Design and characterization of Nanosponges loaded Vaginal gels of Itraconazole

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Objectives: The goal of the current effort is to create and analyse a bioadhesive vaginal gel that is loaded with Itraconazole nanosponges to ensure longer residence time at the infection site, providing a favorable release profile for the drug.

Methods: Nanosponges was prepared by solvent evaporation method in various ratios of Itraconazole to β-cyclodextrin. Physicochemical evaluation of Naosponges includes determination of Zeta potential, polydispersity, particle size analysis, entrapment efficiency and surface morphology by scanning electron microscopy (SEM).

Drug excipient compatibility was established by FTIR and DSC studies. Bioadhesive gel was prepared using Carbopol /Hypromellose /Sodium Carboxymethyl cellulose /HPC, Propyl paraben and methyl paraben was used as a preservative. The pH was adjusted with triethanolamine which resulted in a translucent gel. The optimized Itraconazole nanospoges formulation was dispersed into the gel base.

Nanosponges in gel formulations were evaluated for pH, viscosity, spreadability, extrudability and drug content. Ex vivo diffusion studies of the gel was determined on goat vaginal mucosa. In vitro drug release study was performed using cellophane membrane.

Results: The optimized batch of IDLNS12 Nanosponges (drug-polymer ratio 1:1) showed entrapment efficiency of 90.44%. Particle size of all the formulations was observed below 310 nm. Regular and spherical particles were observed in the SEM photographs. The optimized gel formulation INSG4 (Carbopol and HPC) showed viscosity of 4464 cps at 2-10 RPM, gel strength recorded as 91.76N load, and spreadability of 35.72 g.cm/seconds. INSG4 showed 99.98% drug release at 12.0 hrs and mucoadhesive time of >12 hr.

Conclusion: The study results suggest that Itraconazole-loaded β-cyclodextrin Nanosponges in mucoadhesive gel would provide a mean for sustained treatment of vaginal infections.

Key words: Itraconazole, Nanosponges, mucoadhesive, vaginal gels.

INTRODUCTION

Vaginal drug delivery system is the system where the drug formulations are applied directly in the vaginal cavity for producing systemic and local action, and is considered important because the vaginal membrane has a dense network of blood vessels for effective drug absorption, large surface area, local effect, self-insertion, rich blood supply, and avoidance of the first-pass effect. The vaginal route is particularly used to treat vaginal infections, sexually transmitted diseases, or for contraception. Semisolid and solid dosage forms are preferred for this route and such dosage when tailored with bioadhesion properties prolongs the residence time in the vaginal cavity[1].

Nanosponges are tiny mesh – like nanoporous particular structure in which a large variety of substances can be encapsulated or suspended, and then be incorporated into a dosage form. They have a proven spherical colloidal nature, reported to have a very high solubilization capacity for poorly soluble drugs by their inclusion and non-inclusion behavior. Nanosponges have recently been developed and proposed for drug delivery[2].

Itraconazole, a triazole derivative, is used for the treatment of systemic fungal infection. It is one of the triazole antifungal agents that inhibits cytocrome P-450 dependent enzyme resulting in impairment of ergosterol synthesis. It has been used against histoplasmosis, blastomycosis, cryptococcal meningitis and asperigillosis. Itraconazole inserts preferentially into fungal membranes and disrupts their function. 5-fluorocytosine targets fungal-specific DNA replication. It is a BCS class II drug having low solubility and high permeability[3]. The extremely low solubility results into poor oral bioavailability (55%) of Itraconazole. The work described here is concerned with the formulation of nanosponges loaded vaginal

Itraconazole gels, using Carbopol, Sodium carboxymethyl cellulose, Hydroxypropyl cellulose and Hypromellose (HPMC) as a synthetic polymers.

MATERIALS AND METHODS

Itraconazole API was gift sample obtained from Gland Pharma Ltd., India. β-cyclodextrin, Carbopol 940, Hydroxypropyl cellulose (Klucel HXF), Sodium carboxymethyl cellulose (Aqualon 7H4) Hypromellose (HPMC) K15M were procured from Aurobindo Pharma Ltd, India. All other chemicals were of analytical grade purchased from SD Fine chemicals, Mumbai. Prior approval by Institutional animals ethics committee was obtained for conduction of experiments (CPCSEA Ref: 1371/PO/Re/S/10/CPCSEA, Dated 25-08-2023).

Production of β-cyclodextrin nanosponges:

Nanosponges based on β-cyclodextrin were prepared by a method that was reported by Monika Rao[4]. Nanosponges having β-cyclodextrin and diphenyl carbonate in different ratios such as 1:0.25, 1:0.5, 1:0.75 and 1:1 were prepared as shown in Table: 1. In these nanosponges β-cyclodextrin is the encapsulating polymer and diphenyl carbonate is the cross linking agent. Finely homogenized anhydrous β-cyclodextrin and diphenyl carbonate were placed in a 100 ml conical flask. This flask was heated gradually to a temperature of 100 ^{0}C with magnetic stirrer and maintained there for five hours under magnetic stirring. As the reaction involving the production of nanosponges proceeded, crystals of phenol appeared at the neck of the flask. At the end of five hours the reaction mixture was left to cool down to room temperature and the product obtained was broken down into small lumps by shaking the flask. The lumpy solid was first washed repeatedly with distilled water to remove any unreacted β-cyclodextrin. Then it was washed with acetone to remove any unreacted diphenyl carbonate and any phenol that might have entered the product as a by-product of the reaction. The nanosponges were then purified and stored at 25 ^{0}C in a desiccator. The procedure for the preparation of blank nanosponges was carried out three times at every ratio.

Table: 1 Ratios of β-cyclodextrin (polymer) and diphenyl carbonate (cross linking agent) for the production of nanosponges.

Formulation code	Ratio	β -CD (g)	diphenyl carbonate (g)
NS1	1: 0.25	8	2
NS2	1: 0.5	8	4
NS3	1: 0.75	8	6
NS4	1: 1	8	8

Preparation of drug loaded nanosponges

The drug loaded nanosponges were prepared by solvent evaporation technique. The solvent used was either ethanol or acetone or chloroform. Products for Itraconazole was prepared by using blank nanosponges of all four ratios and using each of the three solvents (acetone, ethanol, chloroform). The procedure for the preparation of drug loaded nanosponges was as follows as shown in Table: 2. Four grams of the Itraconazole was dissolved in 100 ml of the solvent. Then required weight of blank nanosponges (5 grams, 6 grams, 7gram and 8 grams for the products involving NS1, NS2, NS3 and NS4 respectively) was added to the solution .The solutions were triturated in a mortar until the solvent evaporated. As the solution was triturated the nanosponges absorbed the drug solubilised in the solvent and the clumps got segregated. Finally the solid dispersion was obtained. These were dried in an oven over night at 50 ^{0} C in order to remove any traces of solvents. These were sieved through a sieve of 60 # (250 µ). The products of drug loaded nanosponges were stored in a desiccator.

Table: 2 Formulation composition of Itraconazole drug loaded nanosponges prepared by using different solvents.

S. No	Formulation code	Polymer: cross linking agent	Solvent
1	IDLNS1	1 : 0.25	Ethanol
2	IDLNS2	1 : 0.5	
3	IDLNS3	1 : 0.75	
4	IDLNS4	1 : 1	
5	IDLNS5	1 : 0.25	Acetone
6	IDLNS6	1 : 0.5	
7	IDLNS7	1 : 0.75	
8	IDLNS8	1 : 1	
9	IDLNS9	1 : 0.25	Chloroform
10	IDLNS10	1 : 0.5	
11	IDLNS11	1 : 0.75	
12	IDLNS12	1 : 1	

Preparation of drug loaded nanosuspensions

The final product planned was a gel meant for Topical application. Hence the lumpy solids of nanosponges

had to be converted into nanosuspensions for the purpose of incorporation into gels. The nanosuspensions were prepared in the following manner.

The weight of the drug loaded nanosponges required for the preparation of the nanosuspension was calculated based on its encapsulation efficiency. Each 10 ml of suspension was expected to contain 100 mg of the drug. So weight of the drug loaded nanosponges containing 1000 mg of the drug was taken. The weights of nanosponges taken are shown in Table 3 for Itraconazole.

The required weight of the drug loaded nanosponges was taken into a 250 ml volumetric flask containing 100 ml of methanol. The flask was shaken gently and kept overnight. This procedure was carried out to remove free un encapsulated drug. This suspension was then filtered by using a 0.22 µ membrane filter. The un encapsulated drug got dissolved in methanol and was removed as filtrate. The residue on top of the filter bed was washed with distilled water and transferred into graduated test tube and was made up to 5 ml with distilled water. These were the suspension of the nanosponges which were incorporated into gels. This experiment was replicated three times.

Table: 3 Compositions of Itraconazole DLNS containing Carbopol gels.

S. No	Batch Code	Quantity of Miconazole nitrate LNS (mg)	Solvent	Polymer : cross linking agent	Quantity of Carbopol-934 gels (up to)
1	F1	3156.12	Ethanol	1 : 0.25	10 gm
2	F2	3362.92	Ethanol	1 : 0.5	10 gm
3	F3	3504.52	Ethanol	1 : 0.75	10 gm
4	F4	3676.92	Ethanol	1 : 1	10 gm
5	F5	2946.56	Acetone	1 : 0.25	10 gm
6	F6	3104.81	Acetone	1 : 0.5	10 gm
7	F7	3247.59	Acetone	1 : 0.75	10 gm
8	F8	3379.52	Acetone	1 : 1	10 gm
9	F9	2879.07	Chloroform	1 : 0.25	10 gm
10	F10	3039.14	Chloroform	1 : 0.5	10 gm
11	F11	3185.45	Chloroform	1 : 0.75	10 gm
12	F12	3317.11	Chloroform	1 : 1	10 gm

Formulation of different gels containing Itraconazole drug loaded nanosponges

Accurately weighed quantity of Carbopol/Hypromellose/Sodium Carboxymethyl cellulose/ HPC (Klucel HXF) was dispersed in 5 ml of distilled water and was allowed for swelling over night as shown in table: 4. The swollen Carbopol/Hypromellose/Sodium Carboxymethyl cellulose/ HPC (Klucel HXF) was stirred for 60 minutes at 800 rpm using magnetic stirrer. The previously prepared required drug (Itraconazole) equivalent nanosuspensions, methyl paraben and propyl paraben were incorporated into the polymer dispersion with stirring at 500 rpm by a magnetic stirrer for 1 hour. The pH of above mixture was adjusted to 4.5 with triethanolamine (0.5%). The gel was transferred in to a measuring cylinder and the volume was made up to 10 ml with distilled water[5].

Table: 4 Formulation of different gels containing Itracoazole loaded nanosponges:

Ingredients	INSG1	INSG2	INSG3	INSG4
Drug loaded nanosponges equivalent to 1000mg (mg)	4000	4000	4000	4000
HPMC (mg)	1000	--	--	--
Na CMC(mg)	--	1000	--	--
Carbopol (mg)	--	--	1000	--
HPC (mg)	--	--	--	1000
Methyl Parabene (mg)	100	100	100	100
Propyl Parabene (mg)	50	50	50	50
Triethanolamine (0.5%)	Q.S	Q.S	Q.S	Q.S

Propylene glycol (mg)	Q.S	Q.S	Q.S	Q.S
Distilled water (ml) up to	10	10	10	10
Evaluation of different gels containing Itraconazole loaded nanosponges				
Drug content (%)	98.72±0.40	99.83±0.14	99.48±0.19	99.63±0.74
pH	5.42±009	5.47±0.04	5.46±0.29	5.43±0.18
Viscosity (cps)	3498±31	3721±17	3978±36	4464±28
Spreadability (gm.cm/sec)	32.16±1.12	32.44±1.23	33.27±1.51	35.72 ±1.32
Extrudability (N)	90.14±0.05	90.28±0.03	91.64±0.07	91.76±0.03
Mucoadhesive time (hr)	> 12	> 12	> 12	> 12

Evaluation studies for Itraconazole DLNS:

Encapsulation efficiency

The encapsulation efficiency of nanosponges was determined spectrophotometrically. A sample of Itraconazole nanosponges (100 mg) was dissolved in 100 ml of methanol and was kept overnight. One milliliter of the supernatant was taken and diluted to 10 ml with a solution containing 4.5 pH phosphate buffer and the absorbance was measured after suitable dilution at 260 nm respectively for Itraconazole against the corresponding blank solution using UV-visible spectrophotometer. From the absorbance, the free drug content was calculated. The methanol dispersion containing Itraconazole nanosponges was then ultra-sonicated to release the encapsulated drug from the nanosponges structure[6]. Then the solution was filtered by using 0.22µ filter paper and the filtrate was analyzed at 260 nm respectively for Itraconazole against the corresponding blank solution using UV-visible spectrophotometer for the total drug content. The encapsulation efficiency (%) of the nanosponges was calculated by the following equation,

$$\text{Encapsulation efficiency} = \frac{\text{Encapsulated drug content in nanosponges}}{\text{Total drug content}} \times 100$$

Drug content in the drug loaded nanosponges containing gel formulations[7]

Itraconazole nanosponges gel formulation (1 gram) was dissolved in methanol, filtered and the volume was made up to 100 ml with methanol. The drug content was determined by diluting the resulting solution 10 times with a solution containing 4.5 pH phosphate buffer and the absorbance was measured at 260 nm respectively for Itraconazole against the corresponding blank solution using UV-visible spectrophotometer. All measurements were performed in triplicate.

Scanning electron microscopy (SEM)

The morphology and surface texture[8, 9], topography of nanosponges was observed by scanning electron microscope (S-3400 N type II model, Hitachi Ltd., Tokyo, Japan).

FT-IR and DSC analysis

FT-IR spectroscopy[10] and DSC analysis were carried out for prepared Itraconazole loaded Nanosponges formulation as aforementioned.

Particle size analysis (PSA) and Zeta potential measurement

The particle size distribution was determined by using Dynamic Light Scattering (DLS) technique. The equipment used for the particle size distribution is HORIBA particle size analyzer. In this technique the particle sizes of a batch of the nanosponges were observed and from the standard deviation and mean particle size of nanosponges, the poly dispersity index (PDI) was calculated[11]

Zeta potential is a measure of the surface charge of dispersed particles in relation to dispersion medium. It was determined by using HORIBA zeta sizer. The zeta potential value is a measure of the physical stability of the nanosponges[12].

Evaluation studies for Itraconazole DLNS reservoir Gels

pH of the gel formulation[13]

Accurately weighed quantity of 25 mg of gel containing drug loaded nanosponges was solubilised in the distilled water and the pH value of the resulting 1% aqueous solution was measured by a pH meter. The measurement was done in triplicate.

Viscosity of the gel[14]

Viscosity of the prepared gel was measured by Brookfield Viscometer LV-DIII. Previously prepared Carbopol gel containing nanosponges was taken in a

100 ml beaker and suitable helipath T spindle was selected depending on the consistency of the gel and dimensions of 100 ml beaker. T-C spindle was selected as the appropriate spindle for the determination of the viscosity of carbopol gel containing nanosponges. The torque was adjusted in between 10 and 100 and the speed was maintained in the range of 2 to 10 RPM. Viscosity was measured at five points with 30 seconds of time interval. The viscosity shown on the display of the viscometer was absolute and there were no further calculations of shear stress and shear rate relationships.

Spreadability[15]

Spreadability was determined by an apparatus suggested by Multimer, *et al.,* fabricated in-house. The apparatus consists of a wooden block with a fixed glass slide and movable glass slide with one end tied to a weight pan rolled on a pulley, which was in horizontal level with fixed slide. The spreadability of the formulated gel was measured on the basis of 'Slip and Drag' characteristics of gel. An excess of gel (about 2g) under study was placed on this ground slide. The gel was then sandwiched between the two slides. One kg weight was placed on the top of the two slides for 5 minutes to expel air and to provide a uniform film of the gel between the slides. Excess of the gel was scrapped off from the edges. The top plate was then subjected to pull off 50 gm (M) with the help of a string attached to the hook and the time (T in seconds) required by the top slide to move a distance (L) of 7.5 cm was noted. A shorter interval indicated better spreadability. Spreadability (S) was calculated using the following formula:

$S = M \times L / T$

The spreadability measurements were done in triplicate (n=3).

Extrudability[16]

The gel formulations were filled in standard capped collapsible aluminium tubes and sealed by crimping to the end. The weights of the tubes were recorded. The tubes were placed between two glass slides and were clamped. 500 gm was placed over the slides and then the cap was removed. The amount of the extruded gel was collected and weighed. The percent of the extruded gel was calculated (>90% extrudability: excellent, >80% extrudability: good, >70% extrudability: fair).

In vitro drug diffusion study[17, 18]

Modified Franz diffusion cell was used for these studies. Cellophane membrane was used as the simulation for the skin. Cellophane membrane was mounted in a modified Franz diffusion cell. The known quantity (1 gm gel containing 100 mg of Itraconazole) was spread uniformly on the cellophane membrane on donor side. The solution containing 4.5 pH phosphate buffer solution was used as the acceptor medium, from which 1 ml samples were collected every hour and the same amount of fresh medium was replaced to maintain sink conditions. The study was carried out for 12 hours. While taking the samples from the acceptor medium, precautions were taken so that no air bubbles were formed in the acceptor medium. Fresh samples were analyzed at 260 nm respectively for Itraconazole against the corresponding blank solution using UV-visible spectrophotometer and the amount of drug diffused at each time point was calculated. All the samples were analyzed in triplicate.

Ex vivo drug Diffusion Study

Drug diffusion study was conducted using Franz diffusion cell[19]. The receptor compartment was filled with 15 ml of phosphate buffer having pH 4.5 as diffusion media. Goat vagina was mounted on the donor compartment with the help of an adhesive. The gels (1 gm gel containing 100 mg of Itraconazole) were placed in the donor compartment. Magnetic stirrer was set at 50 rpm and whole assembly was maintained at $32 \pm 0.5\ ^0C$. The amount of drug released was determined by withdrawing 1 ml of sample at regular time intervals for 12 hours. The volume withdrawn was replaced with equal volume of fresh buffer solution. Samples were analyzed for drug content using a UV-spectrophotometer by measuring the absorbance at 260 nm respectively for Itraconazole against the corresponding blank solution. The diffusion study was replicated three times on each gel.

RESULTS AND DISCUSSION

Trizole drugs are a group of antifungal drugs which have broad spectrum antifungal activities against a wide range of fungi that cause many mycotic infections. The members of this group are structurally related and have similar physicochemical properties and mechanisms of action.

The FT-IR spectra of the Itracoazole loaded nanosponges were studied by comparing them with the spectra of unreacted β-cyclodextrin and diphenyl carbonate. The FT-IR spectra of unreacted β-cyclodextrin and diphenyl carbonate are shown in Figure: 1-5. The FT-IR spectra of nanosponges formed by reacting β-cyclodextrin with diphenyl carbonate are shown in figures. In the nanosponges of all ratios the major peaks were observed at 940 cm^{-1} which represents the α-1,4 glycoside bond. This bond is the indication that there was no change in the cyclodextrin linkages. The absence of peaks responsible for carbonyl group of the diphenyl carbonate at 1768cm^{-1} in the nanosponges is the indication of the removal of C=O from diphenyl carbonate. The absence of peaks responsible for –C=C- at 1591 and 1497 cm^{-1} in the FT-IR spectra of nanosponges is an indication of the absence of phenol rings which were present in the unreacted diphenyl carbonate. Similarly absence of an intense peak responsible for –C=O group at 1157cm^{-1} in the FT-IR spectra of nanosponges is the indication of removal of -C=O group from the diphenyl carbonate which might be attached to the primary or secondary hydroxyl groups of β-cyclodextrin by leaving phenol as by product. All these changes indicate the formation of nanosponges by the reaction of primary/secondary hydroxyl groups of β-cyclodextrin with the carbonyl groups of diphenyl carbonate.

Figure: 1 FTIR Spectrum Itraconazole

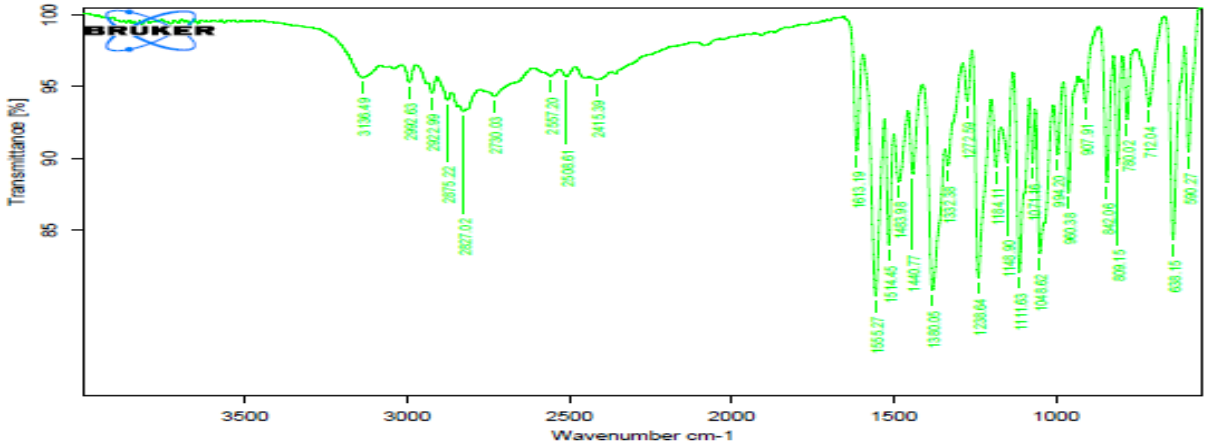


Figure: 2 FTIR Spectrum Itraconazole and β-cyclodextrin

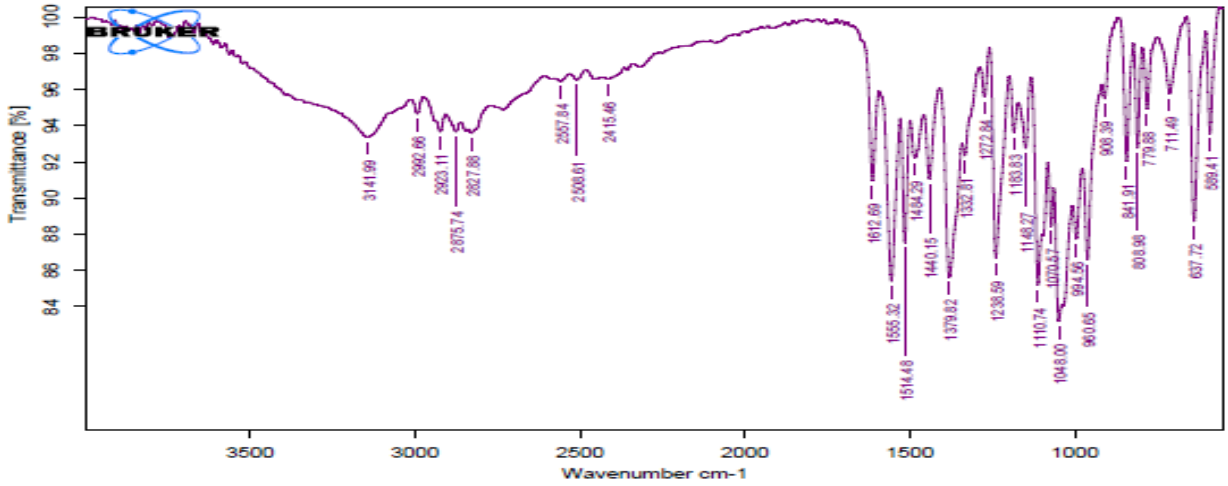


Figure: 3 FTIR Spectrum Itraconazole and diphenyl carbonate

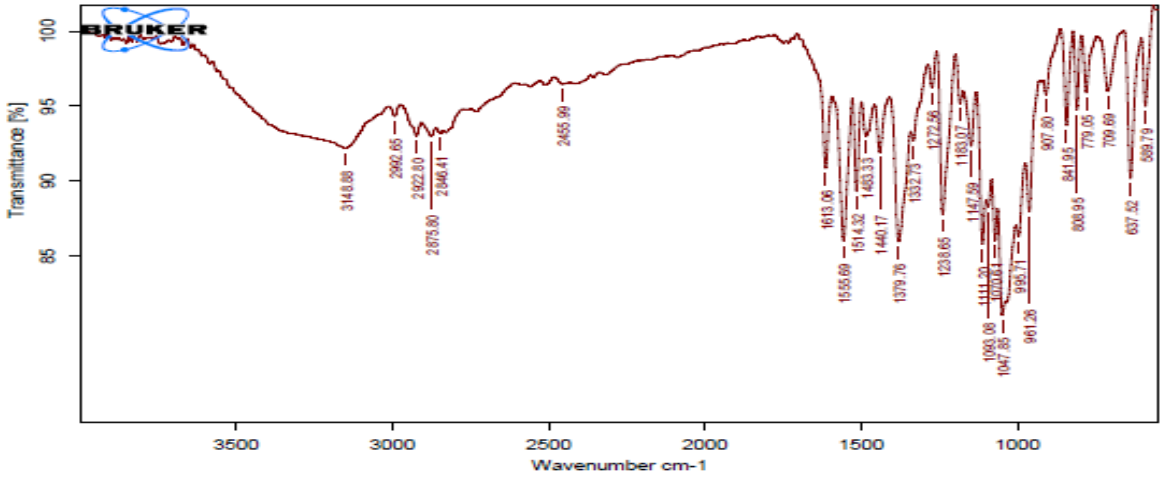


Figure: 4 FTIR Spectrum Itraconazole and carbopol

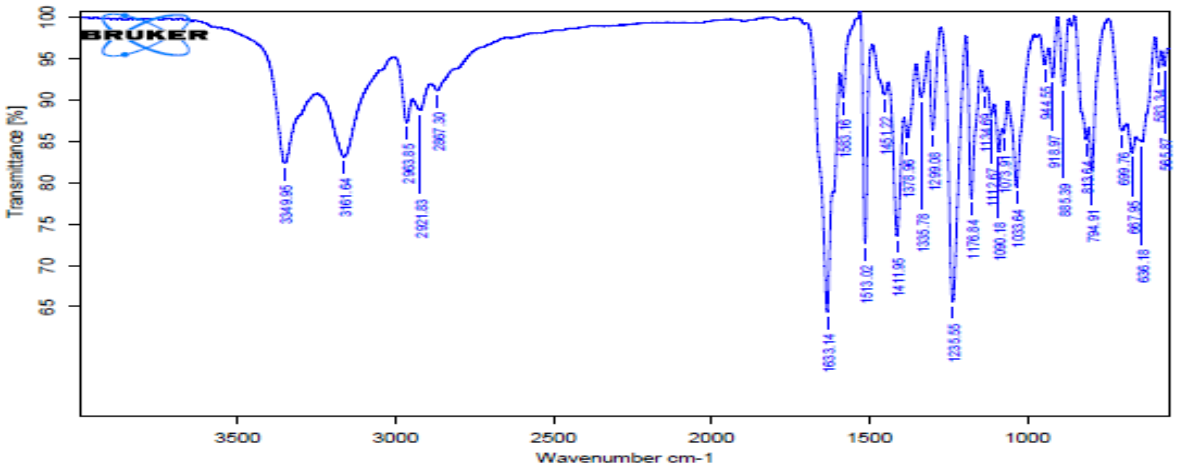


Figure: 5 FT-IR spectra of nanosponges prepared in 1:1 ratio (F12) of β -cyclodextrin and Di phenyl carbonate

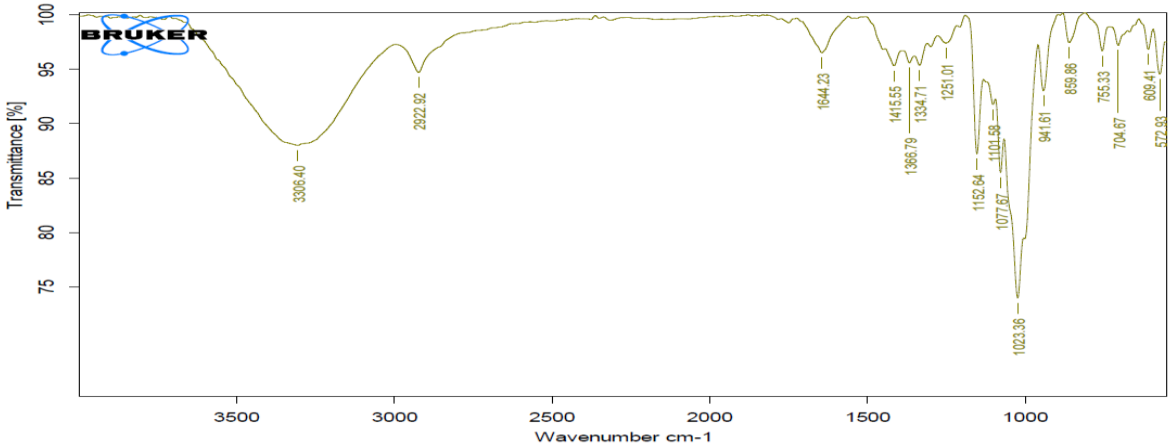


Figure: 6 DSC thermogram of Itraconazole

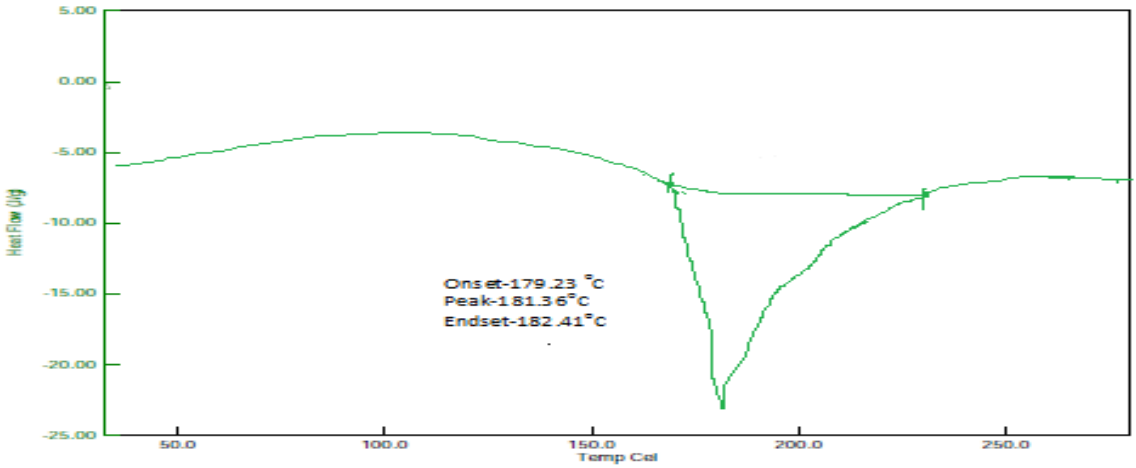
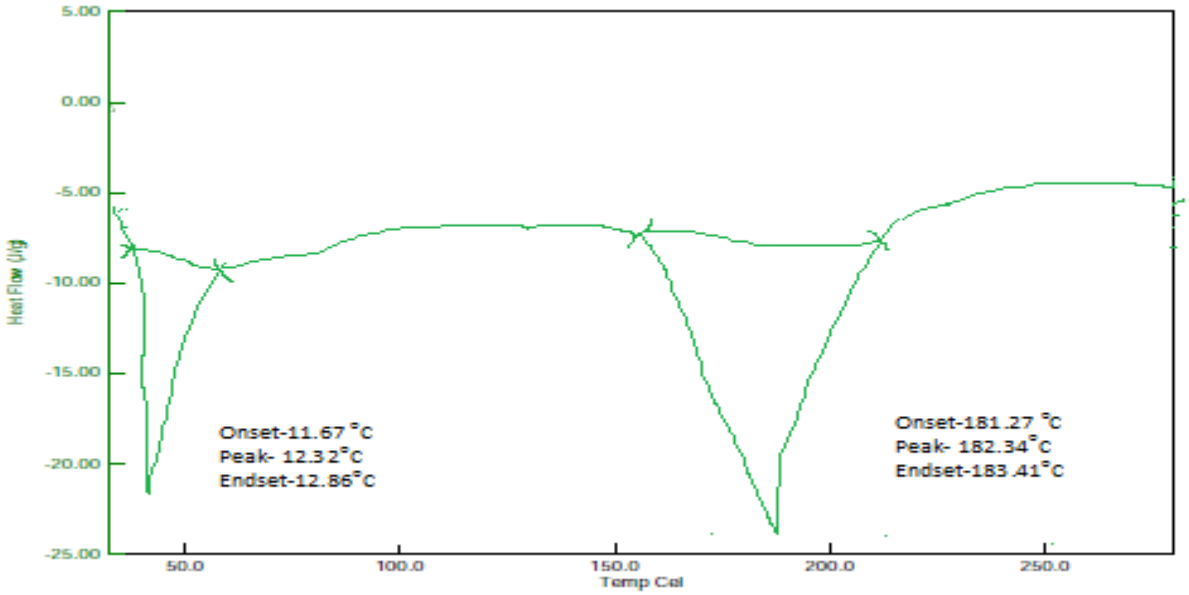


Figure: 7 DSC thermogram of Miconazole nitrate with carbopol



The encapsulation efficiency of the drug Itraconazole loaded nanosponges formulations it was inferred that, as the cross linking ratio increased the encapsulation efficiency was found to be enhanced. The encapsulation efficiency data is shown in Table: 5.

It is also found that the encapsulation efficiencies of drug loaded nanosponges are influenced by the solvent used for drug loading by solvent evaporation technique. Based on the encapsulation efficiency values the solvents can be arranged as

Chloroform >Acetone >Ethanol

The change in the encapsulation efficiency with respect to solvent might be due to the solubility of Itraconazole in the particular solvent.

The DSC spectra of optimized nanosponges show a slight variation in endothermic peak as that of the pure drug while the intensity of peak is slightly reduced as shown in Figure: 6 and 7. This effect may be due to the decrease in the crystal size of the drug. The DSC thermogram of IDLNS12 at 181.27^0C shows a broad endothermic peak. From the peak broadening in the spectra, one can understand that the drug is mostly encapsulated in nanosponges.

The mean particle size, poly dispersity index and zeta potential of the drug Itracoazole loaded nanosponges were found to be good enough to maintain the physical stability of the nanosponges and are shown in Table: 5.

The mean particle sizes of the different Itracoazole loaded nanosponges varied in the range 307.3±3.65 to 238.5±2.25 nm. The mean particle size is 238.50±2.25 nm, standard deviation is 59.2 nm and mode is 231.5 nm. The values are in acceptable range. Histogram of size frequency data and plot of zeta potential versus intensity of this best formulation is shown in Figure: 8 and 9. The histogram indicates that the particle size distribution is a normal distribution. The zeta potential of IDLNS12 is -46.3±1.6 mV and it can be considered as having excellent stability.

The poly dispersity index values of the different Itracoazole loaded nanosponges varied in the range 0.291±0.11 to 0.219±0.06. Their zeta potential varied in the range -36.8±1.6 to -46.3±2.6 mV.

The particle morphology was analysed by scanning electron microscopy. The particles were found to be spherical with good structural composition having a definite boundary as shown in Figure: 10.

Among all the formulations, the formulations IDLNS12 prepared with with β-cyclodextrin and diphenyl carbonate in 1:1 ratio and by using chloroform solvent has shown the highest encapsulation efficiency compared to the other formulations. Hence this formulation IDLNS12 was selected for further studies.

Figure: 8 Histogram of size frequency data

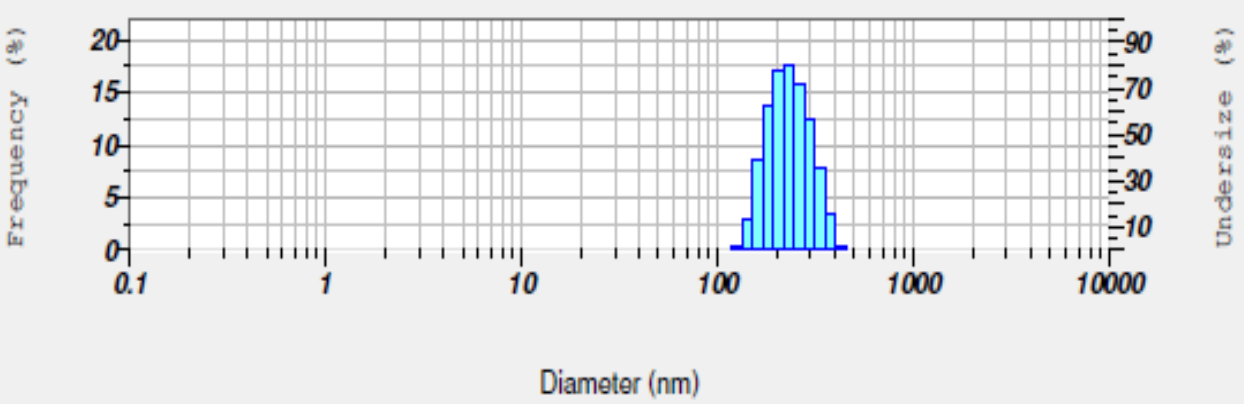


Figure: 9 Plot of zeta potential versus intensity of Itracoazole loaded nanosponges prepared with β-cyclodextrin and di phenyl carbonate in 1:1 ratios and by using chloroform

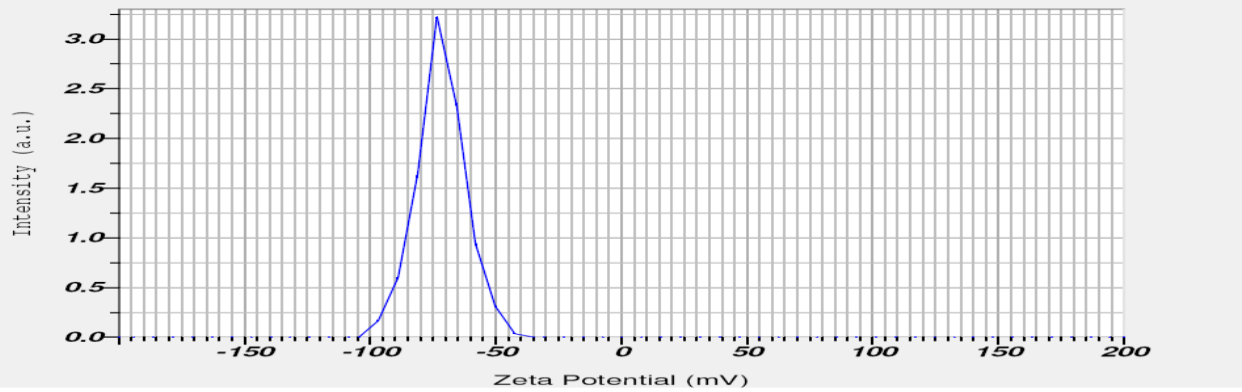


Figure: 10 SEM Photograph showing particle identification with reference to Itracoazole nanosponge (IDLNS12) size

Table: 5 Encapsulation efficiency, Mean particle size, Poly dispersity Index and zeta potential of the Itracoazole drug loaded nanosponges

S.No	Formulation Code	Average Particle size (nm±S.D)	Poly dispersity Index`(X±SD)	Zeta Potential (mV±SD)	%Entrapment efficiency
1	IDLNS1	307.3±3.65	0.291±0.11	+39.8±1.6	71.29±1.07
2	IDLNS2	296.4±2.75	0.274±0.13	+48.9±1.2	75.34±1.03
3	IDLNS3	279.3±2.69	0.266±0.09	+53.7±1.1	78.47±2.57
4	IDLNS4	265.3±3.9	0.253±0.12	+58.4±1.3	81.59±1.37
5	IDLNS5	299.3±2.18	0.277±0.09	+38.6±1.1	76.36±1.05
6	IDLNS6	287.2±2.57	0.268±0.07	+41.5±1.2	80.52±1.91
7	IDLNS7	273.3±2.75	0.252±0.06	+52.6±1.4	85.68±2.20
8	IDLNS8	256.5±1.65	0.238±0.09	+55.1±1.3	88.77±2.09
9	IDLNS9	267.4±2.15	0.251±0.11	+37.4±2.1	78.15±2.06
10	IDLNS10	258.6±2.7	0.245±0.07	+42.3±1.2	82.26±2.27
11	IDLNS11	247.1±2.26	0.229±0.13	+53.4±1.5	86.33±2.84
12	IDLNS12	238.5±2.25	0.219±0.06	+66.3±1.6	90.44±2.29

Physico chemical properties of Carbopol gel formulations containing Itracoazole loaded nanosponges.

In the present study efforts were made to prepare vaginal Carbopol gel containing Itraconazole loaded nanosponges. Drug content values of the formulations were well within the range between 99.13-99.64%. The pH of all the formulations was around the vaginal pH of 5.24 to 5.47, hence there would be no risk of vaginal irritation from these gels. All gels were found to exhibit plastic flow. It was observed that the gel formulations showed good extrudability, homogeneity and spreadability and Mucoadhesive strength. The data are presented in Table: 6.

Table: 6 Percentage of drug content in the Itracoazole loaded nanosponges containing gel formulations

S.No	Batch Code	% Drug content	P^H	Viscosity (Cps)	Spreadability (g.cm/sec)	Extrudability (N)	Muco adhesive time (hours)
1	F1	99.34±0.85	5.26±0.18	3632±21	31.28±1.36	86.24±0.04	> 12
2	F2	99.41±0.21	5.29±0.15	3676±23	31.30±1.23	86.39±0.03	> 12
3	F3	99.23±0.47	5.40±0.10	3732±16	32.38±2.32	86.45±0.07	> 12
4	F4	99.64±0.60	5.46±0.08	3732±14	32.29±2.19	86.49±0.06	> 12
5	F5	99.22±0.89	5.24±0.06	3746±16	31.29±1.27	87.29±0.05	> 12

6	F6	99.30±0.40	5.36±0.09	3743±18	32.29±1.48	87.17±0.04	> 12
7	F7	99.41±0.30	5.39±0.09	3762±21	32.39±1.78	87.27±0.03	> 12
8	F8	99.52±0.40	5.47±0.10	3812±24	33.27±1.49	88.64±0.03	> 12
9	F9	99.53±0.89	5.30±0.08	3803±25	33.29±1.74	88.32±0.02	> 12
10	F10	99.64±0.40	5.39±0.12	3839±18	33.43±1.59	88.46±0.01	> 12
11	F11	99.13±0.21	5.41±0.13	3838±24	32. 56±1.79	90.32±0.06	> 12
12	F12	99.48±0.41	5.46±0.18	3918±26	33.48±1.87	91.79±0.09	> 12

Carbopol gels containing Itraconazole loaded nanosponges prepared with β-cyclodextrin and diphenyl carbonate in 1:0.25, 1:0.5, 1:0.75 and 1:1 ratios by using different solvents such as acetone, ethanol and chloroform. The results of the in vitro diffusion study of Itracoazole from gels as shown in Figure: 11 to 13.

The correlation coefficient values (r) are shown in Table: 7. The data revealed that the diffusion profiles followed zero order kinetics. The drug release data obeyed Peppa's model with the range of n values being 0.6162 to 1.1035. Hence it maybe inferred that the mechanism of drugs release is non Fickian diffusion model.

Figure: 11 *in vitro* drug diffusion profiles of carbopol gels containing Itraconazole loaded nanosponges by using acetone as a solvent

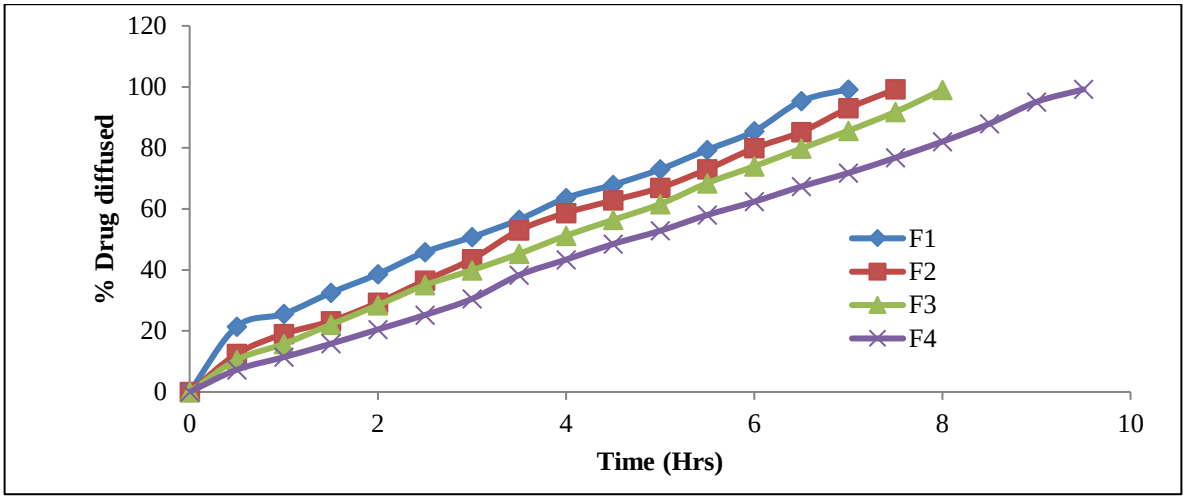


Figure: 12 *in vitro* drug diffusion profiles of carbopol gels containing Itraconazole loaded nanosponges by using ethanol as a solvent

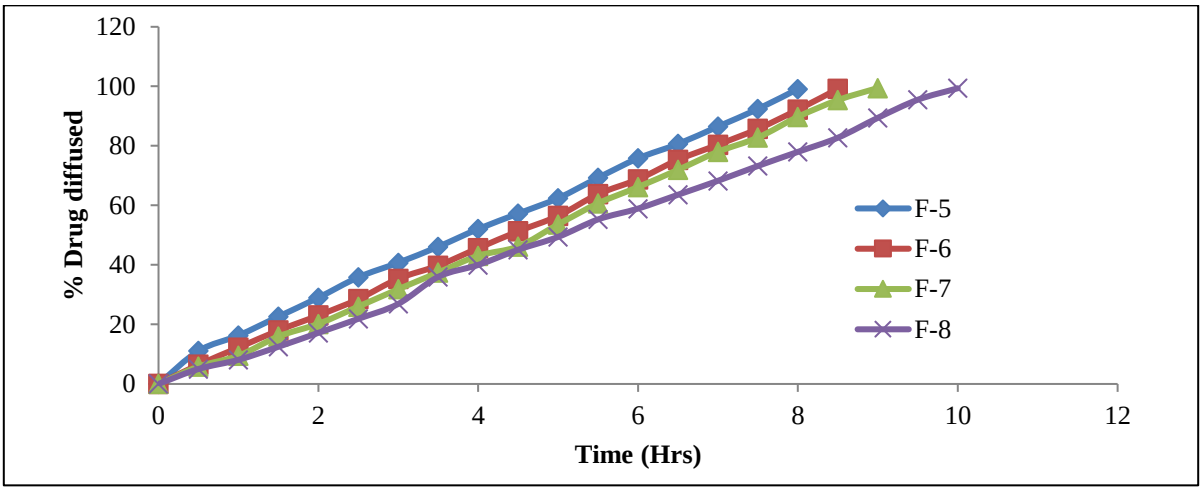


Figure: 13 *in vitro* drug diffusion profiles of carbopol gels containing Itraconazole loaded nanosponges by using chloroform as a solvent

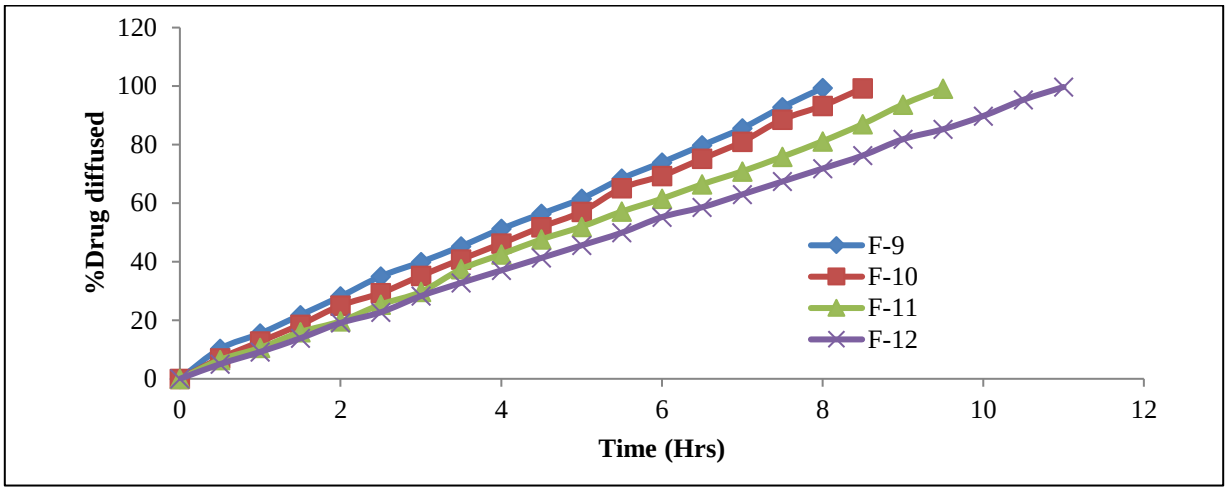


Table: 7 *In-vitro* drug release kinetic data of carbopol gels containing Itraconazole loaded nanosponges by using different solvents

Formulation Code	Correlation Coefficient Values (R^2)				Diffusion Rate Constant (mg/hr) Ko	$t_{50\%}$	$t_{90\%}$	n Value
	Zero Order	First Order	Higuchi Model	Peppas Model				
F1	0.9702	0.8290	0.9741	0.9971	15.8	3.3	6.0	0.6162
F2	0.9939	0.8168	0.9496	0.9946	13.4	3.7	6.6	0.7970
F3	0.9971	0.8138	0.9427	0.9947	12.4	5.0	7.2	0.8301
F4	0.9994	0.8085	0.9271	0.9971	10.4	5.8	8.6	0.9290
F5	0.9963	0.8175	0.9457	0.9972	12.4	5.03	7.25	0.8162
F6	0.9928	0.8020	0.9236	0.9997	11.4	5.3	7.1	0.9650
F7	0.9993	0.7977	0.9136	0.9999	11	5.5	8.2	1.0618
F8	0.9991	0.7984	0.9147	0.9993	9.8	5.1	9.2	1.0840
F9	0.9976	0.7964	0.9404	0.9975	12.5	5.0	7.2	0.8418
F10	0.9998	0.8044	0.9268	0.9995	11.62	5.3	7.8	0.9427
F11	0.9994	0.8068	0.9228	0.9928	10.20	5.9	8.7	1.0301
F12	0.9998	0.7640	0.9261	0.9993	9.09	5.5	10.0	1.1035

Characteristics of Itracoazole gels formulated with different Polymers

Gels (INSG1 to INSG4) were prepared by incorporating the Itraconazole DLNS in different gels (Carbopol/Hypromellose/Sodium Carboxymethyl cellulose/ HPC. The Drug content, pH, viscosity of different formulations (INSG1 to INSG4) are presented in Table 4. All gels were found to exhibit plastic flow. It was observed that the gel formulations showed good spreadability and extrudability and Mucoadhesive strength.

Ex vivo release study of Itraconazole Nanosponges loaded gels and control gel was carried out and compared. Ex vivo drug release was considerably retarded from the gels as compared to drug loaded naosponges alone. Where as in the drug loaded nanosponge gel formulation INSG4 shown the drug release controlled manner upto12 hrs as shown in Figure: 14. The Ex vivo release kinetics were shown in the Table: 8.

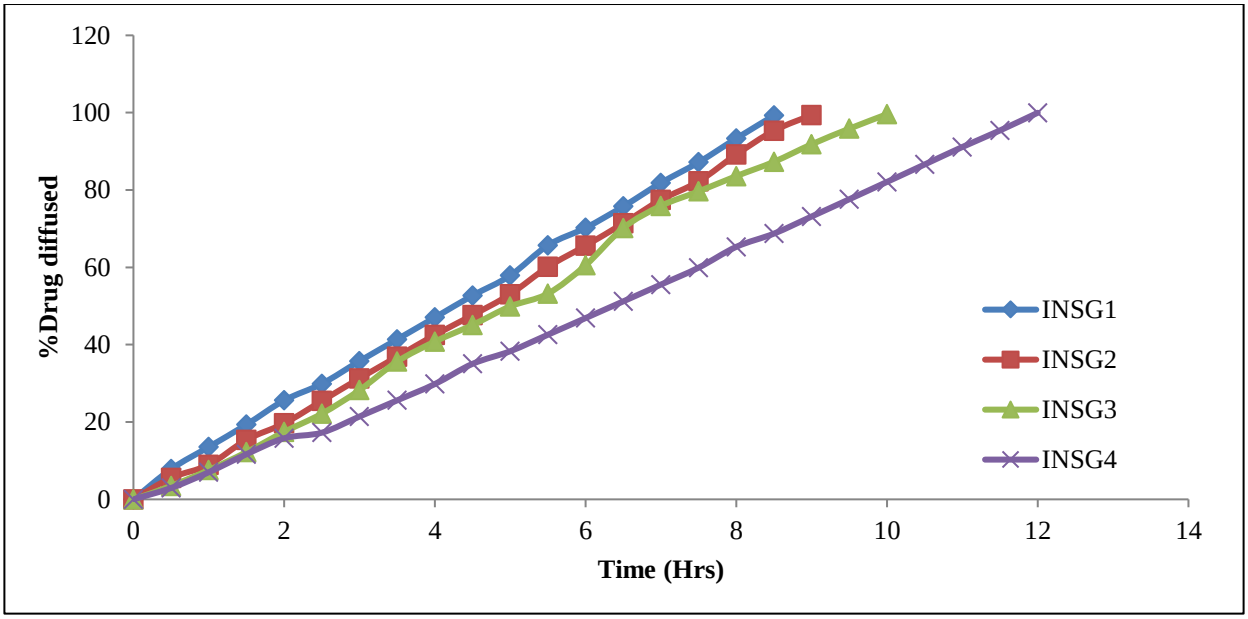
The in vitro release profiles followed zero order kinetics and the mechanism of drug release was governed by peppas mechanism.

Among all the Itracoazole gels prepared, the gels prepared with hydroxypropyl cellulose (INSG4) showed the best prolongation. They gave prolonged and controlled drug release over a period of 12 hours. Hence this formulation, namely, INSG4, was considered as the optimized formulation and was taken up for further studies.

Table: 8 *Ex vivo* drug diffusion kinetic data of different gels containing Itraconazole loaded nanosponges

Formulation Code	Correlation Coefficient Values (R^2)				Diffusion Rate Constant (mg/hr) Ko	$t_{50\%}$	$t_{90\%}$	N Value
	Zero Order	First Order	Higuchi Model	Peppas Model				
INSG1	0.9996	0.8056	0.9303	0.9990	11.62	5.3	7.7	0.9112
INSG2	0.9989	0.7971	0.9107	0.9998	10.86	5.6	8.2	1.082
INSG3	0.9986	0.8073	0.9214	0.9985	9.80	5.1	9.2	1.108
INSG4	0.9983	0.6380	0.9052	0.9991	8.31	6.01	10.83	1.182

Figure: 14 *Ex-vivo* drug diffusion profiles of different gels containing Itraconazole loaded nanosponges



CONCLUSION

Itracoazole loaded nanosponges could be prepared by cross linking β-cyclodextrin and diphenyl carbonate in 1:1 ratio and successfully incorporated into a hydrogel for vaginal application. The solubilization of Itracoazole through nanosponges would improve their vaginal availability. Due to the fast self-cleaning action of the vagina, conventional vaginal dosage forms cannot assure prolonged contact time with mucosa, therefore the mucoadhesive dosage forms are more preferable than conventional dosage forms. This functionality can be imparted by gelling of the Itracoazole loaded nanosponges using bioadhesive agent. The *in-vitro* studies indicate that Itracoazole loaded nanosponges bearing hydrogel provides controlled release of drug over a period of 12 hours. Thus, the Itracoazole loaded nanosponges bearing bioadhesive vaginal gels have good bioadhesive property and enhance the retention & prolong the drug release in the vagina. In conclusion, the developed systems are promising alternative drug carriers for vaginal administration.

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