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Formulation and Evaluation of Voriconazole Loaded Nanosponges for Oral and Topical Delivery

Dr. Prathima Srinivas*, Sreeja. K
Department of Pharmaceutics, Sri Venkateshwara College of Pharmacy and Research Center affiliated to Osmania University, Hyderabad, Andhra Pradesh, India

Abstract

The objective of the present study was to produce controlled release Voriconazole Nanosponges for topical and oral delivery. Nanosponges using three different polymers ethyl cellulose, Poly (methyl methacrylate) and Pluronic F-68 (poloxamer 188) were prepared successfully using PVA as surfactant by emulsion solvent evaporation method. The effects of different drug: polymer ratios, surfactant concentration, stirring speeds and time, sonication time on the physical characteristics of the nanosponges as well as the drug entrapment efficiency of the nanosponges were investigated. Particle size analysis and surface morphology of nanosponges were performed. The scanning electron microscopy of nanosponges showed that they were spherical in shape and spongy in nature. The particle size of the optimized formulations was in the range of 200-400nm and the drug entrapment efficiency was found to be in the range of 69.8 % to 72.5%. These nanosponge formulations were prepared as gel using carbopol 971P and studied for pH, viscosity, *in vitro* drug release, antimicrobial activity. Of the various formulations prepared E2, P2 and F2 were found to show the maximum drug release of 92.76%, 91.84% and 95.88% respectively at 1:2 drug: polymer ratio. The antifungal activity of the optimized formulations was evaluated *in vivo* in Male Wistar rats in comparison with marketed Flucos gel. The optimized nanosponge formulations were selected for preparing nanosponge tablets for controlled drug delivery by oral route. These tablets were prepared using microcrystalline cellulose and were evaluated for hardness, friability, drug content and in vitro drug release studies.

*Corresponding author, Mailing address:
Dr. Prathima Srinivas
E-Mail: drpssvcp@gmail.com

Key words:
Nanosponges, voriconazole, hydrogel, nanosponge tablet

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INTRODUCTION:

Nanosponges are porous polymeric delivery systems that are small spherical particles with large porous surface. These are used for the passive targeting of cosmetic agents to skin, there by achieving major benefits such as reduction of total dose, retention of dosage form on the skin and avoidance of systemic absorption.[1] These nanosponges can be effectively incorporated onto topical systems for prolonged release and skin retention thus reducing the variability in drug absorption, toxicity and improving

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patient compliance by prolonging dosing intervals. Nanosponges can significantly reduce the irritation of drugs without reducing their efficacy.[2]

The nanosponges are solid in nature and can be formulated as Oral, Parenteral, Topical or Inhalation dosage forms. For the oral administration, the complexes may be dispersed in a matrix of excipients, diluents, lubricants and anticaking agents suitable for the preparation of capsules or tablets. For topical administration they can be effectively incorporated into topical hydrogel.[3,4]

Voriconazole is a second-generation azole antifungal agent indicated for use in the treatment of fungal infections including invasive aspergillosis, esophageal candidiasis, and serious fungal infections. Voriconazole works principally, by inhibition of fungal cytochrome P-450-mediated 14 alpha-lanosterol demethylation, an essential step in fungal ergosterol biosynthesis. Voriconazole is designated chemically as (2R,3S)-2-(2,4-difluorophenyl)-3-(5-fluoro-4-pyrimidinyl)-1-(1H-1,2,4-triazol-1-yl)-2 butanol and a molecular weight of 349.31. Compared to fluconazole, voriconazole has an enhanced antifungal spectrum that includes filamentous fungi. Voriconazole was designed to enhance the potency and spectrum of activity of fluconazole used against a broad spectrum of significant clinical isolates like Aspergillus, Candida, Scedosporium and Fusarium.[6]

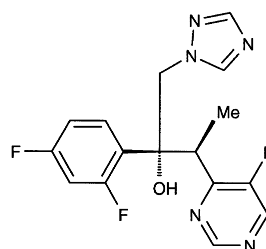

Figure 1.1: Structure of Voriconazole

EXPERIMENTAL METHODS:

MATERIALS:

Voriconazole (VRZ) was the kind gift from Daewoong Pharmaceutical Co., Ltd Hyderabad, India and Pluronic F-68 was a kind gift from Dr. Reddy's Laboratories Ltd, Hyderabad, India. Poly methyl methacrylate was a kind gift from Dr. Reddy's Laboratories Ltd, Hyderabad, India. Ethyl Cellulose, Poly Vinyl Alcohol, Dichloromethane, Triethanolamine, Carbopol 971P and N-methyl-2-pyrrolidone were purchased from SD fine chemicals. Microcrystalline Cellulose from Qualigens fine chemicals, Mumbai. All other ingredients used were of analytical grade.

METHODS:

Preparation of Voriconazole Nanosponges:

Voriconazole Nanosponges were prepared by Emulsion solvent evaporation technique. Three different polymers named ethyl cellulose (EC), poly methyl methacrylate (PMMA) and pluronic F-68 were used for the preparation of nanosponges. Nanosponges were prepared using different proportions of polymers and polyvinyl alcohol.

The disperse phase containing VRZ and polymer in 20ml of dichloromethane was added slowly to a definite amount of PVA in 100mL of aqueous continuous phase with 1000rpm stirring speed using magnetic stirrer for 2hrs. The formed nanosponges were collected by filtration and dried in oven at 40°C for 24hrs and packed in vials. The prepared nanosponge formulations with three different polymers are listed in table 1.1, 1.2, 1.3.

Table 1.1: Formulation of Poly methylmethacrylate Nanosponges

Ingredients	P1	P2	P3	P4	P5	P6	P7	P8
Drug: polymer	25:100	50:100	75:100	100:100	50:150	50:200	50:250	50:300
PVA (%w/v)	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Dichloromethane (ml)	20	20	20	20	20	20	20	20
Distilled Water (ml)	100	100	100	100	100	100	100	100

PVA-polyvinyl alcohol

Table 1.2: Formulation of Ethyl cellulose Nanosponges

Ingredients	E1	E2	E3	E4	E5	E6	E7	E8
Drug: polymer	25:100	50:100	75:100	100:100	50:150	50:200	50:250	50:300
PVA (%w/v)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Dichloromethane (ml)	20	20	20	20	20	20	20	20
Distilled Water (ml)	100	100	100	100	100	100	100	100

PVA- polyvinyl alcohol

Table 1.3: Formulation of Pluronic F-68 Nanosponges

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
Drug: polymer	25:100	50:100	75:100	100:100	50:150	50:200	50:250	50:300
PVA (%w/v)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Dichloromethane (ml)	20	20	20	20	20	20	20	20
Distilled Water (ml)	100	100	100	100	100	100	100	100

PVA-poly vinyl alcohol

Formulation of Nanosponge loaded gel:

The polymer Carbopol 971P was initially soaked in water for the gel for 2 hrs and dispersed by agitation at 600rpm by using magnetic stirrer to get smooth dispersion. Triethanolamine (2% v/v) was added to neutralise the pH. The previously prepared optimized nanosponge suspension was thereby added and permeation enhancers Propylene glycol and N-methyl-2-pyrrolidone were added as ethanolic solution to the aqueous dispersion[7]. The composition of nanosponge gels is shown in table 1.4.

Table 1.4: Formulation of Nanosponge Gel

Ingredients	PMMA	EC	Pluronic F-68
Formulated VRZ Nanosponges	80mg	80mg	80mg
Carbopol 971P	150 mg	150mg	150mg
Distilled Water	10mL	10mL	10mL
Propylene Glycol	0.5mL	0.5mL	0.5mL
Triethanolamine (2%v/v)	1mL	1mL	1mL
N-methyl-2-pyrrolidone	0.2mL	0.2mL	0.2mL

VRZ-voriconazole, PMMA-poly methylmethacrylate, EC-ethyl cellulose

Formulation of Nanosponge Tablet:

Nanosponge tablets were prepared by direct compression method. The composition of the prepared nanosponge tablet formulations is shown in table 1.5. The prepared nanosponges and Excipients were accurately weighed and sieved. Tablet compression was carried out using 9mm flat punches in a rotary tablet punching machine.[8]

Table 1.5: Formulations of Voriconazole Tablets using optimized nanosponges

Ingredients	PMMA	EC	Pluronic F-68
VRZ Nanosponges (mg)	160	160	160
Microcrystalline Cellulose (mg)	35	35	35
Mg stearate (mg)	5	5	5

EVALUATION OF NANOSPONGES:

Particle size Measurement:

The particle size of Voriconazole nanosponges was measured by particle size analyser Horibo scientific nanopartica SZ100. For the measurement 100µl of formulation was diluted with appropriate volume of PBS pH 7.4 and vesicle diameter and zeta potential were determined. Each sample was measured three times, after which the average value was used for further calculations.

Surface Morphology:

Scanning electron microscopy (JSM-5200, Tokyo Japan) was used to analyze particle size and surface topography was operated at 15kV accelaration

voltage. A concentrated aqueous suspension was spread over a slab and dried under vacuum. The sample was shadowed in a cathodic evaporator with a gold layer 20nm thick. Photographs were elaborated by an image processing program and individual NP diameters were measured to obtain mean particle size.

Fourier transform infrared (FTIR) analysis:

Fourier transform infrared analysis was conducted to verify the possibility of interaction of chemical bonds between drug and polymer. Samples were scanned in the range from 400-4000 $cm^{-1}$and carbon black reference. The detector was purged carefully by clean dry helium gas to increase the signal level and reduce moisture.

Entrapment Efficiency:

To calculate the entrapment efficiency, accurately weighed quantity of nanosponges (10mg) with 5ml of methanolic HCl (HCl: Methanol-10:1) in a volumetric flask was shaken for 1min using vortex mixer. The volume was made upto 10ml with Methanolic HCl. Then the solution was filtered and diluted and the concentration of Voriconazole was determined spectrometrically at 256nm.

$$\text{Loading efficiency} = \frac{\text{actual drug content in nanosponges}}{\text{theoretical drug content}} \times 100$$

Physical parameters:

Nanosponge formulations containing Voriconazole were characterized for pH using Digital pH meter (Systronics India). The viscosity of different nanosponge gel formulations was determined using cone and plate viscometer with spindle 61 (Brookfield programmable rheometer DV-III).

***In vitro* Drug Release studies of nanosponge gel formulations:**

In vitro evaluation studies of topical gel were performed using dialysis membrane method. The membrane was soaked for 24hr in 0.1NHCl and the receptor compartment was filled with 6.8pH phosphate buffer. Test vehicle equivalent to 100mg was applied evenly on the surface of the membrane. The prepared membrane was mounted on the cell carefully to avoid entrapment of air bubbles under the membrane. The whole assembly was maintained at 37°C, and the speed of stirring was kept constant (600 rpm) for 12 hrs. Aliquots of drug sample (4mL) was taken at 1hr time intervals and replaced with equal amount of freshly prepared buffer. Each experiment was performed in triplicate. The drug analysis was done using UV spectrophotometerically at 256nm.

Anti microbial activity:

The antimicrobial studies ascertained the microbial activity of the optimized formulations and of the marketed gel (Flucos gel® Mfg.by Cosme Farma laboratories ltd.) against *Candida albicans* fungus. This was determined by sabouraud dextrose agar diffusion test employing standard paper disc diffusion method using previously sterilized petridish. Nutrient agar (20 mL) seeded with the test organism (0.5mL) was allowed to solidify in the Petri plate. Paper discs (Wattman filter no. 42, diameter of 4mm), which were soaked in an antibiotic solution were carefully placed on the surface of sabouraud dextrose agar at suitable distance with the help of sterile pointed forceps. Then the plates were incubated at 27°C for 48 hrs. The zones of inhibition were obtained. The diameters of the zones of inhibition were measured. Readings were taken in triplicate.

***In vivo* study:**

Nanosponge gel containing Voriconazole as an active ingredient was prepared, and their antifungal activity is tested. Male Wistar rats were used in the experiment. The hair was removed from their flanks with electrical clipper. The area of skin (20mm diameter) on each flank was scarified with coarse sandpaper. Scarified skin was infected with few drops of culture of *Candida albicans*. Infected rats were housed individually in wire bottom cages and were provided with food and water. The fungal infection was induced on the rats for first 3 days. On the 4th day, the skin of rat was scraped and was cultivated in sabouraud dextrose agar media plates. The

inoculated plates were incubated at 27°C for 48 hrs. The colonies were measured after incubation. On the 4th day, treatment was initiated by topical application to the infected sites with gel formulation for another 4 days. On the 8th day the skin was again scraped and cultured on sabouraud dextrose agar plate respectively and further treatment was done. The inoculated plates were incubated at 27°C for 48 hrs and examined for growth of colonies. The number of colonies were counted using Cintex colony counter.

Evaluation of nanosponge tablet formulations:

The nanosponge tablets of Voriconazole were prepared by direct compression method. The prepared nanosponge tablets were evaluated for their hardness, friability, drug content and in vitro drug release. The Monsanto hardness tester was used to determine the tablet hardness. The Roche friability test apparatus was used to determine the friability of the tablets.

Drug Content Estimation:

Ten Voriconazole nanosponge tablets were accurately weighed, finely powdered and mixed. A portion of the powder equivalent to 20mg of Voriconazole was then transferred into a 100mL volumetric flask and 60mL of methanol was added. The contents of the flask were sonicated for 15 min and diluted to volume with methanol. 2ml of this solution was then diluted to 100ml volume with methanol. Absorbance of the resulting solution was measured at 256nm using UV spectrophotometer. Drug concentration was determined from standard graph.

***In Vitro* Drug Release Studies of nanosponge tablet formulations:**

In-vitro drug release studies were carried out using USP XXIII dissolution test apparatus Type II, paddle apparatus (100 rpm/min, 37 ±0.5°C). Voriconazole Nanosponge tablets were evaluated by exposing them to 900 ml 7.4pH phosphate buffer (simulated gastric fluid, SGF) for 12hrs. The drug release at different time intervals was analyzed by UV double beam spectrophotometer at 256nm.

RESULTS AND DISCUSSIONS:

Effect of variables on the preparation of nanosponges:

The effect of various variables like drug/polymer ratio, stirring rate, stirring speed, volume of internal phase, surfactant concentration and sonication time on the particle size and drug entrapment efficiency of nanosponges was studied.

The effect of drug: polymer on the physical characteristics of the formulated nanosponges was examined for various drug: polymer ratios nanosponges at stirring speed of 1000rpm for 2hrs. The mean particle size of nanosponges can be influenced by drug: polymer ratio. It was observed that as drug: polymer ratio increases, the particle size is decreased. This is probably due to the fact that at higher relative drug content, the amount of polymer available per nanosponge to encapsulate the drug becomes less, thus reducing the thickness of the polymer wall and hence smaller nanosponges.

The effect of stirring rate on the physical characteristics of the formulated nanosponges was examined for 1:2 drug: polymer ratio nanosponges. The stirring rate was varied in the range of 500 to 2000 rpm. The dispersion of the drug and polymer into the aqueous phase was found to be dependent on the agitation speed. As the speed was increased, the size of nanosponges was reduced and the nanosponges were found to be spherical and uniform. When the rate of stirring was increased up to 1000 rpm the spherical nanosponges were formed with mean particle size of about 300nm. It was noted that at higher stirring rate the production yield was decreased. Possibly, at the higher stirring rates the polymer adhered to paddle due to the turbulence created within the external phase, and hence production yield decreased.[9]

The stirring speed was varied from 1hr to 6hrs. The results of the study showed that 2hrs is the optimum

stirring time for the formation of nanosponges. It was found that stirring time also played a crucial role in the formation of nanosponges with reduced particle size.

Dichloromethane as internal phase varied in volume 10 to 40ml and the impact on particle size and %drug entrapment was studied. As the volume of internal phase increased particle size and drug entrapment in the polymers did not follow any particular pattern. This may be due to the decrease in viscosity of internal phase. The result suggests that the amount of dichloromethane need to be controlled within an appropriate range to effect not only the formation of emulsion droplets at the initial stage but also the solidification of drug and polymer in the droplets. The nanosponges with better entrapment efficiency were produced when 20 mL of dichloromethane were used.

By varying the concentration of surfactant from 0.1% to 1% w/v of the external phase the %entrapment efficiency was found to vary from 31% to 62% for PMMA, and from 26% to 60% for EC and from 22% to 65% for F-68. The minimum concentration of surfactant found to be required for the formation of nanosponges was 0.1% w/v of external phase for pluronic F-68, 0.2% w/v for ethyl cellulose and 0.3%w/v for poly methylmethacrylate. The particle size was found to increase with the increase in the surfactant concentration, higher amounts of surfactant resulted in foaming. This resulted in the formation of aggregates. At increased surfactant concentrations, the drug entrapment efficiency was also found to be reduced. This may be due to insufficient polymer concentrations for Voriconazole for particle encapsulation.

Particle size Measurement:

The particle size distributions of Nanosponges were characterized. The mean size of nanosponges were found to 400-800nm. Particle size of the different nanosponge formulations are tabulated in table 1.6, 1.7 and 1.8. The mean particle size was found to increase with the decrease in polymer amount.

Surface Morphology:

The morphology of the nanosponges prepared by emulsion solvent evaporation method were investigated by SEM. The representative SEM photographs of the nanosponges are shown in Fig. It was observed by SEM analysis that the nanosponges were uniformly spherical in shape. The spongy and porous nature of the nanosponges can be seen in figures 1.11 and 1.12.

Fourier transform infrared (FTIR) analysis:

All characteristic peaks of VRZ were observed in the IR spectra of nanosponge formulations (Figure 1.7, 1.8, 1.9 and 1.10). No significant shifts are observed in the positions of wave numbers when compared to that of pure drug. These results showed that there was no chemical interaction or changes during nanosponge preparation and VRZ was stable in all nanosponge formulations.

Physical Parameters:

The hydrogels were found to have smooth appearance and texture[10]. The pH of all hydrogels was found between 5.84 to 7.35 that lie in the normal pH range of skin 4.0 to 6.8. Hence the preparations are non irritant in nature[11]. The viscosities of optimized formulations P2, E2 and F2 are found to be 3870, 4220 and 3640 cps respectively.

Entrapment Efficiency:

The drug entrapment efficiency of Voriconazole Nanosponge formulations are given in Table 1.6, 1.7 and 1.8. The loading efficiency calculated for all nanosponges ranged from 42.7 to 72.5 %. The entrapment efficiency was effected by drug: polymer and internal phase (solvent) and external phase (non solvent) ratio. The entrapment efficiency was changed when drug and polymer ratio has been changed. The highest loading efficiency was found for

the F2 formulation 72.5%, and 70.4% for P2 formulation and 69.8% for E2 formulation where a greater amount of drug was encapsulated. The highest loading efficiency, greater the amount of drug was encapsulated. Percentage entrapment depends on internal phase and external phase volume. It suggests that change in phase volume changed the entrapment efficiency.

***In Vitro* Diffusion Studies:**

The nanosponge formulations were subjected to *in vitro* release studies using Franz diffusion cell at the stirring rate at 100rpm and temperature at 37±0.5°C. The results obtained in *in vitro* release studies were plotted in cumulative percent drug release Vs Time. The release data obtained for formulations P1 to P8 and E1 to E8 and F1 to F8 are tabulated in Table 1.6, 1.7, and 1.8. Figures 1.2, 1.3 and 1.4 show plots of cumulative percent drug released as a function of time for different formulations respectively. The total amount of drug released for the 1:2 of Drug: Polymer ratios were 92.76%, 91.84% and 95.88% observed at different time intervals for a period of 12 hours for optimized formulations P2, E2 and F2. The release rate was related to drug: polymer ratio. Increase of drug release was observed as a function of drug: polymer ratio. It was observed that the drug release decreased with an increase in the amount of polymer for each formulation. This may be due to the fact that the release of drug from the polymer matrix takes place after complete swelling of the polymer and as the amount of polymer in the formulation increases the time required to swell also increases. The release showed a bi-phasic pattern with an initial burst effect may be due to the unentrapped drug adsorbed on the surface of the nanosponges. In the first hour drug release was found to be 17-30%. In general, all nanosponge formulations showed a prolonged release and no burst effect was observed.[12]

Table 1.6: Evaluation of Poly methylmethacrylate Nanosponges

Formulation Code	Drug:PMMA:PVA	Particle size (nm)	Entrapment Efficiency (%)	%Drug Release
P1	0.25:1:3	650	57.6	-
P2	0.5:1:3	285	70.4	92.76
P3	0.75:1:3	570	64.6	85.64
P4	1:1:3	800	53.2	80.12
P5	0.5:1:2	Micro range	61.2	82.36
P6	0.5:2:2	Micro range	56.7	80.05
P7	0.5:2:3	Micro range	47.4	79.42
P8	0.5:3:3	Micro range	42.8	77.58

Table 1.7: Evaluation of Ethyl cellulose Nanosponges

Formulation Code	Drug:EC:PVA	Particle size (nm)	Entrapment Efficiency (%)	% Drug Release
E1	0.25:1:2	800	58.2	-
E2	0.5:1:2	370	69.8	91.84
E3	0.75:1:2	510	62.2	85.54
E4	1:1:2	750	59.4	82.06
E5	0.5:1:1	Micro range	65.4	87.31
E6	0.5:1:3	Micro range	52.8	79.44
E7	0.5:2:2	Micro range	55.6	81.32
E8	0.5:2:3	Micro range	44.5	74.36

Table 1.8: Evaluation of pluronic F-68 Nanosponges

Formulation Code	Drug:F-68:PVA	Particle size (nm)	Entrapment Efficiency (%)	%Drug Release
F1	0.25:1:1	750	62.3	-
F2	0.5:1:1	310	72.5	95.88
F3	0.75:1:1	620	64.8	89.74
F4	1:1:1	780	60.1	86.02
F5	0.5:1:2	Micro range	67.4	91.54
F6	0.5:1:3	Micro range	56.3	83.92
F7	0.5:2:2	Micro range	48.6	81.14
F8	0.5:2:3	Micro range	42.7	76.28

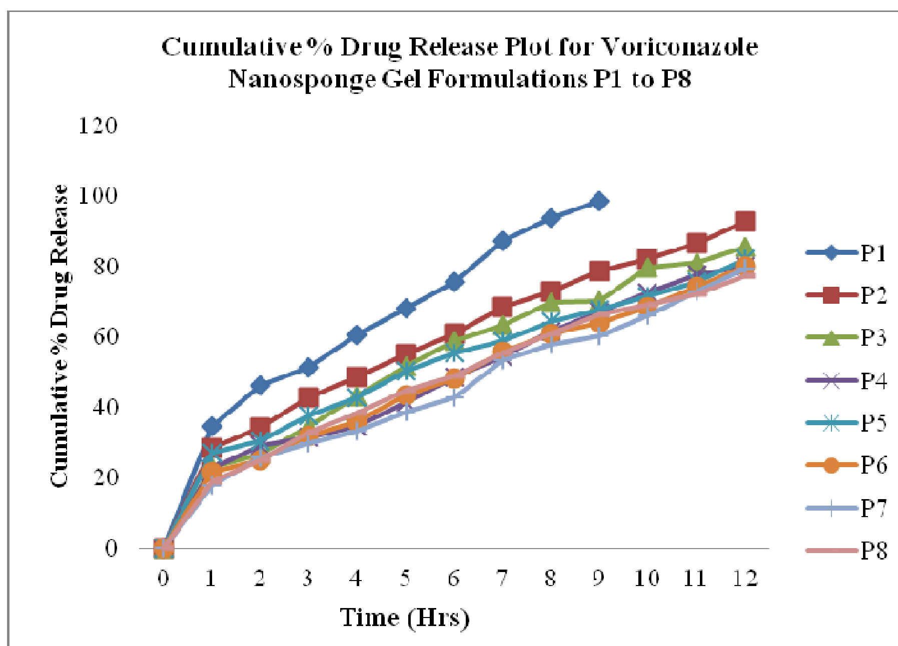

Figure 1.2: *In vitro* diffusion profile of PMMA Nanosponge Gel P1 to P8 in 6.8pH phosphate buffer

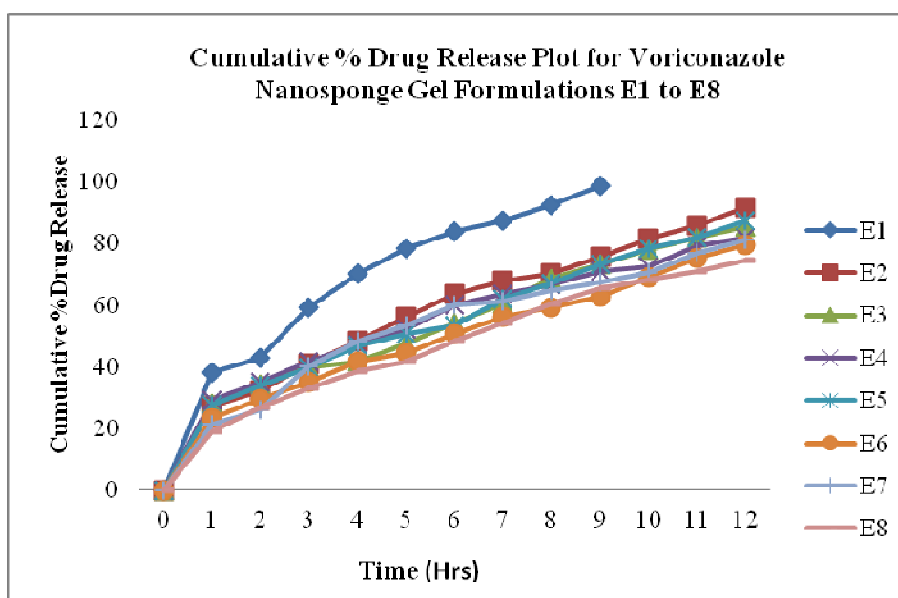

Figure 1.3: *In vitro* diffusion profile of EC Nanosponge Gel E1 to E8 in 6.8pH phosphate buffer

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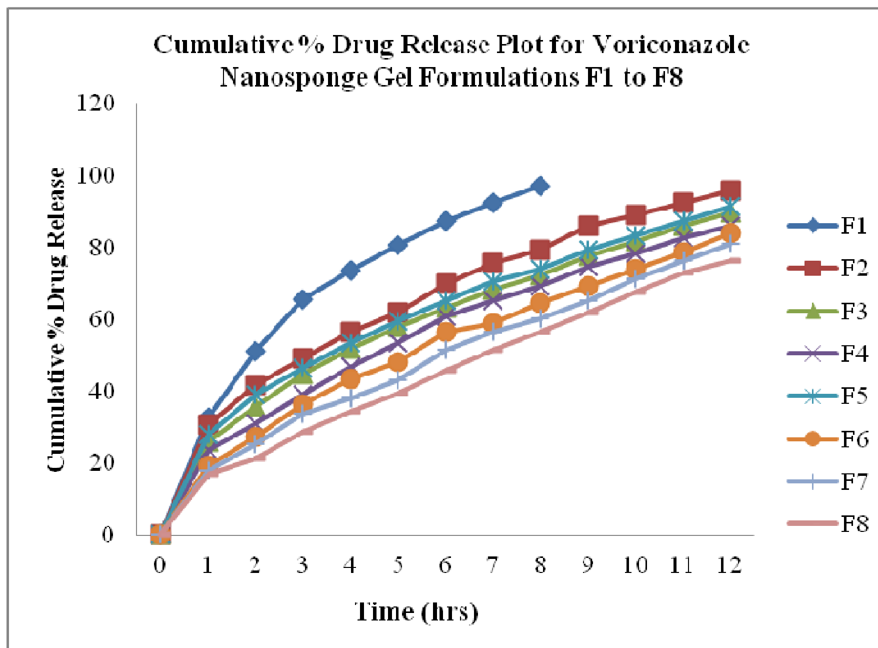


Figure 1.4: *In vitro* diffusion profile of Pluronic F-68 Nanosponge Gel F1 to F8 in 6.8pH phosphate buffer

Table 1.9: Release kinetics data from Voriconazole nanosponges

Formulation	Zero order R^2	First order R^2	Higuchi model R^2	Korsmeyer-peppa's model R^2
P2	0.9916	0.9433	0.9925	0.9857
E2	0.9856	0.9449	0.9911	0.9844
F2	0.9754	0.9541	0.9978	0.9878

The drug release from nanosponges data were fitted into drug release kinetic models such as zero order, first order, higuchi and korsmeyers-peppa's equation[9]. The *in vitro* release data of nanosponge formulations fitted best to the Higuchi model (Table 1.9). The release data from the Higuchi equation nanosponges obeyed diffusion controlled process as shown in the table 1.9.

Evaluation of nanosponge tablet formulations:

The nanosponge tablets of Voriconazole were prepared by direct compression method. The prepared nanosponge tablets were evaluated for their hardness, friability, drug content and in vitro drug release and the results are presented in Table 1.10. Hardness was in the range of 4.1 to 4.2 kg/cm2. Friability was less than 1% in all the formulations. Drug content of the nanosponge tablet formulations ranged between 91±0.5 to 92±0.5%. It is clear from the above said factors that the physical parameters evaluated for the different batches of tablets were satisfactory.[13]

Table 1.10: Evaluation of Nanosponge tablets P2, E2 and F2 formulations

Formulations	Hardness Kg/cm²	Thickness mm	Friability (%)	Drug Content (%)
P2	4.1	1.8	0.42	92.0±0.5
E2	4.1	1.8	0.38	91.5±0.5
F2	4.2	1.8	0.45	91.8±0.5

Anti microbial study:

Antimicrobial activities for gels are shown in Table 1.11. Formulation P2, E2 showed 18mm and F2 showed 18.5 mm inhibition which is similar to the zone of inhibition of marketed Fluconazole Gel 19mm. Further P2, E2 and F2 formulations were used for *in vivo* antifungal activity study on rats for topical study.

Table 1.11: Antimicrobial activity of Voriconazole nanosponge Gel P2, E2 and F2 comparing with Marketed Fluconazole Gel

Organism	Formulation	Zone of inhibition diameter in mm
Candida albicans	P2	18mm
Candida albicans	E2	18mm
Candida albicans	F2	18.5mm
Candida albicans	Marketed Fluconazole gel	19mm

Figure 1.5: Antimicrobial activity of optimised formulations E2, P2 and F2 in comparison with Marketed formulation

***In vivo* study:**

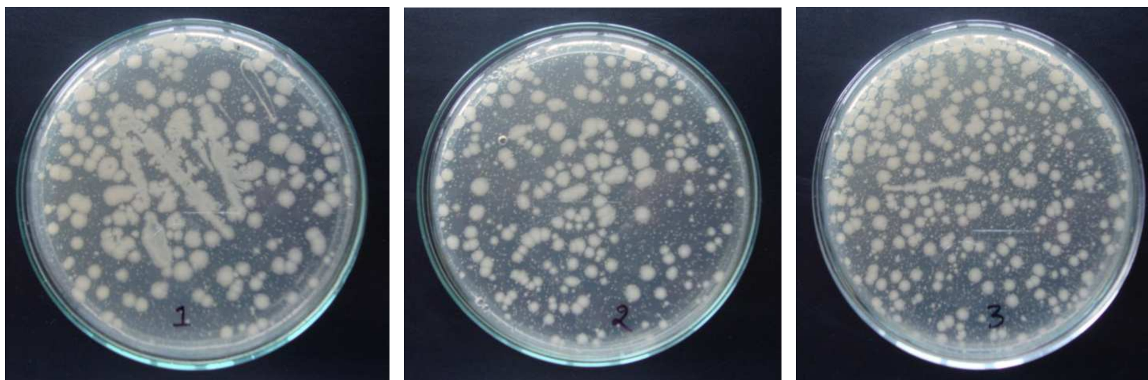
The results of the *in vivo* antifungal activity on rat skin are shown in Table 1.12. The fungal infection was induced on the rat for first 3 days, on the 4th day, the skin of rat was scraped and was cultivated in sabouraud dextrose agar media plates and colony count was observed more than 130 for formulation E2 gel, P2 gel and Marketed Flucos gel respectively. The treatment of the gel formulation was started from 4th day applying for next 4 days and studied with the effectiveness of gel on fungal inhibition. After 8th day the skin of the rat was again scraped and checked for the growth of colony in sabouraud dextrose agar plate. Colony count was observed 90, 70 and 75 for formulation E2, P2 and marketed formulation respectively. Similarly on 11th day the skin of rat was again scraped and checked for the growth of colony in sabouraud dextrose agar plate. Colony count was observed 43, 32 and 38 for formulation E2, P2 and marketed formulation respectively.[14]

Antimicrobial study and *in vivo* study results showed that formulation E2 and P2 showed effective antifungal action. However, of all the formulations P2 was found to show better antifungal activity indicating poly methylmethacrylate is a good carrier for topical nanosponge gel.

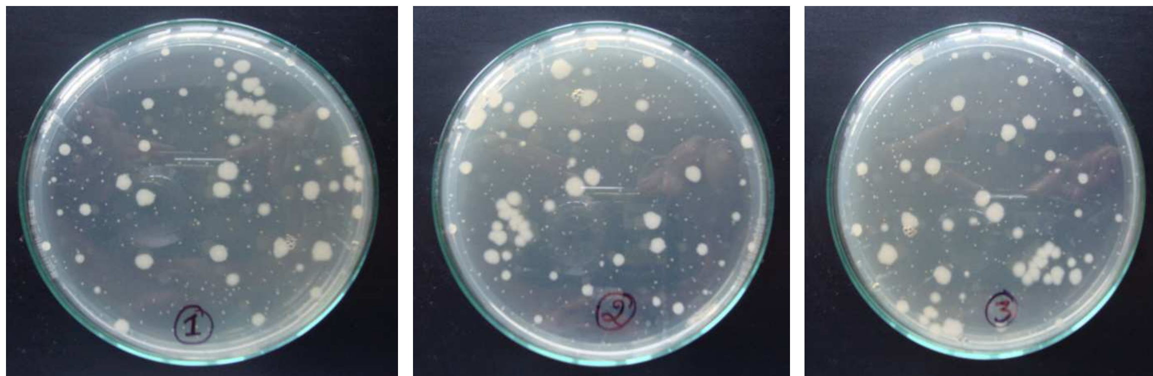
Table 1.12 Results of *in vivo* antifungal study on rat skin

Formulation	Colony count After fungal infection	Colony count on 8th day after treatment	Colony count on11th day after treatment
E2	> 130	90	43
P2	> 130	70	32
Marketed Flucos Gel	> 130	75	38

After induction of fungus for formulations 1. E2 Gel 2. P2 Gel 3. Marketed Flucos Gel



On 8th Day Inhibition of colony after application of formulated gel 1. E2 Gel; 2. P2 Gel; 3. Marketed Flucos Gel



On 11th Day Inhibition of colony after application of formulated gel 1. E2 Gel; 2. P2 Gel; 3. Marketed Flucos Gel

Figure 1.6 *In vivo* antifungal study on rat skin

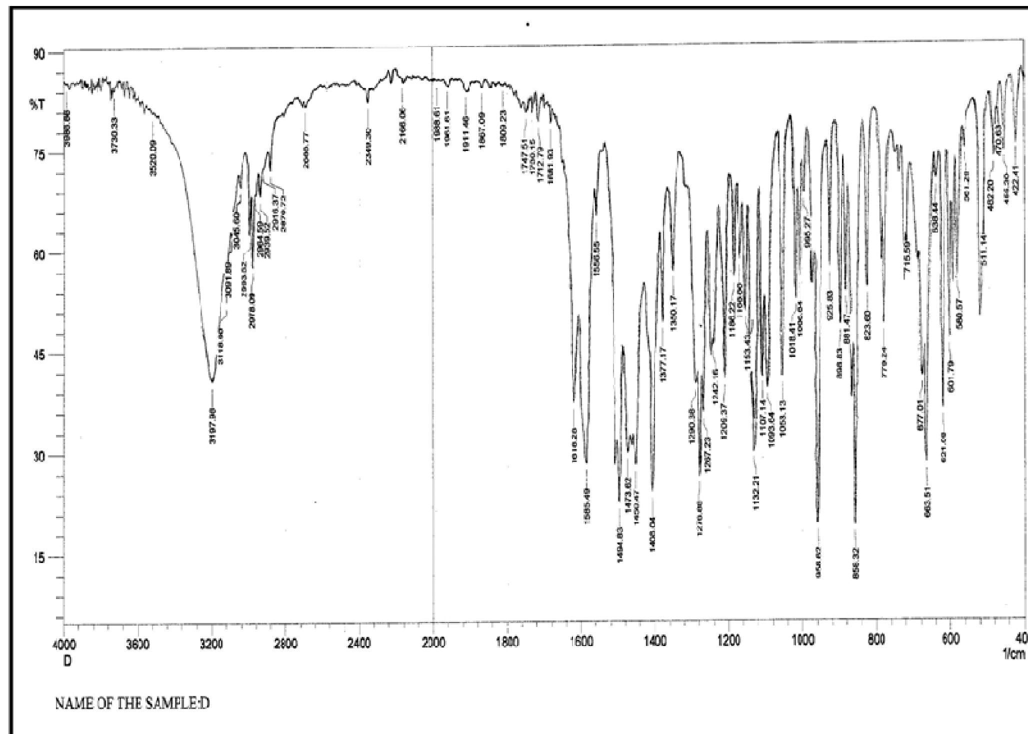


Figure 1.7: FTIR image of Voriconazole

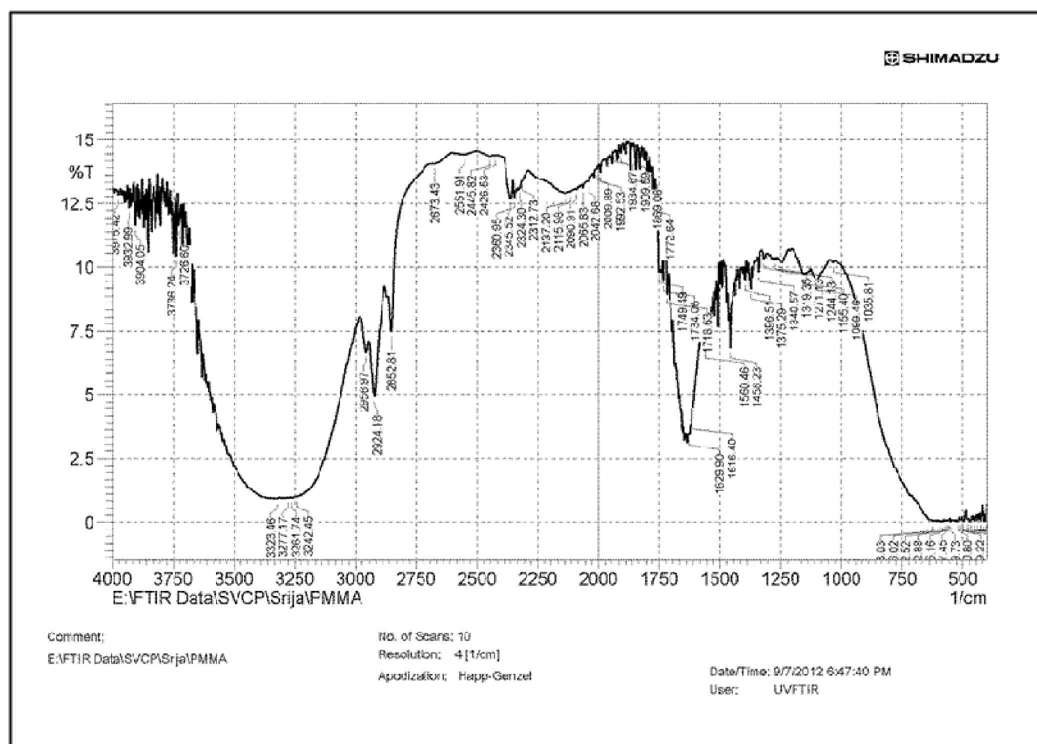


Figure 1.8: FTIR image of P2 Formulation

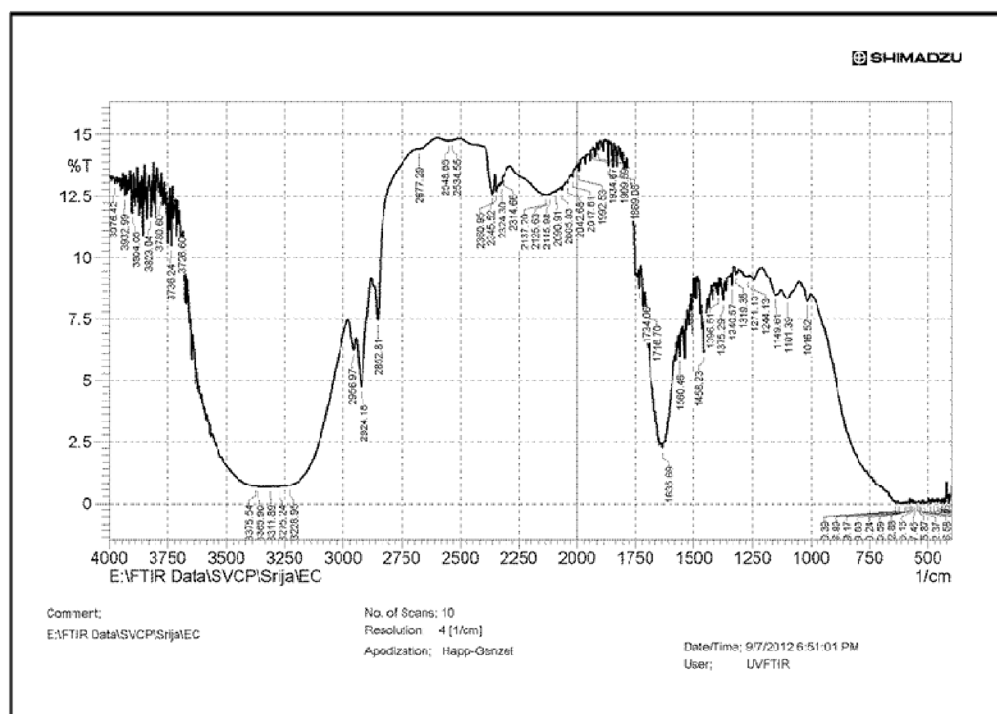


Figure 1.9: FTIR image of E2 Formulation

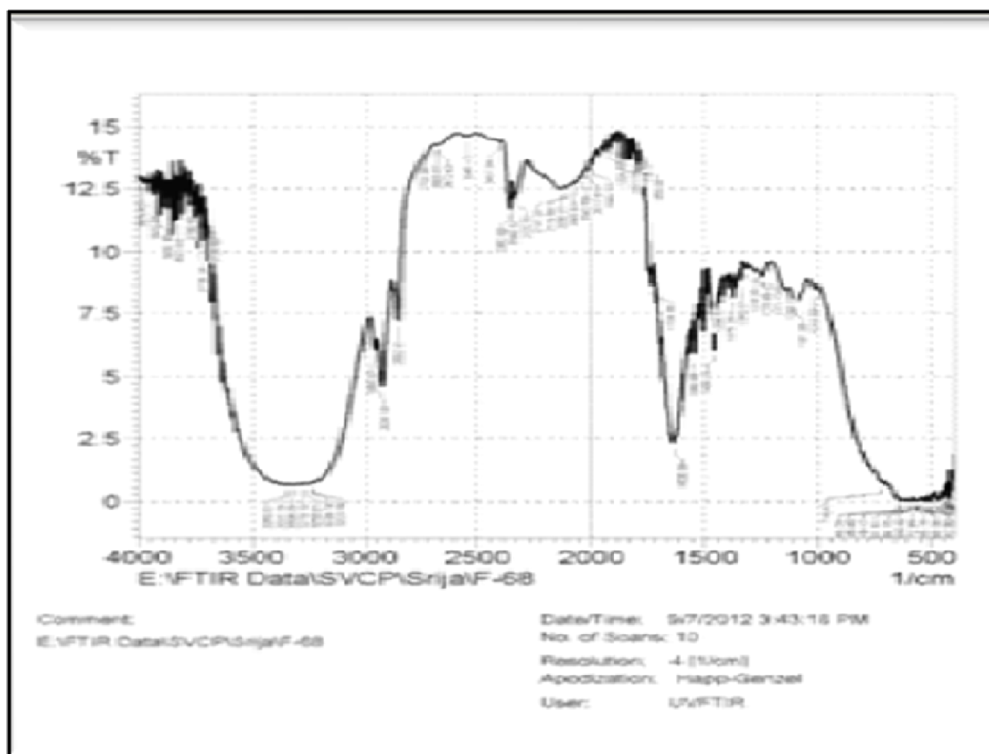


Figure 1.10: FTIR image of F2 Formulation

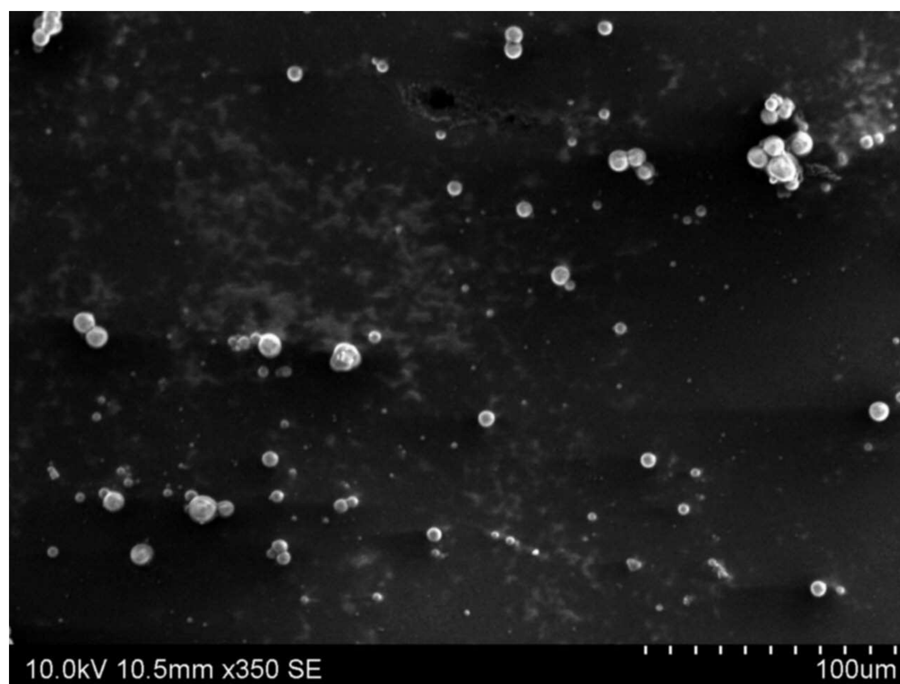


Figure 1.11: Scanning Electron Microscopy of E2 nanosponge formulation

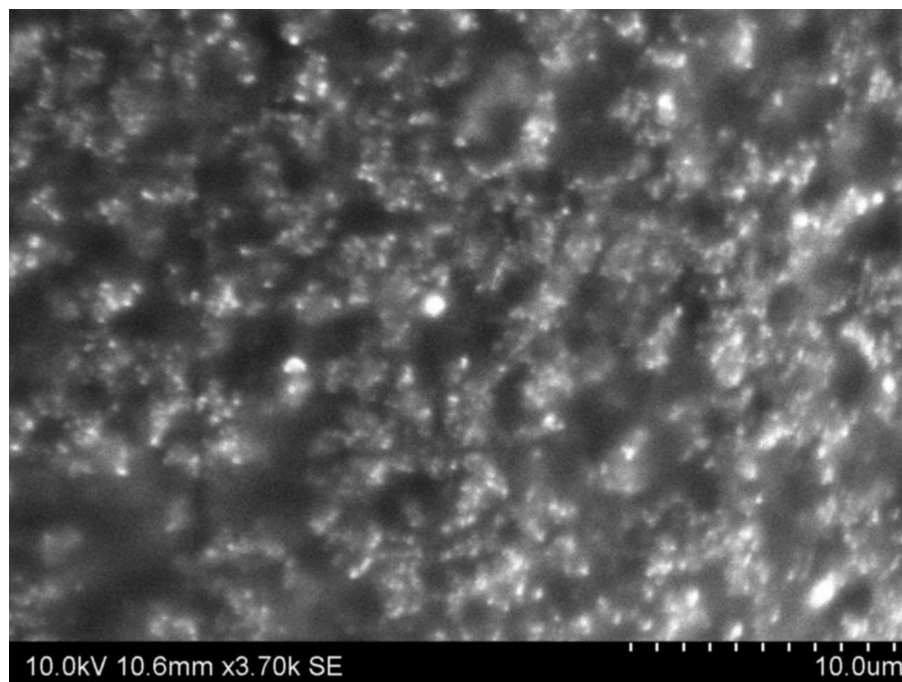


Figure 1.12: Scanning Electron Microscopy of P2 nanosponge formulation

CONCLUSION:

The nanosponges prepared with ethyl cellulose, poly methylmethacryate and pluronic F-68 were successfully incorporated into topical hydrogel as well as tablet for oral administration. The polymers studied were found to be the efficient carriers for Voriconazole nanosponges showing diffusion controlled release. The nanosponge systems have been found to have good potential for prolonged drug release and therefore can be beneficial for use in the treatment of various chronic fungal infections. Additional benefits such as dose reduction, reduced frequency of administration and avoiding related systemic side effects can be produced.

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