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FORMULATION AND EVALUATION OF NANOSPONGES LOADED HYDROGEL OF ITRACONAZOLE

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ABSTRACT

The objective of present work was to formulate and evaluate Itraconazole nanosponges using emulsion solvent diffusion technique and nanosponges loaded hydrogel of Itraconazole by using carbopol 934. Itraconazole nanosponges were formulated successfully with different proportions of ethyl cellulose, polyvinyl alcohol and poloxamer 188. The physical characterization of nanosponge formulations showed better entrapment efficiency and production yield. Surface morphology of nanosponges evaluated by scanning electron microscopy showed nanosized spherical particles with numerous pores on surface. The formulations were prepared as hydrogel by using 0.5% w/w carbopol and studied for drug content, pH, spreadability, viscosity and i*n –vitro* release study by using Franz diffusion cell. Optimized batch of nanosponges hydrogel showed better results like drug content 95.6%, pH 7.15, Spreadability 19.61 cm/s, viscosity 12093 cps and *in – vitro release* 96.16%.

Keywords: Itraconazole, nanosponges, carbopol, emulsion solvent diffusion, hydrogel.

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INTRODUCTION

A conventional topical drug delivery suffers some problems such as aesthetically unpleasing, greasiness and stickiness that often leads to lack of patient compliance. In the formulation point of view, uncontrolled drug release, unpleasant odour and incompatibility of drugs with vehicles are main drawbacks faced by Pharmaceutical inventors (1). Thus, there is huge demand for novel drug delivery Systems. Nanosponge drug delivery is one of the potential and promising drug delivery systems to overcome those hurdles. Additionally, it may improve stability, reduced side effect, further it helps to modify drug release in predetermined manner (2). Nanosponges are a new class of materials, made of microscopic particles with few nanometer wide cavities, in which large variety of substances can be encapsulated. This tiny sponge can circulate around the body, they encounter the specific target site and stick on the surface and began to release the drug in a controlled and predictable manner(3). Nanosponges are prepared by four different methods namely, emulsion solvent diffusion method, hypercross-linked β- cyclodextrins, solvent method and ultrasound – assisted synthesis. Itraconazole is a synthetic triazole

antifungal drug for the treatment of superficial and systemic fungal infections. Itraconazole is a selective inhibitor of the fungal cytochrome P450 system and also an inhibition of lanosterol 14-α-demethylase. Inhibition of this enzyme blocks the conversion of lanosterol to ergosterol, which disrupts fungal cell membrane synthesis (4). Itraconazole is available as oral and topical preparations and oral administration of Itraconazole often produces nausea, vomiting, dark urine, rash and there is less patient compliance with long term therapy. Furthermore, oral administration of Itraconazole is reported to interact with a number of co-administered drugs, especially oral hypoglycemic. Thus aim of the present study is to formulate and evaluate Itraconazole nanosponges and also evaluate the *in-vitro* performance of prepared hydrogel.

MATERIALS AND METHODS

Materials

Itraconazole was obtained as a gift sample from Macleod Pharmaceuticals, Mumbai, India. Ethyl cellulose, polyvinyl alcohol, poloxamer 188, dichloromethane were purchased from Modern science, Nashik, India. All other chemicals and solvents used were of analytical grade.

Calibration curve of Itraconazole

Stock solution of Itraconazole was prepared by dissolving 10 mg of accurately weighed amount of Itraconazole in 10 ml of methanol and sonicated for 5 min and then final volume was adjusted to 100 ml with phosphate buffer pH 5.5.

Procedure

Working standard solutions of strengths 2, 4, 6, 8, 10 and 12 µg/ml were made from the stock solution by appropriate dilutions. The above solutions were analyzed by UV spectrophotometer at λ_{max} 255 nm (5). Phosphate buffer was used as blank during spectrophotometric analysis. The standard calibration curve was obtained by plotting absorbance vs. concentration.

Solubility studies

The solubility of Itraconazole was determined in methanol by forming complex with Poloxamer 188 using solid dispersion technique. Solid dispersion of Itraconazole was characterized by saturation solubility study, drug content and FTIR spectroscopy (6).

Preparation of nanosponges by emulsion solvent diffusion method

Itraconazole nanosponges were prepared by different proportions of ethyl cellulose, polyvinyl alcohol and Poloxamer 188 by emulsion solvent diffusion technique. The disperse phase consisting of 100 mg Itraconazole and specified quantity of ethyl cellulose dissolved in 30 ml of dichloromethane was slowly added to a definite amount of polyvinyl alcohol in 100 ml of aqueous continuous phase. The mixture was stirred at 1000 rpm on a magnetic stirrer for two hours. The formed Itraconazole nanosponges were collected by vacuum and dried in an oven at 40^0C for 24 hours (7, 8). Formulation was coded as F1 to F9 respectively (Table-1). The formed nanosponges were evaluated for their physical characteristics, % entrapment efficiency, porosity, FTIR, DSC, SEM analysis, particle size, zeta potential, *in-vitro* release study (9).

Determination of Entrapment efficiency, Percentage yield and Porosity

Itraconazole nanosponges equivalent to 10 mg of drug was dissolved in 10 of methanolic phosphate buffer pH 5.5. Sonicated for 20 minutes, filtered and analyzed concentration from calibration curve data after appropriate dilutions. Entrapment efficiency was calculated as follows.

Entrapment efficiency = Actual drug content in the nanosponges/Theoretical drug content x 100

Percentage yield = Weight of nanosponges/ Total solid weight x 100

Porosity = (Bulk volume – True volume)/ Bulk volume x 100

Table-1 Formula for Itraconazole nanosponges

Formulation Code	Ingredients (mg)						Total Quantity (mg)
	Drug	Polyvinyl Alcohol	Ethyl cellulose	Poloxamer 188	Dichloro-methane	Distilled Water	
F1	100	600	400	200	40	100	1440
F2	100	600	800	200	40	100	1840
F3	100	600	600	200	40	100	1640
F4	100	800	400	200	40	100	1640
F5	100	800	600	200	40	100	1840
F6	100	800	800	200	40	100	2040
F7	100	1000	400	200	40	100	1840
F8	100	1000	600	200	40	100	2040
F9	100	1000	800	200	40	100	2240

Fourier Transform Infrared spectroscopy (FTIR) and Differential Scanning Calorimetric (DSC)

The infrared spectra of Itraconazole nanosponges were recorded by SHIMADZU FTIR Affinity 1S spectrometer, equipped with a inferometer, detector. Samples were prepared by KBr disc method (2 mg sample in 100 mg KBr) and examined in the transmission mode. Each spectrum was measured over a frequency range of 4000–400 cm^{-1}. DSC analysis was performed using Shimadzu-Thermal Analyzer DSC 60 on 2 - 5 mg samples. Sample was heated in an open nitrogen pan at a rate of 10^0C/min conducted over a temperature range of 30 to 400^0C for Itraconazole nanosponges nitrogen flow of 2 bar pressure.

Scanning Electron Microscopy (SEM)

The shape and morphology of nanosponges was examined using Scanning Electron Microscopy (SEM). Sample was deposited on a glass slide, and was kept under vacuum. The samples were coated with a thin gold/palladium layer using a sputter coater unit. The scanning electron microscope was operated at an acceleration voltage of 15kV.

Particle Size and Zeta potential Determination

The average particle size and zeta potential of Itraconazole nanosponges were determined by photon correlation spectroscopy (PCS) using a Nano plus instrument. Sample was diluted 10 times with distilled water and then it was analyzed for particle size and zeta potential.

***In-Vitro* Diffusion study**

In – Vitro release study was performed using USP paddle apparatus at 100 rpm and 37 ± 2^0C in 900 ml of phosphate buffer (pH 5.5). Equivalent weight of formulated nanosponges containing 50 mg Itraconazole was used for each experiment. Samples were withdrawn at appropriate time intervals for half hour for first 3 hours and then hourly for up to 12 hours. The samples were measured spectrophotometrically at 255 nm (10).

Preparation of nanosponge loaded Hydrogel of Itraconazole

In a separate container gel forming polymer Carbopol 934 was taken and to it sufficient quantity of purified quantity was added and allowed to soak for 24 hours. After 24 hours to this remaining ingredients i.e. glycerine as a moistening agent, methyl and propyl paraben as a preservative were added. The methanolic dispersion of Itraconazole nanosponges (1mg/ml) from optimized batch F6 was added to it and made the volume up to 100ml with distilled water. Finally pH of Carbopol 934 was adjusted by adding sufficient quantity of triethanolamine. The formulation was allowed to stand for 24 hours at room temperature. The pH of the hydrogel was maintained to neutral and store in well closed container. [11] Formula for formulation of hydrogel was given in Table-2.

Table-2 Formula for Hydrogel formulation

S. No.	Ingredients	Formulation Code			
		G1	G2	G3	G4
1	Itraconazole (gm)	0.1	0.1	0.1	0.1
2	Carbopol 934 (gm)	0.25	0.5	0.75	1
3	Glycerine (ml)	10	10	10	10
4	Methanol (ml)	5	5	5	5
5	Methyl paraben (gm)	0.1	0.1	0.1	0.1
6	Propyl paraben (gm)	0.05	0.05	0.05	0.05
7	Triethanolamine (ml)	q.s.	q.s.	q.s.	q.s.
8	Distilled water (ml)	Up to 100	Up to 100	Up to 100	Up to 100

The formulated hydrogel should be evaluated for different tests namely, pH, spreadability, viscosity, extrudability, percentage yield, drug content, *in-vitro* release, skin irritation test (12, 13).

Physical properties of the prepared gel

The visual examination, pH and viscosity

The test considered a series of visual characteristics (consistency, colour and homogeneity). The pH of the prepared formulation was measured by dissolving 1 gm of gel in to 100 ml water using digital pH meter. A sample of 1 gm was pressed between 2 slides with 125 gm weights and left for about 1 – the time in seconds needed to separate the two slides is taken as measure of spreadability. Spreadability was determined by using formula.

Spreadability = Weight tied on upper slide x Length of glass slide / Time in seconds

Viscosity of the gel formulation was determined by using Brook-field viscometer, Spindle LV- 61. For determinations of extrudability gel formulations were filled into collapsible metal tube and the excessive weight was applied to extrude material from the tube.

Extrudability = Weight applied in gm / Area in cm^2

Percentage yield = Practical yield / Theoretical yield x 100

Drug Content

Formulation containing 10 mg Itraconazole was transferred into 100ml volumetric flask containing 10ml methanol and sonicated for 20 minutes. The final volume was made upto 100 ml with phosphate buffer pH 5.5 and filtered. From this 1ml of solution is pipette out and further diluted to 10 ml with phosphate buffer pH 5.5. The solution was analyzed by UV- Spectrophotometrically at 255 nm.

$$\text{Drug content} = \frac{\text{Absorbance}}{\text{Slope}} \times \text{Dilution factor} \times \frac{1}{100}$$

In-Vitro **release study**

In – Vitro release studies were performed using modified Franz diffusion cell and Cellophane membrane which is soaked in dissolution medium for 12 hours before carrying out experiment. In modified Franz diffusion cell 0.6 gm of gel was placed in donor compartment of cell. The entire surface of membrane was in contact with the receptor compartment containing 28 ml of phosphate buffer pH 5.5. The receptor compartment was continuously stirred at 50 rpm using magnetic stirrer with temperature maintained at 37 ± 2^0C. The study was carried out for 12 hours, with the interval of half hour for first 3 hours and then hourly for up to 12 hours. The sample was withdrawn at predetermined time interval and same volume was replaced with fresh phosphate buffer. The absorbance of withdrawn sample was measured by spectrophotometrically at 255 nm.

Skin irritation test

Skin irritation test was performed on Swiss albino mice as per CPCSEA protocol. Swiss albino mice (25 – 30gm) 3 male and 3 female were selected for skin irritation test. Test solution (1ml/kg) of optimized hydrogel is applied once to the site where hairs are removed by using depilatory cream. Animals were observed for 7 days to check the skin reddening and inflammation on animal skin (14).

RESULT AND DISCUSSION

Calibration curve of Itraconazole

Calibration curve of Itraconazole was prepared in phosphate buffer pH 5.5 since evaluation studies of formulation were carried out in phosphate buffer. A linear relationship was obtained in between concentration (2-12 µg/ml) and absorbance of Itraconazole in phosphate buffer with R^2 value 0.998 at 255 nm is shown in Figure-1 and Table-3.

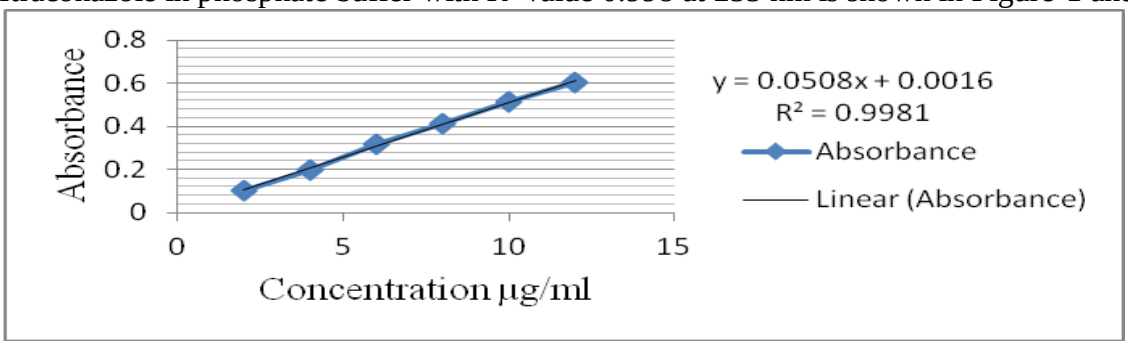

Fig-1 Calibration curve of Itraconazole in phosphate buffer pH 5.5

Table-3 Absorbance of Itraconazole in phosphate buffer pH 5.5

S. No.	Concentration (µg/ml)	Absorbance
1	2	0.099
2	4	0.198
3	6	0.319
4	8	0.412
5	10	0.515
6	12	0.602

Solubility studies

Solid dispersion was prepared using a carrier like poloxamer 188. This carrier was selected for the reason that carrier by virtue of its properties may make a poorly water-soluble drug more soluble than its inherent solubility.

Saturation solubility

Solid dispersion with poloxamer 188 increases solubility of drug 4 times that of single drug. Correlating the solubility data with respect to drug, it was observed that solubility increased with increasing concentration of drug carrier. The increase in solubility of Itraconazole observed with increase in carrier concentration may be attributed to the increase amorphising efficiency of carrier in higher concentrations. Data for saturation solubility study was summarized in Table-4.

Table-4 Saturation solubility data for Itraconazole and carrier combination in methanol

Sr. No.	Itraconazole: carrier combination	Saturated solubility (mg/ml)
1	Itraconazole	0.099
2	Itraconazole: Poloxamer 188 (1:1)	0.110
3	Itraconazole: Poloxamer 188 (1:2)	0.264
4	Itraconazole: Poloxamer 188 (1:3)	0.377

Drug content

The drug content in all the tested combinations was found to be in the range of 98 to 101 % (Table-5). Almost negligible loss was observed probably because preparation of combinations was confined to the very small area of a mortar. Hence, the drug content was close to 100% in most of the cases.

Table-5 Drug content data for Itraconazole and carrier combinations

S. No.	Itraconazole: Poloxamer 188	% Drug content
1	1:1	101.6
2	1:2	100.9
3	1:3	97.8

Fourier Transform Infrared (FTIR) Spectroscopy

The solid dispersion of Itraconazole with poloxamer was subjected to FTIR analysis. IR spectrum was shown in Figure-2 shows peaks observed at different wave numbers and the functional group associated with these peaks. The major peaks were identical to functional groups of Itraconazole. The results indicating that there is no probable chemical interaction between drug and carrier when formed as solid dispersion.

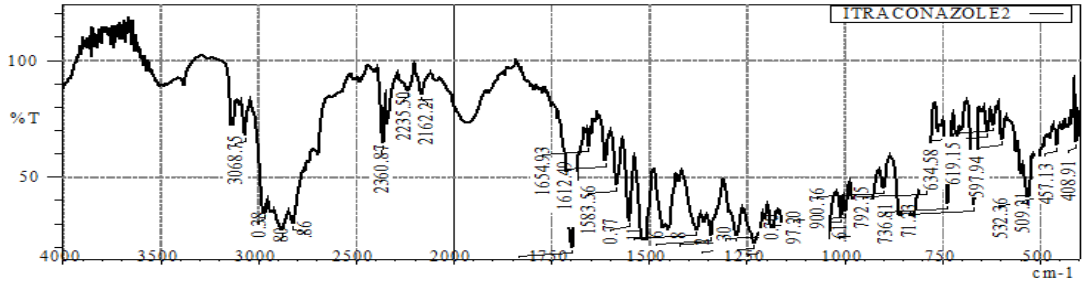

Fig-2 FTIR spectrum of Itraconazole: Poloxamer solid dispersion

Percentage yield, entrapment efficiency and porosity of Itraconazole nanosponges

Percentage yield value of nanosponges was found to be best for F6 (Table-6). Further increasing the concentration polymer the % yield was found to be decreased due to the sticky nature of the product which cannot be filtered. The entrapment efficiency was found to be best for formulation F6. Further increasing concentration of the polymer, entrapment efficiency was found to be decreased due to low solubility of polymer in aqueous phase. Porosity study was performed to check the extent of nanochannels and nanocavities formed. Owing to their porous nature, nanosponges exhibit higher porosity compared to parent polymer used to fabricate the system. Percentage yield, entrapment efficiency and porosity of nanosponges was summarized in Table-6.

Table-6 Percentage yield, entrapment efficiency and porosity of Itraconazole nanosponges

Formulation Code	Percentage yield (%)	Entrapment efficiency (%)	Porosity (%)
F1	43.75	81	10.52
F2	36.95	82.6	13.63
F3	27.43	91.66	10.00
F4	40.24	87.30	16.66
F5	35.32	82.33	07.69
F6	58.82	95.4	11.29
F7	26.08	89.23	05.00
F8	34.80	89.6	05.55
F9	34.37	91.58	03.33

Fourier Transform Infrared spectroscopy (FTIR) and Differential Scanning Calorimetric (DSC)

FTIR spectra of pure Itraconazole (Figure-3) demonstrated the characteristic absorption peaks of 3128 cm^{-1} for aromatic H stretching, at 1697 cm^{-1} C=N stretching, 669 cm^{-1} for aromatic chloride, 1557 cm^{-1} for C=C stretching. Itraconazole nanosponges also showed almost similar absorption peaks indicates good compatibility with polymers.

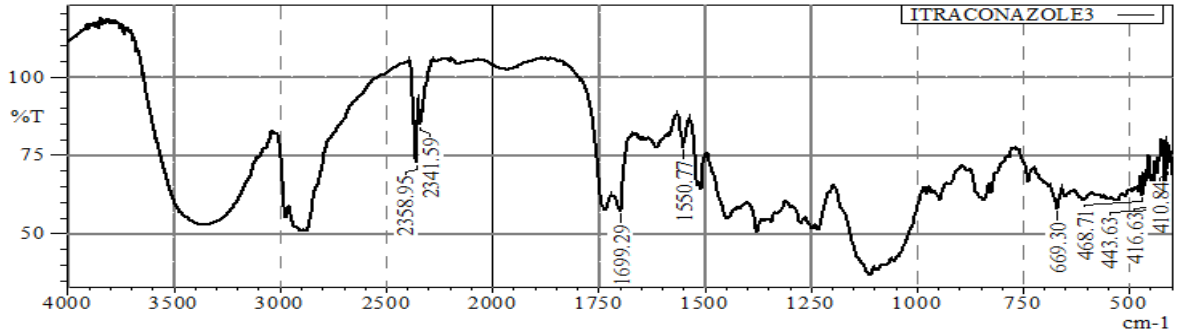

Fig-3 FTIR spectrum of Itraconazole nanosponges

DSC thermogram of pure Itraconazole (Figure-4) shows sharp peak at 164.82^{0}C corresponding to its melting point. Itraconazole nanosponges showed a similar endothermic peak at 166.28^{0}C which confirms no polymer drug interaction.

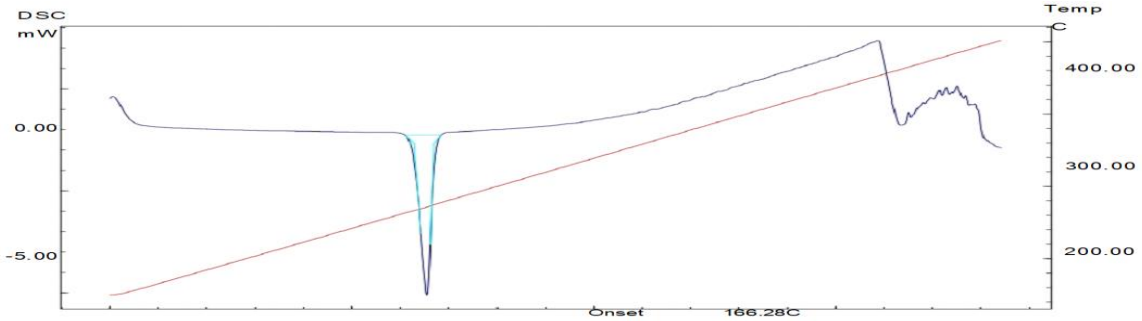

Fig-4 Thermogram for Itraconazole nanosponges

Scanning electron microscopy (SEM)

SEM analysis revealed that nanosized spherical particles with numerous pores on surface (Figure-5). The pores are tunneled inwards which may be due to diffusion of dichloromethane from the surface of nanosponges (15).

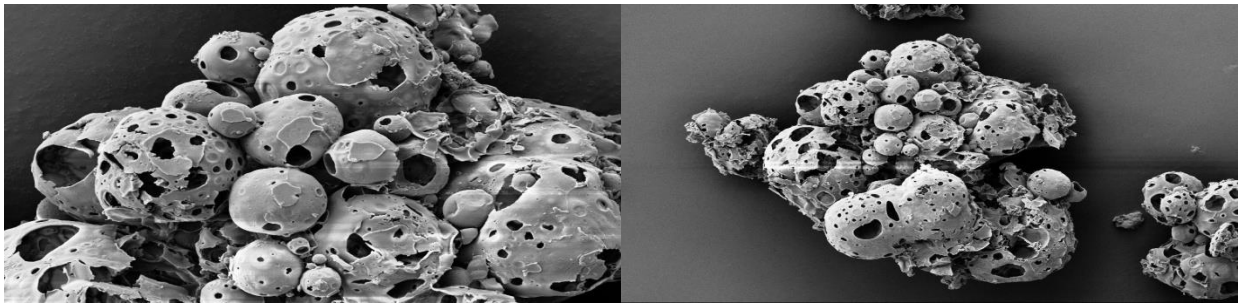

Fig-5 SEM images of Itraconazole nanosponges

Particle size, Zeta potential and Polydispersity determination

The particle size, zeta potential and polydispersity index (PDI) of the nanosponges of optimized formulation F6 was found to be in the range of 2 nm - 95 nm, 52.51 mV and 0.004 respectively. Drug delivery from nanosponges works best when size is below 100 nm (16). In current study 2-95 nm size nanosponges were formulated.

Zeta potential in range of 40 ± 60 mV indicating that the good stability behaviour of the nanosponges of optimized formulation batch F6. Also the PDI 0.05 – 0.08 indicate that the formulation is nearly monodisperse. In current study optimized formulation batch F6 have PDI 0.04 indicating that the formulation was nearly monodisperse. Data for particle size, zeta potential and PDI was shown in Figure-6 & 7 and Table-7.

Table-7 Table for Particle size, Zeta potential and Polydispersity index

S. No.	Parameter	Observed	Reference
1	Particle size	2-95 nm	< 100 nm
2	Zeta potential	52.51 mV	40 ± 60 mV
3	Polydispersity index	0.04	0.05 – 0.08

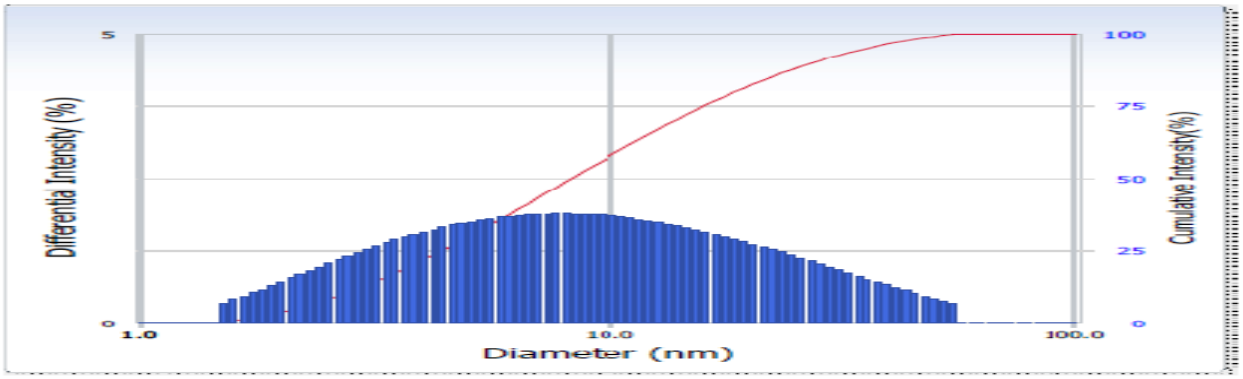

Fig-6 Particle size distribution of optimized formulation batch F6 of Itraconazole nanosponges

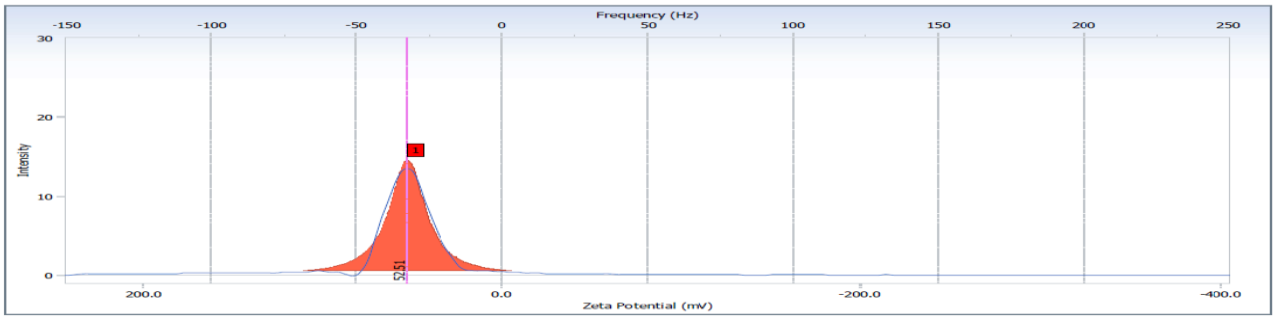

Fig-7 Zeta potential measurement of optimized formulation batch F6 of Itraconazole nanosponges

***In-Vitro* release study**

In – Vitro release study of Itraconazole nanosponges were carried out in duplicate for formulation batches F6 and F9, to determine the optimized formulation batch of Itraconazole nanosponges. Evaluation of *In – Vitro* release study gives the information about release pattern of the nanosponges. Results shown in Table-8 indicate that the drug release from nanosponges was controlled and in predictable manner.

Table-8 Table for *In – Vitro* release study of Itraconazole nanosponges

S. No.	Formulation batch	% Drug release
1	F6	96.18
2	F9	92

Evaluation of nanosponges loaded hydrogel of Itraconazole

The formulated nanosponges loaded hydrogel of Itraconazole were evaluated for Percentage yield, drug content, pH, spreadability, viscosity, *in-vitro* drug release, extrudability and skin irritation test. Data for all these test were summarized in Table-9.

Table-9 Evaluation of Nanosponges loaded Hydrogel formulation G1 to G4

Batch code	Percentage yield (%)	Drug Content (%)	pH	Spreadability	Viscosity (Cp)	% Drug release	Extrudability (gm/cm^2)
G1	88.7	88.8	7.02	14.57	12104	90.54	9.1
G2	89.8	95.6	7.15	19.61	12093	96.16	12.3
G3	90.0	94.2	6.87	24.38	12012	89.89	11.55
G4	93.2	92.4	6.88	31.8	12023	92.16	10.70

All batches of prepared hydrogels were evaluated for the different parameters as shown in above Table. From the results it is clearly evident that all the hydrogel formulations showed good gelling property and homogeneity. The pH of all formulations was in the range compatible with normal pH range of the skin. The drug content release was also found to be moderate releases drug with in optimum range of time period (Figure-8). The rheological behaviors of the hydrogel formulations were studied with Brookfield viscometer. The result indicates the viscosity of hydrogel formulation was consistent. A comparative study of viscosity and spreadability showed that with increase in viscosity of the formulation, the spreadability decreased and vice versa. The FTIR spectra of optimized batch hydrogel formulations did not show the presence of any additional peaks for new functional groups. The major peaks of the drug remain unchanged in the mixture were observed in FTIR spectra, the formulation batch G2 reveals all essential peaks of Itraconazole which confirm no drug excipient interaction (Figure-9).

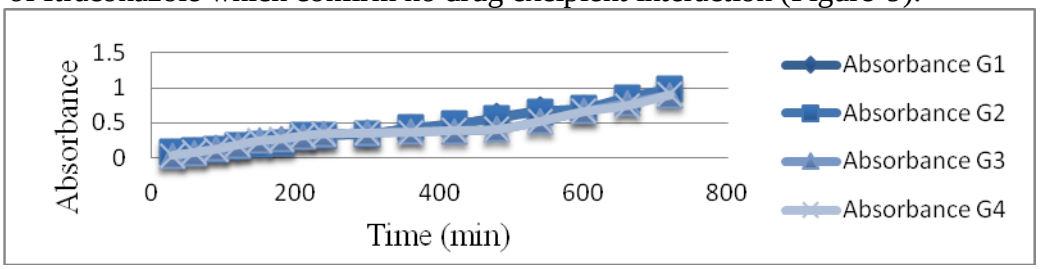

Fig-8 Graph of percent drug release for formulation batches G1 toG4

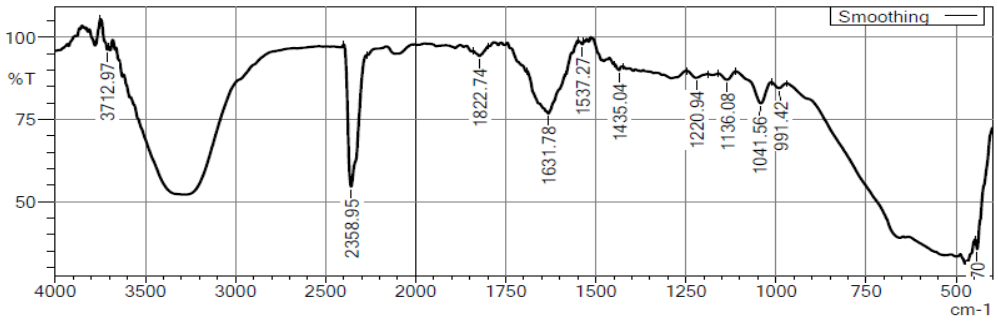

Fig-9 FTIR spectrum of optimized formulation batch G2

Extrudability

Extrudability test is based upon the determination of weight required to extrude 0.5 cm ribbon of Hydrogel in 10 sec from lacquered collapsible aluminum tube. The test was performed in triplicate and the average values were calculated.

Skin irritation test

Skin reddening or inflammation on the animal skin was not observed when skin irritation test is performed, which is indicative that the prepared formulation is safe to apply on skin. Results for skin irritation test are shown in Figure-10 & 11.

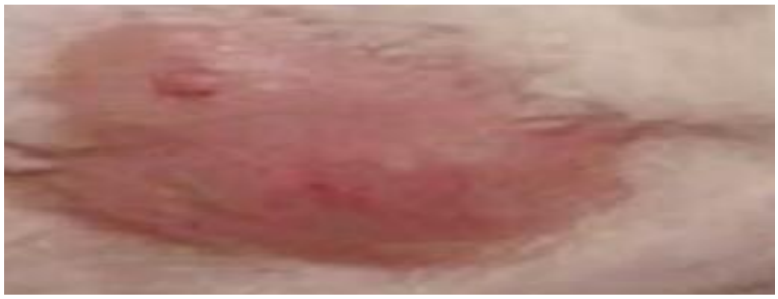

Fig-10 Skin irritation observation at 24 hours

Fig-11 Skin irritation observation at 84 hours

CONCLUSION

In the present work solubility enhancement of Itraconazole with polymer poloxamer 188 was investigated. The solid dispersions were prepared in various ratios of drug to polymer. Saturation solubility analysis aided the selection of optimized ratio of solid dispersion (SD) form carriers used. After drug content analysis studies of nanosponges it was found that almost 81 % to 95.5 % of drug was incorporated in the nanosponges indicated almost uniform dispersion of drug. FTIR studies of nanosponges not showed shifts in principle peaks of drug in nanosponges, suggesting the absence of interaction between drug and polymers. DSC studies of nanosponge shows a sharp endothermic peak at 166.28^0C which is characteristic peak of Itraconazole. Hydrogel was evaluated for percentage yield, drug content, pH, spreadability, viscosity, *in vitro* drug release, extrudability, FTIR, skin irritation test. Dissolution profile of formulated hydrogel of optimized batch indicated the drug release in controlled and predictable manner up to 12 hours is 96.16%.

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