International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2019.436.440

Research Article
Fluconazole Nano-particles Loaded Gel for Improved Efficacy in Treatment of Oral Candidiasis

Hibah Mubarak Aldawsari

Department of Pharmaceutics, Faculty of Pharmacy, King Abdulaziz University, P.O. Box 80260, 21589, Jeddah, Saudi Arabia

Abstract

Background and Objective: Nano-particles gained a new attention in the last decades for control of fungal infections, resistance and mutations. This work aimed to formulate fluconazole (FLU) in form of nano-particles (NPs) using ethylcellulose and eudragit RS 100 polymers in different ratios to reach to smallest particle size. **Materials and Methods:** Six formulae were prepared using solvent evaporation technique. The prepared formulae were characterized for size analysis, the ability to encapsulate drug, NPs photographs, drug release, from the smallest particulate size and antifungal activity against *Candida albicans* in comparison with raw FLU. **Results:** Revealed that FLU-eudragit RS 100 NPs had smaller sizes than ethylcellulose NPs. Formula of FLU:eudragit RS 100 ratio (1:1.5, F6) achieved size 212±21 nm with highest encapsulation of 69.3% ±2.1. Transmission electron microscope image confirmed the spherical nature of the prepared NPs. Drug release study from NPs showed prolonged FLU release (90% after 8 h) in comparing with raw FLU release of 90% after 1 h. Data of anti-fungal activity revealed that improvement of anti-fungal activity by about 1.45 fold of formula 6 in comparing with raw FLU. **Conclusion:** Finally, FLU-eudragit RS 100 NPs achieved smaller NPs sizes in comparing with FLU-ethylcellulose NPs. In addition to FLU release was extended which decreasing dosing frequency moreover, anti-fungal activity was improved significantly, that could enhance patient's convenience and acceptance.

Key words: *Candida albicans*, fungal infection, fluconazole (FLU), nano-particles, polymers, drug release, ethylcellulose

Received: October 10, 2018 Accepted: November 30, 2018 Published: March 15, 2019

Citation: Hibah Mubarak Aldawsari, 2019. Fluconazole nano-particles loaded gel for improved efficacy in treatment of oral candidiasis. Int. J. Pharmacol., 15: 436-440.

Corresponding Author: Hibah Mubarak Aldawsari, Department of Pharmaceutics, Faculty of Pharmacy, King Abdulaziz University, P.O. Box 80260, 21589, Jeddah, Saudi Arabia Tel: +966-0126400000-23010

Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The FLU, used for the treatment of oral, esophageal and vaginal candidiasis has been selected as a model drug in this investigation[1]. Nano-particles (Nps) formulations have received the attention of drug delivery laboratories for their ability to overcome barriers that hinder delivered drug's bioavailability and release drug more uniformly[2]. Moreover, Nps represent a multiarticulate (unit) system that spreads over a large mucosalarea that offers improved absorption and reduce the concentration of drug exposed to mucosa in specific regions of the gastrointestinal tract, offering advantage over the conventional single unitchronic dosing dosage form. Among the techniques used for NPs preparation is the solvent evaporation methods. In these methods, the drug is either solubilized, emulsified or dispersed into an organic polymer solution[3]. The micro/nano-structures are formed after solvent diffusion/evaporation and the drug is precipitated within the polymer. Solvent evaporation showed a versatile, easy and practical method. Moreover, this technique makes possible the entrapment of a wide range of drugs having different physical properties and solubility characteristics. On the other hand, final product characteristics prepared by solvent evaporation technique depends mainly on the formulation and the process variables. To formulate NPs with reproducible sizes, drugs distribution (surface/core) and drug release profiles[4], several factors as emulsifier concentration, stirring rate and organic phase volume should be controlled. The NPs containing water soluble drugs have been prepared using various polymers such as poly (lactide-co-glycolide) (PLGA), eudragit and polymethyl methacrylate. In addition, modified procedures have been proposed for the solvent evaporation methods to achieve target properties[5] of NPs. The modifications include the use of enteric wall material in oil phase, addition of non-solvent into external aqueous phase, incorporation of solid lyophilized powder during primary emulsification and the addition of electrolytes into external aqueous phase[6]. Also, the research efforts have been made to incorporate slightly water-soluble drugs into polymeric micro/nano-spheres by the conventional w/o/w emulsion solvent evaporation technique. Recently, it has been stated that the poly (ε-caprolactone) microparticles resulted in poor encapsulation efficiency (2.89 ± 0.20 μg mg^{-1}) for melarsoprol. An encapsulation efficiency of only 5-17% has also been reported for zidovudine into PLGA nano-particles[7]. Ethyl cellulose and eudragit RS100 are biocompatible polymers that extensively studied encapsulating material for the controlled release of pharmaceuticals. However, both polymers have been little investigated by modified solvent evaporation technology[8]. Thus, the objective of this work was to improve FLU efficacy via loading on NPs buccal gel to extend FLU release which enhance the anti-fungal activity. This will enhance patient's convenience.

MATERIALS AND METHODS

Formulation of FLU-nano-particles: Six formulae were prepared using modified solvent evaporation technique to prepare FLU-NPs. Ethanol with each polymer were mixed together to form the organic phase, then FLU (50 mg) was added to 20 mL of the organic solvent (Table 1). Aqueous phase was composed of 30 mL of 0.5, 1 and 1.5% w/v Tween 80 solutions, the organic phase was dropped slowly for a long 30 min in to aqueous phase under stirring. The organic phase was allowed to evaporate for 24 h under stirring. Once the organic solvent got evaporated, the nano-particles were collected by centrifuging at 50000 rpm for 40 min and then washed with deionized water and freeze-dried[9]. All experiments were carried out at pharmaceutical technology lab, Faculty of Pharmacy, King Abdulaziz University, from February-May, 2018.

Particle size measurement: All formulated nano-particles were subjected to size measurement using Zetatrac (Nano-trac Wave, Microtrac, USA), each sample was diluted to 10% of its original concentration by deionized water then measured directly.

Entrapment efficiency (EE %): Indirect method was used to determine free FLU which did not entrapped inside the nano-particles. After centrifugation of nano-particles for collection, 1 mL of the supernatant was injected directly to the HPLC at 260 nm using Abdel-Moerty *et al.*[10] method. The following equation was used to estimate:

$$EE\ (\%) = \frac{TFLU - FFLU}{TFLU} \times 100 \quad (1)$$

where, FFLU is the free FLU and TFLU is total amount of FLU in the formula.

Table 1: Formulae components

Formula	Ethylcellulose (mg)	Eudragit RS 100 (mg)	Tween 80 (%)
F1	450		0.5
F2	450		1.0
F3	450		1.5
F4		450	0.5
F5		450	1.0
F6		450	1.5

Nano-particles morphology by Transmission Electron Microscope (TEM): Surface morphology of the smallest particle sized was imaged by TEM.

Hydrogel formation: Forming polymer hydroxypropyl methylcellulose was dispersed in water by agitation at with the aid of magnetic stirrer (1% w/v). Specified amounts of nano-particles (F6) was incorporated into the dispersion. The dispersion was allowed to stand for 15 min in water bath sonicator to expel entrained air.

Diffusion study: A Franz diffusion cell apparatus (Microette Plus; Hanson Research, Chatsworth, CA, USA) was used to assess the diffusion of prepared hydrogels (100 µL) of each gel was added to the donor part and allowed to diffuse throw the silica membrane to pass to receptor chamber. The receptor medium was distilled water kept at 37±0.5°C and 400 rpm stirring rate. The aliquots collected by autosampler at 0.5, 1, 2, 4, 8 and 12 h.

Anti-fungal activity: Agar diffusion method was used to evaluate the efficacy of anti-fungal activity of formula 6 against raw FLU. Agar dilution method was used with *C. albicans* (ATCC 10231) to determine the minimum inhibitory zone. About 10 mm gaps were made in Petri dishes (200 mm) containing 30 mL Muller-Hinton agar, 1 mL *C. albicans* of number (1×106 CFU mL^{-1}). About 100 µL of the raw FLU and FLU-Eudragit RS 100 NPs solutions with concentration 0.5 mg mL^{-1} FLU of each sample. Dishes were then incubated for 24 h at 37°C, inhibitory zone was characterized as the non-attendance of bacterial development in the territory encompassing the gaps. All tests were triplicates and expressed with mean±SD.

RESULTS

Data of Table 2 revealed NPs size and EE (%). In general NPs of eudragit RS 100 achieved lower particle size which allocated in range (212±21-432±11) while ethylcellulose range size was from 343±23-652±32. In concern to EE (%), it was observed that increase FLU entrapped in NPs with increasing Eudragit RS 100 ratio, generally Eudragit RS 100 NPs gave higher EE (%) in comparing with ethylcellulose Nps.

For the reasons mentioned before, formula No. 6 was chosen for imaging as this formula is the smallest particle size with highest EE (%) by TEM, (Fig. 1), spherical nano-particles were produced with homogenous sizes which control drug release.

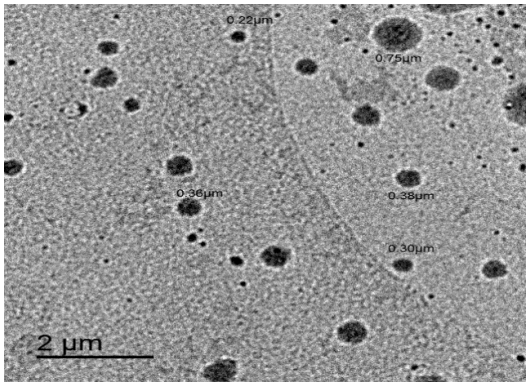

Fig. 1: TEM image of formula No. 6

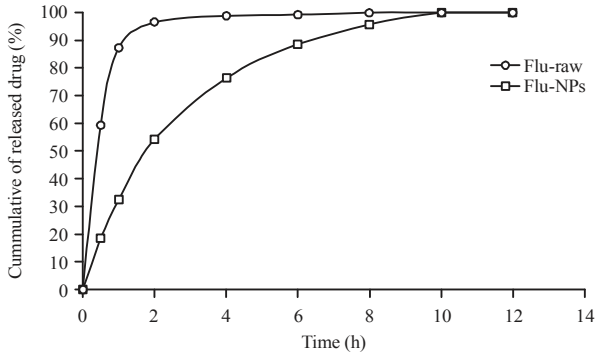

Fig. 2: Comparative Flu release of FLU-raw and FLU-NPs (formula No. 6)

Table 2: Size and EE % of FLU-NPs

Formula No.	Size (nm)	EE (%)
F1	652±32	50.4±3.1
F2	432±12	54.2±1.1
F3	343±23	48.2±2.1
F4	432±11	59.2±0.2
F5	321±37	61.4±1.1
F6	212±21	69.3±2.1

Table 3: Average of inhibition zone of FLU-raw and FLU-NPs ±SD

	FLU-raw	FLU-NPs
Inhibition zone (mm)	122.2±3.4	176.9±6.1

The results in Fig. 2 showed significant delay in FLU release in case of FLU-NPs from the hydrogel, about 100% of FLU was diffused after 2 h in FLU-raw, while in case of FLU-NPs it took about 10 h to reach to 100% FLU release.

Anti-fungal activity was carried out to evaluate the enhancement of FLU activity after incorporation with eudragit RS 100, data revealed that FLU-NPs had wider inhibition zone (176.9±6.1 mm) against *C. albicans* than raw-FLU (122.2±3.4 mm) as represented in Table 3, data was represented in millimeter of the inhibition zone.

DISCUSSION

Data of this work achieved extended FLU release with efficient anti-fungal activity against *C. albicans* in comparing with FLU-raw that could decrease dosing frequency. Eradicates the *C. albicans*'s resistance and decreases the treatment period which reach to several weeks. Further clinical studies are required. Nano-encapsulation by the solvent diffusion and evaporation method is a simple method. However, to manufacture nano-particles with reproducible desired properties i.e., high encapsulation efficiency, suitable release profile and proper particle size and distribution, could represent a challenge especially as the method rely one several factors that affect the produced nano-particles[6]. These factors include solvent system type and composition, total volume and phase volume ratio, polymer concentration, stirring speed and time. The effect of each of these parameters has to be determined empirically. Ethylcellulose polymer has higher viscosity grade compared with eudragit[11] RS 100. These are two copolymers synthesized from acrylic and methacrylic acid esters, containing a low level of quaternary ammonium groups. The improved permeability of eudragit RS 100 compared with ethylcellulose is attributed to the increased number of quaternary ammonium substitutions in eudragit RS 100 which affects the release behavior of the investigated drug[12]. Due to decrease in solubility of FLU in organic phase, the drug loss was reduced and enhanced the encapsulation efficiency. In this study, ethylcellulose as drug carrying polymer was used. Ethyl cellulose showed a hydrophobic nature that encapsulated larger amount of the slightly soluble drug. When organic phase diffused in the aqueous solution containing surfactant, ethylcellulose matrix immediately started to precipitate because of fast diffusion of organic solvent (ethanol). Due to polymer saturated solvent polymeric matrix was immediately precipitated out as solvent starts to evaporate and gives maximum encapsulation efficiency[12-15]. Tween 80 is a better surfactant in terms of encapsulation efficiency, drug content and particle size as a result of the surfactant (Tween 80) ability to migrate towards the surface of ethylcellulose formed particles. Accordingly, the saturated polymeric solution and faster diffusion rate of solvent enhance the encapsulation efficiency of the prepared formula. The increase in polymer concentration led to increase in both particle size and encapsulation efficiency of the prepared NPs. This was attributed to the increase viscosity that helps to enlarge the size and maximize encapsulation efficiency. Viscosity also influenced percentage yield of recovered nanoparticles[16,17]. The increased polymer concentration also improved the binding capacity of polymer with FLU. Accordingly, more drug was entrapped in the polymeric core that improved both encapsulation efficiency and percentage yield of the produced NPs. Ethylcellulose also acted as a self-emulsifier that helped FLU got entrapped into solid NPs due to ethylcellulose hydrophobic nature. The obtained results concluded that particle size of the prepared NPs was directly proportional to polymer concentration as a result of increase in viscosity of the internal phase[12,18].

Formulation prepared using eudragit RS 100 showed higher encapsulation efficiency than those prepared using ethylcellulose. It was also found that release of drug was slower than formulation prepared from eudragit RS 100 compared with raw FLU. This porous nature of eudragit RS 100 was responsible for the enhancement of the holding capacity for FLU and extended the drug release[8,19,20].

CONCLUSION

Nanoparticles showed improvement of several medications efficacy. *C. albicans* is causing resistant in oral and vaginal infections. FLU-eudragit RS 100 NPs achieved smaller NPs sizes in comparing with FLU-ethylcellulose NPs. FLU-Eudragit RS 100 NPs of (1:1.5) drug to polymer ration sustained FLU release and enabled better reactivity with fungi cell components, anti-fungal activity was improved by about 1.45 folds compared with raw-FLU. The study proved that eudragit RS 100 NPs loaded with FLU is an effective way to improve FLU efficacy against *C. albicans* .

SIGNIFICANCE STATEMENT

This research found a new strategy for improvement of FLU anti-fungal activity via sustaining FLU release. Furthermore, achieve deep penetration of nano-particles through infected tissue that will eradicate the candida resistance. This research also helps researchers to uncover the potential use of FLU as nano-particulate loaded gel in clinical models to support its antifungal use in case of *C. albicance* resistance.

REFERENCES

1. Darwesh, B., H. Aldawsari and S. Badr-Eldin, 2018. Optimized chitosan/anion polyelectrolyte complex based inserts for vaginal delivery of fluconazole: *In vitro*/*in vivo* evaluation. Pharmaceutics, Vol. 10, No. 4. 10.3390/pharmaceutics10040227
2. Rex, J.H., M.A. Pfaller, J.N. Galgiani, M.S. Bartett and A. Espinel-Ingroff *et al*., 1997. Development of interpretive breakpoints for antifungal susceptibility testing: Conceptual framework and analysis of *in vitro-in vivo* correlation data for fluconazole, itraconazole and *Candida* infections. Clin. Infect. Dis., 24: 235-247.

3. Aher, N.G., V.S. Pore, N.N. Mishra, A. Kumar, P.K. Shukla, A. Sharma and M.K. Bhat, 2009. Synthesis and antifungal activity of 1, 2, 3-triazole containing fluconazole analogues. Bioorg. Med. Chem. Lett., 19: 759-763.
4. Amaral, A.C., A.L. Bocca, A.M. Ribeiro, J. Nunes and D.L. Peixoto *et al*., 2009. Amphotericin B in poly (lactic-co-glycolic acid)(PLGA) and dimercaptosuccinic acid (DMSA) nanoparticles against paracoccidioidomycosis. J. Antimicrob. Chemother., 63: 526-533.
5. Gomez-Sequeda, N., R. Torres and C. Ortiz, 2017. Synthesis, characterization and *in vitro* activity against *Candida* spp. of fluconazole encapsulated on cationic and conventional nanoparticles of poly (lactic-co-glycolic acid). Nanotechnol. Sci. Applic., 10: 95-104.
6. Mainardes, R.M. and R.C. Evangelista, 2005. PLGA nanoparticles containing praziquantel: Effect of formulation variables on size distribution. Int. J. Pharm., 290: 137-144.
7. Barichello, J.M., M. Morishita, K. Takayama and T. Nagai, 1999. Encapsulation of hydrophilic and lipophilic drugs in PLGA nanoparticles by the nanoprecipitation method. Drug Dev. Ind. Pharm., 25: 471-476.
8. Bilati, U., E. Allemann and E. Doelker, 2005. Development of a nanoprecipitation method intended for the entrapment of hydrophilic drugs into nanoparticles. Eur. J. Pharm. Sci., 24: 67-75.
9. Ahmed, O.A., U.A. Fahmy, A.S. Al-Ghamdi, B.M. Aljaeid and H. Aldawsari *et al*., 2017. Finasteride-loaded biodegradable nanoparticles: Near-infrared quantification of plasma and prostate levels. J. Bioact. Compatible Polym., (In Press). 10.1177/0883911517694397
10. Abdel-Moety, E.M., F.I. Khattab, K.M. Kelani and A.M. AbouAl-Alamein, 2003. Chromatographic determination of clotrimazole, ketoconazole and fluconazole in pharmaceutical formulations. II Farmaco, 57: 931-938.
11. Murakami, H., M. Kobayashi, H. Takeuchi and Y. Kawashima, 1999. Preparation of poly(DL-lactide-co-glycolide) nanoparticles by modified spontaneous emulsification solvent diffusion method. Int. J. Pharm., 187: 143-152.
12. Sakai, H., A. Sekita, K. Tanaka, K. Sakai, T. Kondo and M. Abe, 2011. Preparation and properties of nanosized biodegradable polymer capsules. J. Oleo Sci., 60: 569-573.
13. Quintanar-Guerrero, D., A. Ganem-Quintanar, E. Allemann, H. Fessi and E. Doelker, 1998. Influence of the stabilizer coating layer on the purification and freeze-drying of poly (D, L-lactic acid) nanoparticles prepared by an emulsion-diffusion technique. J. Microencapsulation, 15: 107-119.
14. Surolia, R., M. Pachauri and P.C. Ghosh, 2012. Preparation and characterization of monensin loaded PLGA nanoparticles: *In vitro* anti-malarial activity against *Plasmodium falciparum*. J. Biomed. Nanotechnol., 8: 172-181.
15. Panariti, A., G. Miserocchi and I. Rivolta, 2012. The effect of nanoparticle uptake on cellular behavior: Disrupting or enabling functions? Nanotechnol. Sci. Applic., 5: 87-100.
16. Mandal, B., N.K. Mittal, P. Balabathula, L.A. Thoma and G.C. Wood, 2016. Development and *in vitro* evaluation of core-shell type lipid-polymer hybrid nanoparticles for the delivery of erlotinib in non-small cell lung cancer. Eur. J. Pharm. Sci., 81: 162-171.
17. Wang, Y.C., Y.T. Wu, H.Y. Huang and C.S. Yang, 2009. Surfactant-free formulation of poly (lactic/glycolic) acid nanoparticles encapsulating functional polypeptide: A technical note. Aaps PharmSciTech, 10: 1263-1267.
18. Bhatt, Y. and D. Shah, 2012. Influence of additives on fabrication and release from protein loaded microparticles. Iran. J. Pharm. Sci., 8: 171-179.
19. Fahmy, U.A., 2015. Nanoethosomal transdermal delivery of vardenafil for treatment of erectile dysfunction: Optimization, characterization and *in vivo* evaluation. Drug Des. Dev. Ther., 9: 6129-6137.
20. El-Say, K.M., T.A. Ahmed, S.M. Badr-Eldin, U. Fahmy, H. Aldawsari and O.A. Ahmed, 2015. Enhanced permeation parameters of optimized nanostructured simvastatin transdermal films: *Ex vivo* and *in vivo* evaluation. Pharm. Dev. Technol., 20: 919-926.