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Research Article

Design and Characterization of Fluconazole Loaded Nanosponges Containing Topical Gel Preparation

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Abstract: The main aim and objective of this research was to develop and evaluate Fluconazole loaded nanosponges and formulate them as suitable topical gels for delivering the drug systemically after topical application. Fluconazole (FLZ) is a potent triazole anti fungistatic drug; topical administration of novel Fluconazole resulted in systemic absorption, for improved therapeutic effect and better dispersibility as compared to conventional topical formulation. Fluconazole (FLZ) nanosponges prepared by using emulsion solvent diffusion method to improve topical permeation. Nanosponges were formulated successfully using ethyl cellulose and Eudragit RS 100 as polymer, polyvinyl alcohol as the surfactant, and dichloromethane as solvent. The optimised batch of fluconazole loaded nanosponges used to prepare topical gel using different concentrations of carbopol gel. The prepared nanosponges were evaluated by various tests like production yield, drug entrapment efficiency, FTIR, particle size, zeta potential, SEM study. The entrapment efficiency and production yield were excellent. Based on the entrapment efficiency and production yield,out of ten formulations batches of nanosponges was evaluated and from that batch F3 was optimised.Optimized batch F3 containing FLZ and EC in the ratio of 1:3 showed optimum physicochemical and release characteristics. The optimised batch F3 of ethyl cellulose shows highest entrapment efficiency as 97.97% and production yield as 93.85% resp. A complex Fourier transform infrared (FTIR) study examines the formation in the nanosponge structure. The nanosponges had particle size in the range of 234.95 to 374.26 nm. The Zeta Potential was strong enough to produce stable formulations. The zeta potential of optimised batch nanosponges was found to be -25.05. SEM analysis of optimized batch F3 confirms that nanosponges were spherical in size with a porous and smooth surface. The formulated fluconazole topical gel were characterized for pH, actual drug content, viscosity, spreadability, *In -Vitro* drug release study. The *in -vitro* drug release showed maximum drug release of topical gel i.e. 88.34% in 8 hours. The present study demonstrates that, an antifungal drug Fluconazole is formulated in the form of nanosponges topical gel for the antifungal activity and can be best suitable approach in novel drug delivery system than conventional gel. Hence it is concluded that above topical gel formulation of fluconazole loaded nanosponges can be used in treatment of fungal infection.

Keywords: Fluconazole, Nanosponge, Emulsion solvent diffusion, fungal infection, Topical gel, Scanning electron microscopy

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1. INTRODUCTION

Nanotechnology have been advanced to enhance the bioavailability and dissolution rate of numerous drugs with poor solubility in water. Some of these approaches involve altering the crystallinity of the drug or developing new nanomaterial that can act as a drug carrier to attain controlled release.[1] Nanotechnology is a critical and interesting strategy because existing formulations have a number of concerns, including significant side effects, inaccurate targeting, and solubility and stability complications. To overcome the above disadvantages, nanosized (10–1000 nm) drug carriers can be used with site-specificity and continuous drug release, improved dissolution rate, absorption, bioavailability, and drug half-life in biological systems.[2] Nanosponge belongs to the new class of nanosponge having nano-sized particles that are filled with therapeutic agents with specific substances.[3] Nanosponges are spongy polymeric delivery systems that are tiny sphere-shaped particles with a highly porous surface. Passive targeting of drug molecules as well as cosmetics can be achieved by nanosponge. The Nanosponge has various advantages, including dose reduction, avoidance of first-pass metabolism, prolonged drug retention on the skin, and retention of dosage form topically.[4] The nanosponge delivery system will try to precisely regulate release rates or focus treatments on specific body areas, which would have a huge effect on the healthcare system. The main aim of this study is to develop drug-loaded nanosponges for topical gel preparation. The nanosponges are prepared by using different polymers based on the emulsion solvent diffusion method. Its great stability, high carrier potential, and ability to include both hydrophilic and hydrophobic substances make it a great choice. This novel delivery system has clear advantages for topical drug delivery. The use of nanosponges for selective and dispersed therapeutic agent delivery is the driving force behind research in this field.[5] Fluconazole (FLZ) is a potent antifungal and belongs to the BCS II class. It is a triazole derivative and used in the treatment of various fungal infections such as candidiasis, blastomycosis, coccidioidomycosis, cryptococcosis, histoplasmosis, dermatophytosis. Fluconazole (FLZ) generally acts by inhibiting 14-sterol demethylase, which converts lanosterol to ergo sterol to remove lanosterol from the 14-methyl group. It has fungistatic activity because of the reduction of typical fungal sterols. Being an antifungal agent, it also requires long-term treatment and may increase the risk of side effects after systemic administration. To prevent these side effects, a topical fluconazole preparation should be made. A topical novel drug delivery device based on nanosponges has the potential to reduce side effects associated with traditional delivery systems.[6]

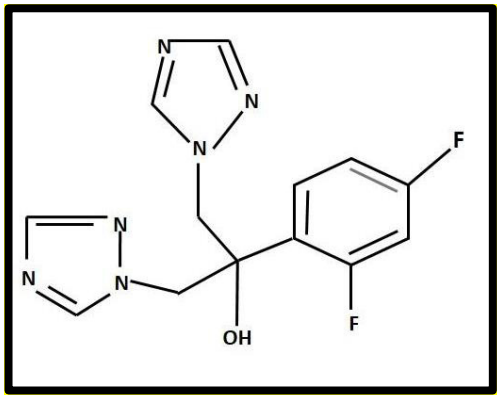

Fig 1: Structure of Fluconazole

Fungal infections have become an issue of great concern around the world; it was estimated that over 40 million people do in fact suffer fungal infection both in developed and in growing countries. The high rate of morbidity and mortality caused by fungal infection are associated with the current limited antifungal compound. Another important issue is the development of new formulation for antifungal agents, and interest in the Nanoformulation. The synergistic effect was achieved by combining antifungal nanosized particles as a type of drug carrier. The objective of the present study is to evaluate formulate nanosponge and nanosponge loaded topical gel.[7]

2. MATERIALS AND METHODS

2.1. Materials

Fluconazole was obtained as a gift sample from Wintech Pharmaceutical Limited, Musalgaon MIDC, Sinnar, Nashik, and Maharashtra, India. Other excipients like ethyl cellulose, Eudragit RS 100, Dichloromethane (DCM), polyvinyl alcohol (PVA), Carbopol 934, Methylparaben, Propylparaben, and Propylene glycol were procured from Modern Science lab, Nashik, Maharashtra, India. All the chemicals used were analytical grade and were used as obtained.

2.2. Methods

2.2.1. Preformulation Studies

Preformulation is the initiative in designing or development of a rational dosage form of drug. Pre-formulation studies were performed to determine the physicochemical properties of the drug moiety that would affect the stability, safety, and efficacy of the dosage form.[8-10]

2.2.1.1. Organoleptic properties

The drug samples were studied for external appearance like color, odour and texture by using visual method.[9,10]

2.2.1.2. Melting point

The melting point of fluconazole was determined by taking a small amount of sample into a sealed capillary tube, tying the thermometer with a rubber band, and immersing the end of the tube in a Thiele's tube. Heating is initiated and thus the

temperature range at which the sample melts can be observed. During continuous heating, the purpose of melting is observed, and hence the constant temperature is the freezing point of the sample.[11-14]

2.2.1.3. Solubility

The solubility of Fluconazole in methanol, ethanol, distilled water, phosphate buffer pH 7.4 and chloroform was determined by shake flask method. The examined compound was dissolved in solid excess in 1-10 ml respective solvent. The solutions were stirred for 48 hours in the magnetic stirrer under thermostated circumstances until the solubility equilibrium. To separate phases the solutions were left to sediment under thermostated circumstances. The solution was filtered. Aliquots were taken from clear part of the solution. The aliquots were diluted the absorption was measured with UV-Spectrophotometer (Shimadzu UV-2600). The concentrations of the aliquots were calculated.[15]

2.2.1.4. Ultraviolet -visible spectrophotometer

Determination of λ -max in Phosphate Buffer pH 7.4

The stock solution of 100µg/ml was prepared in a pH 7.4 phosphate buffer, used to prepare different dilutions in the range of 5-25 µg/ml. The absorbance of resulting solutions ware measured at 264 nm by UV-visible spectrophotometer. The solution was then scanned in the range 200-400nm using Shimadzu-2600 UV/Vis Spectrophotometer to determine the Absorption maximum.

Construction of Beer's-Lambert's plot in pH 7.4 phosphate buffer

A series of dilutions with concentration 2, 4, 6, 8 and 10ppm were prepared from 100ppm stock solution of Fluconazole in Phosphate buffer (pH 7.4) and were scanned at 264 nm. Absorbance vs. Concentration graph was plotted and linearity was obtained with an R^2= 0.99

2.2.1.5. Fourier Transform Infra-Red (FT-IR) Spectroscopy

The qualitative identification of the compound was performed using FT-IR Spectroscopy in which the necessary information regarding the groups present in the compound was obtained. The IR absorption spectrum of fluconazole was recorded with KBr. The dried sample of fluconazole compound was mixed with KBr in the ratio of 1:1, the sample triturate and finally placed in the sample holder. The spectrum was run over the wavenumber 4000 to 650 cm-1 using Fourier Transform Infrared Spectrophotometer Shimadzu, 8400S, Japan. The FTIR spectral study was done by using standard absorbance of the functional groups.[18] The FTIR peaks observed are shown in the spectrum Figure no.4,5,6 and 7.

2.2.2. Preparation of Fluconazole loaded nanosponges by using Emulsion Solvent Diffusion method

Table 1: Formulation of Fluconazole loaded nanosponges

Formulation code	Fluconazole (mg)	Ethyl Cellulose(mg)	Eudragit RS100(mg)	Polyvinyl Alcohol (%w/v)	DCM (ml)	Polymer-Drug Ratio
F1	100	100	-	0.5	20	1:1
F2	100	200	-	0.5	20	1:2
F3	100	300	-	0.5	20	1:3
F4	100	400	-	0.5	20	1:4
F5	100	500	-	0.5	20	1:5
F6	100	-	100	0.5	20	1:1
F7	100	-	200	0.5	20	1:2
F8	100	-	300	0.5	20	1:3
F9	100	-	400	0.5	20	1:4
F10	100	-	500	0.5	20	1:5

Table 1 illustrates the formulation of fluconazole nanosponges by using the emulsion solvent diffusion method. In this formulation, two different polymers like ethylcellulose and eudragit RS100 with different ratios were used for the nanosponge formulation. A total of ten formulations were prepared for the further optimization process. Two phases were used; one is organic and the other is the aqueous phase. The organic phase, which contained the drug and polymer mixture, was placed in 20 ml of DCM, and the aqueous phase, which contained PVA, was placed in 100 ml of distilled water. The aqueous phase was added in a dropwise manner to the dispersed phase on a magnetic stirrer at 1000–5000 rpm. After two hours of stirring, nanosponges were collected by the filtration method and dried in an oven at 40 °C for 24 hours. Nanosponges are stored in a vacuum desiccator for the removal of moisture.[19-21]

2.2.3. Preparation of fluconazole nanosponge topical gel

Table 2 broadly explains the composition of the topical gel of fluconazole loaded nanosponges. The topical gel was prepared by using different amounts of gelling agents like Carbopol 934 was dissolved and soaked overnight in insufficient amounts of water. After 24 hours, to this remaining ingredient i.e. propylene glycol as a permeation enhancer, methyl paraben and propyl paraben as a preservative was added. In another beaker, the optimised batch (F3) formulation of nanoaponges were dispersed in water. This was added to the previous beaker containing other excipients. Finally, triethanolamine was added to neutralize the pH of the formulation.[22,23]

Table 2: Composition of Fluconazole nanosponge gel

Sr. No	Ingredient	Formulation Code			
		C1	C2	C3	C4
1.	Nanosponges(mg)	0.2	0.2	0.2	0.2
2.	Carbapol 934(mg)	0.25	0.5	0.75	1
3.	Propylene Glycol(ml)	10	10	10	10
4.	Methyl Paraben(mg)	0.1	0.1	0.1	0.1
5.	Propyl Paraben(mg)	0.05	0.05	0.05	0.05
6.	Triethanolamine (ml)	q.s	q.s	q.s	q.s
7.	Distilled Water(ml)	20	20	20	20

2.2.4. Evaluation of nanosponge

2.2.4.1. Production yield

Production yield can be determined by calculating the initial weight of raw material and the final weight of drug-loaded nanosponges[24] The production yield was determined by the following formula.

$$Production\ Yield\ (\%) = \frac{Practical\ mass\ of\ nanosponges}{Theoretical\ mass\ of\ nanosponges} \times 100$$

2.2.4.2. Drug entrapment efficiency

Accurately weighed 10 mg of nanosponges were suspended in 100 ml of phosphate pH 7.4 buffer solution. After that, the solution was filtered through filter paper and after appropriate dilution, absorbance was measured at 264 nm by using a UV-visible spectrophotometer (Shimadzu UV-2600).[25] The drug entrapment efficiency formula are given below:

$$Drug\ entrapment\ efficiency = \frac{Actual\ drug\ content\ in\ nanosponge}{Therotical\ drug\ loading} \times 100$$

2.2.4.3. Fourier Transform Infrared spectroscopy (FTIR)

FTIR study was performed to override the possibility of interaction between drug and polymer. FTIR studies were performed on the optimized nanosponges F3 formulation. The nanosponges were mixed with potassium bromide (KBr) in 1:90 ratios, compressed in the form of the pellet. The FTIR spectrum of Fluconazole nanosponges' formulation batch was recorded in the wavelength range of 4000 to 400 cm-1. The changes in the main peaks of spectra of optimised nanosponges batch were recorded.[26]

2.2.4.4. Particle size

The average particle size of optimised nanosponge formulation was determined by a dynamic light scattering analyser by using Brookhaven Zetasizer, (Brookhaven Instrument Ltd.). The dried nanosponges were added in distilled water to get proper light scattering intensity for Fluconazole nanosponges.[27]

2.2.4.5. Polydispersibility index

The Polydispersity index (PDI) is an index showing the particle size distribution range. PDI can be determined by a dynamic light scattering instrument. Lower PDI values are observed in the monodisperse sample, whereas higher PDI values indicate wide particle size distribution and the polydisperse nature of the sample.[28]

2.2.4.6. Zeta potential

The zeta potential was analysed for the determination of the movement of the particles in an electric field and the particle charge. In the present work, nanosponges were diluted 10 times with distilled water and analysed by Brookhaven Zetasizer, (Brookhaven Instrument Ltd.).[29]

2.2.4.7. Scanning electron microscopy (SEM)

SEM is used for detailed morphological structure characterization of nanosponges. The sample was kept on a glass slide under a vacuum. The samples were coated with a thin gold/palladium layer using a sputter coater unit of scanning electron microscopy . The scanning electron microscope was operated at an acceleration voltage of 15Kv.[28]

2.2.5. Evaluation of Gel

2.2.5.1. Physical evaluation

The clarity and Homogeneity of the formulation was observed. PH of the formulated gel was measured using the digital pH meter.

2.2.5.2. Actual Drug content

10 mg of the gel was dissolved in 100 ml of phosphate buffer pH 7.4, a sample (5ml) was taken from this solution and diluted

to 10ml. Fluconazole concentration was determined by measuring the absorbance at 264 nm using UV-visible Spectrophotometer (Shimadzu, UV2600).[30]

2.2.5.3. *Spreadability*

1gm of prepared topical gel was used to analyse the spreadability. The prepared topical gel was placed between the 2 glass slides of the spreadability apparatus, weight was tied to the upper glass slide and the time required to slide over both slides from gel was noted.[31] The spreadability of the formulated gel was calculated using the following formula –

$$S = M*L/T$$

Where, S = Spreadability, M = weight tied to the upper slide, L = length of the glass slide (L = 7.5cm), T = time taken to separate the 2 slides (sec.).

2.2.5.4. *Viscosity*

The viscosity of the formulated Topical gel of optimized batch (F3) was used to prepared by using four kind of concentration of gelling agent like – Carbapol 934.The viscosity of formulated Topical gel was measured using the Brookfield viscometer spindle LV6 at different rpm and noted.[31]

2.2.5.5. *In-vitro drug release study*

In-vitro diffusion study of nanosponge loaded topical gel was performed through the cellulose membrane by using Franz diffusion cell. The receptor compartment was filled with a 7.4 pH phosphate buffer and kept at 37 ± 0.5 °C with continuous stirring with help of a magnetic stirrer. 0.1gm of the gel was placed over the cellulose membrane. An interval of 1, 2, 3, 4, 5, 6, 7, and 8-hour , 1 ml sample was withdrawn and suitably diluted. The withdrawn sample was replaced with the same amount of phosphate buffer pH 7.4 to maintain the sink condition. Diluted samples were analysed for fluconazole content with help of UV-visible spectrophotometer at 264 nm.[32]

3. RESULT AND DISCUSSION

3.2. *Preformulation Studies*

3.2.1. *Organoleptic properties*

The sample of drug received was studied for its organoleptic characters which shows white colour, odourless in nature and crystalline in nature.[9,10]

3.2.2. *Melting Point*

Melting point of pure Fluconazole is found in 134°C which is found to be nearly standard melting range of Fluconazole. That indicates the gifted samples obtained were of pure quality.[11-14] Melting point of Fluconazole is given in Table 3.

Table 3: Melting point of Fluconazole against reference value

Sr. No.	Obeservation	Reported Standard
1.	134°C	134°C-136 °C

3.2.3. *Solubility*

Figure 2 represented the solubility curve of fluconazole in the different kind of solvent. The solubility of Fluconazole was done by using various solvent like distilled water, methanol, ethanol, chloroform, and phosphate buffer pH 7.4. The drug Fluconazole was found practically insoluble in distilled water, the solubility of pure Fluconazole in methanol, ethanol, chloroform, and in phosphate buffer pH 7.4 was found to be 165.00mg/ml, 129.09mg/ml, 106.9mg/ml and 14.45mg/ml respectively. It indicates that the drug is freely soluble in ethanol, methanol as well as sparingly soluble in phosphate buffer pH 7.4.The drug Fluconazole was found practically insoluble in distilled water, the freely soluble in ethanol and methanol, slightly soluble in chloroform, and sparingly soluble in phosphate buffer pH 7.4. The solubility of sample was observed by visual inspection.[15]

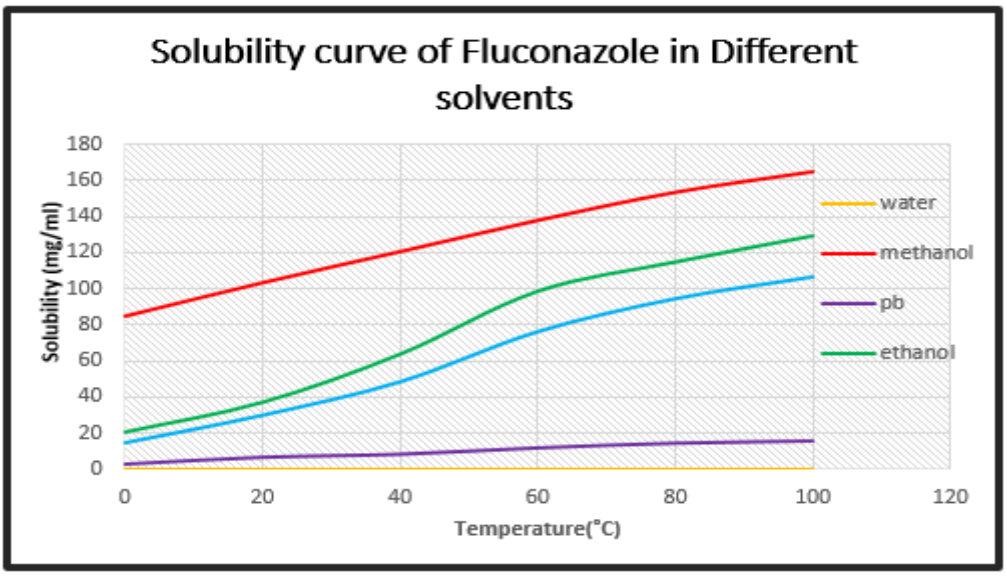

Fig 2: Solubility graph of Fluconazole in different solvent

3.2.4. *Ultraviolet-Visible Spectroscopy Study*

Determination of λ max of Fluconazole in pH 7.4 phosphate buffer

Figure 3 illustrated that the UV spectrum of Fluconazole solution in phosphate buffer pH 7.4 exhibited wavelength of absorbance maximum at 264.75. This is near to the reported value. However, carrying out *In-vitro* drug release studies and considering the predicted theoretical λ max involved, the working λ max was decided as 264nm in phosphate buffer pH 7.4.[16,17]

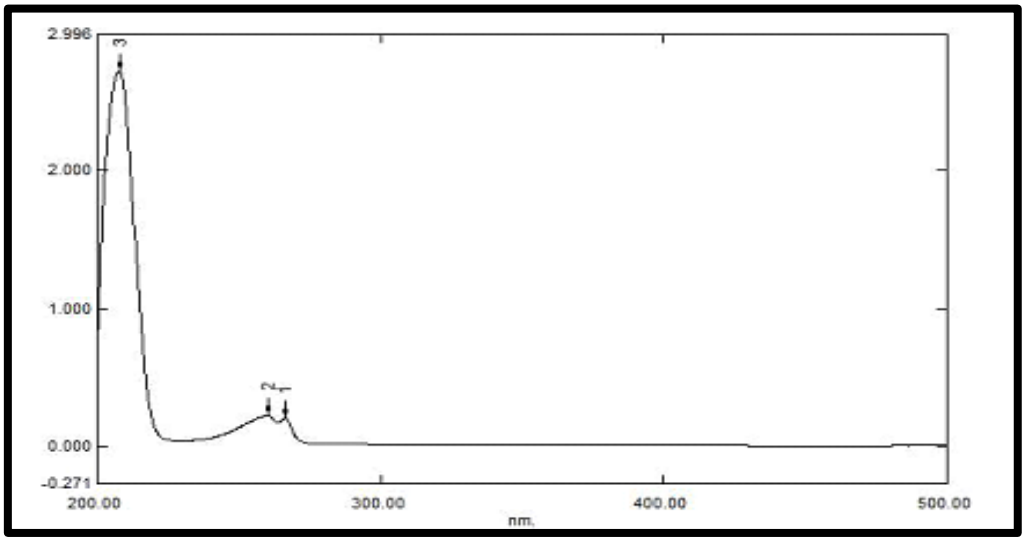

Fig 3: UV absorption spectrum of Fluconazole in Phosphate buffer (pH 7.4)

Construction of Beer's-Lambert's Plot in pH 7.4 phosphate buffer

Figure 4 illustrates that the calibration curve was found to be linear in the concentration range of 2 to 10µ g/ml (Table 3). From that, the coefficient of regression value R^2 =0.9952 and Slope y = 0.0033x - 0.0007 in pH 7.4 phosphate buffer.

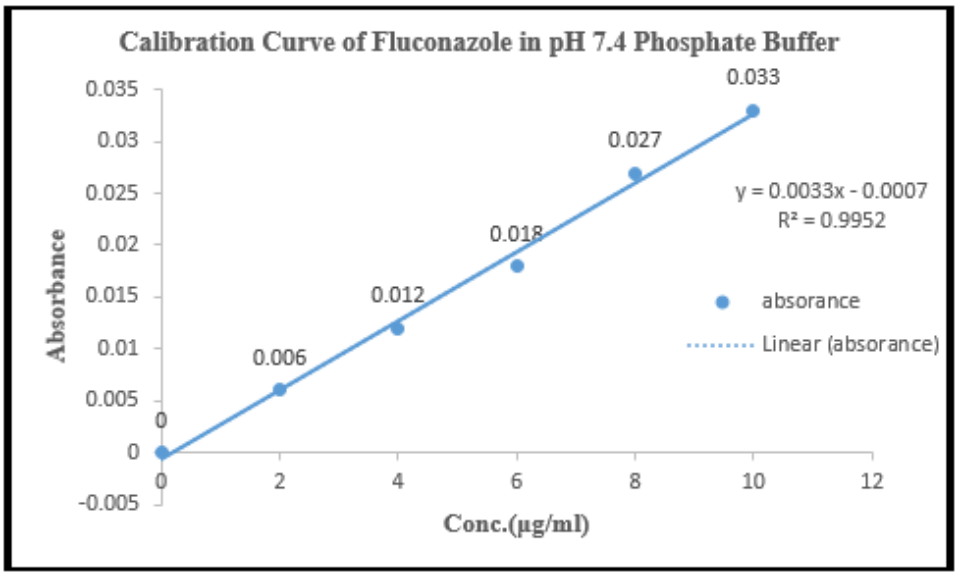

Fig 4: Calibration curve of Fluconazole in Phosphate buffer (pH 7.4)

3.2.5. *Fourier transforms infrared spectrophotometer (FT-IR) studies of Fluconazole*

The powdered mixture of Fluconazole and KBr was taken in a sampler and the spectrum was recorded by scanning in the wavelength region of 4000-700 cm^{-1} using an IR spectrophotometer. The FT-IR spectrum of Fluconazole and the combination of Fluconazole and polymer was shown in (figure 5),(figure 6) and (figure 7) respectively and principle peaks were obtained at wave numbers 3121cm^{-1} for C – H Stretching Aromatic, 1136 cm^{-1} for C- N Stretching, 672 cm^{-1} for C – Cl Stretching, 959cm^{-1} for S-O Stretching . The Fluconazole nanosponges showed peaks at 3121 cm^{-1},1619 cm - 1 ,1500 cm^{-1} , and 1412cm^{-1} which confirmed that there were no changes in drug peak at both spectra. FT-IR spectra of prepared formulation showed there are significant changes in the fingerprint region ,i.e. 600 to 1500 cm^{-1}. This confirmed the formation of a bond between ethyl cellulose and Fluconazole. The presence of absorption bands corresponding to the functional groups present in the structure of Fluconazole confirms the identification and purity of gifted Fluconazole drug.[18]

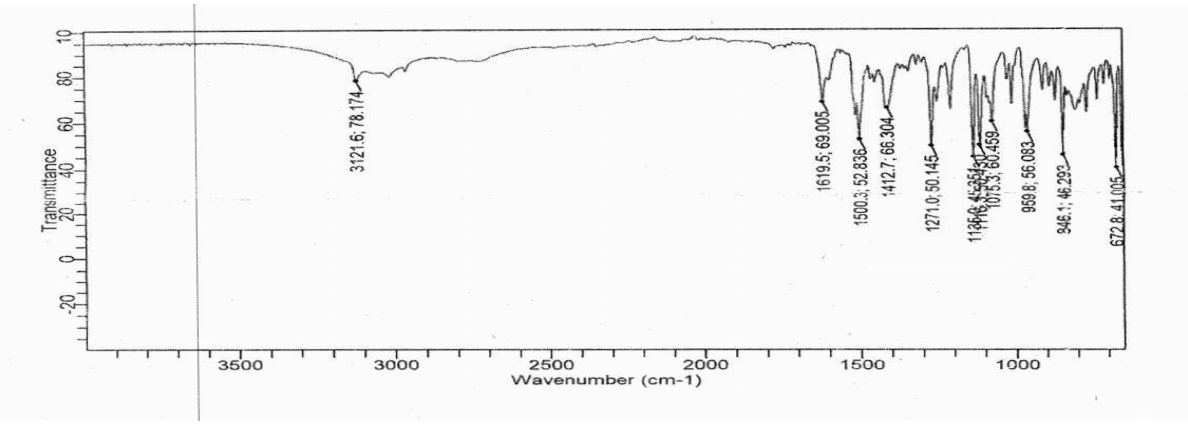

Fig 5: FTIR of Fluconazole pure Drug

In Figure 5, the peaks present in the FTIR spectra of pure fluconazole are present in the FTIR spectra of the optimised batch formulation (F3). The FTIR interpretations indicated that fluconazole is compatible with the excipients ethyl cellulose, eudragit RS 100 and polyvinyl alcohol, and no interactions were observed in all formulations of nanosponges. The principle peaks were obtained at wave number 3121cm^{-1} for C–H Stretching. Aromatic, 1136 cm^{-1} for C–N stretching, 672 cm^{-1} for C–L stretching, and 959 cm^{-1} for S–O stretching.[18]

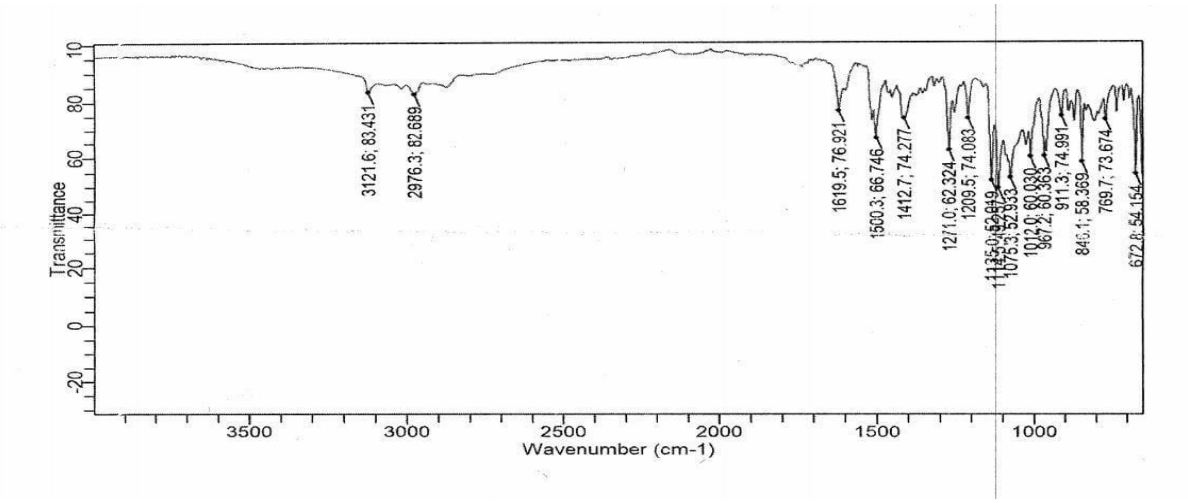

Fig 6: FTIR of Fluconazole and Ethyl cellulose

In Figure 6, the peaks present in the FTIR spectra of fluconazole and ethyl cellulose mixture. are present in the FTIR spectra of the pure fluconazole. The FTIR interpretations indicated that fluconazole is compatible with the polymer ethyl cellulose. The principle peaks were obtained at wave number 1500 cm^{-1} for C–C Aromatic. 1135 cm^{-1} for C–N stretching, 672 cm^{-1} for C–CL stretching.[18]

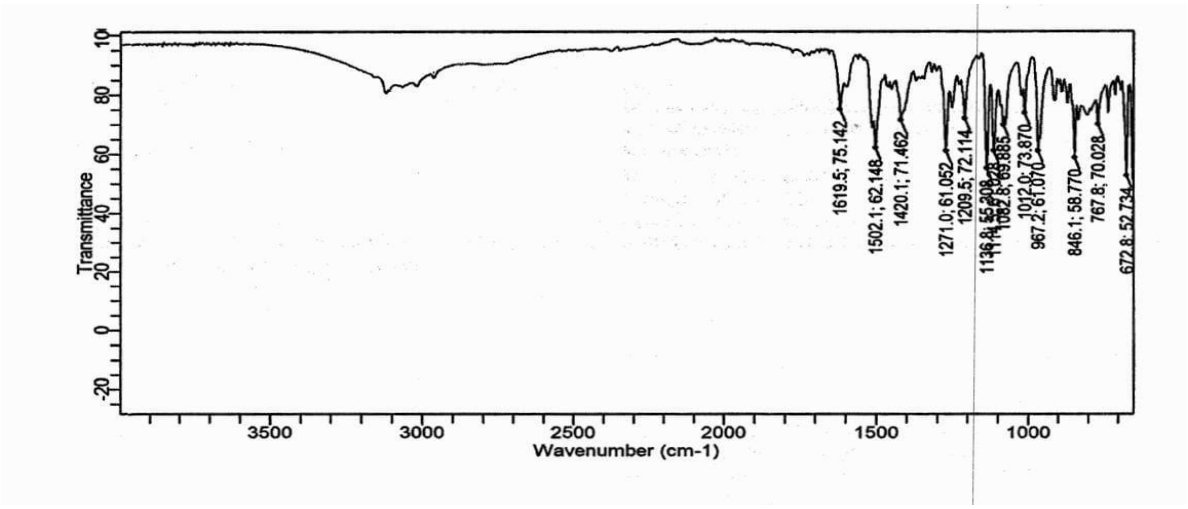

Fig 7: FTIR of Fluconazole and Eudragit RS 100

In Figure 7, the peaks present in the FTIR spectra of pure fluconazole and Eudragit RS 100 mixture. The FTIR interpretations indicated that fluconazole is compatible with the polymers eudragit RS 100.The principle peaks were obtained at wave number 1136 cm^{-1} for C–N stretching, 672 cm^{-1} for C–CL stretching, 1502 cm^{-1} C=O stretching and 967cm^{-1} for S–O stretching.[18]

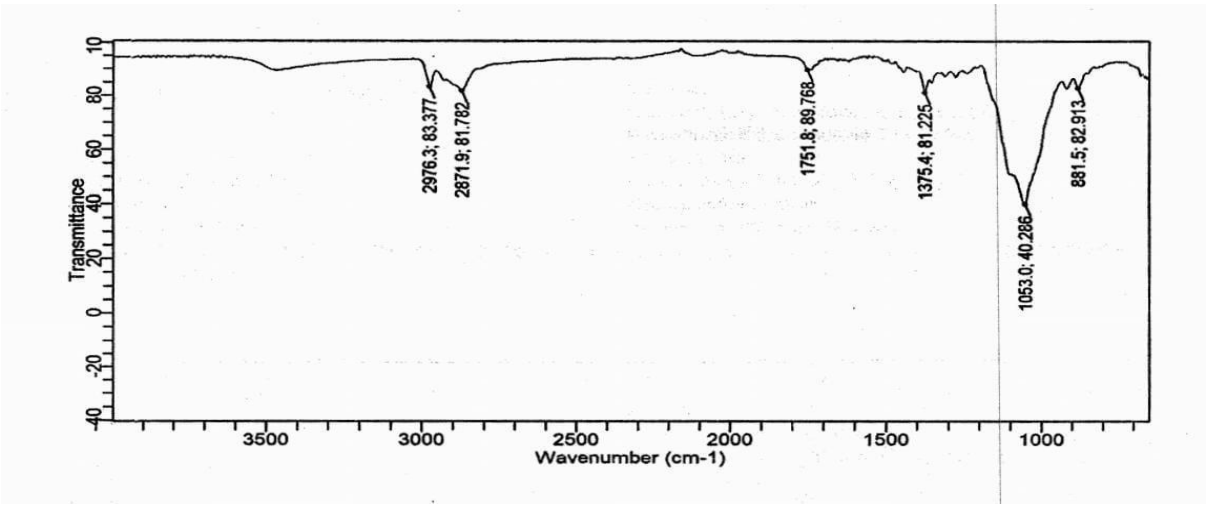

Fig 8: FTIR of Fluconazole Loaded Nanosponge (Batch F3)

In Figure 8, the peaks present in the FTIR spectra of optimised formulation batch F3 of fluconazole loaded nanosponges. The FTIR interpretations indicated that fluconazole nanosponges are compatible with the polymer ethyl cellulose, eudragit rs 100. The principle peaks were obtained at wave number 1335 cm^{-1} for C=C Aromatic. 1035 cm^{-1} for C–N stretching, 2871 cm^{-1} for C–H stretching, and 881 cm^{-1} S-O stetching.[18]

3.3. Evaluation of Fluconazole Loaded Nanosponges

3.3.1. Production yield

Table 5 illustrates the percentage of production yield of all ten batch formulations. The percentage production yield of F1 to F10 batches was observed in a wide range from 57.52% to 94.52%. From this, batch F3 has a good % production yield 94.52%. It was concluded that ethyl cellulose concentration and cross-linking time affects the production yield of nanosponges. The production yield may vary due to the change in polymer concentration.[24]

Entrapment efficiency

Table 5 presents the entrapment efficiency (%) of all formulation batches. The percentage Entrapment efficiency of batches F1 to F10 was in the range from 87.67%±0.03 to 97.97%±0.02. Highest % entrapment efficiency shown in batch F3 was 97.97%±0.02. From this, it was concluded as polymer concentration increases, percentage entrapment efficiency increases. [25]

Table 5: Production yield and Entrapment efficiency

Formulation code	Production Yield (%)	Entrapment Efficiency (%)
F1	91.31%	93.43±0.02
F2	86.70%	94.94±0.04
F3	**94.52%**	**97.97±0.02**
F4	90.26%	94.04±0.01
F5	93.44%	96.76±0.01
F6	57.52%	87.67±0.03
F7	62.36%	90.12±0.04
F8	68.38%	89.43±0.02
F9	75.24%	93.35±0.06
F10	73.25%	90.33±0.07

Figure 9 illustrates the graphical representation of the percentage entrapment efficiency. From that it was clearly observed the entrapment efficiency of the all ten batch formulation of the formulated nanosponges loaded drug batches. In this graphical representation, the x-axis consists of percent entrapment efficiency and the y-axis belongs to formulation batches.

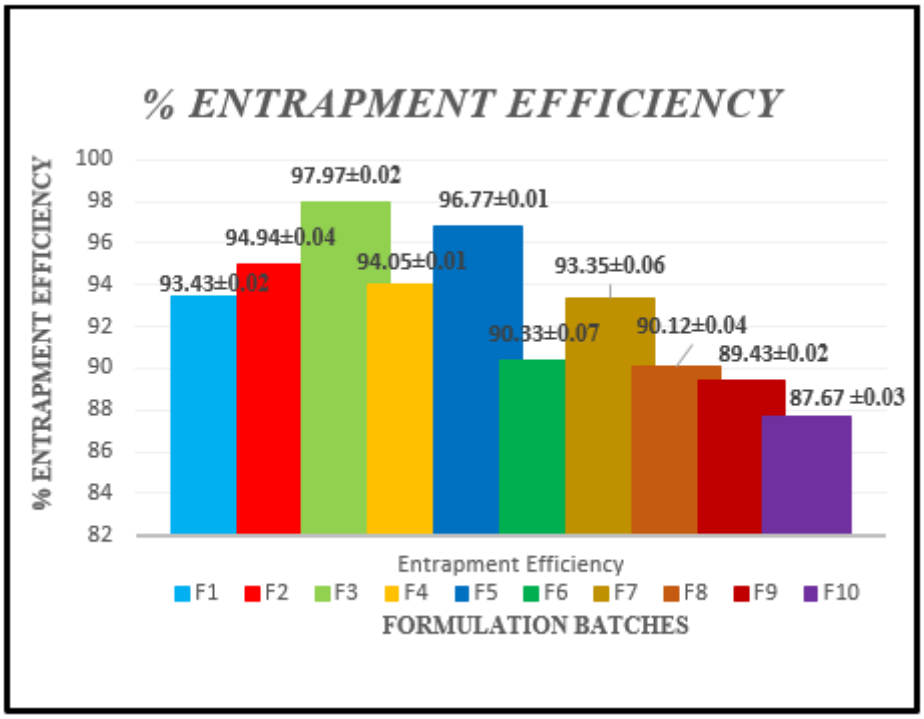

Fig 9: Graphical representation of nanosponge's entrapment efficiency

3.3.2. Particle size

Figure 10 shows the particle size analysis of the fluconazole loaded nanosponges. Particle size analysis was performed by Brookhaven instruments of all batches. The fluconazole nanosponges were successfully prepared by using different concentrations of polymers. The particle size was found to be between 234 nm to 374 nm which is in increasing order due to an increase in the concentration of polymer, but after a certain concentration, the ratio of drug to polymer was increased the particle size decreased. This may be because of the high drug to polymer ratio; the amount of the polymer available was less. Hence, it was concluded that particle size varies with the concentration of the drug-polymer ratio. Hence, the result of the average particle size of optimized batch F3 was observed 234.95nm.[27]

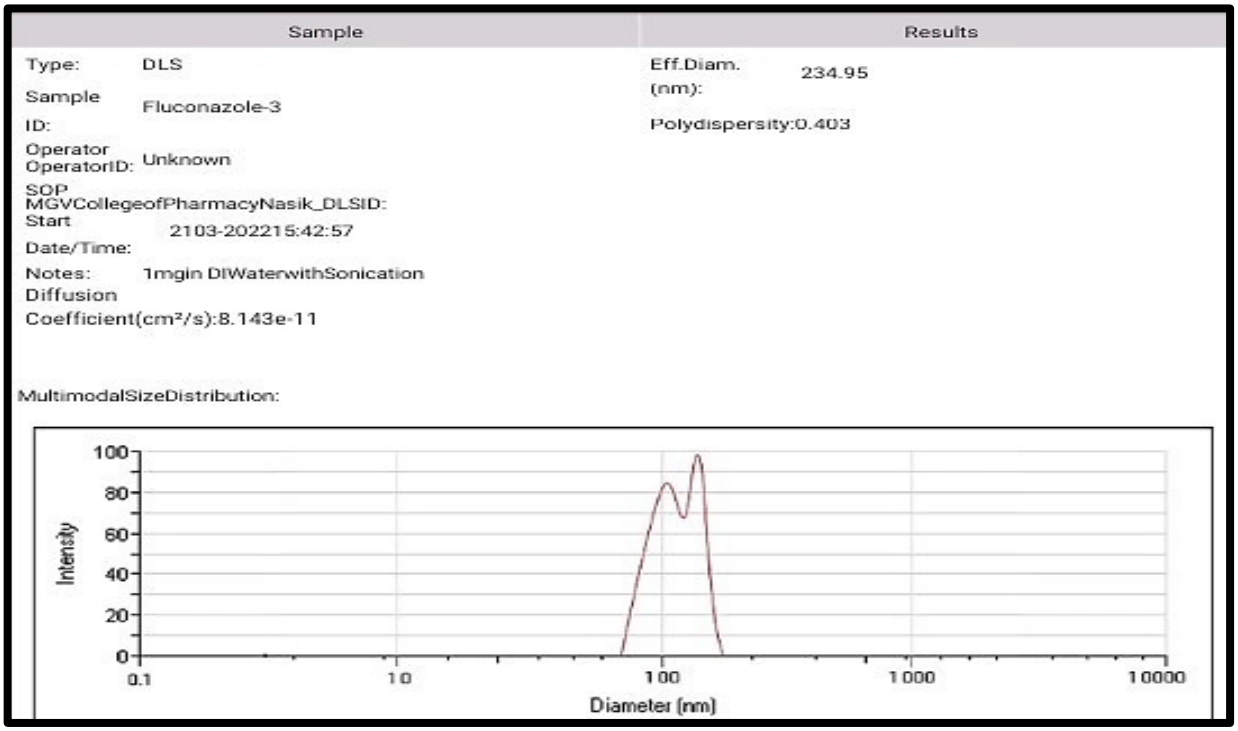

Fig 10: Particle Size of Fluconazole loaded Nanosponge (F3)

3.3.3. *Poly dispersibility index (PDI)*

PDI is an index of width, spread, or variation within the particle size distribution. Monodisperse samples have a lower PDI value, whereas the PDI of the higher value indicates a wider particle size distribution and polydisperse nature of the sample. Table 6 present the nature of nanosponges' formulation for the optimised batch shows mid-range monodisperse. The Polydispersity indices of nanosponges were found to be 0.4. Therefore, it can be stated that the ethyl cellulose-based nanosponges prepared exhibited a homogeneous size distribution.[28]

Table 6: Polydispersibility index according to its type of dispersion

Polydispersity index	Type of dispersity
0-0.05	Monodisperse Standard
0.05-0.08	Nearly Monodisperse
0.08-0.7	Mid- Range monodisperse
> 0.7	Very Polydisperse

3.3.4. *Zeta potential*

The zeta potential was analysed for the determination of the particle movement in an electric field and the particle charge. Zeta potential gives the type of charge present on the surface of the nanosponges and stability of the prepared formulation. Figure 11 shows the zeta potential graph of optimised F3 batch formulation. The zeta potential of the F3 batch is -25.04. The nanosponges of the optimized batch are having moderate stability.[28,29]

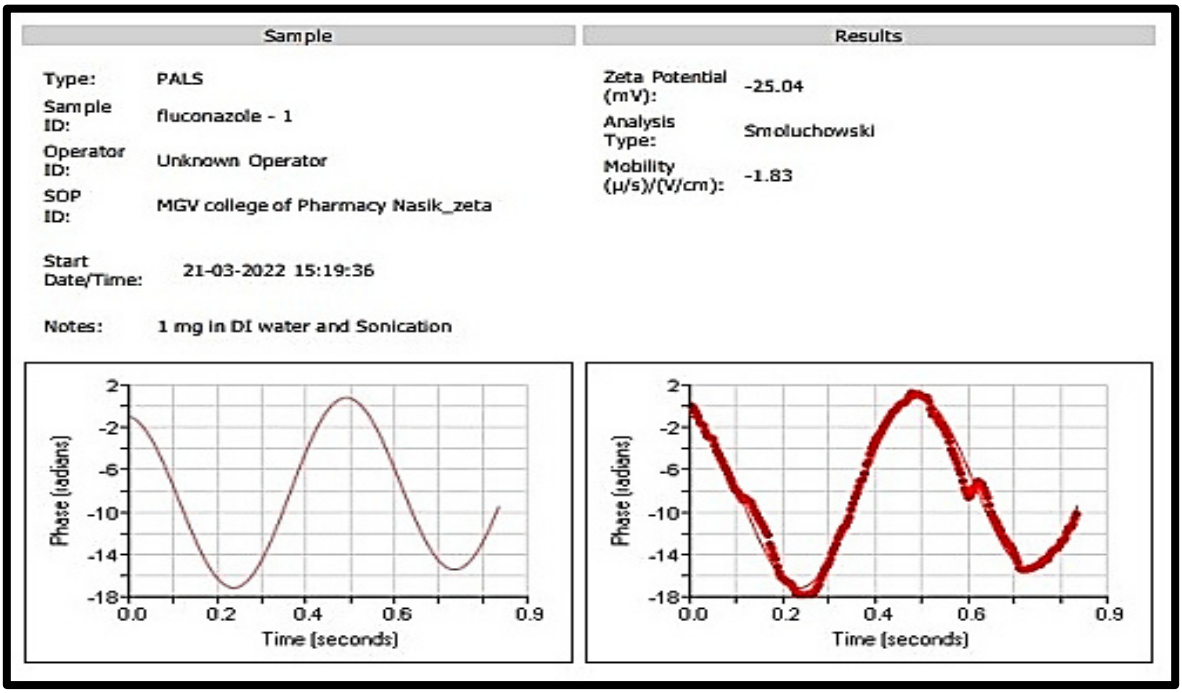

Fig 11: Zeta Potential of Fluconazole loaded Nanosponge (F3)

3.3.5. *Scanning Electron Microscopy (SEM)*

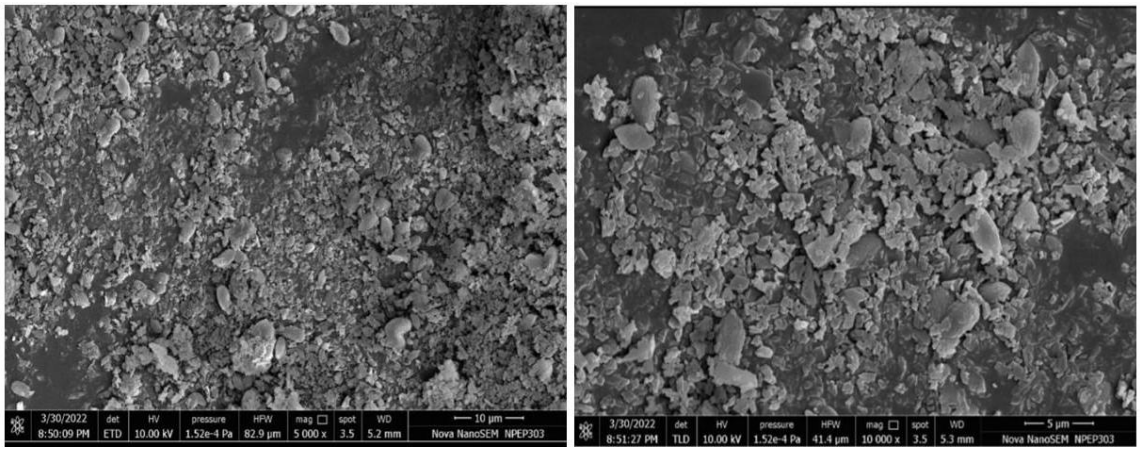

Fig 12: SEM images of pure Fluconazole

Figure 12 present the SEM image of pure Fluconazole at different magnification level. With help of scanning electron microscopy, surface morphology of the pure drug.[28]

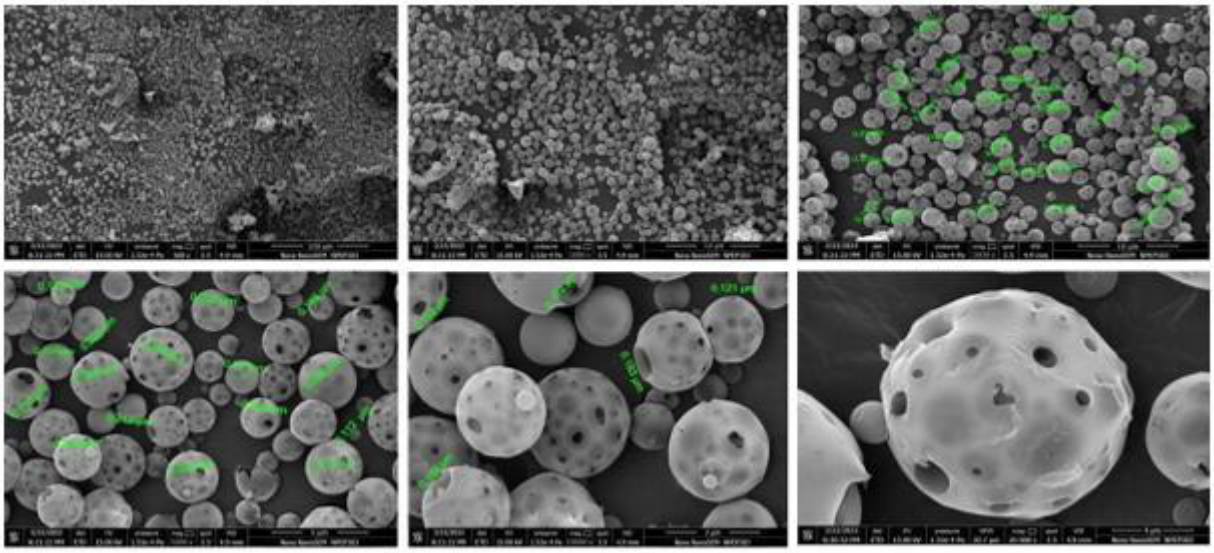

Fig 13: SEM images of Fluconazole loaded nanosponge (F3)

Figure 13 shows the SEM images of the optimised batch (F3) of fluconazole loaded nanosponges. Through Scanning Electron Microscopy, the surface morphology of nanosponges can be studied. From SEM images, it was observed that the optimized batch (F3) of nanosponges were spherical in size with a porous surface and no drug crystals on the surface of the nanosponges. SEM analyses of the formulated fluconazole nanosponges were performed to evaluate the surface morphology of the nanosponges.[28]

3.4. Evaluation of Topical Gel

3.4.1. Physical Evaluation

The topical gel formulations were visually evaluated to derive their appearance, including their homogeneity, color, and consistency. All of the batches have a good appearance and a homogeneous viscosity. Formulated batches were transparent and exhibited a smooth consistency.

3.4.2. pH of topical gel

Table 5 lists the formulation batch pH. The pH of the topical gel formulation was determined by using the pH meter. The pH of all formulations was in a range compatible with the normal pH range of the skin. The pH of all gel formulations was in the range of 6.68–6.82. Hence, the topical gel preparation was non-irritant.

3.4.3. Drug content in topical gel

The prepared formulation was analysed for drug content. It was observed that the drug content in the prepared nanosponges gel was satisfactory and the drug was uniformly distributed in all the formulation.[29],[30] The percentage of drug content was found in the range of 84.34 % to 88.67%. The actual drug content of topical gel was listed in Table 5.

3.4.4. Spreadability of topical gel

Spreadability is an important characteristic of topical formulation and it's responsible for correct dosage transfer to the target site. Spreadability is an important factor to consider in the formulation of gel. Viscosity and spreadability are inversely proportional to each other. The spreadability of prepared nanosponges gel formulation was in the range between 19.73-23.44 gm.cm/sec.[31] The spreadability of topical gel was listed in Table 7.

Table 7: pH, Spreadability and actual drug content of topical gel Evaluation of Topical Gel

Formulation Batch	pH	Spreadability(gm.cm/sec)	Actual Drug Content (%)
C1	6.82	23.44	84.34
C2	6.83	21.42	88.67
C3	6.62	20.83	85.45
C4	6.77	19.73	86.67

3.4.5. Viscosity of topical gel

Table 8 illustrates the viscosity of formulated topical gel. The viscosity of gel was measured by Brookfield viscometer with spindle LV6. The result shows a decrease in viscosity as a shear rate (RPM) is increased which indicates Gel has pseudo plastic flow. The result indicates the viscosity of gel formulation was consistent.[32]

Table 8: Viscosity of formulated topical gel		
Formulation Batch	**RPM**	**Viscosity**
C1	10	16738
	25	8765
	50	6542
	70	4478
	100	2897
C2	10	19564
	25	9747
	50	7612
	70	5679
	100	3456
C3	10	22723
	25	10234
	50	9236
	70	6529
	100	4239
C4	10	26543
	25	14734
	50	9843
	70	7723
	100	5674

3.4.6. *In-vitro* drug release study

Table 9 illustrated the *In-vitro* cumulative drug release of topical gel . *In-vitro* drug release data of formulation C1 to C4 showed that as the concentration of ethyl cellulose increased in nanosponge gel formulation drug release decreased. The in –vitro release of fluconazole from the nanosponges loaded topical formulations containing different concentrations of polymer, carbapol 934 was examined as given in Table 9. The obtained results indicated that the release rate of fluconazole from these nanosponges loaded topical gel formulation. Based on Table 9, at 0.25% w/w,0.5%w/w,0.75%w/w and 1% w/w carbapol 934,the percent amount of drug released after 8 hrs. was 79.12, 74.12, 71.37, 63.45 respectively.[33] Formulated nanosponge gel showed drug release over 8 hours.[32]

Table 9: *In-vitro* cumulative drug release of topical gel batch C1 to C4					
Sr.No.	**Time (hrs.)**	**Formulation Batch**			
		C1	**C2**	**C3**	**C4**
1.	0.	0	0	0	0
2.	1.	28.12	22.05	20.05	16.34
3.	2.	39.16	32.48	29.87	25.99
4.	3.	47.89	42.15	40.34	32.99
5.	4.	58.93	51.98	48.76	41.89
6.	5.	69.45	65.23	62.09	52.23
7.	6.	76.76	71.56	66.46	58.19
8.	7.	79.12	74.62	71.37	63.45
9.	8.	88.34	83.29	79.79	73.46

Figure 14 shown the graphical representation of the *In-vitro* cumulative drug release. In that the y-axis represent the % cumulative drug release and x- axis represent the time (hrs.) ,from that it was discussed that the batch C1 was having the greater the release of drug after that in order to C1 >C2> C3> C4.[33]

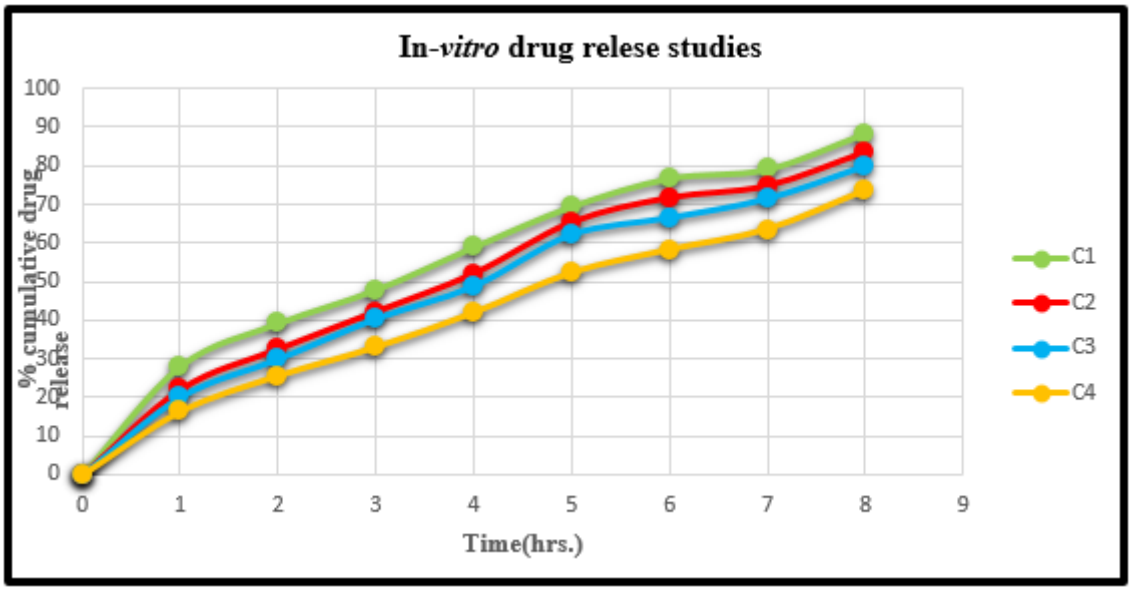

Fig 14: In-vitro cumulative drug release of topical gel batch C1 to C4

4. CONCLUSION

From the above study, it was confirmed that fluconazole loaded nanosponges were prepared by the emulsion solvent diffusion method by using the different kind of polymers. Also, the topical gel preparation of Fluconazole loaded nanosponges successfully developed and evaluated. Formulation batch F3 of nanosponges showed better results among ten nanosponge formulations. Formulation batch F3 showed 97.97 % entrapment efficiency and 94.52% production yield. FTIR spectra of the F3 formulation batch indicated minimum interaction between drug and polymer. The particle size of optimised batch (F3) was found to be 234.95nm. PDI indicated mid-range monodisperse. Zeta potential of the F3 batch is found to be -25.04, which indicates better stability. SEM images confirmed the formation of nanosized spongy and porous nanosponges. The topical gel was prepared from optimized batch formulation F3. Among formulated batches of nanosponge gel batch C2 showed maximum drug content i.e 88.67% with 21.42 gm.cm/sec spreadability. In-vitro drug release showed sustained drug dissolution for up to 8 hours.

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6. AUTHOR CONTRIBUTION STATEMENT

All authors conceived and designed the study. Khanderao Jadhav and Ashish Pawar conceptualized and gathered the data with regard to this work. Ashwini Sanap and Sharayu Rathod performed the research work and discussed methodology. Prashant Malpure, Rishikesh Bachhav contributed in writing final draft of the manuscript.

7. CONFLICT OF INTEREST

Conflict of interest declared none.

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