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Formulation and Evaluation of Nanoparticles for The Treatment of Oral Candidiasis

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ABSTRACT

This work aimed to formulate the nanoparticles containing fluconazole as an antifungal by using Eudragit RS100 and Eudragit RL100 polymer and Tween 80 surfactants for preparation. Fluconazole Nanoparticles were prepared by Solvent evaporation technique using different percentages of polymers. These nanoparticles were evaluated for particle size, percentage drug entrapment, in vitro drug release and stability studies. The percentage of drug entrapment observed in all formulations was between 80.3% – 90.3%. In vitro, release of nanoparticles in all formulations was between 79.2%-94.6%. The particle size obtained for formulation was in the range of 418.2 nm. Studies showed that the release of fluconazole from the nanoparticles was mainly influenced by the polymer concentration. Stability studies suggested that the formulation was stable at 4℃ and is the most suitable temperature for storage of prepared nanoparticles. It could be concluded from the present investigation that nanoparticles are promising oral candidiasis release carriers for fluconazole.

Keywords: Fluconazole, Eudragit RS100, Eudragit RL100, Tween 80, Nanoparticles, Oral candidiasis.

I. INTRODUCTION

Oral candidiasis (OC), commonly referred to as "thrush" encompasses infections of the tongue and other oral mucosal sites and is characterized by fungal overgrowth and invasion of superficial tissues[1]. The colloquial term "thrush" refers to the resemblance of the white flecks present in some forms of candidiasis with the breast of the bird of the same name. The etymology of oral thrush dates back to the time of Hippocrates (around 400 Before Christ (BC)) who, in his book "Of the Epidemics," described OC as "mouths affected with aphthous ulcerations[2].

The oral cavity is an exceptionally complex habitat harboring unique and diverse microbial communities that co-exist in an equilibrium crucial for maintaining oral health. Any disturbances in this ecosystem that result in the dominance of one pathogenic species (dysbiosis) may lead to the development of oral disease. In the oral cavity, the co-adhesion of C. albicans with bacteria is essential for C. albicans persistence and, therefore, these interactions may enhance colonization in the host[3].

Nanotechnology is a rapidly growing field with a wide range of applications in various fields like medicine, pharmacy, engineering and biotechnology for manufacturing of new materials at the nm scale level[4]. Nanoparticles are particles of clusters of atoms with a size of at least 100 nm. Nanoparticles exist within the wildlife and also are created as a result of human activities. These are ultrafine units with dimensions measured in nanometres (nm; 1 nm = 10−9 meters)[5].

Fluconazole (FLZ) is a bis-triazole antifungal agent used as the primary treatment of OC (generally 100 mg/day during 1 or 2 weeks) in both immune-competent and immune compromised patients. As with other triazoles, FLZ inhibits ergosterol synthesis of the fungal cell walls. Oral administration of FLZ results into disturbances in the gastrointestinal tract (vomiting, bloating and abdominal discomfort), causes irritation and serious hepatotoxicity.

By above literature review to formulation by fluconazole based solvent evaporation method using by Preparation of nanoparticles. Nanoparticles for the treatment of oral candidiasis to have improved drug utilization, reduce dose frequency and cast effective.

Therefore, the current study is formed formulation and evaluation nanoparticles for the treatment of oral candidiasis for Fluconazole drug using by solvent evaporation method. Invention also includes influence of electrical factor, physio chemical factor under other physicochemical evaluation and characterization of optimize formulation will be conducted. Stability studies

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will perform for optimize formulation as per ICH guideline.

II. MATERIALS AND METHOD

Fluconazole was the gift sample from Aarti pharma Mumbai. Eudragit RS100, Eudragit RL100 and Tween 80 was provided by Karnataka college of pharmacy, Bangalore. All the solvents used were of analytical grade.

Preparation of Fluconazole Nanoparticles

Solvent evaporation technique is used to prepare Fluconazole Nanoparticles. Ethanol with each polymer (Table 1) were mixed together to form the organic phase, then Fluconazole was added to 20 mL of the organic solvent. 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 cooling centrifuging at 8000 rpm for 2 hr.

Table 1: Formulation for preparing Fluconazole Nanoparticles.

Formulation	Drug (mg)	Eudragit RS100 (mg)	Eudragit RL100 (mg)	Tween 80 (ml)	Ethanol (ml)
F1	256	500	-	0.1	20
F2	256	1000	-	0.2	20
F3	256	1500	-	0.3	20
F4	256	-	500	0.1	20
F5	256	-	1000	0.2	20
F6	256	-	1500	0.3	20
F7	256	500	500	0.1	20
F8	256	1000	1000	0.2	20
F9	256	1500	1500	0.3	20

III. RESULT AND DISCUSSION

Identification of Pure Drug

Fluconazole (pure drug) was examined by FT-IR (Shimadzu -8400S Japan) and was compared with the reference spectrum of Fluconazole.

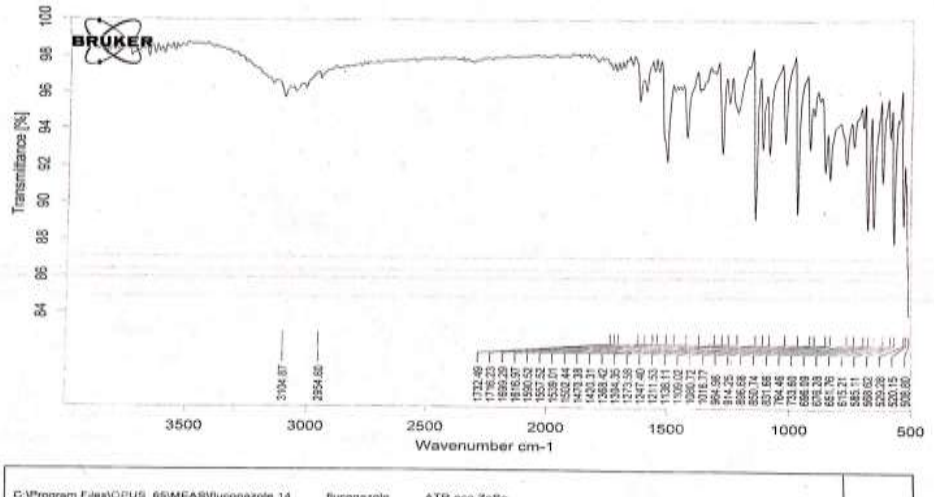

Figure 1: FTIR Fluconazole

Solubility studies

Solubility studies was done to select suitable solvents/ solvent system to dissolve the drug, polymer as well as various excipients used for the formulation of nanoparticles.

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Table 2: Solubility of Fluconazole

Drug	Solubility (%)
Fluconazole	89.43

Table 3: Solubility Studies of fluconazole in different solvents and polymers

Drug	Solubility (%)
Ethanol	78
Methanol	72.20
Tween 80	64.08
0.1M HCl	52.04

Melting Point

Melting point of the drug was determined by using melting point apparatus (Thale's tube).Melting point of Fluconazole was found to be 139.32^{0}C.

Table 4: Melting point

Range	Melting point
138-140^{0}C	139.32^{0}C

Compatibility studies

Compatibility of the Fluconazole drug with Eudragit RS 100, Eudragit RL 100, Ethanol & Tween 80 used to formulate Nanoparticles was established by FT-IR.Spectral analysis of Fluconazole, Eudragit RS 100, Eudragit RS 100 and combination of the Fluconazole with Eudragit RS 100 & Eudragit RS 100 was carried out to investigate any changes in chemical composition of the drug after combining with the excipients.

STANDARD CALIBRATION CURVE FOR FLUCONAZOLE

Standardization/ Method of estimation for Fluconazole:

Methanol

Accurately weighed 10mg of Fluconazole is transferred into a 100ml volumetric flask and dissolved in 30ml of methanol. It was then sonicated for 10 minutes, and made up to the mark with methanol to give a stock solution having 100 µg/ml concentration. For calibration curve, serial dilutions were made for Fluconazole in the range of 2, 4, 6, 8, and 10µg/ml concentrations were prepared by diluting the stock solution with methanol. The absorbance values of above solutions were measured in the wavelength at λ max 260 nm.

Table 5: Concentration and absorbanceobtained for calibration curve of Fluconazole in Methanol

Concentration (µg/ml)	Absorbance (Mean ±SD)
2	0.17±0.004
4	0.19±0.012
6	0.22±0.021
8	0.27±0.022
10	0.32±0.029

0.1 M HCL

About 100 mg of accurately weighed standard fluconazole was dissolved and made up to mark with 0.1M HCl solution, in a 100 ml volumetric flask, to give primary stock solution of 1000 µg/ml. From this stock solution, dilutions were made to obtain 50, 100, 200, 300 and 400 µg/ml using 0.1M HCl solutions.

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Table 6: Concentration and absorbance obtained for calibration curve of Fluconazole in 0.1M HCL

Concentration (µg/ml)	Absorbance (Mean ±SD)
0.5	0.094 ±0.03
1	0.183 ±0.015
2	0.365 ±0.062
3	0.519 ±0.023
4	0.703 ±0.032

pH 7.4 Phosphate buffer

50 ml of 0.2 M potassium dihydrogen phosphate solution was taken in 200 ml volumetric flask. To this 39.1 ml of 0.2 M NaOH was added and the volume was made to 200 ml distilled water.A standard curve for the Fluconazole was obtained by measuring absorbance atλ_{max} 260 nm and the concentration was taken between 2-12µg/ml ranges. The graph was plot between the concentrations versus absorbance. The regression equation generated was y = 0.0296x - 0.0057 of phosphate buffer 7.4 pH as shown in the table no 7

Table 7: Concentration and absorbance obtained for calibration curve of Fluconazole in 7.4 pH

Concentration (µg/ml)	Absorbance (Mean ±SD)
2	0.062±0.002
4	0.122±0.009
6	0.160±0.012
8	0.212±0.020
10	0.288±0.023
12	0.367±0.032

EVALUATION OF NANOPARTICLES

PERCENTAGE YIELD

Nanoparticles recovered at the end of the preparation were weight and the yield was calculated as % of total theoretical weight of the material taken for the preparation. The yield of the Nanoparticles was calculated as below.

$$\% \text{ Yield} = \frac{\text{Practical}}{\text{Theoretical yield}} \times 100$$

Table 8: Percentage yield of Fluconazole Nanoparticles

Formulation	**Total amount of Ingredient (mg)**	**Practical Yield (mg)**	**% Yield Mean ± SD**
F1	756	692	91.5 ±0.37
F2	1256	978	77.86 ±0.51
F3	1756	1623	92.42 ±0.73

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F4	756	532	70.37 ±0.64
F5	1256	942	75.00 ±0.80
F6	1756	1143	65.09 ±0.84
F7	1256	1134	90.20 ±1.13
F8	2256	1826	80.09 ±0.73
F9	3256	2289	70.03 ±0.83

Standard deviation= SD

DRUG ENTRAPMENT EFFICIENCY

Weighed amount of the nanoparticles (100mg) with phosphate buffer pH 7.4 (10 ml) was added in a vial. The solution was stirred vigorously for 24 hours with mechanical stirrer. Supernatant was collected by centrifugation and drug content in supernatant was determined by using UV spectrophotometer at wavelength 359 nm. Efficiency of drug entrapment was calculated by the following formula.

$$\% \text{ Drug Entrapment} = \frac{\text{Particle content}}{\text{Theoretical content}} \times 100$$

Table 9: Drug Entrapment efficiency for prepared formulation from F1-F9

Formulation	Entrapment efficiency %
F1	81.3
F2	84.9
F3	82.5
F4	80.3
F5	88.5
F6	90.3
F7	83.7
F8	81.3
F9	84.1

DRUG CONTENT

Drug loaded Nanoparticles (100mg) were powdered and suspended in 100 ml methanol. The resultant dispersion was kept for 20 minutes for complete mixing with continuous agitation and filtered through a 0.45µm membrane filter. The drug content was determined spectrophotometrically at 359 nm.

Table 10: Drug content of Fluconazole Nanoparticles

Formulation	**Drug content (%) (Mean± SD)**
F1	94.25 ±0.80
F2	93.87 ±0.83
F3	90.21 ±0.82
F4	90.35 ±0.82
F5	94.05 ±0.50
F6	92.07 ±0.47
F7	95.25 ±0.82

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F8	92.21 ±0.82
F9	95.26 ±0.82

IN VITRO DRUG RELEASE STUDIES

In vitro release study of Nanoparticles was performed in pH progression medium at 37℃ ±0.5℃. The drug dissolution test of Nanoparticles was performed by the Paddle method. nanoparticles (100 mg) were weighed accurately and filled into Dialysis bags. The Dialysis bags were tied using thread with paddle and loaded into the basket of the dissolution apparatus. The content was rotated at 100 rpm. The simulation of GI transit condition was achieved by altering the pH of the dissolution medium at different time intervals. The pH of the dissolution medium was kept at 1.2 pH for 2 hours using 0.1 N HCl. The release was carried out 7.4 pH and the release rate study were continued and maintained up to 8 hours. The final volume in all case was kept at 900 ml. The samples were withdrawn from the dissolution medium at various time intervals. The rate of drug release was analysed by UV spectrophotometer (Shimadzu).

Table 11: In vitro drug release studies for prepared formulation from (F1-F9)

Formulation	Drug release %
F1	81.1
F2	91.5
F3	87.3
F4	79.2
F5	92.7
F6	94.6
F7	88.3
F8	82.9
F9	84.7

CHARACTERIZATION OF NANOPARTICLES

PARTICLE SIZE ANALYSIS

The particle size distribution of Nanoparticles is measured by using Malvern Zeta sizer. The average particle sizes of the individual batch of nanoparticles were reported.

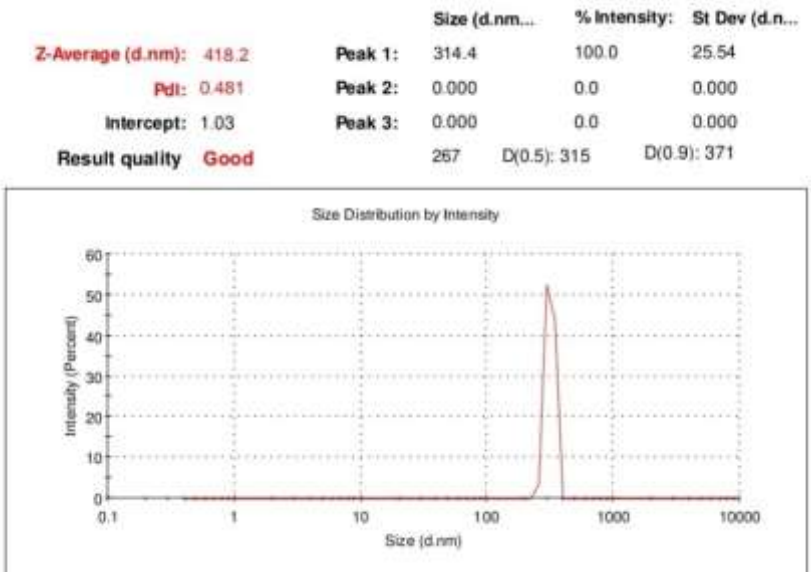

Figure 2: Particle size of Fluconazole

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ZETA POTENTIAL

The Zeta potential of prepared nanoparticles is commonly used to characterize the surface charge property of nanoparticles. Zeta potential is measured by Malvern zeta analyser.

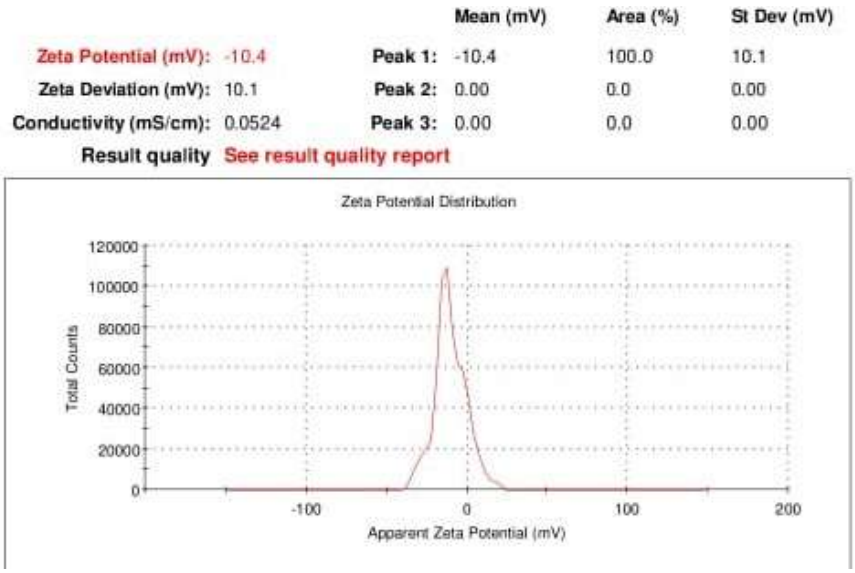

Figure 3: Zeta potential of Fluconazole

IV. DISCUSSION

In this study, fluconazole nanoparticles were formulated using different combinations of Eudragit RS100, Eudragit RL100, Tween 80, and ethanol. Pre-formulation studies confirmed compatibility between the drug and excipients. Solubility studies indicated fluconazole's limited solubility in water and the polymers' solubility properties. A UV spectrophotometric method was used for fluconazole estimation, showing a high correlation coefficient. Nanoparticle evaluation revealed high drug entrapment efficiency (80.3% to 90.37%) and uniform drug content (90.35% to 95.25%). In vitro drug release studies demonstrated cumulative release ranging from 79.2% to 94.6%, with higher polymer concentrations leading to decreased drug release.

V. CONCLUSION

In this study, fluconazole nanoparticles were formulated and evaluated for treating oral candidiasis. Preformulation studies confirmed compatibility between the drug and polymers. Nine formulations were prepared and tested for various parameters including particle size, drug entrapment efficiency, in vitro drug release, and in vivo organ distribution. The results indicate that these nanoparticles could potentially reduce the side effects associated with conventional dosage forms by minimizing drug absorption in the upper gastrointestinal tract.

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