Development and Characterization of Itraconazole Nanocrystals for the treatment of Ophthalmic Fungal Infection

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ABSTRACT:

One of the longest-standing and most fruitful fields of pharmaceutical research and development has been in ophthalmic medications. The issues of reduced absorption of medicinal material after application to the eyeball are the primary cause for the persistent attention of scientists in these medication forms. Adding polymers to the formulation of standard ophthalmic medications increased the bioavailability of the drug by increasing the amount of time the active component spent in contact with the cornea. Drug pure solid medication crystals in the nanoscale range, including just the active pharmaceutical ingredient and necessary stabilizers, are known as nano crystals. Nano crystals, with particle sizes in the nanometer range (100-1000nm), can improve drug absorption and bioavailability more effectively than conventional formulation by increasing the saturation solubility and dissolution velocity of poorly soluble drugs using the Noyes– Whitney and Ostwald–Freundlich equations.

Keywords: Ophthalmic, Nano-crystals, Ostwald–Freundlich, Absorption, Bioavailability.

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INTRODUCTION

One of the longest-standing and most fruitful fields of pharmaceutical research and development has been in ophthalmic medications [1]. The issues of reduced absorption of medicinal material after application to the eyeball are the primary cause for the persistent attention of scientists in these medication forms [2]. Tear formation, blinking, and the flow of the substance through the nasolacrimal duct all play a role in this phenomenon because of the eye's complex structure, the cornea's small absorptive surface and low transparency, the epithelial cell's lipophilicity, and the drug's bonding with proteins contained in tear fluid [3]. Oedema of the cornea, cellular infiltration, and cilliary congestion are all symptoms of keratitis, an infection of the cornea. It has the potential to be infectious or noninfectious [4]. The symptoms of keratitis change based on the source of the infection (bacterial keratitis, fungal keratitis, viral keratitis, acanthamoeba keratitis) [5]. Mycotic corneal ulcers are most often caused by the fungus Aspergillus flavus, Aspergillus fumigates, Aspergillus niger, Candida, and Fusarium. Pure solid medication crystals in the nanometer size range that exclusively contain the active pharmaceutical ingredient (API) and any necessary stabilisers are known as drug nanocrystals [6]. Particles in the nanometer range (100-1000nm) have been shown to increase the saturation solubility and dissolution velocity of poorly soluble pharmaceuticals using the Noyes– Whitney and Ostwald–Freundlich equations, resulting in enhanced drug absorption and bioavailability. In the 1990s, drug nanocrystals were introduced, which are pure solid drug particles with a nano dimension. Water, water-reduced mixes, or non-aqueous media can be used as the dispersion medium [7]. Surfactants or polymers typically stabilize nano-suspension via electrical repulsion or steric effect. By reducing the size into the nano-range (below 1 μm), physicochemical properties of a substance might change. The drug nanocrystals (less than 100 nm) are immediately taken up by the cells via endocytosis, delivering the drug to the cell compartment or diffusing to the underlying tissues [8]. The concentration gradient of the medication between the top and lower layers of the skin controls passive diffusion. Drug nanocrystals have been shown to collect in hair follicles, forming a depot of nanocrystal suspension that gradually enhances drug penetration into the surrounding epidermal layers in a concentration-dependent way [9].

MATERIAL

Itraconazole sample was obtained from Sun Pharma, Ahemdabad, Gujrat, India. Poloxamer 407 (Pf127) BASF corporation, New Jersey USA, Methanol and Ethanol, Central Drug house Ltd. New Delhi, India. Acetone, Qualikems FineChem.Pvt.Ltd., Mumbai, India.

METHODS

Preparation of Itraconazole loaded nano-crystals

For the production of stable Nano crystals of Itraconazole a low energy anti-solvent precipitation method was employed. The Itraconazole solution (mg/ml) was prepared in solvent and this solution was placed in a syringe. This solution was poured at constant flow rate of 2-8 ml/min using a syringe into an anti-solvent solution consisting of a stabilizer (1% w/v). Here water is used as an anti-solvent phase. The amount of Anti-solvent phase was 27ml for a 30ml batch size. The solutionwas stirred at 600-1000rpm continuously during this phase. Different polymers were utilized to evaluate their impact on itraconazole nanocrystals produced. The itraconazole nanocrystals produced were filtered and then they were subjected to drying using rotary evaporator. The formulation design for a 30ml batch size is given below consisting of 3ml of solvent phase and 27ml of anti-solvent phase [10].

Table 1: Formulation design for Itraconazole loaded nanocrystals carrier system

FormulationCode	Amount of Itraconazole drug (mg/ml)	Stabilizer (1% w/v)	Solvent(3 ml)	Anti-Solvent (27 ml)
NC1	5	Pf 127	Ethanol	Water
NC2	10	Pf 127	Ethanol	Water
NC3	15	Pf 127	Ethanol	Water
NC4	5	Pf 127	Acetone	Water
NC5	10	Pf 127	Acetone	Water
NC6	15	Pf 127	Acetone	Water
NC7	5	Pf 127	Methanol	Water
NC8	10	Pf 127	Methanol	Water
NC9	15	Pf 127	Methanol	Water

Characterization of drug loaded nanocrystals

Drug content

Drug Content was determined by dissolving 5 mL ethanol in 1mL nanocrystals suspension to ensure their drug release. To bring the volume up to 10mL, phosphate buffer, pH 6.8, was utilized. The total drug (Itraconazole) content was measured spectrophotometrically at 261 nm, and the percent drug content was determined using the equation below [11]:

%Drug Content = Amount of drug present in nanocrystals/ Total drug incorporated × 100

FTIR

The FTIR spectrum of itraconazole nanocrystals was determined using the sodium pellet method and a Fourier transform infrared spectrophotometer (Shimadzu- 8400S, Kyoto, Japan) equipped with a DRS attachment, where the spectrum of sodium was used as a background and the spectrum of the sample was superimposed on top of it. It scanned from 400 to 4000 cm-1. A comparison was made between the FTIR spectra and the reference spectrum of pure itraconazole [12].

Differential Scanning Calorimetry

DSC was used to record the thermogram of the sample. Sample weighing 2 milligrams was directly weighed in an aluminum pan under dynamic atmosphere of dry nitrogen with 10ºC/mint heating rate, and scan in the range 138- 175 ºC temperature. The test was done with API, excipients and physical mixture [13]

In-vitro Permeation

A Franz diffusion cell was constructed with a donor and receptor compartment separated by a dialysis membrane to test the nanocrystals loaded with itraconazole for penetration in vitro (Hi Media Ltd., India). A volume of phosphate buffer, pH 6.8, 3 mL, and a volume of Itraconazole-loaded nanocrystal formulation, 1 mL, were each introduced to the receptor and donor compartments, respectively. For this reason, a piece of dialysis membrane (pore size 0.22 m) was used to partition off the donor and receptor compartments. The Franz diffusion cell was placed on a magnetic stirrer and kept at 37^0 C to mimic internal body temperature. At 0, 1, 2, 3, 4, 6, 8, 10, and 12 hours, 1 ml of sample was taken from the diffusion cell via the sampling port and analyzed using a UV spectrophotometer set at 348 nm. Phosphate buffer (pH 6.8) was added to the receptor compartment in a constant, measured volume after each sample [14].

Particle Size

The Zetasizer was used to determine the particle size and polydispersity index (PDI) of drug-loaded nanocrystals (Malvern Instruments Ltd, Worcestershire, UK). The temperature was held at a steady 25^0 C, and the scattering angle was fixed to 173^0 C. Particle size was determined by filtering diluted samples of Itraconazole-loaded nanocrystals over a membrane with a pore size of 0.45 μm. We used a disposable, transparent sizing cuvette to get an accurate measurement of the particles' dimensions. [15]

Selection of optimized formulation

The optimized formulation was chosen based on high drug content, percentage of cumulative drug release with lowest Polydispersity index, optimal particle size and TEM was performed of optimized formulation.

Transmission electron microscopy

The morphology of Itraconazole-loaded nanocrystals was studied using transmission electron microscopy. Excess droplets were collected using filter paper after 1 drop of nanocrystal suspension was deposited on a carbon-coated copper cxzgrid with a 400-mesh size. The negatively stained sample was dried at room temperature after a drop of a phosphotungstic acid solution (4% w/v) was inserted into the grid [16].

Stability Study

Both visual control and analytical drug content measurement were determined for the nanocrystals stability study. In this study Itraconazole loaded nanocrystal was placed in a climatic chamber for 3 months at 25 ± 2°C temperature and 60% relative humidity. This nanocrystal was put in an amber container with a lid. The drug content was measured from the beginning and also at the end of the 3 months spectrophotometrically at λmax 263 nm [12].

RESULT AND DISCUSSION

The most appropriate method for preparing nanocrystals was chosen, i.e. anti-solvent precipitation. This technique has also been shown to enhance drug loading in laboratory settings, and as a final process, it's utilized to make Itraconazole loaded nanocrystals. Because the organic solvent can be virtually eliminated, this technique is appropriate for both the production of nanocrystals and as well as their topical administration. With the inclusion of Itraconazole and Pluronic F-127 polymer as a stabilizer, several types of nanocrystal formulations were formed. All the formulations were made using formulation design and the effect of polymer with three different solvents i.e. Methanol, Ethanol and Acetone, was studied with varying drug concentrations. The final optimal formulation will be chosen based on numerous factors such as percentage drug content, particle size distribution, TEM, and *in-vitro* permeation of these several formulations.

Drug Content

The drug content was found to be in the range of (43.24± 0.25%- 95.24± 0.24%) as shown in (Table 2). As the ratio of Itraconazole with Pluronic F-127 was increases in acetone (NC4-NC6) was increased the drug content was also increased from (78.29±0.33%– 95.24±0.24%), drug content with Ethanol (NC1-NC3) was increased from (43.26.79±0.25% – 68.35±0.54%) and in case of methanol (NC7-NC9) it was increased with (72.53±0.25% – 81.20±0.44%). Using acetone as organic solvent (NC4-NC6) shows higher drug content as compared to other formulations. Type of organic solvent slightly affected the drug and polymer ratio used in nanocrystal. The nanocrystal formulated using acetone as a solvent resulted better drug loading than Ethanol and methanol.

FTIR

FT-IR spectrum of Itraconazole loaded nanocrystals was determined using KBr Pellet technique. Itraconazole Nanocrystals showed characteristic peaks in the range 500-4000 $cm^{-1.}$ It was found that peak at 3130 cm^{-1} and 3068 cm^{-1} are due to absorption of $-NH_2$ group. Sharp peak observed at 1698 cm^{-1}indicates –C=O –stretching vibration. The characteristic peak at 1510 cm^{-1} and 1451 cm^{-1} showed C-H deformation. Pure drug showed characteristic peaks at 400-1800 cm-1 showed stretching and vibration of –C=C- of aromatic ring. Peak for alkane and amine was found at 2800 cm^{-1} and 3200 cm^{-1} respectively matched with reference spectra. The FTIR spectra is shown in Figure no. 1.

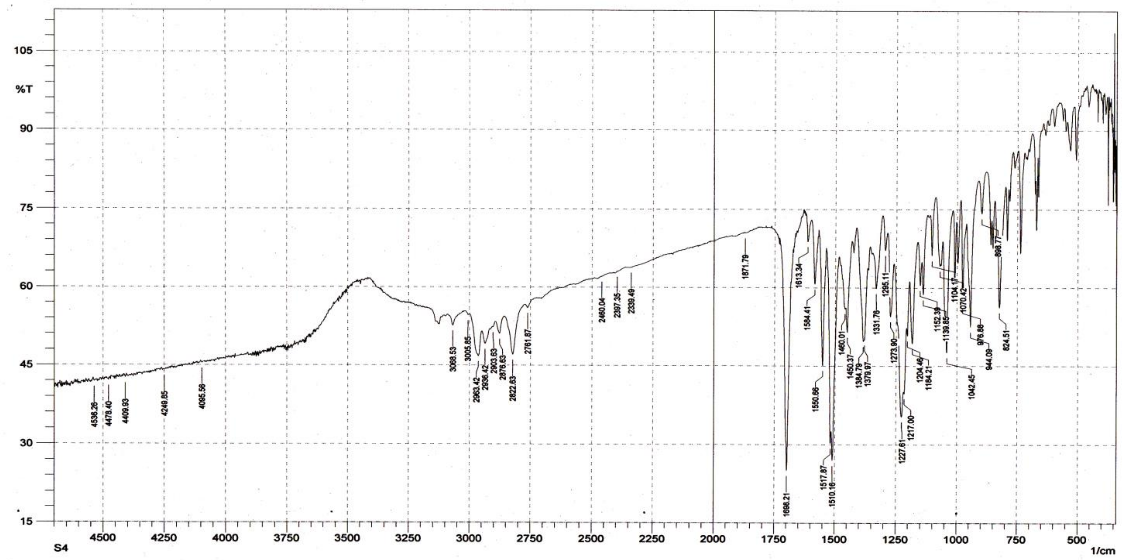

Figure 1: FTIR Spectra of Itraconazole loaded Nanocrystals.

Differential Scanning Calorimetry (DSC)

DSC patterns of Itraconazole, Pluronic F-127, and Itraconazole Nanocrystals were shown in Figure 2. The thermogram of raw Itraconazole powder showed a characteristic peak at 178.86 ^{0}C corresponding to drug melting endotherm, Pf127 displayed an endothermic peak at 54.31 ^{0}C and in case of (NC5) Itraconazole nanocrystal were 54.91^{0}C and at 178.86^{0}C showed an endothermic peak which was little less in intensity as compared to raw drug powder.

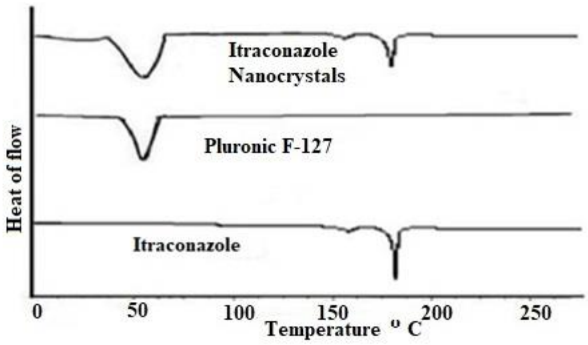

Figure 2: DSC of Itraconazole loaded Nanocrystals, Pluronic F-127 and Itraconazole Drug

***In-vitro* permeation**

The % cumulative drug permeated in 10 hours was determined by the *in-vitro* drug permeation profiles and it was varied between 62.25±0.19%-94.50±0.28%. The maximum % cumulative drug permeated was shown in formulation (NC5) with 94.50±0.28%, because of their small particle size and high drug content. The drug permeation variation might be due to particle size and PDI of nanocrystals and that was affected by type of organic solvent used and drug: polymer weight ratio. Therefore NC1-NC3 and NC7-NC9 was prepared by using Ethanol and methanol as solvent and produced comparatively large nanocrystals than the acetone (NC4- NC6). Various kinetic model was used to indicate the result of in vitro permeation profiles. The formulations were fitted into Higuchi model (Table 2) which indicated that, the release drug in the shell was rapidly replaced by the drug available in the core of the reservoir means the concentration of drug will not change as a function of time. While, the percent cumulative drug permeation of (NC5) was more than 90% and best fitted to Higuchi model and it was selected as optimized formulation with maximum drug content (95.24±0.24%). The comparitive study is shown in figure no. 3.

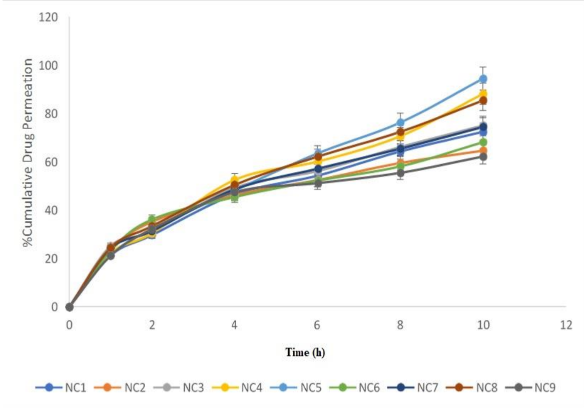

Figure 3: Comparison of *in-vitro* drug permeation profiles of drug loaded nanocrystals(NC1-NC9)

Particle size and Polydispersity index

The particle size of various Itraconazole loaded nanocrystal formulations (NC1-NC9) is shown in Table 2 and particle size ranged from 114.2 to 164.2 nm, with PDI values ranging from 0.2320.275. As demonstrated by the results, the amount of drug and stabilizer used has a significant impact on the particle size of the nanocrystal produced. Uniform size distribution and particle size of the drug are the most important parameters for delivery of drug through topical route. The optimum particle size for topical delivery was observed in formulation (NC5) i.e. 114.2 nm with low PDI value of 0.232 as shown in figure no. 4 The mean particle sized of developed Itraconazole loaded nanocrystals by using ethanol as solvent (NC1-NC3) ranged from 143.5-164.2 nm and by using acetone (NC4-NC6) ranged from 114.2-132.9 nm and by utilizing methanol as solvent ranged from 138.1-156.4 nm. The Ratio of drug and stabilizer used has great impact on the size of nanocrystals produced and the produced nanocrystals showed lower particle sizes with acetone as a solvent in comparison to other solvents. Low weight of the drug to polymer (stabilizer) ratio (NC3, NC6, NC9) resulted in comparatively high particle size as stabilizer helps in creation of adequate particle–particle repulsion to prevent the growth of the particles during the nucleation process. Out of all formulations (NC1-NC9), the formulation (NC5) had optimal particle size range of nanocrystals for topical delivery with low PDI value indicating a narrow and mono-disperse pattern.

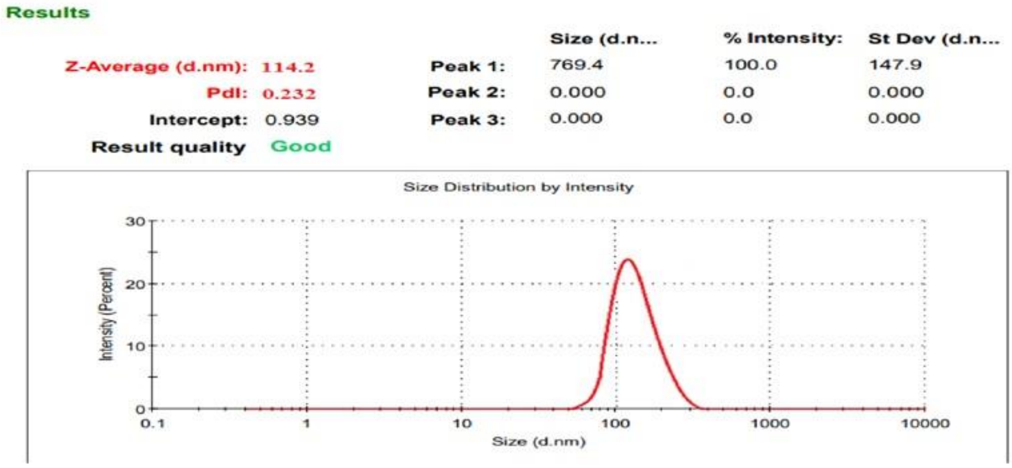

Figure 4: Particle size and Polydispersity index

Table 2: Characterization of drug loaded nanocrystals (NC1-NC9)

Formulation code	Particle Size (nm)	Polydispersity index	% Drug content ±(SD)	% CDP at 10 hr. ±(SD)	Bestfit model	r^2 value
NC1	152.6	0.239	43.19±0.35	72.5±0.32	Higuchi	0.9845
NC2	143.5	0.247	56.26±0.25	64.7±0.24	Higuchi	0.9823
NC3	164.2	0.264	68.35±0.54	75.1±0.18	Higuchi	0.9851
NC4	125.7	0.275	78.29±0.82	88.2±0.35	Higuchi	0.9914
NC5	114.2	0.232	95.24±0.24	94.5±0.28	Higuchi	0.9980
NC6	132.9	0.265	90.14±0.62	68.2±0.25	Higuchi	0.9835
NC7	140.5	0.262	72.53±1.12	74.5±0.27	Higuchi	0.9854
NC8	138.1	0.251	76.40±0.57	85.5±0.24	Higuchi	0.9906
NC9	156.4	0.237	81.20±0.44	62.25±0.19	Higuchi	0.9804

Selection of optimized formulation

The Formulation (NC5) exhibited the greatest percentage of cumulative drug permeation (94.5±0.28%). Drug content (95.24± 0.24%) with optimum particle size (114.2 nm). The (NC5) formulation was regarded as the best of the nine nanocrystal formulations, and this improved formulation was then integrated into a topical gel after evaluation.

Characterization of optimized formulation

Transmission Electron Microscopy

As demonstrated in the Transmission electron microscopic picture of formulation (NC5), the particles have a limited size distribution (Figure 5). The particles were discovered to be in the appropriate size range for dermal drug delivery.

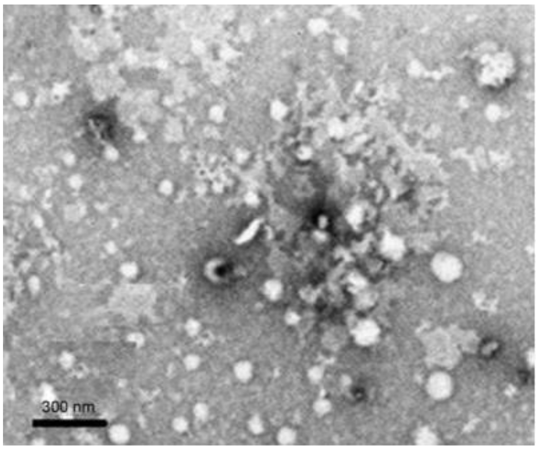

Figure 5: TEM

Skin permeation

The cumulative amount of Itraconazole permeated across the porcine skin from nanocrystals gel was studied. The plot of *ex vivo* skin permeation of formulation NG1 was compared against the plots of Formulation NC5 and the market preparation of Itraconazole. NG1 showed the sustain release of drug and achieved 68±0.43% in 10 hours. Meanwhile the market preparation was able to obtain only 49.38±60%. So prepared gel formulation showed better cumulative drug release than the market formulation. The ex-vivo drug study is shown in figure no. 6.

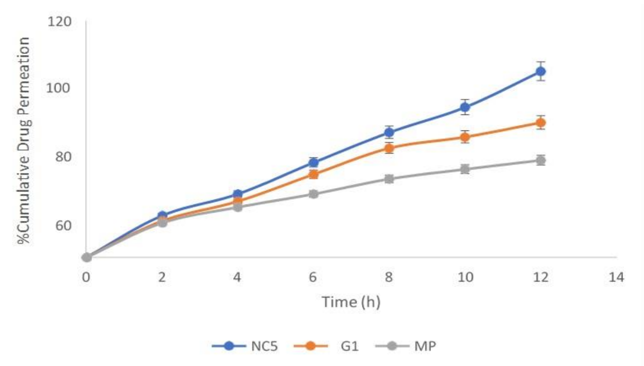

Figure 6: Comparison of ex-*vivo* drug permeation profiles of drug loaded nanocrystal(NC5), and market preparation of Itraconazole cream and gel.

Stability Study

The stability of an optimized Itraconazole loaded nanocrystals formulation (NC5) was tested, there was no significant change in drug content. i.e. (85.25±0.34%) and after three months (82.56± 0.64%), indicating that no chemical degradation, phase separation and sedimentation at the time of stability study. So, the result was found to be the formed Itraconazole loaded nanocrystals gel maintained their integrity.

CONCLUSION

Itraconazole loaded nanocrystals were successfully produced using anti-solvent precipitation technique. The method was found to be very simple, economic and reproducible in nature. The Itraconazole loaded nanocrystals of desirable size were produced. Furthermore *in-vitro* release study of produced nanocrystals showed sustained release profile. Drug loaded Nanocrystal (NC5) can be considered effective drug delivery system as compared to other delivery systems, it overcomes the drawbacks related to oral administrations of Itraconazole without any compromise with safety and patient's compliance. The developed formulation showed high drug content and other characteristics (pH, viscosity and clarity) were also found to be optimal. Nanocrystal formulation (N5) containing Itraconazole showed sustained release of drug over time as compared to marketed product. Topical formulations show better results in ocular infection treatments as compare to other formulations, because the topical formulations show less toxicity in body. The method demonstrated greater permeability rather than market formulations.

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