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Formulation and Evaluation of Antifungal Nanosponge Loaded Hydrogel for Topical Delivery

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ABSTRACT

The objective of the present study was to formulate Clotrimazole nanosponges for topical delivery. Nanosponges using ethyl cellulose polymer were prepared successfully using PVA as surfactant by an emulsion solvent diffusion method. The obtained nanosponges have been evaluated for physicochemical characteristics and *in vitro* release studies. Particle size analysis and surface morphology of nanosponges were performed. The scanning electron microscopy of nanosponges showed that they were spherical in shape. The prepared nanosponges were formulated to hydrogels using carbopol 934 as a gelling agent and studied for pH, spreadability and in vitro drug release. Of the various formulations prepared, F4 was found to show maximum drug release. Overall this study resulted in porous nature of nanosponges which provides a channel for the release of the drug and the method is quick and reproducible.

INTRODUCTION

Efficacy of a drug can be altered by the method of drug delivery into the body. Some drugs have an optimum concentration range within which maximum benefit is derived, and concentration above or below this range can produce toxic or no therapeutic benefit. The aim of Novel drug delivery system is to provide a therapeutic amount of drug to the appropriate site in the body to accomplish promptly and maintain the desired drug concentration[1].

Topical preparations are used for the localized effects at the site of their application by virtue of drug penetration into the underlying layers of skin or mucous membranes. The main advantage of the topical delivery system is to bypass first pass metabolism. Avoidance of the risks and inconveniences of intravenous therapy and of the varied conditions of absorption, like pH changes, the presence of enzymes, gastric emptying time are another advantage of topical preparations[2].

Nanosponges are a tiny mesh-like nanoporous particular structure in which a large variety of substances can be capsulated or suspended, and then be incorporated into a dosage form. Nanosponges are more like a three- dimensional network or scaffold. The predictable release is one of the major advantages of this system compared to other nanoparticle delivery systems under development[3,4].

Clotrimazole is a topical, azole group of synthetic fungistatic agents with a broad spectrum of activity based on the imidazole or triazole nucleus. The primary mechanism of action of Clotrimazole is against the division and growing of fungi. It is used in the topical treatment of tinea infection like ringworm; 60-100% cure rates are reported with 2-4 weeks of application an, a twice-daily schedule. It is also effective in skin infection caused by corynebacteria**.** In order to improve solubility, dissolution and sustain the release of Clotrimazole. It was proposed to prepare nanosponges of Clotrimazole and incorporate them in a suitable gel base.

The proposed work involves the formulation of Clotrimazole nanosponges using emulsion solvent diffusion method. The prepared nanosponges will be loaded into a hydrogel base.

MATERIALS AND METHODS

MATERIALS

Clotrimazole was supplied from Yarrow Chem Products, Mumbai. All other excipients and solvents used were of the analytical pharmaceutical grade.

METHODS

Compatibility studies using FT-IR Spectroscopy

The pure drug, drug and polymer were prepared and scanned from 4000-400 cm^{-1} in FTIR spectrophotometer. The results are shown in fig 1 and 2.

Preparation of standard calibration curve[5]

A stock solution of Clotrimazole was prepared by dissolving the required amount in ethanolic PBS 7.4. Standard solutions of the analyte (2-12µg/ml) were prepared by serial dilution of the stock solution. The absorbances of resultant solutions were measured at 261nm by UV spectrophotometer. A graph of concentration Vs absorbance was plotted.

Preparation of Clotrimazole nanosponges by emulsion solvent diffusion method[6]

The organic phase consists of the accurately weighed amount of Clotrimazole and ethyl cellulose dissolved in dichloromethane. The aqueous phase which consists of polyvinyl alcohol dissolved in warm water was used as the emulsifying or stabilizing agent. The organic phase was gradually added into an aqueous phase and stirred mechanically at 1200 rpm for 2 hrs at room temperature to remove the solvent dichloromethane from the mixture. Nanosponges formed were filtered and dried at room temperature and stored in a tightly closed container.

Table 1: Composition of Clotrimazole nanosponges by emulsion solvent diffusion method

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
Clotrimazole (mg)	100	100	100	100	100	100	100	100
Ethyl cellulose (g)	0.15	0.2	0.25	0.3	0.15	0.2	0.25	0.3
Dichloromethane (ml)	20	20	20	20	20	20	20	20
Polyvinyl alcohol (% w/v)	0.2	0.2	0.2	0.2	0.3	0.3	0.3	0.3

Preparation of Clotrimazole nanosponges loaded hydrogel

Accurately weighed amount of carbopol 934 was taken and soaked in water for 24hrs for complete swelling of the polymer. To the weighed amount of carbopol gel base, Clotrimazole nanosponges equivalent to 1 %w/w were uniformly dispersed. Propylene glycol was added as a penetration enhancer. Methylparaben and propylparaben were added as a preservative. Triethanolamine was added dropwise with gentle stirring using a homogenizer for adjusting the pH. The drug-loaded plain gel was also prepared in the same way using drug instead of nanosponge formulation.

Table 2: Preparation of Clotrimazole loaded nanosponge

Ingredients	Quantity
Clotrimazole nanosponges	1% w/w
Carbopol 934	1 % w/v
Propylene glycol(ml)	1
Methylparaben(g)	0.02
Propylparaben(g)	0.01
Triethanolamine	q.s

EVALUATION STUDIES OF PREPARED NANOSPONGES

Particle size analysis[7]

The particle size of Clotrimazole nanosponges was determined using a laser light diffractometry or Malvern Zeta sizer. From this, the mean diameter can be measured. Measurements were made at the fixed angle of 90° for all the samples. The samples were suitably diluted with distilled water for every measurement.

Scanning electron microscopy

For the evaluation of the surface morphology of nanosponges, the sample was analyzed in a scanning electron microscope after preparing the sample by lightly sprinkling on a double adhesive tape stuck to an aluminum stub. The stubs were then coated with platinum. The stub containing the coated sample was placed in a scanning electron microscope. The samples were then randomly scanned and photomicrographs were taken at the acceleration voltage of 20 kV. From the resulting image, average particle size was determined.

Production yield (%)

For calculating production yield, the theoretical mass was calculated initially by taking the mass of solid ingredients added. All the prepared nanosponge formulations were accurately weighed and the weight was recorded. The production yield of the nanosponges was then determined using the following equation:

$$\text{Production yield (\%)} = \frac{\text{Practical mass of nanosponges} \times 100}{\text{Theoretical mass (polymer + drug)}}$$

Drug entrapment efficiency (%)[8]

The accurately weighed quantity of prepared Clotrimazole nanosponges was taken and crushed in a mortar and pestle. Added 5 ml of ethanol and contents in the mortar are transferred to a 100 ml standard flask and made up to the volume with PBS. Kept aside for 1hr with frequent shaking for extracting the drug from the nanosponges. Then it is filtered and the absorbance of the filtrate was measured at 261 nm after suitable dilutions.

The drug content was calculated from the calibration curve and expressed as actual drug content in nanosponge. The drug entrapment efficiency (%) of the nanosponges was calculated according to the following equation:

$$\text{Drug entrapment efficiency (\%)} = \frac{\text{Experimental drug loading} \times 100}{\text{Theoretical drug loading}}$$

EVALUATION STUDIES OF PREPARED NANOSPONGE LOADED GEL

Physical Examination

Gels should have a pleasant appearance with respect to color, consistency etc. The prepared nanosponge loaded hydrogels were inspected visually for their color, homogeneity, and consistency.

Determination of pH

The pH of the prepared nanosponge loaded hydrogel formulations were determined by using a digital pH meter. One gram of gel was dissolved in 100 ml of distilled water and stored for two hours. Then, pH measurement was performed. The measurement of pH of each formulation was done in triplicate and average values were calculated.

Drug content[10] (%)

1 g of prepared Clotrimazole nanosponge loaded hydrogel formulation containing drug equivalent to 100 mg was extracted with 30 ml of ethanol. The volume was made up to 100 ml with phosphate buffer saline 7.4. The solution was filtered. The absorbance of the resulting solution was measured at 261 nm using a UV spectrophotometer after suitable dilutions. The drug content of the drug-loaded plain gel was also determined in the same manner. The drug content of the formulation was determined using the following equation:

$$\% \text{ Drug content} = \frac{\text{Actual concentration of drug in the formulation x } 100}{\text{Theoretical concentration of drug}}$$

Skin irritancy study

Skin irritation test was performed for the final nanosponge gel formulations on human volunteers to find out any irritation problems which could make it unsuitable for topical use. About 1 g of final formulation to be tested was applied to the sensitive part of the skin (like wrist portion of the hand). The site of application was inspected for irritancy, erythema, and edema.

Spreadability studies[12]

Spreadability is a term expressed to denote the extent of the area to which the gel readily spreads on application to the skin. The therapeutic efficacy of a semisolid formulation also depends on its spreading value.1 g of the formulation was placed within a circle of 1cm diameter pre-marked on a ground glass slide. The gel formulation was sandwiched between this slide and the second slide having the same dimension. A weight of 500 g was allowed to rest on the upper glass slide for 5 min. The increase in the diameter due to gel spreading was noted. The spreadability was then calculated from the following formula:

$$\text{Spreadability} = M \times L/T$$

Where, M = mass in grams

L =distance traveled by gel

T =time taken in seconds

***In vitro* drug release studies**

In vitro release study of Clotrimazole nanosponges, the loaded hydrogel was carried out by using Franz diffusion cell. The formulation was taken in the donor compartment and phosphate buffer saline was taken in the receptor compartment. The cellophane membrane previously soaked overnight in the diffusion medium (PBS 7.4) was placed between the donor and receptor compartment. 1 g of the formulation was spread uniformly on the cellophane membrane, which is in contact with the receptor medium. The whole assembly was placed on the thermostatically controlled magnetic stirrer with continuous stirring and the temperature of the medium was maintained at 37± 0.5^{o}C. At specific intervals, 1 ml of sample was withdrawn from the receptor compartment and replaced with an equal volume of PBS 7.4. The *in vitro* drug release of drug loaded nanosponge hydrogel was compared with drug-loaded plain gel. After suitable dilutions, the absorbance of the sample was determined at 261 nm by UV-visible spectrophotometer.

Kinetics of *in vitro* drug release[13]

The results obtained from *in vitro* release studies were attempted to be fitted into various mathematical models as follows:

1. Cumulative percent drug released Vs. Time (Zero order kinetics)

2. Log cumulative percent drug retained Vs. Time (First order kinetics)

3. Cumulative percent released Vs. The square root of Time (Higuchi model)

Log cumulative percent drug released Vs. Log Time (Korsmeyer-Peppas model)

Peppas model, the value of 'n' characterizes the release mechanism of the drug as described in table 2

Table 3: Interpretation of diffusional release mechanism

Release exponent (n)	Diffusion release mechanism
0.45	Fickian diffusion
0.45 <n<0.89	Anomalous(Non-Fickian) diffusion
0.89 - 1.0	Case II transport (Zero order release)
>1.0	Super case II transport

Viscosity

The viscosity of the formulations was determined using Brookfield viscometer with small sample adapter, spindle no. 64. Speed was increased from 10 to 100 rpm and viscosity was noted on cps.

Evaluation of antifungal activity[15]

The microorganism used in this study was fungus *Candida albicans*.

Disk Diffusion Method

An antimicrobial assay was performed by using the Kirby-Bauer disk diffusion agar plate method. Agar plates were prepared by pouring freshly prepared agar medium to the sterilized Petri dishes after autoclaving. The microbial suspension of *Candida albicans* was applied onto the solidified agar by using sterile cotton swabs and allowed to dry for 10 minutes. Formulated gel containing drug loaded nanosponges impregnated discs were aseptically transferred onto the inoculated agar plates and left to be incubated for 2 days. The clear zones of inhibition around the test sample disc were shown for any indication of antimicrobial activity. All assays were carried out in triplicate.

Stability studies[16]

Stability testing plays a crucial role in the drug development process. The purpose of stability testing is to provide evidence on how the quality of drug product varies with time under the influence of different environmental factors, such as temperature, humidity, and light to recommend shelf life for the drug product and recommended storage conditions. Stability studies were carried out on the optimized formulation according to ICH guidelines. The optimized formulation was packed in a tightly closed container and was stored in the ICH certified stability chamber maintained at 40 ± 2^{o}C and 75% ± 5% RH for one month. The formulation was evaluated before and after at periodic intervals for change in appearance, pH, drug content and *in vitro* drug release.

RESULTS AND DISCUSSION:

Compatibility studies using FT-IR Spectroscopy

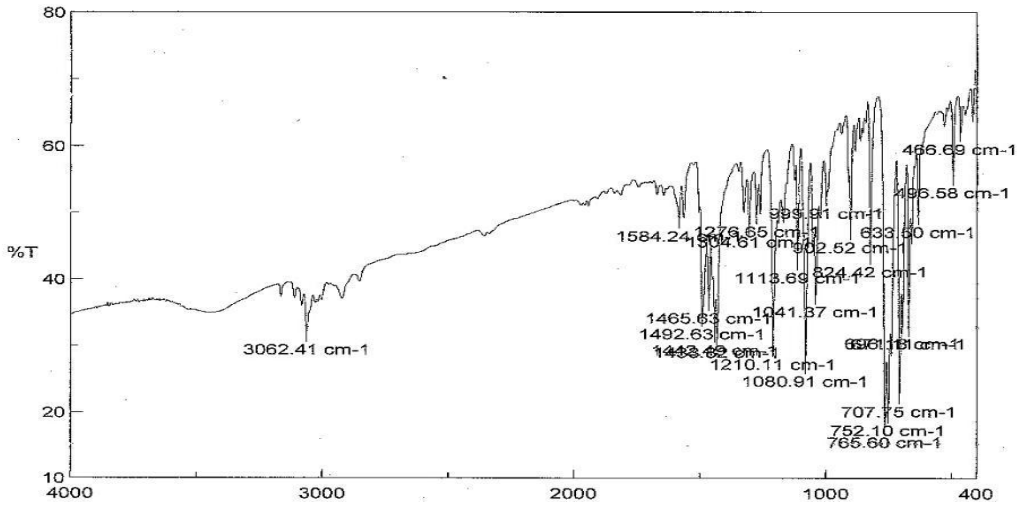

Figure 1: FTIR spectra of drug

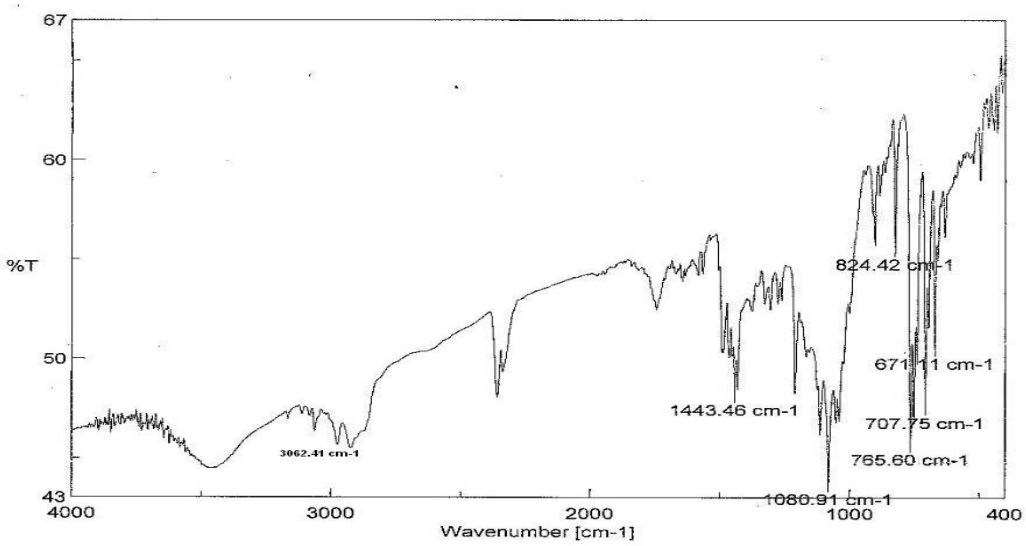

Figure 2: FTIR spectra of the drug with an excipient

After the compatibility study of Clotrimazole with excipients, the IR spectra of pure drug and drug-excipient physical mixture were analyzed. Fig 1 and 2 indicate no interaction between drug and excipients when compared with spectra of the pure drug as all functional groups were present.

Preparation of a standard calibration curve of Clotrimazole

The calibration was found to be linear in the concentration range of 2-12 µg/ml at 261 nm

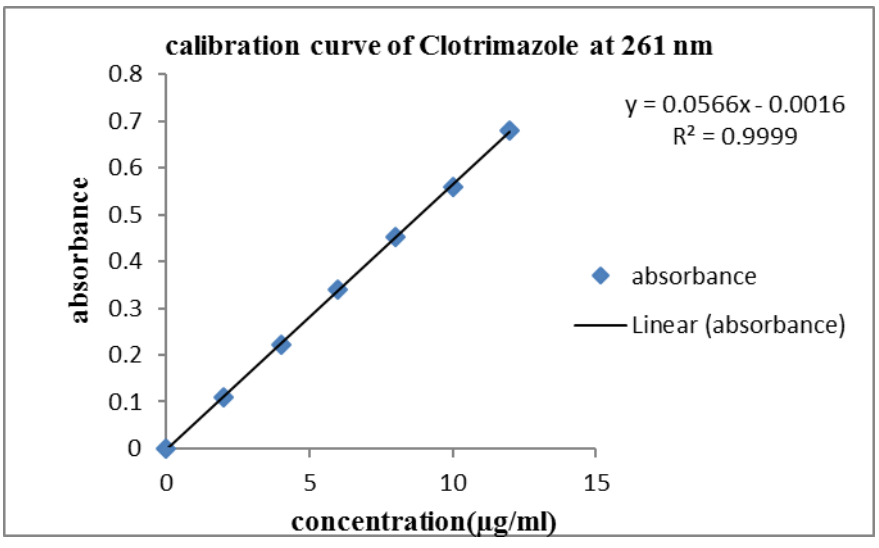

Figure 3: Calibration curve of Clotrimazole in PBS 7.4

Evaluation of Clotrimazole nanosponges

Table 4: Evaluation parameters of nanosponge formulations

Formulation code	Particle size (nm)	Production yield %	Drug entrapment efficiency %
F1	561	51.51± 0.13	45.82 ± 0.42
F2	566	60.18±0.23	51.04 ± 0.44
F3	571	64.24±0.17	55.43 ± 0.52
F4	578	77.86±0.14	61.72 ± 0.54
F5	623	47.12±0.13	42.30 ± 0.41
F6	628	65.24±0.17	49.04 ± 0.43
F7	643	67.50±0.12	55.95 ± 0.42
F8	701	73.65±0.18	55.70 ± 0.40

Scanning electron microscopy

The SEM images showed that the surface of prepared nanosponges was spherical in shape and uniform in size and its surface was porous in nature.

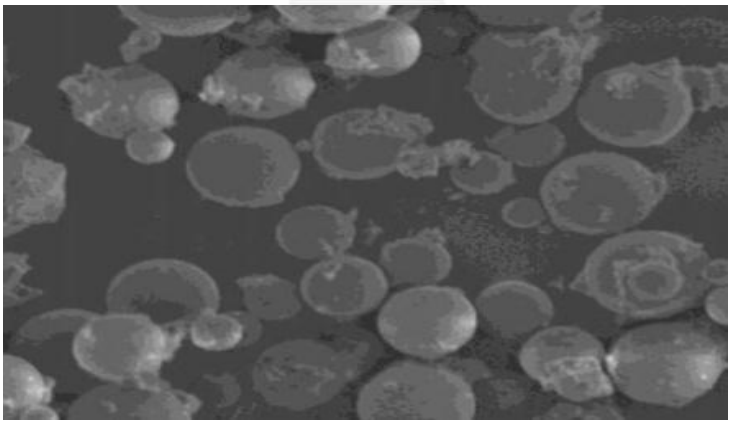

Figure 4: SEM of nanosponge F4

Evaluation of nanosponge loaded hydrogel

Table 5: Evaluation parameters of nanosponge loaded hydrogel formulation

Formulation	pH	Drug content	Spreadability (gm-cm/sec)
F1	5.7 ± 0.03	90.26 ± 0.12	9.82
F2	5.8 ± 0.02	90.4 ± 0.11	9.37
F3	5.7 ± 0.04	90.81 ± 0.16	9.82
F4	5.9 ± 0.02	91.94 ± 0.12	10.33
F5	5.8 ± 0.05	90.45 ± 0.15	8.82
F6	5.9± 0.04	90.75 ± 0.10	9.37
F7	6.0± 0.12	91.1 ± 0.13	9.33
F8	6.2 ± 0.06	91.34 ± 0.14	9.82
Drug-loaded plain gel	5.8 ± 0.07	92.27 ± 0.15	10

***In vitro* drug release study**

The *in vitro* drug release studies were carried out using Franz diffusion cell for 12 hrs. The percentage of cumulative drug released from the formulations was tabulated in Table 5.

Table 6: Percentage of cumulative drug release data for all formulations

Time (hrs)	F1 %CDR	F2 %CDR	F3 %CDR	F4 %CDR	F5 %CDR	F6 %CDR	F7 %CDR	F8 %CDR	Drug-loaded plain gel
0	0	0	0	0	0	0	0	0	0
1	15.32± 0.63	16.16± 0.11	16.7± 0.21	19.7 ± 0.41	13.64± 0.32	16.92± 0.11	16.75± 0.34	17.73± 0.22	20.13± 0.55
2	27.11± 0.36	23.61± 0.12	28.5± 0.17	29.5± 0.45	27.82 ± 0.15	27.15± 0.28	26.25± 0.19	26.36± 0.55	36.54± 0.41
3	37.88± 0.10	29.96± 0.37	36.65± 0.23	36.65± 0.34	34.41 ± 0.13	31.61 ± 0.10	31.31± 0.16	38.85± 0.67	52.42± 0.36
4	42.95± 0.11	34.75± 0.29	47.34± 0.32	45.34± 0.21	40.7 ± 0.24	37.86± 0.10	40.7± 0.62	43.66± 0.20	70.98± 0.25
5	46.55 ± 0.55	46.57± 0.61	52.27± 0.41	52.27± 0.11	48.33 ± 0.15	49.57± 0.21	49.12± 0.36	53.88± 0.81	83.1± 0.12
6	50.96± 0.24	53.99± 0.51	56.64± 0.54	56.64 ± 0.20	54.65 ± 0.56	58.82± 0.15	55.39± 0.15	61.33± 0.73	96.56± 0.65
7	56.75± 0.16	60.17 ± 0.26	63.6± 0.62	63.59± 0.58	59.81 ± 0.22	66.18 ± 0.48	62.78± 0.23	64.86± 0.13	-
8	62.98 ± 0.31	65.64 ± 0.34	67.59± 0.53	70.56± 0.66	64.91 ± 0.24	69.02± 0.11	67.25± 0.37	70.55± 0.10	-
9	66.83 ± 0.18	69.73± 0.22	74.65± 0.41	75.53± 0.56	68.35 ± 0.26	74.85± 0.61	73.26± 0.11	74.95± 0.15	-
10	72.65 ± 0.33	74.56 ± 0.14	78.53± 0.15	81.12± 0.17	73.11 ± 0.31	79.34± 0.45	78.05± 0.75	78.12± 0.37	-
11	76.11± 0.28	80.8± 0.55	84.52± 0.55	87.64± 0.61	77.53± 0.22	82.21± 0.55	83.71± 0.36	85.86± 0.10	
12	83.02± 0.19	84.92± 0.32	87.65± 0.37	91.73± 0.45	84.85 ± 0.12	85.83±0.67	86.69±0.11	89.13± 0.21	

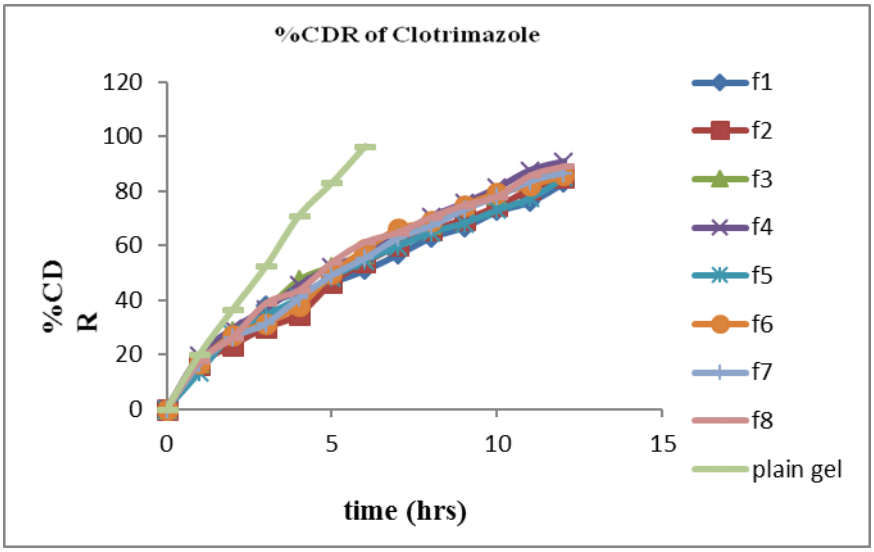

Figure 5: Comparison of percentage cumulative drug release profile of formulations

Kinetics of *in vitro* drug release

The *in vitro* drug release data of all the Clotrimazole nanosponge loaded hydrogel formulations and the drug-loaded plain gel was subjected to the goodness of fit test by linear regression analysis according to zero order and first orders kinetic equations, Higuchi's and Korsmeyer–Peppas models to ascertain mechanism of drug release

Table 7: Kinetic study of nanosponge based gel formulations and drug-loaded plain gel

Formulation	Drug release kinetics				
	Zero-order R^2	First order R^2	Higuchi R^2	Peppas	
				R^2	n
F1	0.979	0.970	0.992	0.977	0.672
F2	0.988	0.977	0.985	0.985	0.683
F3	0.977	0.976	0.977	0.995	0.654
F4	0.992	0.944	0.993	0.998	0.624
F5	0.982	0.972	0.979	0.971	0.655
F6	0.989	0.977	0.990	0.994	0.663
F7	0.987	0.980	0.987	0.996	0.682
F8	0.973	0.972	0.995	0.994	0.658
Drug loaded plain gel	0.995	0.855	0.940	0.998	0.886

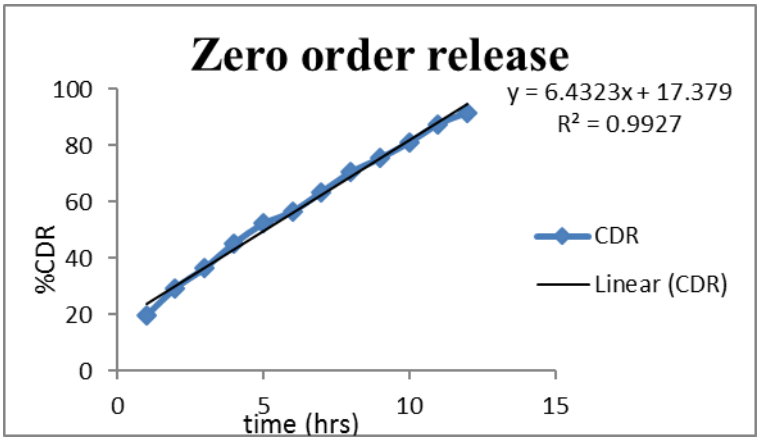

Figure 6: Zero-order release kinetics profile of optimized formulation F4

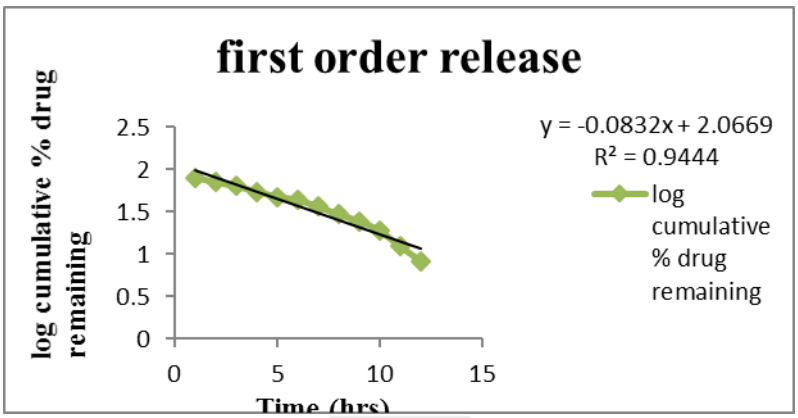

Figure 7: First order release kinetics profile of optimized formulation F4

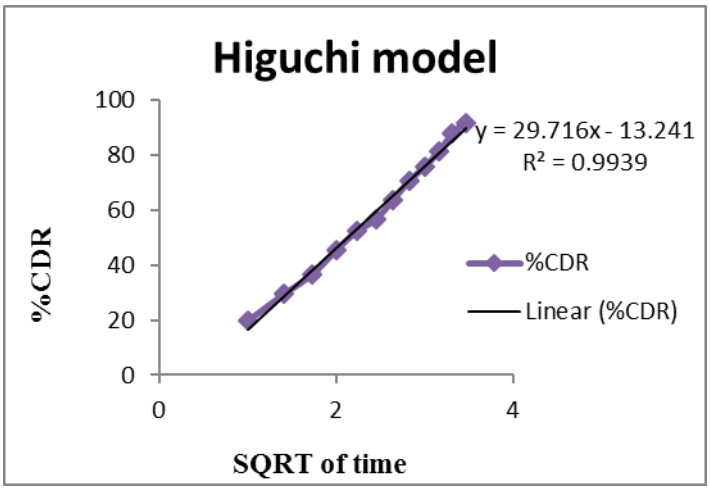

Figure 8: Higuchi release kinetics profile of optimized formulation F4

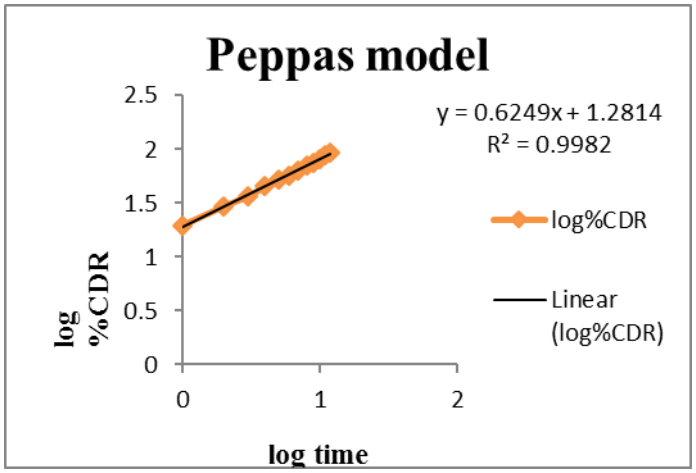

Figure 9: Peppas release kinetics profile of optimized formulation F4

Viscosity study

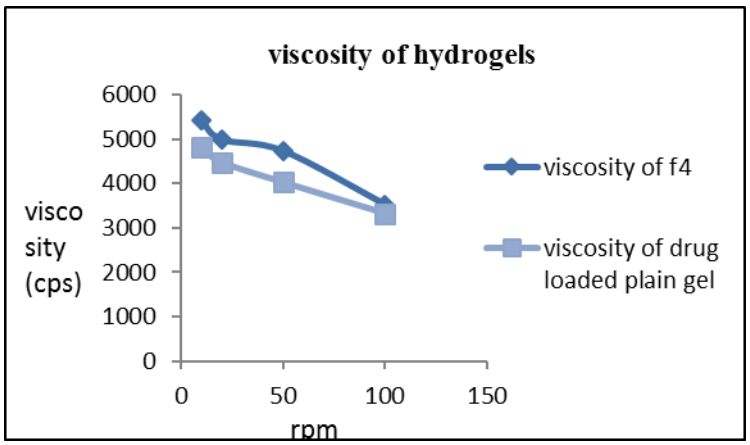

Figure 10: Comparison of viscosities of optimized formulation f4 and drug-loaded the plain gel

Evaluation of antifungal activity by disk diffusion method

The optimized formulation showed a clear zone of inhibition around the sample disc and it is shown in Figure 11

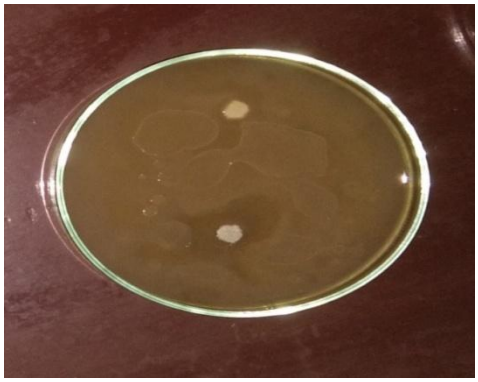

Figure 11: Clear zone of inhibition showed by formulation F4

Stability studies

Stability studies were carried out on optimized formulation F4 for a period of 1 month. The comparison of the parameters before and after stability studies was represented in Table 7 and 8.

Table 8: Comparison of parameters before and after stability studies

Parameters	**Before stability studies**	**After stability studies**
Appearance	white colored nanosponges suspended in a transparent gel base	white colored nanosponges suspended in a transparent gel base
pH	5.9	5.8
Drug content (%)	91.94	90.53

Table 9: *In vitro* drug release determination after stability studies

Time (hrs)	**Before stability studies % CDR**	**After stability studies % CDR**
1	19.7	18.97
2	29.5	28.67
3	36.65	35.42
4	45.34	44.69
5	52.27	51.64
6	56.64	55.78
7	63.59	62.45
8	70.56	69.38
9	75.53	74.97
10	81.12	80.44
11	87.64	86.52
12	91.73	90.8

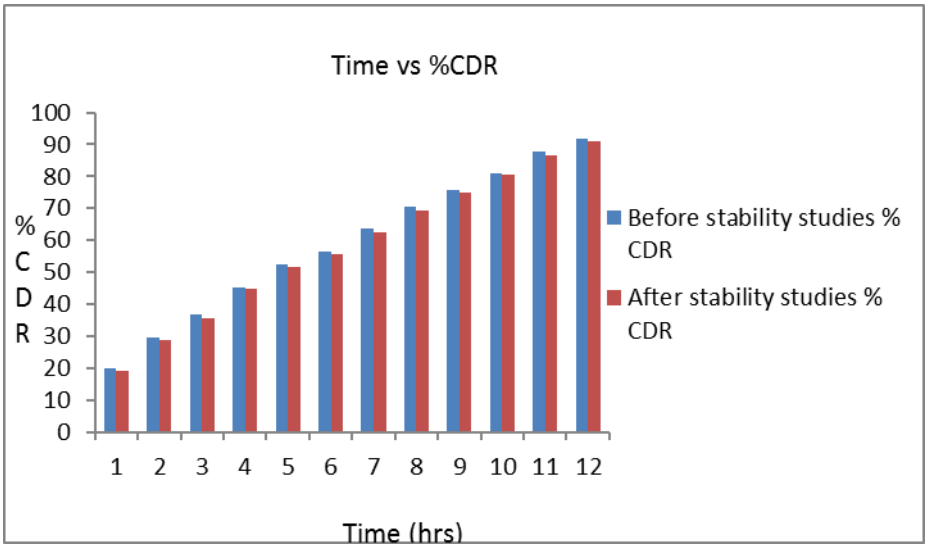

Figure 12: Comparison of % CDR before and after stability studies

CONCLUSION

All the prepared Clotrimazole nanosponges were white in color and had a rigid structure. The mean particle size of all nanosponge formulations was found in the range of 561-701 nm. Surface morphology of optimized nanosponges was evaluated by scanning electron microscopy and concluded that nanosponges were spherical in shape and uniform in size and its surface was porous in nature.

The nanosponge based gel formulation prepared using carbopol 934 and estimated for pH, viscosity, spreadability, *in vitro* drug release. Based on the observations the optimized formulation was safe and effective for topical use as Clotrimazole nanosponge loaded hydrogel and shows a controlled release effect with reduced side effects.

ACKNOWLEDGMENT

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