Original Article

Formulation and optimization of fluconazole polymer-lipid hybrid nanoparticles

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ABSTRACT

Background: Fluconazole is an antifungal drug that is effective both through systemic and topical route. Its bioavailability through the oral route is limited due to its solubility. Polymer-lipid hybrid-based alternative drug delivery system will result in improved oral bioavailability. **Objective**: The present study deals with the development and evaluation of fluconazole-loaded polymer-lipid hybrid nanoparticles for oral administration. **Materials and Methods:** Fluconazole-loaded polymer-lipid hybrid nanoparticles were prepared by the solvent emulsification method using dichloromethane as the solvent, stearic acid as lipid, and polyvinyl alcohol as the polymer. The prepared nanoparticles were characterized for particle size, polydispersity index, entrapment efficiency, and in-vitro drug release. **Results:** Average size and polydispersity index of optimized formulation F5 were 237.0 ± 3.3 nm and 0.345, respectively. The entrapment efficiency of formulation F5 was found to be 86.70%. **Conclusion**: These results reveal the potential application of novel fluconazole-PLH in the treatment of fungal infections.

Keywords: Fluconazole, polymer-lipid carriers, Factorial design, polydispersity index, solvent emulsification method.

INTRODUCTION

The delivery of active pharmaceutical ingredients is often limited by their poor aqueous solubility. Among other approaches aimed at increasing solubility of such molecules, their formulation into colloidal nanoparticles is often encouraged. Nanoparticles (NPs) have attracted much attention due to their ability to deliver drugs to the therapeutic targets at relevant times and doses. Polymer-based systems include polymeric nanoparticles, polymeric micelles, and polymer-drug conjugates, to name a few, while lipid-based systems include liposomes, solid lipid nanoparticles (SLNs), and nanostructured lipid carriers (NLCs). On comparing these two different matrices, it was observed that lipid-based carriers show advantages such as cost-effective manufacturing and better drug entrapment efficiency. However, they suffer from limitations in terms of their stability, tedious sterilization process, a burst release of the drug, and high polydispersity index. Further, there is limited scope for chemical modification of the system to control properties such as surface functionalization for targeting, improving drug entrapment, and modifying drug release pattern.[1] On the other hand, polymeric systems provide an excellent diversity of chemical modifications and prove to be helpful in designing the nanocarrier systems with tailored requirements. Polymeric carriers also offer benefits such as small particle size with narrow size distribution, diversity of synthesis procedures, easy and reproducible manufacturing process, better control over surface functionalization, and stability. However, polymeric systems have their own limitations, such as the use of organic solvents in the manufacturing process, toxicity due to polymer degradation products, and limited drug loading capacities.[2]

Polymer-lipid hybrid nanoparticles (PLHs) are an interesting alternative to polymeric nanoparticles. The biomimetic characteristics of lipids and architectural advantage of polymer core are combined to yield a theoretically superior delivery system. PLH is solid and sub-micron particles composed of at least two components; one being the polymer and the other being the lipid. Various bioactive molecules such as drugs, genes, proteins, and targeting ligands can be entrapped,

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adsorbed, or covalently attached in the hybrid system. The common choices of biodegradable polymers include polylactic-*co*-glycolic acid (PLGA), polylactic acid (PLA), polycaprolactone (PCL), dextran, or albumin due to their biocompatibility, biodegradability, non-toxicity, and previous use in approved products.[3-5]

Fluconazole is an antifungal agent of the triazole category. It is slightly soluble in water and saline. It causes inhibition of cytochrome P-450-dependent 14α-sterol demethylase. Mammalian demethylase is less effective to fluconazole than fungal demethylase. This causes the conversion of lanosterol to ergosterol.[6]

The aim of this study was to prepare and evaluate polymer-lipid hybrid nanoparticles of fluconazole using stearic acid as lipid and polylactic acid as a polymer.

MATERIALS AND METHODS

Materials

Fluconazole was received as a gift sample from Syncom Healthcare, Dehradun, India. Stearic acid, polylactic acid, and dichloromethane were procured from SD Fine Chemicals, Mumbai, India. All other reagents and chemicals used were of analytical grade.

Methods

Identification of drug

The received drug sample was subjected to identification tests as per Indian Pharmacopoeia.[7] The melting point was determined by the open capillary method.

Recording of FTIR spectrum

The drug and its physical mixture with lipid and polymer were subjected to IR spectrum determination. The samples were weighed and homogeneously mixed in dried KBr using a glass pestle and mortar and compressed under vacuum to obtain a pellet. The pellet was placed in the IR light path, and spectra were recorded using FT-IR spectrophotometer [Perkin Elmer spectrum version 10 03 05]. The samples were scanned between 400 and 4000 cm^{-1} at a resolution of 4 cm^{-1}.

Preparation of standard curve

Solutions of fluconazole in dichloromethane were prepared in the concentration range of 10–100 μg/ml and absorbance was measured at 261 nm using a Shimadzu double-beam UV spectrophotometer. The standard curve was plotted by taking absorbance on the Y-axis and concentration of analyte on the X-axis.

Preparation of polymer-lipid hybrid nanoparticles of fluconazole

A total of eight batches were prepared using 2^3 factorial design[8] as per the details given in Table 1. PLH was prepared by the solvent emulsification method. PLA, stearic acid, and fluconazole were dissolved in dichloromethane taking a lipid to polymer ratio of 1:10. This solution was added dropwise to a continuously stirred aqueous solution of polyvinyl alcohol which acts as a stabilizing agent. The contents were stirred for 15 min and then kept aside for 1–2 h to facilitate evaporation of the solvent. Later, they were centrifuged at 1200 rpm for 30 min at room temperature. This was followed by the redispersion of the contents in distilled water and sonication step. The solutions were frozen for 3 h and then finally freeze-dried.

Evaluation of Polymer-lipid hybrid Nanoparticles

Particle size determination

Particle size was measured by dynamic light scattering using the particle size analyzer (Malvern Zetasizer) at room temperature. All measurements were taken by scattering light at 90°. The dispersion was centrifuged at 1200 rpm for 30 min at room temperature. The supernatant was discarded and solution was redispersed in double-distilled water. The dispersion was then taken for the particle size measurement. Samples were sonicated before estimation.

Entrapment efficiency (EE) and drug content

The dispersion was centrifuged at 1200 rpm for 30 min at room temperature, supernatant was discarded and the aqueous solution was dissolved in DCM and then the drug concentration was analyzed by UV spectrophotometer (261nm). Drug loading was identified by a direct method for entrapment efficiency EE.

Zeta potential

The zeta potential of the solution was measured by determining the electrophoretic mobility using the Zetasizer (Malvern Zetasizer). Supernatant was discarded and the resultant pellet was redispersed in distilled water using an ultrasonic probe system for 1 min with 50 s pulse at 200 V. Dispersion was then exactly diluted and zeta potential was measured.

In vitro drug release study

Franz diffusion cell system was used with a cellulose-based membrane. The semipermeable membrane was dipped in phosphate buffer overnight,

Table 1: Design values and characterization parameters

Formulation Code	PLA (ml)	FLZ (mg)	DCM (ml)	PVA (mg)	Stearic acid (mg)	Particle Size (nm)	PDI	EE (%)	% CDR (6h)
F1	2.5	150	3.7	1.0	250	791.2	0.696	68.3	61.6
F2	2.5	150	3.7	2.5	250	693.2	0.717	66.9	57.3
F3	2.5	150	3.7	1.0	250	647.9	0.599	67.8	63.1
F4	2.5	150	7.9	1.0	250	809.4	0.634	72.1	53.5
F5	1.0	150	7.9	1.0	100	237.3	0.345	86.7	67.5
F6	1.0	150	7.9	1.0	100	323.8	0.458	80.7	50.2
F7	1.0	150	7.9	2.5	100	297.8	0.662	76.2	62.6
F8	1.0	150	3.7	2.5	100	408.9	0.698	73.6	51.9

and later, it was put between the donor compartment and the receptor compartment of the diffusion cell. The receptor compartment contained phosphate-buffered saline (PBS) maintained at 37 ± 1°C. After giving 30 min to the system to equilibrate, 20 mg of prepared PLH were introduced in the donor compartment. Aliquots (1 ml) were withdrawn from the receptor chamber at regular intervals of 15, 30, 60, 90, 120, 150, and 180 min. The volume of the receiving solution was maintained by replacing the amount withdrawn with an equal volume of PBS. Samples were analyzed spectrophotometrically at a wavelength of 261 nm.

RESULTS AND DISCUSSION

Identification of the drug

The supplied sample of the drug exhibited a melting point of 138°C, which was within the reported literature value (137–141°C). Further, the peaks observed in the IR spectrum of pure drug matched well with the reported peaks (Indian Pharmacopoeia, 2018). The UV scanning of the supplied sample exhibited a peak at 261 nm.

Compatibility studies

These studies were carried out by recording the IR spectra of pure drug and its physical mixture with other excipients. The characteristic peaks of the drug[9] were also visible in the IR spectrum of the physical mixture [Figure 1], thereby indicating the absence of any chemical interaction between the drug and excipients.

Preparation of standard curve

The absorbance of the samples was found to vary linearly with concentration. An equation of a straight line (Y = 0.0246 X + 0.3629) with a good correlation coefficient (R2 = 0.9641) was obtained, and the same equation was used for the purpose of calculation of drug amount throughout the experiments.

Preparation of PLH of fluconazole

Eight batches of PLH were successfully prepared using factorial design. In the present work, FLZ-PLH consisting of the polymeric core and lipid were easily prepared by the solvent emulsification evaporation

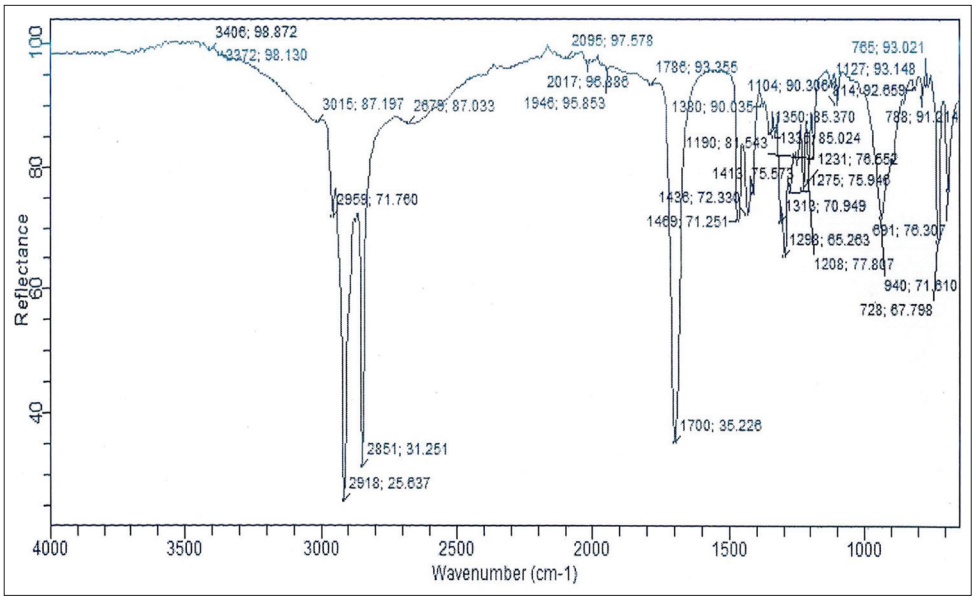

Figure 1: FT-IR spectrum of a physical mixture of fluconazole and excipients

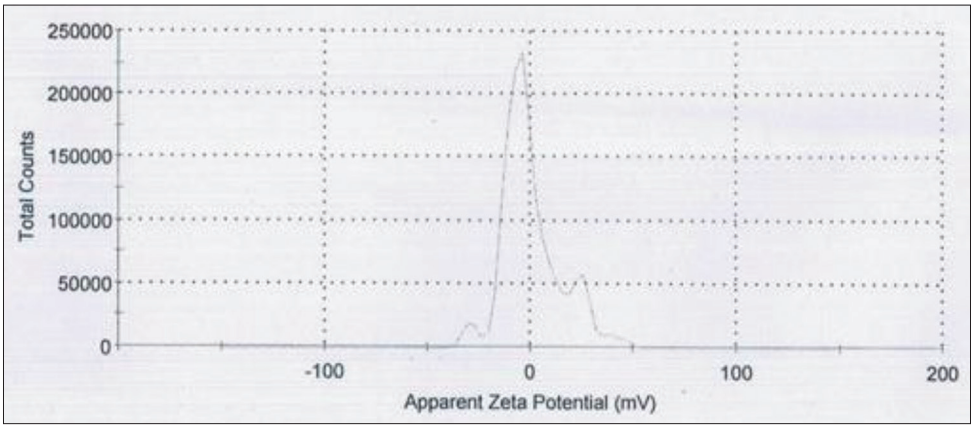

Figure 2: Zeta potential curve of optimized batch F5

method[10] with notable particle size and entrapment efficiency [Table 1]. PLH may help to improve the oral bioavailability because they directly penetrate into the systemic circulation by the help of lymphatic uptake, M cells of Payer's patch, and paracellular pathway that reduce the effect of food and hepatic first-pass metabolism.

Particle size and distribution

The size of the prepared PLH was found to vary between 237.3 nm to 809.4 nm. The nanoparticles had reasonable size distribution with a polydispersity index varying between 0.345 and 0.717. The batches having less amount of PLA (1.0 ml) invariably gave particles with less particle size.

Entrapment efficiency, drug content, and zeta potential

The entrapment efficiency of prepared PLH was found to vary between 66.9 and 86.7%. The optimized batch F5 possessed the highest entrapment efficiency. This could be due to a lower concentration of PLA and higher concentration of stabilizing agent. The zeta potential of this batch was found to be −3.75 mV indicating the stability of the prepared PLH [Figure 2].

In vitro drug release

All batches of prepared PLH were subjected to *in vitro* drug release studies using a Franz diffusion cell. The studies were carried out for a period of 6 h. The percent cumulative drug release (CDR) was recorded [Table 1]. It was found to vary between 50.2 and 67.5%.

CONCLUSION

The distinct advantage of this PLH has been demonstrated to include the unique advantages of both liposomes and polymeric nanoparticles while excluding some of their intrinsic limitations, thereby holding great promise as a delivery vehicle for various drugs.

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