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DESIGN AND EVALUATION OF TOPICAL FORMULATION OF VORICONAZOLE USING CROSS LINKED CYCLODEXTRIN NANOSPONGES

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ABSTRACT

The solubility of voriconazole a triazole antifungal drug in water is very low. The molecule is semi-polar (log D=1.8), making it difficult to solubilize with oils, surfactants, or water miscible co-solvents. It also breaks down by a retro-aldol reaction to compounds A and C. This instability is pronounced not only in aqueous media but also in solid state.Nanosponges are hypercross-linked cyclodextrin polymers that have recently been produced and are assembled into three-dimensional networks via nanostructure. Cyclodextrin undergoes a reaction with a cross-linking agent, like diphenyl carbonate or carbonyldiimidazole, to produce these polymers. Nanosponges based on cyclodextrin were able to complex a greater number of molecules than natural cyclodextrins. They limit the release of the weakly soluble actives, protect the labile groups, and improve solubility. The present study investigated whether cyclodextrin-based nanosponges may improve voriconazole chemical stability and water solubility. The results, indicate that the cyclodextrin nanosponges-based gel formulation of voriconazole offers great topical administration potential due to its delayed and consistent drug release, enhanced skin penetration, and good storage stability.

Keywords: Voriconazole, nanosponges, cyclodextrin, solubility, stability

INTRODUCTION

Azole antifungals often cure superficial and systemic fungal infections **[1]**. Voriconazole, a newer triazole drug, offers superior effectiveness and safety than the others **[2]**. Fluconazole, itraconazole, flucytosine, and amphotericin B are weaker than it **[1, 3]**. This antifungal drug treats filamentous fungal infections systemically **[4]**. Over the last decade, nanosponges based on cyclodextrin have developed into a potential medication delivery system for synthesizing complex molecules **[5, 6]**. Nanosponges are hypercross-linked cyclodextrin polymers that have recently been produced and are three-dimensional networks created by nanostructure **[7]**.

These polymers are created when cyclodextrin reacts with a cross-linking agent, such as diphenyl carbonate or carbonyldiimidazole **[8]**. Nanosponges based on cyclodextrin couldwere able to complex agreater number of molecules than natural cyclodextrins **[9]**. They prevent the release of the poorly soluble actives, improve solubility, and shield the labile groups. They protect the labile groups, improve solubility make the solubility better, and restrict the release of the weakly soluble actives **[10]**. Recent research conducted by Darandale and colleagues found that using cyclodextrin nanosponges increased curcumin's solubility while simultaneously lowering the rate of hydrolytic degradation and biotransformation **[11]**.

The need for the study stems from the fact that nanosponges lack an uninterrupted membrane in their surroundings due to their open structure and surface holes. The encapsulated active medicinal ingredient is introduced to the vehicle **[12]**. The active ingredient that has been encapsulated can freely flow from the particles into the vehicle until the vehicle is saturated and equilibrium is reached **[13]**. Upon application When the product is applied to the skin, the drug's active is released from the vehicle, disrupting the equilibrium. This will initiate the drug's release, allowing the active ingredient to flow from the nanosponge particle into the vehicle and then onto the skin until the vehicle is either absorbed or dries out. The release of active medication material lasts for a considerable amount of time even after the stratum corneum, or nanosponge particles, on the skin's surface are eliminated. The release of the active medicinal ingredient into the skin lasts for a considerable amount of time even after the nanosponge particles on the stratum corneum, the skin's outermost layer, are removed. The most effective technique for a regulated and extended release of medication goods onto the skin is nanosponges technology. Medications with antifungal and anti-inflammatory properties

are frequently utilized in topical treatments. The medicine is released in a sustained and predictable manner by the nanosponge drug delivery system, in contrast to conventional products that release the drug in a relatively high concentration that may cause substantial side effects. It is possible to formulate the nanosponges into creams, lotions, gels, ointments, and other dosage forms **[8]**.

EXPERIMENTAL

Materials

Cyclodextrin-based carbonate nanosponges were prepared in our laboratory as reported elsewhere **[14]** (Singireddy A *et al.,* 2014). Hetero Labs, Hyderabad, provided voriconazole and tazarotene. S.D. Fine Chem. Pvt. Ltd., Mumbai supplied Carbopol 934, propylene glycol, triethanolamine, and N-methyl-2-pyrrolidone.

Quantitative determination of voriconazole by HPLC

Development of chromatographic method

Standard stock solution preparation

10 mL of acetonitrile was poured to a 25 mL standard flask that had been cleaned and dried, containing 25 mg of voriconazole, followed by shaking to dissolve the drug upto 10min and made upto mark with ACN. The concentration of the solution was 1mg/mL. A standard solution of 100 ug/mL was prepared by serial dilution from standard stock solution. Both the solutions were stored at -20 °C.

Selection of chromatographic method

Hamilton PRP C-18 (150×4.6mm 5micronThe mobile phase of the column was adjusted to facilitate chromatographic separation flow of1mL/min at 273 nm. Column temperature maintained at room temperature was usedThe stationary phase is less polar than the mobile phase in an RP-HPLC system. Typically, it consists of a blend of two or more distinct solvents, either with or without organic solvent modifiers to adjust pH. At a pH of 4, the mobile phase consists of 0.1% formic acid in water and 30% acetonitrile v/v. The flow rate is 1 mL/min, and the sample is measured at 254 nm. The mobile solvent system was degasified by sonication is performed after vacuum filtration with a 0.45µ membrane filter.

Five different concentrations over the 50 to 250 µg/mL range were produced, and calibration curves were used to measure linearity **[15]**. Each concentration of linearity was infused into the LC-system thrice by taking the same infusion volume.

Solubilisation efficiency of nanosponges

In this study, the solubilization-enhancing ability of nanosponges (NS1-NS3) and their efficacy in solubilizing substances were studied **[16, 17]**. A constant amount of NS, also 20 mg, was mixed with an excessive quantity of voriconazole, also 20 mg, and The mixture was then suspended in Milli-Q water (20 milliliters). A mechanical shaker

was used to mix the contents of the volumetric flasks while they were left at room temperature. Following the establishment of equilibrium for twenty-four hours, the suspensions were centrifuged for ten minutes at a speed of ten thousand rpm was the speed at which the free voriconazole was extracted from the collected colloidal supernatant. Following establishing equilibrium for twenty-four hours, the suspensions were centrifuged for ten minutes at a speed of 10000rpm to separate the free voriconazole from the colloidal supernatant, collected. The voriconazole contained in the nanosponges was extracted from the supernatant by adding 10 milliliters of methanol to the above mentioned mixture. Following this, the colloidal supernatant solution was examined by high-performance liquid chromatography (HPLC) using a calibration curve to determine the concentration of voriconazole.

Preparation of voriconazole loaded β-cyclodextrin nanosponges

Voriconazole was freeze-dried into nanosponges **[14]**. A magnetic stirrer was used to suspend accurately weighed nanosponges in fifty milliliters of Milli Q water. After the addition of excess voriconazole, for 10 minutes, the mixture was sonicated, and for twenty-four hours, it was stirred. The uncomplexed medication was separated as a residue that is lower than the colloidal supernatant by centrifuging at 2,000 rpm for 10 min. A LARK INDIA lyophilizer set at -20 °C and 13.33 mbar was employed and freeze-dried the supernatant. In desiccators, the freeze-dried powder was kept. Desiccators held the powder. Depending on the kind of nanosponges, the developed voriconazole-loaded nanosponge formulations were given the abbreviations VONS2 and VONS3, respectively.

Drug payload and entrapment efficiency

Voriconazole-loaded nanosponge complex was dissolved in methanol, broken up by sonication for ten minutes, diluted, and then subjected to HPLC analysis to assess the voriconazole content in the formulation. Calculations were made using the following formulas 6.1 and 6.2 to determine the percent drug payload and the percent drug entrapment efficiency **[18]**.

$$\%\, Drugpayload = \frac{Weightofdrugencapsulatedin NSformulation}{WeightoftheNSformulationtakenforanalysis} \times 100 \quad (6.1)$$

$$\%\, Drugentrapmentefficiency = \frac{Weightofdrugentrappedin NSformulation}{Initialweightofthedrugfedforloading} \times 100 \quad (6.2)$$

Characterisation of voriconazole-loaded nanosponges

Zeta Potential

To determine the formulation's particle size range and homogeneity, a Mastersizer 2000 was used. Each measurement was diluted with Milli Q water. Zetasizers were used to measure its zeta potential (Malvern Instruments Ltd, Worcestershire, UK). A transparent disposable zeta cell kept the instrument at 25°C **[19]**. After averaging three data, cumulant analysis computed the particles mean diameter and PI.

Transmission Electron Microscopy

The researched voriconazole-loaded nanosponges' particle sizes were examined using transmission electron microscopy. A drop of diluted voriconazole nanosponge suspension was applied to a film-coated copper grid to enhance contrast and dyed with 2% (w/v) phosphotungstic acid and dried. Transmission electron microscopy examined the samples. At a resoulution of 45000X, the particle size of the nanosponges were studied.

FTIR studies

Voriconazole pure drug, plain nanosponges and voriconazole-loaded nanosponges were analyzed by potassium bromide disc technique utilising Tensor 27 FTIR from 4000 to 600 cm^{-1}.

Differential Scanning Colorimetry

Voriconazole, nanosponges and nanosponge complexes were studied utilising a Perkin Elmer DSC/7. Melting point and heat of fusion were calibrated using Indium. 30–400°C temperatures were heated at 10°C/min. The reference standard was an empty Perkin-Elmer aluminium sample pan. Nitrogen-purged 5 mg samples were analysed in triplicate.

XRD studies

Voriconazole, nanosponges, and voriconazole nanosponge complexes were measured at 5°/min in the 2° range of 2.5° to 60° using a Bruker D8 Advance X-ray diffractometer **[20]**. ***In-vitro*** **release studies**

In-vitro release studies used dialysis membrane-equipped multicompartment (n = 6) rotating cells (Sartorius cut off 12,000 Da). In the donor phase, there were Voriconazole 10 mg in 100 milliliter buffers with a pH of 6.4. Same media was used in receptor phase. After predetermined time intervals, the receptor phase was removed, distilled water used to dilute it, then HPLC analysis done at 254 nm. Triplicated experiments were performed. Several kinetic models were fitted with the dissolution study's data to assess the mechanism of drug release.

Preparation of gel formulation of voriconazole nanosponges

Carbopol 934 was used to make voriconazole nanosponges gel base **[21]**. For a homogenous 1% w/w gel basis, the polymer wassteeped in water for 2 hours and then dispersed in distilled water using a

magnetic stirrer. 1% Triethanolamine, 2% N-methyl-2-pyrrolidone and 2% w/w Propylene glycol were added with stirring into the gel base **[22]**. In the end, nanosponges loaded with voriconazole were added to the prepared gel base to achieve a concentration of 1% w/w voriconazole in the gel base (drug-to-carrier ratio of 1:99). Free voriconazole was added to the gel base to produce the control formulation.

Evaluation of gel formulations [23, 24]

pH determination

Digital pH metres were utilized to measure the gel compositions pH. 0.25 g nanosponge-based gel was carefully weighed and dispersed in 25 ml purified filtered waterThe pH meter was calibrated using buffer solutions with pH values of 4.0, 7.0, and 9.0 prior to use. Formulation pH was measured in triplicate and the mean values were computed.

Skin permeation studies

The Central Animal House of Vijaya College of Pharmacy, Hyderabad, India, furnished 6–8-week-old Wistar Albino rats weighing 120–150 g for in-vitro permeation investigations **[25]**. Ventilated animal chambers at 25°C with a 12/12 light/dark cycle was the condition for housed the animals. Before the trial, animals were acclimatized for a week. The Vijaya College of Pharmacy institutional animal ethics committee accepted all animal experiment protocols. Three groups of rats were administered with free voriconazole gel (Control), nanosponge encapsulated voriconazole, or both (VONS2 & VONS3).

***In vitro* skin permeation and deposition studies**

To chloral hydrate sedated Wistar Albino rats and abdominal hair was meticulously shaved. Subcutaneous fat, connective tissue and a 5 cm^2 of abdominal skin were also removed. The removed skins were cleaned and checked for flaws. Research on the in vitro skin penetration and deposition were employed using Franz diffusion cells having a 3.14 cm2 effective diffusion area. The donor chamber was positioned facing the stratum corneum, and the removed dorsal skin samples were clamped between the Franz diffusion cell **[26]**. The stratum corneum was thinly coated with 0.05 g of the test gel with an effective diffusional area of 1 cm^2. The receptor compartment was filled with 20 milliliters of physiological saline (pH 7.2) with 1% Tween80 in order to increase voriconazole solubility to 155.8 µg/mL and preserve the sink condition for skin penetration. Throughout the experiment, the diffusion cells were swirled at 300 rpm and 32±1°C. At specified intervals (0.5, 1, 2, 4, 6, 8, 10, or 12 hours), 0.5 milliliters of receptor media were obtained for each research and promptly replaced with the same amount of fresh physiological saline solution to maintain preserve sink conditions **[27, 28]**. The

amount of voriconazole in the samples were determined using HPLC.

The skin was properly cleansed with distilled water at hours 3, 6, 9, and 12 following dorsal application. Following dorsal application, the skin was properly cleansed with distilled water at hours 3, 6, 9, and 12h to eliminate excess formulation. The stratum corneum voriconazole was removed by stripping the epidermal layer using cellophane sticky tape. Homogenized skin sections were cut and methanol extracted voriconazole from the tape and homogenised skin. HPLC was used to measure voriconazole content in the supernatant following a 10-minute centrifugation at 4000 rpm.

***In vivo* permeation study in mice**

The dorsal hair was shaven before to the experiment started and cleansed with physiological solution 24 hours in advance. 0.05 g of Test gel were applied in the form of a thin coating to the dorsal surface (3.14 cm^2) **[29]**. Cervical dislocation was used to end the mice's life. The skin that was treated was examined after the mice's deaths at hours 3, 6, 9, and 12h.The methanol extracted voriconazole from skin samples were treated using the same methods that were used to extract it. HPLC was utilized to measure the concentrations of voriconazole in the dermis and epidermis **[28]**.

Assessment of Anti-fungal activity

Candida albicans and *Asperigillus fumigatus* were tested for nanosponge-encapsulated voriconazole antifungal efficacy **[30]**. Sabouraudddextrose was dissolved in hot distilled water to make the nutritional agar medium, which was autoclaved at 121°C for 15 minutes. Test organisms were seeded (10 CFU/mL) in a nutrient agar medium using a diffusion technique **[31]**. Aliquot Test samples were pipetted onto petri plates with nutrient agar medium. The plates were incubated for 24 hours at 25°C after 30 minutes. After 24 hours, the zone of inhibition diameters for *Candida albicans* and *Aspergillus fumigatus* were assessed **[2]**.

Stability studies

Carbopol gel formulations with free or nanosponge-encapsulated voriconazole (1%, w/w) underwent stability testing. All formulations were kept in closed amber glass bottles in a dark environment in room temperature conditions (25°C±2°C; 60% RH±5% RH). Aliquots (40–50 mg) were taken and placed in calibrated flasks at suitable times (20 ml). The samples were sonicated in methanol, diluted to volume, membrane filtered, and HPLC-analyzed for residual voriconazole. Triplicate measurements were taken.

Statistical analysis

At least three separate trials were conducted for each experiment. The statistical comparisons were carried out with the help

of the SPSS 12.0 programmeThe data are presented as mean values with standard deviation (mean S.D.), with statistical significance indicated by a value of P less than 0.05.

RESULTS AND DISCUSSION

Cyclodextrin nanosponges, a new nanoscale polymer delivery method, might carry drugs for parenteral, oral, ocular, and topical administration. They also improve stability, permeability, bioavailability, and drug release. Their highly porous structure traps many hydrophilic and lipophilic chemicals **[9]**.

Solubilisation efficiency

The voriconazole solubilization efficiency of all three nanosponges was compared to free voriconazole in distilled water. **Figure 1** shows that all nanosponges (NS1-NS3) increased solubility. NS2 solubilizes better than voriconazole (13.482 µg/ml). Nanosponges may have better solubilization capacity owing to drug inclusion complex development and matrix entrapment.

The freeze-drying method was used in order to load voriconazole into three distinct kinds of nanosponges. Depending on the kind of NS that was used, the drug-loaded NS formulations that were developed were given the names VONS1, VONS2, and VONS3, respectively. All the manufactured formulations were discovered to be powders that were very fine and easily flowed. **Table 2** contains information on the entrapment efficiency as well as the drug pay load that was calculated in order to determine the percentage of drug to the overall weight of the carrier and the amount of medication connected with nanosponges. It was determined that the average medication payload across all of the batches was 278.92 mg of voriconazole per gram of lyophilized powder.The high encapsulation efficiency of voriconazole (73.56 ± 2.32%) indicated the formationof inclusion complex formation. Nanosponges might have accommodated the drug within the internal cavities of cyclodextrin.The results show a good encapsulation of voriconazole into CDNS2 (1:4) in comparison with CDNS1 (1:2) and CDNS3 (1:8). Based on the high loading capacity, VONS2 and VONS3 were selected for further studies.

Sizes and morphology of the crosslinked CDs loaded with voriconazole

The average size of particles that scatter laser light from nanosponges and nanosponge complexes is 80-130 nm with low polydispersity index. **Table 3** and **Figure 2** show a limited, unimodal particle size distribution. A high zeta potential makes complexes stable and unlikely to agglomerate. Homogenous colloidal suspensions have narrow PIs. All formulae were fine, free-flowing powders. **Figure 3** shows that both nanosponges retain their normal spherical form and size following drug encapsulation.

FTIR analysis of voriconazole-nanosponge complexes

Figure 4 shows FTIR spectra of voriconazole-nanosponge complexes (VONS2 & VONS3In the voriconazole FTIR spectra, the C-N, C-F, and C-C stretching bands peaked at 3197.8–3047.3, 1498.6–1454.2, and 1589.2–1454.2 cm-1. The plain nanosponge's FTIR spectra showed a normal carbonate bond peak at about 1780 cm-1 as well as additional characteristic peaks at 2918 cm-1 from C-H stretching vibrations, 1418 cm-1 from C-H bending vibrations, and 1026 cm-1 from C-O stretching vibrations in primary alcohol. Nanosponge formulations featured solely nanosponge peaks in their FTIR spectra. The nanosponge complex showed no drug peaks, indicating no non-encapsulated drug.

DSC analysis of voriconazole-nanosponge complexes

Figure 5 shows DSC thermograms of free voriconazole, plain nanosponges and complexes. Voriconazole's DSC thermogram exhibited a prominent endothermic peak at 130°C—its melting temperature. Nanosponges DSC thermograms indicated an exothermic peak of about 350°C. The voriconazole compound revealed only nanosponge peaks. Freeze-drying eliminated the drug endothermal peak. This suggests formulation component interactions. This suggests drug amorphousization or inclusion complex development.

XRPD analysis of voriconazole-nanosponge complexes

XRPD patterns of pure voriconazole, cyclodextrin nanosponges and voriconazole-loaded nanosponges were examined to determine its physical properties. **Figure 6** shows voriconazole's high-crystalline peaks. NS complexes showed no pure voriconazole peaks. The nanosponges contained voriconazole since there were no complicated crystalline peaks. FTIR, DSC, and XRD revealed voriconazole inclusion compound with nanosponges.

Voriconazole's *In vitro* release from nanosponge formulations

Figure 7 depicts The properties of voriconazole and voriconazole-loaded nanosponges' dissolution in simulated gastric media. After 24 hours, pure voriconazole dissolves only 12% **(Table 4)**, whereas nanosponges accelerate medication release. Nanosponge complexes released 30% of medication in two hours. Because nanosponges solubilise well. In vitro voriconazole release from nanosponges with low cross linking density was complete after 12 hours (VONS2). VONS2 (1:4) released medication for 24 hours. Nanosponges with altered mesh sizes cause this. VONS3 released drugs slowly and incompletely. Due to VONS3's hypercross-connecting

structure. Complex formulas released gradually after the first action. Diffusion of voriconazole entrapped in nanosponges as inclusion complex slows and sustains release.

***In vitro* skin permeation study**

Both of the gel formulations have increased voriconazole skin penetration substantially 12 h after application **(Figure 8)**. At 12 h after dose, voriconazole gel, VONS2 gel, and VONS3 gel had cumulative levels of 3.07 ± 0.1, 46.22 ± 1.22, and 55.33 ± 2.12 µg/cm2. At 12 h, gel formulations with encapsulated voriconazole penetrated the skin more than 15-20 times more than those with free voriconazole.

Significant difference was observed between both the Test formulations. Compared to VONS2 gel formulation, VONS3 gel formulation enhanced voriconazole skin penetration 12 h after application. The cumulative amount of voriconazole penetrating through the skin from VONS3 gel was 1.2 times more than that from VONS2 gel formulation.

Figure 8 shows voriconazole dispersion in excised rat skin. In-vitro skin penetration demonstrated that voriconazole in nanosponges increased SC concentration at 3h, 6h, 9h, and 12 h ($P< 0.05$). Voriconazole concentration in [E + D] increased after 3h, 6h, 9h, and 12 h ($P<0.05$). Nanosponge encapsulation and carbopol gel formulation may increase voriconazole permeability and deposition.

***In vivo* skin permeation study**

Figure 9 shows the cumulative voriconazole in SC and [E+D] from VO, VONS2, and VONS3 gel formulations after 3, 6, 9, and 12h following treatment in in-vivo experiments. At 12 h, VONS2 gel formulation penetrated SC and [E+D] 18.1 and 23.9 times more voriconazole than VO gel ($p < 0.05$). At 12h, VONS3 formulation penetrated SC and [E+D] 23.5 and 29.6 times more voriconazole than VO gel ($p < 0.05$). VONS3 gel formulation penetrated SC and [E+D] 1.3 and 1.2 times more voriconazole than VONS2 gel formulation at 12 h ($p < 0.05$).

In vivo investigations demonstrated that voriconazole nanosponge gel formulation improves skin penetration. Due to their unique structureVoriconazole encapsulated in nanosponges only diffused in the epidermis, particularly the stratum corneum, with little ability to penetrate the dermis. The findings show that adding nanosponge complex to gel formulation increases voriconazole skin penetration without altering transdermal administration, improving topical delivery.

Assessment of Anti-fungal activity

Voriconazole was tested against *Candida albicans* and *Aspergillus fumigatus*. **Table 5** shows zone of inhibition mean diameters. Agar diffusion microbiological assays

showed that nanosponge-based voriconazole gel formulation suppressed *Candida albicans* and *Aspergillus fumigatus* growth, whereas VO gel did not. After 24 hours, inhibitory zones showed microbial growth decrease.

Finally, gel formulations maintained pH and clarity at ambient temperature. **Figure 10** shows VO gel formulation destroyed 13% of the medication during 3 months. VONS2 and VONS3 gel formulations showed no significant voriconazole content decline over the same time span. Cyclodextrin nanosponges guard against these effects.

Table 1: Optimized chromatographic conditions

Parameter	Condition
HPLC	LC Solutions LC-20AWater 2669 separation module with PDA detector
Mobile phase	Water : acetonitrile (30:70) pH 4
Column	Hamiltin PRP C-18 (150 x 4.6mm) 5µm.
Column Temperature	Room temparaure
Wavelength	254 nm
Injector volume	20µL
Flow speed	1.0 mL/min
Run time	20.0 min
Retention (min)	5.56
No. of theoretical plates	6325
Tailing factor	1.029

Table 2: Drug pay load and entrapment efficiency

Type of formulation	Drug:Polymer	Encapsulation efficiency (%)	percent drug load
VONS1	22.32:77.68	46.78 ± 1.67	22.32 ± 1.35
VONS2	31.26:68.74	73.56 ± 2.32	31.26±1.72
VONS3	26.45:73.55	59.34 ± 2.35	26.45 ± 1.23

The average values of three replicate experiments given as mean ± SD

Table 3: Mean particle size distribution of the voriconazole loaded crosslinked CDs

Sample	particle diameter	PDI	ZP (mV)
NS2	80.56 ± 3.67	0.22 ± 0.005	-25.78 ± 2.6
NS3	81.37 ± 11.45	0.18 ± 0.005	-23.47 ±1.8
VONS2	127.2 ± 9.72	0.227 ± 0.005	-23.7 ± 6.17
VONS3	129.8 ± 6.34	0.215 ± 0.005	-28.9 ± 7.79

The average values of three replicate experiments given as mean ± SD

Table 4: Results of *in vitro* skin permeation study

Sample	Voriconazole (µg/cm^2)	
	Stratum corneum (SC) (µg/cm^2)	(Epidermis + Dermis) (µg/cm^2)
Voriconazole in gel formulation (control)	2.86 ± 0.3	0.21 ± 0.1
VONS2 in gel formulation (test 1)	41.32 ± 1.32*	5.36 ± 1.24*
VONS3 in gel formulation (test 2)	47.87 ± 1.68**	7.51 ± 1.12**

The average values of three replicate experiments given as mean ± SD* p<0.05; **p<0.01

Table 5: Antifungal activity of Voriconazole gel formulation

S. No.	Sample	Zone of inhibition (mm) ± SEM
Candida albicans	Voriconazole in gel formulation	11.24 ± 0.38
	VONS2 in gel formulation	33.23 ± 0.56
	VONS3 in gel formulation	45.23 ± 0.12
Aspergillus fumigates	Voriconazole in gel formulation	10.78 ± 0.12
	VONS 2 in gel formulation	41.61 ± 0.34
	VONS 3 in gel formulation	46.78 ± 0.23

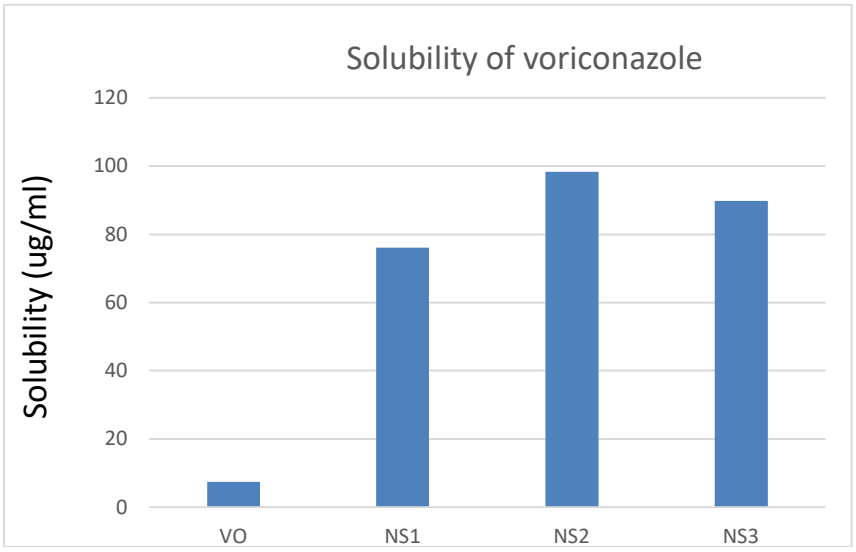

Figure 1: Solubilization efficiency of Nanosponges

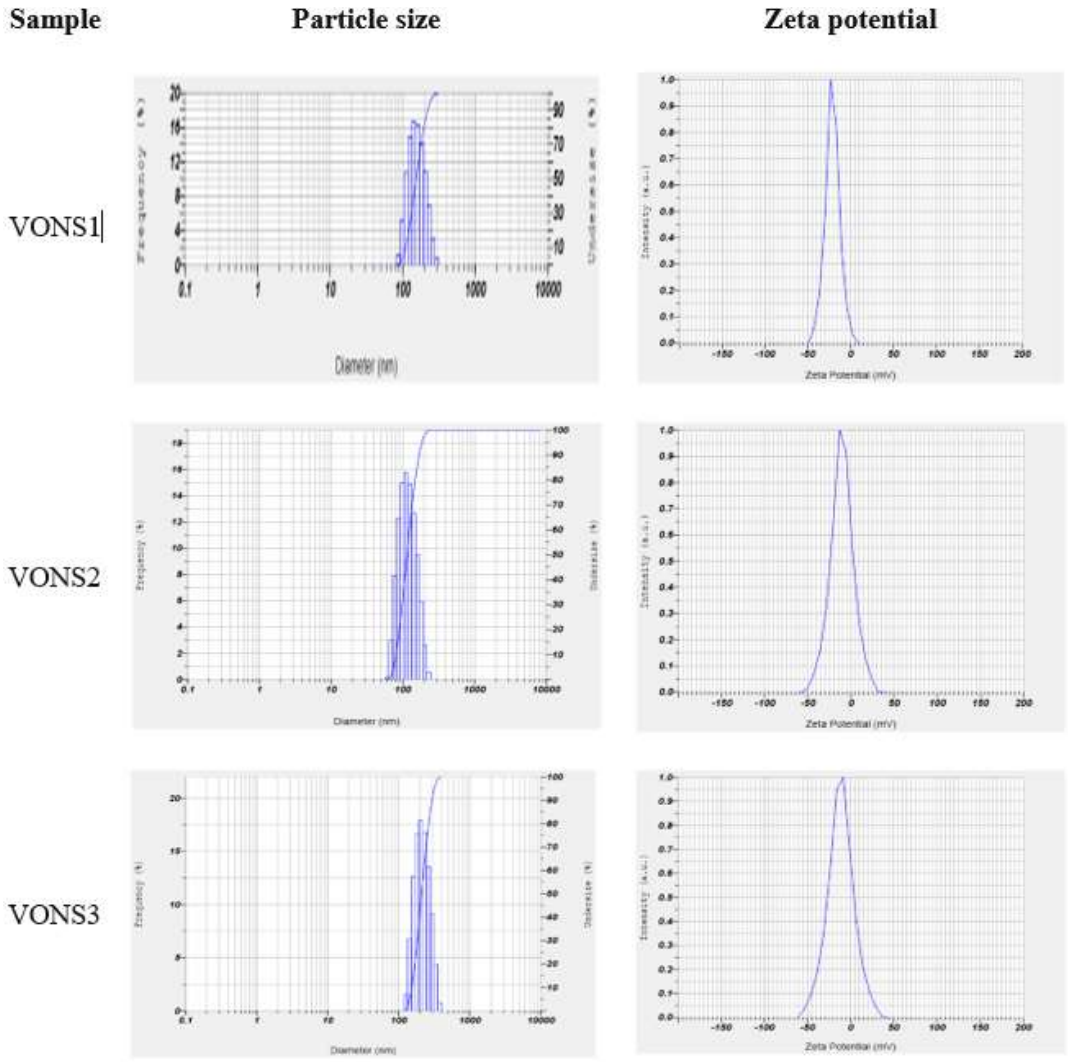

Figure 2: Particel size distribution and zeta potential of drug loaded nanosponges

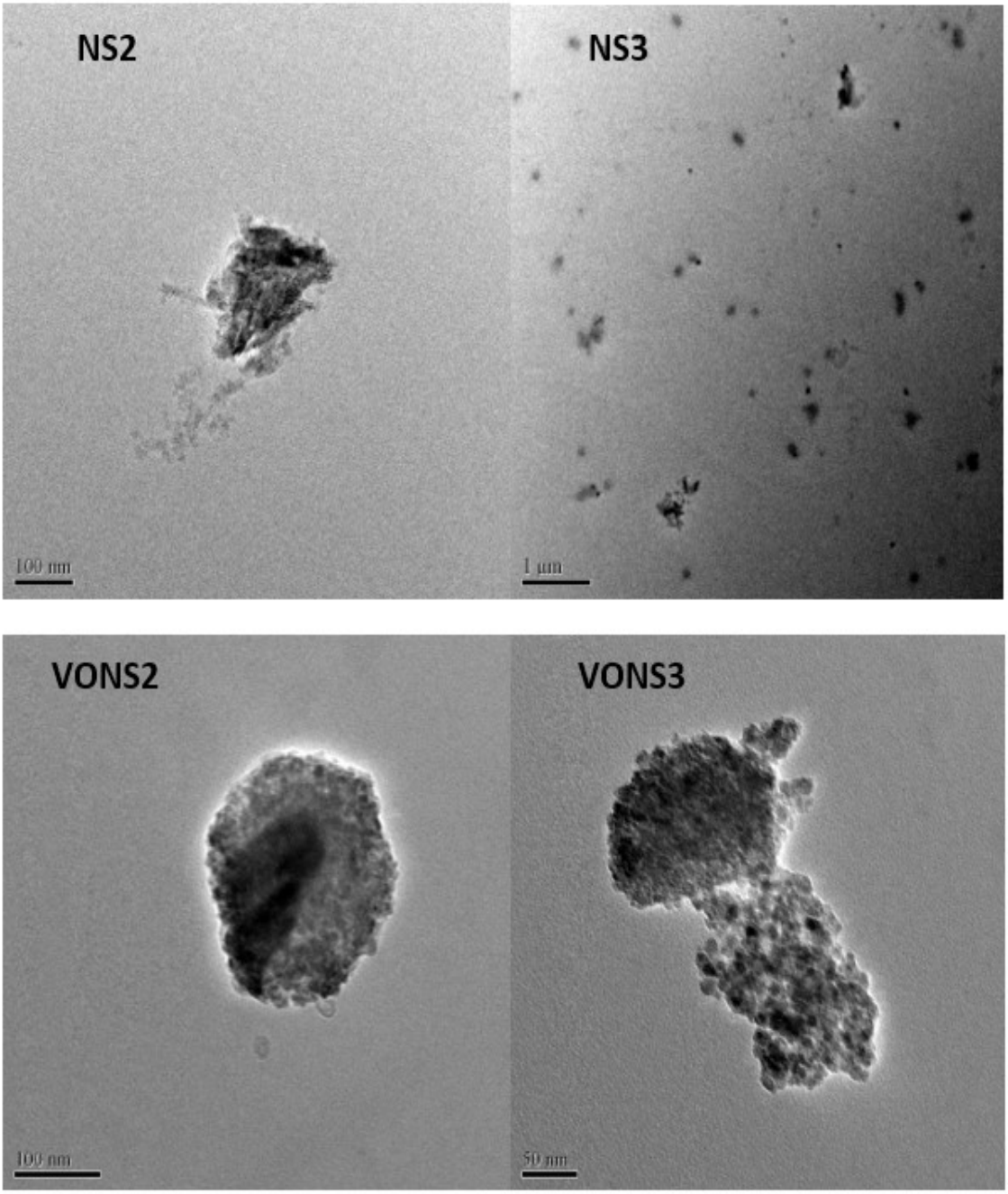

Figure 3: TEM images of cross lined cyclodextrin nanosponges and drug loaded nanosponges

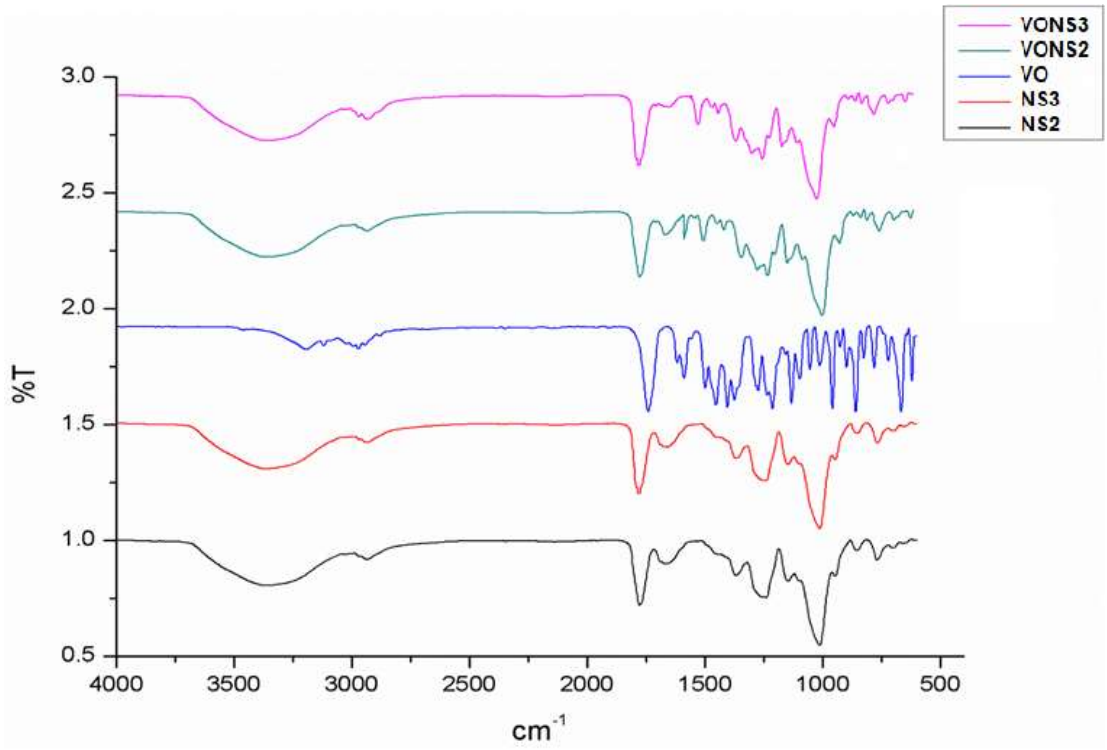

Figure 4: FTIR spectra of cross lined cyclodextrin nanosponges and drug loaded nanosponges

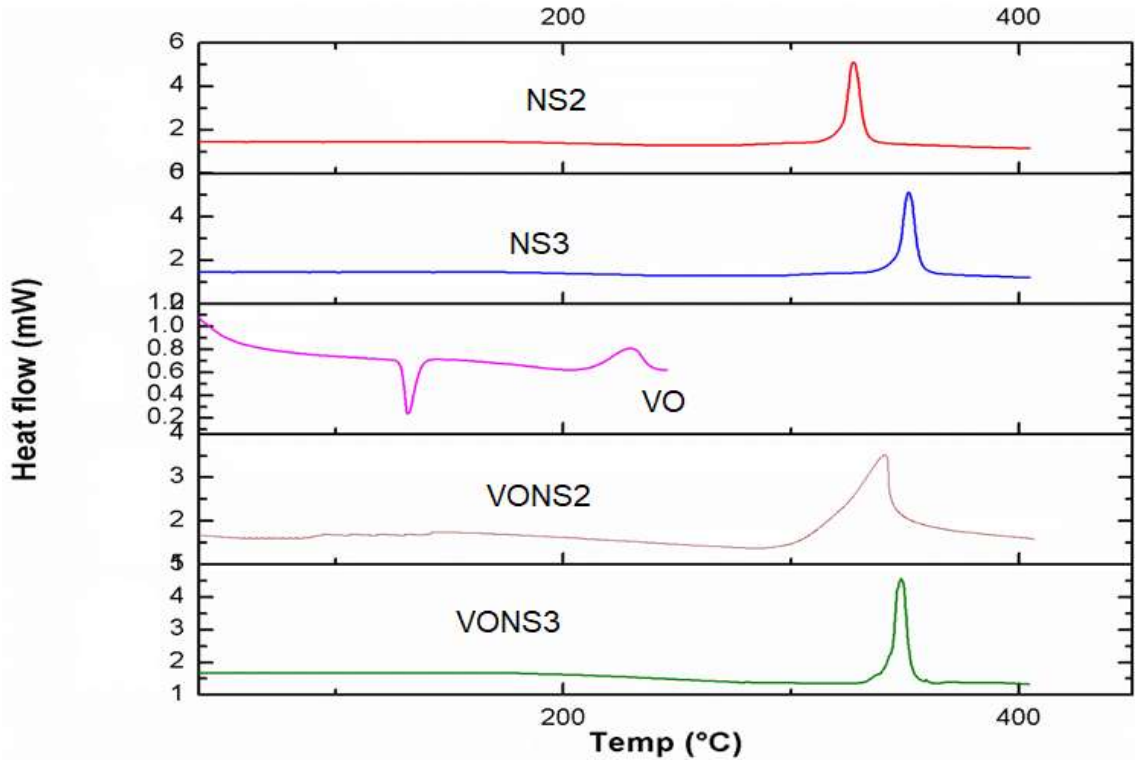

Figure 5: Differential scanning calometry of cross lined cyclodextrin nanosponges and drug loaded nanosponges

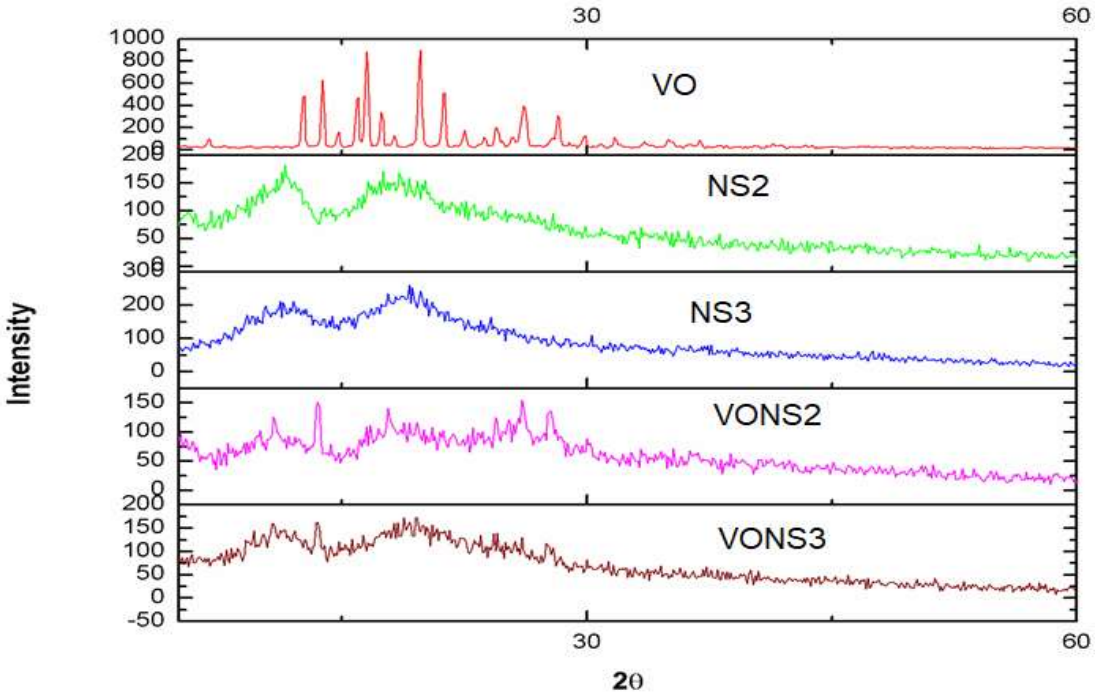

Figure 6: XRPD pattern of cross lined cyclodextrin nanosponges and drug loaded nanosponges

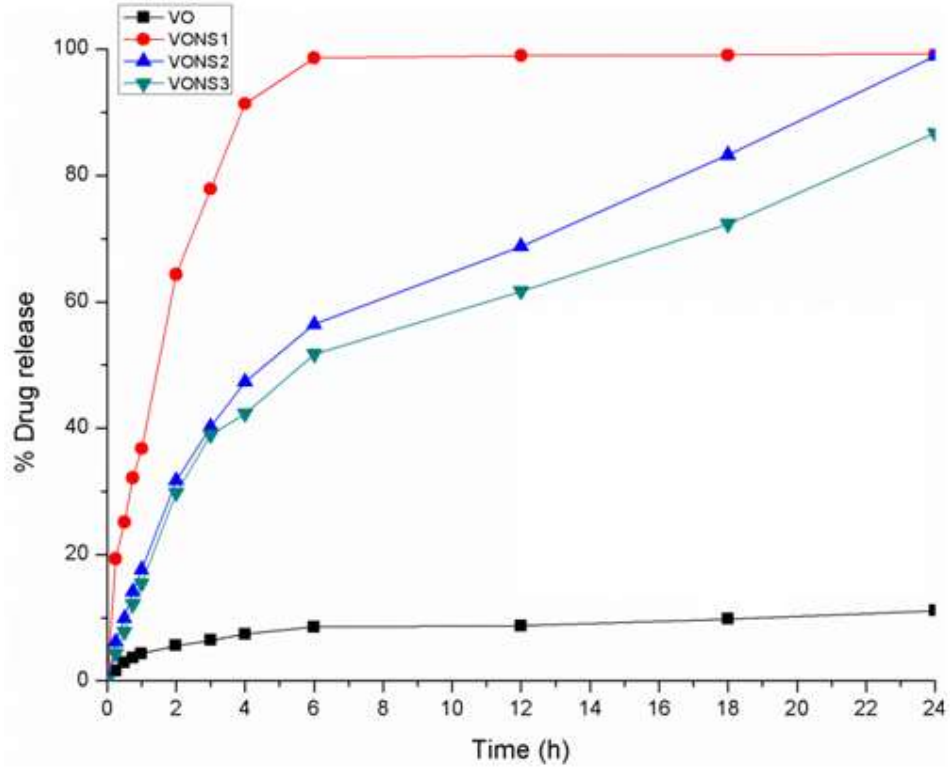

Figure 7: *In vitro* release profile of plain voriconazole and voriconazole loaded cross linked cyclodextrins

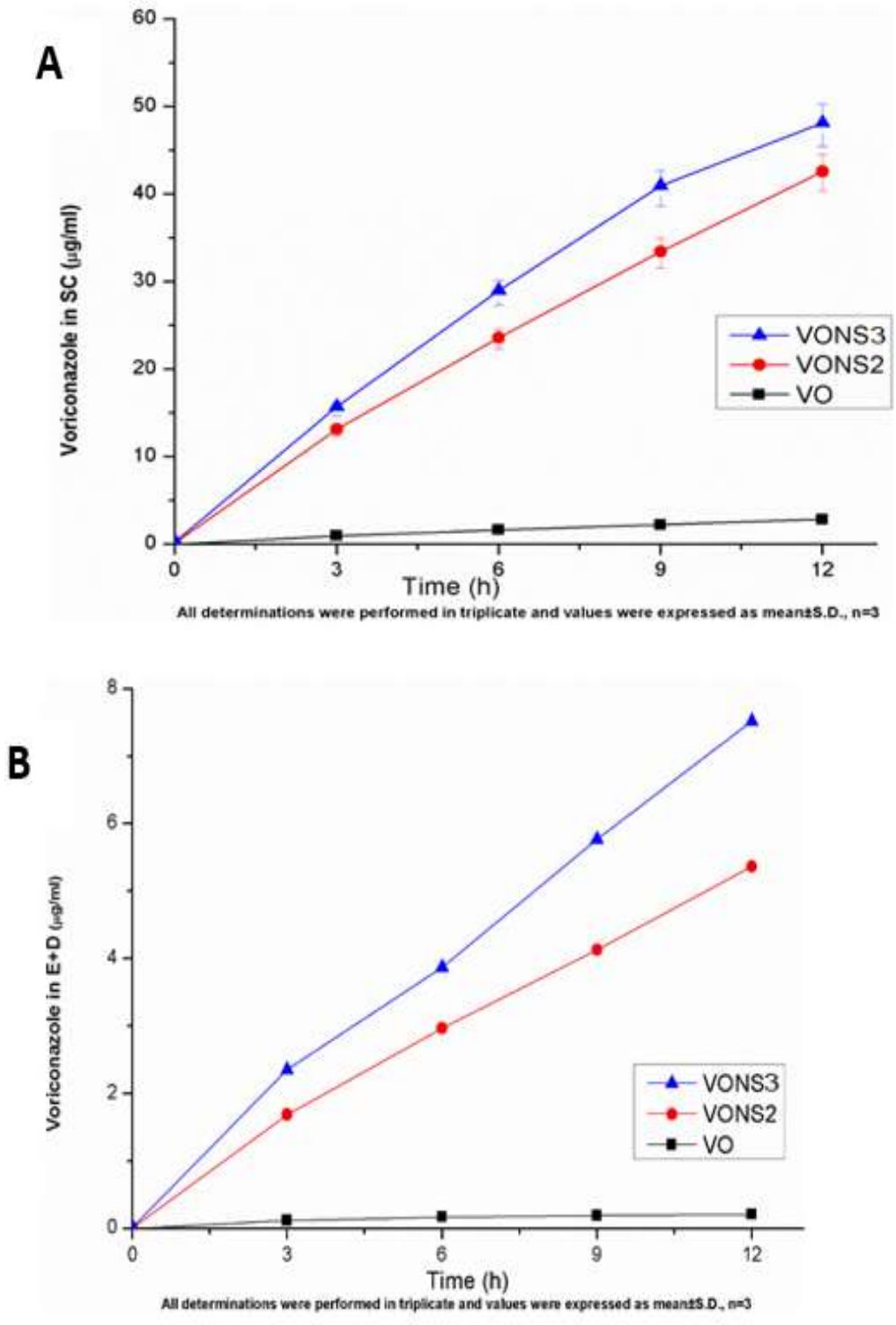

Figure 8: The Results of *in vitro* skin permeation study

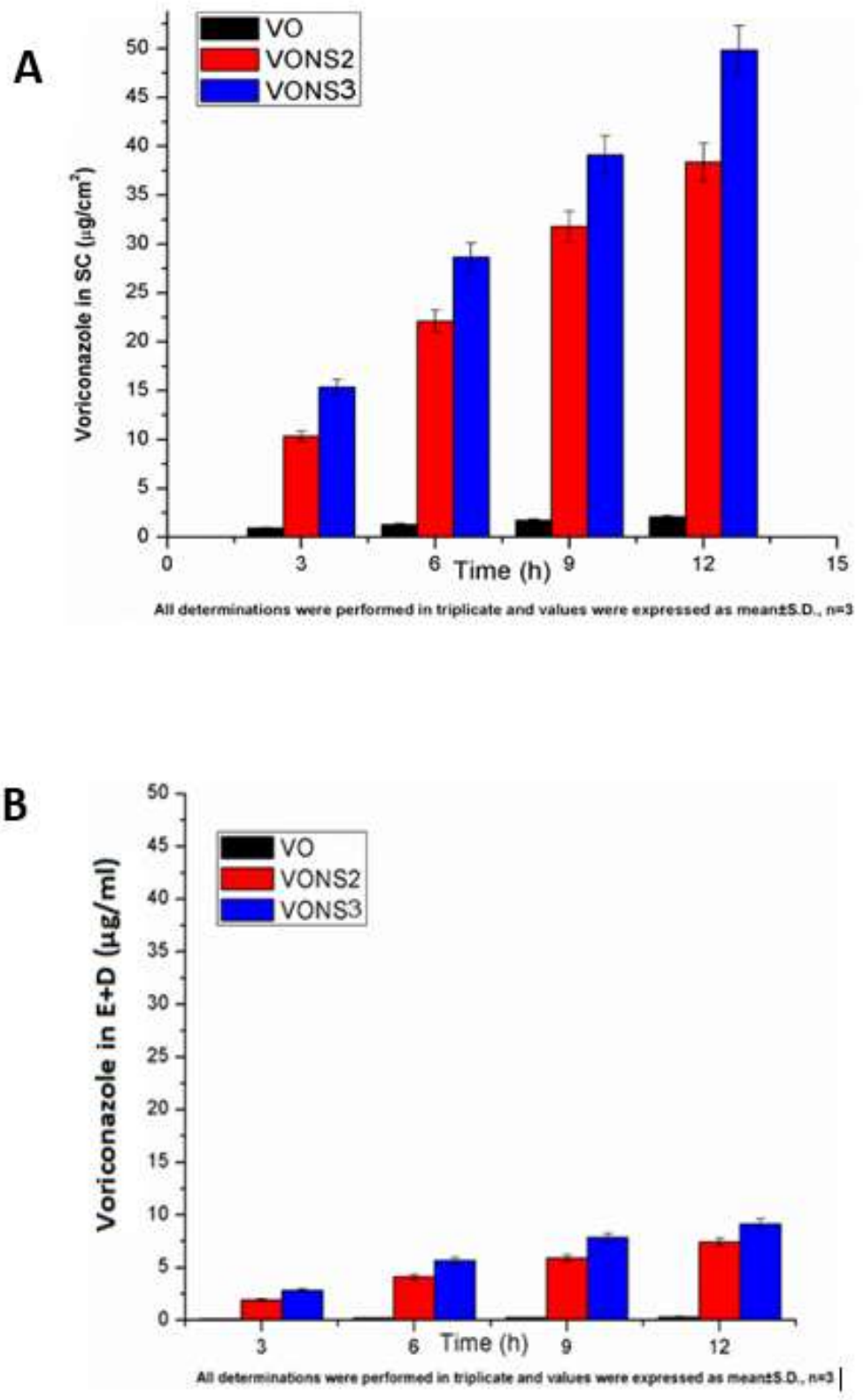

Figure 9: Amount of voriconazole in different layers of skin by *in vivo* experiments

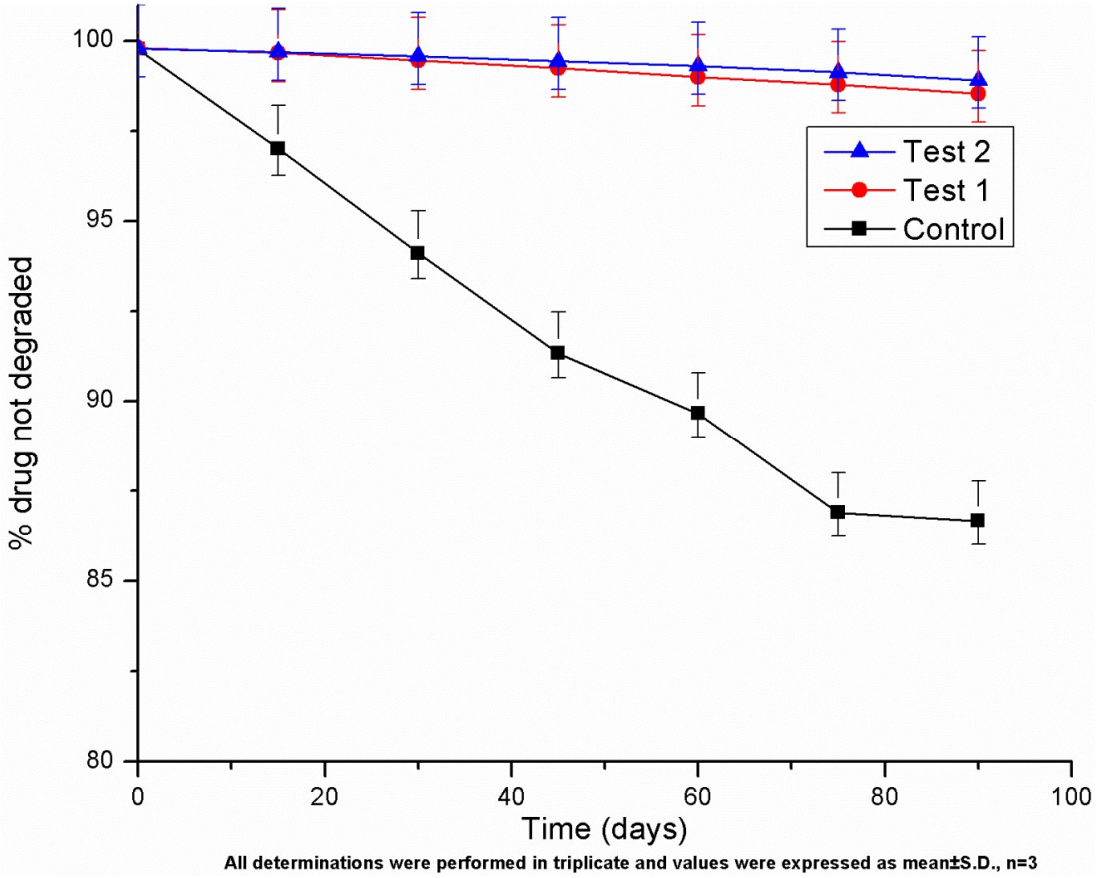

Figure 10: Results of stability study

CONCLUSION

This study showed that freeze-drying can create voriconazole-loaded nanosponges. 278.92 mg of voriconazole was found per gramme of lyophilized powder. VONS2 and VONS3 load voriconazole similarly. Voriconazole's 98.4% encapsulation efficiency indicated an inclusion complex. FTIR, DSC, and XRD investigations confirmed voriconazole-nanosponges inclusion complex formation. Due to the reduced drug particle size, intermolecular hydrogen bonding, and high-energy amorphous state voriconazole nanosponges diffused faster than the pure drug. Skin penetration, antifungal effectiveness, and stability were examined after integrating nanosponge formulations into a model carbopol gel formulation. Skin permeation experiments showed that nanosponge-encapsulated voriconazole released slowly over 12 h over rat skin. The nanosponge formulation dispersed roughly 20-fold more medication than free voriconazole gel. Four to five times more antifungal activity than free voriconazole gel formulation. Over three months of storage at ambient temperature and in the dark, the nanosponge-based gel formulation substantially eliminated voriconazole's chemical instability. According to the results, the cyclodextrin nanosponges-based

gel formulation of voriconazole offers great topical administration potential due to its delayed and consistent drug release, enhanced skin penetration, and good storage stability.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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