9]. Also retention time in the vagina can be optimized by using bioadhesive polymers.

Bioadhesion has received considerable attention in the medical literature due to the potential applications of drug delivery and wound care systems. The inclusion of mucoadhesive polymers into formulations has increased the residence time of the desired drug in the area, thereby boosting the efficacy of the treatment [10]. Apart from prolongation of drug release at the site of absorption, drug targeting to the affected site can also be realized [11] [12]. Fundamental thermodynamic quantities, such as surface free energies of both adhesive and substrate, determine the magnitude of the adhesive forces. However, this assembly and thus, effective bioadhesion is also a function of good wetting [13].

Hence an objective of the present study was to develop and produce vaginal mini matrices containing natamycin utilizing hot-melt extrusion technique to optimize drug delivery. Furthermore, the study objectives included characteriz-

ing and evaluating this antifungal treatment modality using analytical, bioadhesion, dissolution, and thermal studies.

2. Materials

Natamycin (Shandong Freda Biotechnology Co., Ltd., Shandong, China), hydroxypropylcellulose (Klucel HF, Ashland Inc., Kentucky, USA), triethyl citrate (TEC, Aldrich Chemical Co., Inc., Milwaukee, Wis.) and polyethylenglycol 400 (PEG400, Merck, Darmstadt, Germany).

3. Methods

3.1. Preparation of Hot Melt Extruded Matrices

Based on previous studies, high molecular weight grades of Klucel™ hydroxypropylcellulose (HPC) were used as a thermoplastic polymer to develop the bioadhesive hot melt extruded formulations for vaginal delivery [13] [14]. TEC, PEG400 were chosen as plasticizer. Firstly, HPC and PEG400/TEC were mixed using a pestle and a mortar and then natamycin were added to this mixture (Table 1). The extrusion temperature is preferred as 90°C with a screw speed of 25 rpm. This raw material was fed into the hopper and transferred inside the heated barrel by rotating screws. The raw material was extruded with a co-rotating twin screw hot-melt extruder (Minilab HAAKE Rheomex CTW5, Thermo Fisher Scientific, Karlsruhe, Germany). The die diameter was preferred as 1.75 mm. The torque values were determined.

3.2. Melt Viscosity Measurements

Melt viscosity values were measured by hot melt extruder automatically during the manufacturing process. The torque values were revealed depends on extrusion temperature and screw rotation speed. The following table consists of these values.

3.3. Thermogravimetric Analysis of NT

The thermal stabilities of natamycin was determined by the thermogravimetric analysis method (TGA) (Perkin Elmer TGA-4000). Sample weighed approximately 3 mg were placed in a porcelain bowl and heated at a rate of 10°C/min between 30°C - 400°C. The change in weight was determined.

Table 1. The compositions of formulations.

The codes of formulations	Natamycin (%)	HPC (%)	PEG400 (%)	TEC (%)	Processing Temperature (°C)
F1	20	72	-	8	90
F2	20	72	8	-	90
F3	40	54	6	-	90
F4	60	36	4	-	90

3.4. *In Vitro* Drug Release

Extrudates of 5 mm length were used in drug release studies. The drug release was determined using 250 mL of pH 4.5 citrate buffer containing 0.1% SDS; 70 rpm; 37°C in a horizontal shaker incubator (Thermo Fisher Scientific Max Q™ 6000) according to the sink conditions. At predetermined time intervals, samples were collected and assayed by using UPLC (Accela, Thermo Fisher Scientific, Karlsruhe, Germany) at 304 nm. The release medium was replaced with fresh medium every 24 hours.

3.5. *In Vitro* Mucoadhesion Test

The mucoadhesive strength of the formulations was evaluated by measuring the force required to detach the formulation from a 8% mucin solution using a 500 g load cell TPA in tension mode. The extrudates were attached to the lower end of the probe (P 10 Perspex, θ 10 mm). The probe holding the extrudate was lowered on to the surface of the mucin with a constant speed of 0.1 mm·s⁻¹ and a contact force of 0.005 N were applied. After keeping in contact surfaces for 180 s, the probe was then moved vertically upward at a constant speed of 0.1 mm·s⁻¹. Maximum detachment force (F) was obtained from the force-distance graph. The area under the curve (AUC) was calculated from force-distance plot as the mucoadhesion (M). The tests were conducted at 37°C and each experiment was carried out three times [15].

4. Results and Discussion

4.1. Preparation of Extrudates

The formulations and the processing conditions for the preparation of the vaginal bioadhesive mini matrices containing natamycin are listed in **Table 1**. HPC was chosen as the matrix-forming polymer since it is the only water-soluble cellulose derivative that is thermoplastic. HPC has a softening temperature in the range of 100°C - 150°C, depending on its molecular weight [16]. PEG400/TEC were used as plasticiser to facilitate the hot-melt extrusion process. Also, plasticisers provide die lubrication, reduce melt viscosity and improve melt uniformity [17]. Therefore, the extrudates could be produced at 90°C successfully.

In this study, four formulations have been tried but a colour change has been detected at F4 and it was concluded that drug loadings up to 40% could be extruded. So the characterization studies have been revealed with F1, F2, F3.

4.2. Melt Viscosity Measurements

Pharmaceutical polymers utilized in hot-melt extrusion typically require a plasticizer in order to reduce the T_g of the polymer and to facilitate the HME process [8]. According to the obtained results of melt viscosity measurements, the maximum torque values of F1 and F2 were close to each other and the mini matrices containing NT and HPC could be produced with low torque values us-

ing both of the plasticisers. However the maximum torque of extrudates prepared using PEG400 increased with increasing drug loading (increased resistance to the flow of dispersed drug particles) [7] (Table 2).

4.3. Thermogravimetric Analysis of NT

The results obtained from the TGA studies showed that natamycin rapidly degraded from 198 °C and weight loss increased (Figure 1).

4.4. In Vitro Drug Release

Comparing the release profiles of F1 and F2, the release profile of the extrudate containing PEG 400 is closer to the zero order release (Figure 2). F2 has 2 hours

Table 2. Maximum torque values obtained for different formulations and temperatures at screw speed of 25 rpm during HME operation.

The codes of formulations	Maximum tork (Nm)
F1	0.38
F2	0.33
F3	0.56
F4	0.75

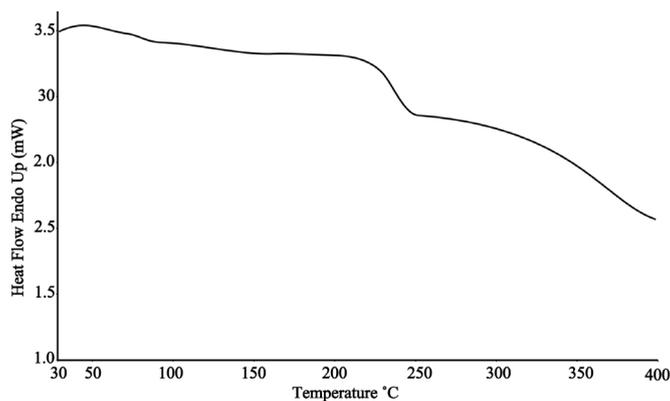


Figure 1. TGA curve of natamycin.

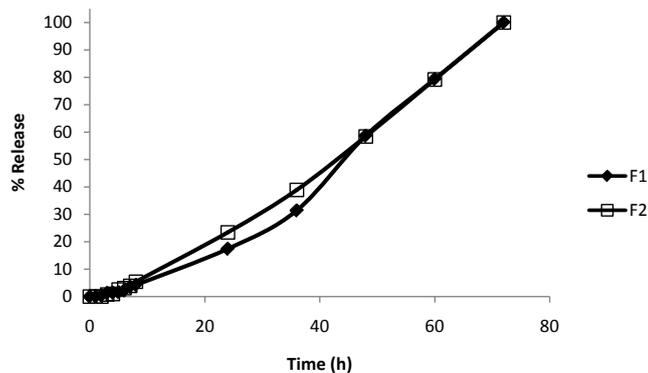


Figure 2. Effect of plasticiser type on the in vitro drug release profiles of HPC extrudates.

lag time, and it released approximately 5% and 23% of drug at the 8. and 24. hours respectively, whereas F1 reached 4% and 17% at the same time zones. Formulation F1 has just 1 hour lag time and its release is faster than formulation F2 for the first 4 hours. However, it was seen that the release rate of F2 is higher than formulation F1 up to 48 hour and than the release rates of two formulations were almost same.

Comparing the release profiles of F2 and F3, it was seen that drug release rate increased with increasing drug loading (Figure 3). The lag time is shortened on the extrudates containing 40% NT. First 8 hours, F3 has faster release than F2. At the 24 hour, F2 has slightly more release rate than F3 and later two of formulations showed same release rates. According to the *in vitro* drug release results, the release was extended up to 72 hours.

4.5. *In Vitro* Mucoadhesion Test

The mucoadhesion results work of mucoadhesion and peak detachment force were calculated from the resulting force-distance plot obtained from the mucoadhesion test and given in Figure 4.

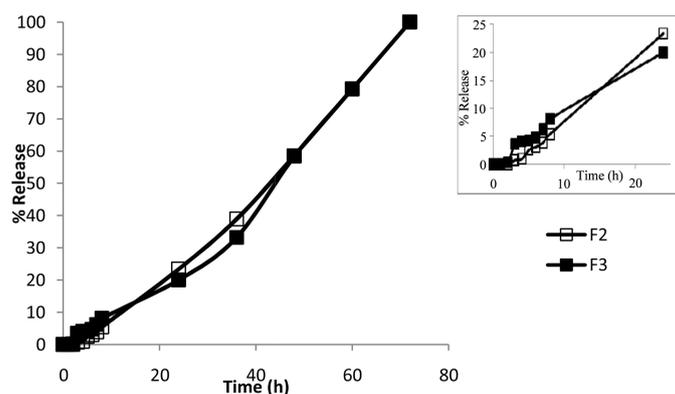


Figure 3. Effect of drug loading on the *in vitro* drug release profiles of HPC/PEG400 extrudates.

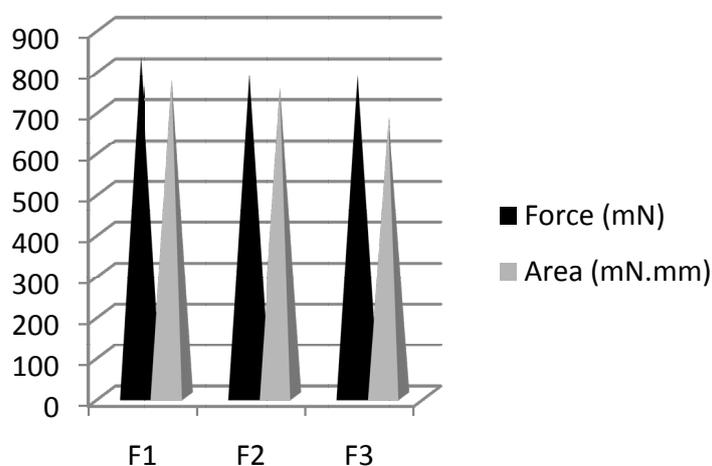


Figure 4. Results of mucoadhesion studies of the formulations with 8% mucin.

The values of work of mucoadhesions were found as 771,977 mN·mm, 753,199 mN·mm, 686,356 mN·mm and peak detachment force values were found as 830,574 mN, 786,693 mN, 783,931 mN for the F1, F2, F3 respectively. These results showed that, the convenient mucoadhesion for the vaginal mucosa could be obtained by using hot melt extruded mini matrices produced with the matrix-forming bioadhesive polymer HPC. Additionally, it was observed that with increasing of natamycin ratio in the formulations, mucoadhesion was decreased.

5. Conclusion

This preliminary study demonstrated that NT loaded mini matrices for vaginal delivery can be produced by HME successfully and this could be a promising alternative formulation for vaginal candidiasis. These are important initial results that need to be developed. Further studies are required to determine whether this novel approach to treatment should be recommended.

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