Invitro, *Ex vivo* permeation of lemon grass oil and Voriconazole nanosponges loaded herbal gel and evaluation of antifungal and antimicrobial potential

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Abstract

Background

The present study aimed to formulate and evaluate nanosponges-based herbal gels of lemon grass oil (*Cymbopogan citratus)* and Voriconazole using Ethyl cellulose, P.V.A., D.C.M., and other excipients. Formulated optimized nanosponges of Chinese lemon grass oil and Voriconazole were added to the Carbopol-940 gel base, propylene glycol, and other excipients. The antifungal and antibacterial activity of optimized nanogel was determined against different microbial strains, *Candida albicans, Staphylococcus aureus,* and *Escherichia coli*. The herbal oil extracted from Chinese lemon grass has excellent therapeutic potential in the Chinese system of medicine. Pharmacological activity suggests that Chinese lemon grass has a potent antibacterial, antifungal, anti-tumor, antimicrobial, and neuroprotective effect. Furthermore, Voriconazole has potent antifungal activity and antibacterial effects.

Method

The lemon grass and voriconazole nanosponges loaded with Ethyl cellulose were formulated using different excipients. Optimized formulation (F4) of nanosponges was characterized for particle size, zeta potential, entrapment efficiency, viscosity, *in vitro* drug release, *ex vivo* permeation, drug content, CLSM, S.E.M., and T.E.M. characterization. Nanogel was formulated using polymer Carbopol-934, Triethanolamine, Propylene glycol as a permeation enhancer, and sodium benzoate as a preservative. Formulated nanogels of Chinese lemon grass oil and Voriconazole were studied for antifungal and antimicrobial activity against *Candida albicans, Staphylococcus aureus, and Escherichia coli*.

Result

The prepared nanosponges showed uniform particle size and Z.P., indicating the physical stability of nanosponges. S.E.M. and T.E.M. surface morphology showed discrete, spongy particles of prepared optimized nanosponges. The herbal gel of Chinese lemon grass and Voriconazole met all properties and complied with the pharmacopeial standards. The *ex vivo* study of the optimized formulation was subjected to a CLSM (confocal laser scanning microscopy) analysis to confirm the permeation of Chinese lemon grass oil and voriconazole nanosponges into deeper skin tissues. The gel formulations showed therapeutic efficacy against

S. aureus, E. Coli, and *C. albicans*. Furthermore, the gel was effective for topical delivery and had antifungal and antibacterial potential.

Conclusion

The prepared nano gels have potent antifungal and antibacterial effects that need exploration for research in future studies in clinical trials. Formulation E showed the best results in ZOI 34±3mm against *E. coli*, suggesting a potent antibacterial effect for *Cymbopogan citratus*. Furthermore, the formulation E, measured ZOI (25±3mm), showed a promising antifungal effect against *C. albicans.* All the prepared herbal gels are in good condition till today (August 2024). Carbopol-934 gelling agent showed promising results when combined with paraben-free preservatives to avoid side effects like skin and eye irritation and environmental hazards to aquatic animals. Prepared nanogel reported no toxicity in the absence of parabens. It can be used for topical application. CLSM photographs (Figure 9) for the *ex vivo* permeation study suggested that the formulation could penetrate deeper tissues and was effective for topical delivery.

Keywords: *Cymbopogan citratus*; CLSM; Carbopol-934; Voriconazole; Antifungal; Antibacterial

1. Introduction

L.G. (lemon grass), commonly known *as Cymbopogan citratus* has a pungent, sweet, warm taste and is employed for managing epigastric cold pain, headache, traumatic injuries, and diarrhea [1-3]. It is mainly cultivated in southern parts of China, Malaysia, and South America. Further, in traditional Chinese medicine, citronella is popularly used. *Cymbopogan mekongensis* leaf extract and decoction have potent antimicrobial, antifungal, antipyretic, antidiabetic, and anti-epileptic effects. The potent antibacterial activity of *Cymbopogan* species contributes to inhibitory effects against fungi and bacteria. Gram-positive bacteria like *S. aureus* and *Staphylococcus albus* and gram-negative like *E. coli* and *pseudomonas* microbial strains are employed for antimicrobial potency. Fungi (*Candida albicans* and *Candida tropicalis*) also showed inhibitory effects for *C. mekongensis* due to active ingredients like geraniol, citral, linalool, and citronellol. Traditionally, *Cymbopogon* acts as an insect repellent and analgesic manages flu, and acts as a potent antioxidant in tea. Furthermore, lemon grass essential oil is a traditional remedy for antimicrobial, anti- inflammatory, and antifungal effects by inhibiting inflammatory responses in mice [4-7]. It has potent antifungal potential against *Candida, Aspergillus*, and *Fusarium* sp. Chinese lemon grass, or *Cymbopogan mekongensis* has potent antimicrobial and antifungal effects [7]. Voriconazole is a potent triazole antifungal medication that causes interruption of ergosterol and binds with enzyme 14α-demethylase. Furthermore, this leads to the accumulation of sterol precursors and the depletion of ergosterol, resulting in cell death and inhibiting fungal growth. Voriconazole treats invasive fungal infections like candidiasis and aspergillosis in immunocompromised patients [8].

Nanosponges are tiny solid nanoparticles having porous voids and are spongy. They are like virus structures with a diameter of less than 1μm. They are capable of entrapping both hydrophilic and lipophilic drugs. They are widely employed for topical, oral, and parenteral delivery. The spongy nature makes these nanoparticles a promising carrier for drug delivery. They can improve solubility, in vitro permeability, bioavailability, skin retention, and patient compliance. They become futuristic nanotechnology-based carriers for the delivery of antifungal agents and topicals, improving the solubility of poorly water-soluble drugs and providing target-based delivery in a controlled manner [9].

Fungal infections have become prominent worldwide and mainly affect immune-compromised patients requiring corticosteroid management. It primarily affects the toes, eyes, nails, and hair. Thus, it becomes challenging to treat with conventional formulations like tablets, creams, ointments, gels, and shampoos due to side effects of penetration, itching, solubility, and

bioavailability. So, it is essential and challenging for researchers to explore other alternatives where topical nano herbal gels are prepared to overcome drawbacks of poor penetration, solubility, bioavailability, and providing controlled release [10-12]. One scientist has prepared nanosponges loaded with lemon grass oil using ethyl cellulose and polyvinyl alcohol using the emulsion solvent evaporation method for antimicrobial and antifungal effects. The study results concluded that prepared nanosponges were spongy and had reduced in vivo skin toxicity when tested in albino mice. Further, the research findings suggested a potent antifungal effect, and topical gels showed improved penetration and provided controlled release. Another research was carried out using Voriconazole-loaded nanolipid carriers-based hydrogel formulated using the homogenization method for treating fungal candidiasis and reducing skin toxicity, promoting controlled release [13,14]. The present research work was focused on the fabrication of Chinese lemon grass and Voriconazole-loaded ethyl cellulose nanosponges and their incorporation into a gel matrix for antifungal and antibacterial effects.

2. Materials and Methods

Lemongrass oil was purchased from Veda oil (Order No. V67941), Voriconazole was procured from Chemland India, and other excipients like Carbopol 934, Propylene glycol, sodium benzoate, and triethanolamine were obtained from S.D. Fine Chem Limited. All the reagents were of analytical grade and used throughout the study. Different microbial strains for antibacterial and antifungal activity were procured from the Institute of Microbial Technology, Chandigarh, India.

2.1. Phytochemical Screening of (lemon grass oil)

Phytochemical screening of lemon grass oil and different gels confirmed the presence of active metabolites such as saponins, flavonoids, tannins, terpenoids, steroids, and coumarins [15] presented in Table 1.

2.2. Formulation of Nanosponges

In varying ratios, Lemon grass oil and Voriconazole were used for nanosponges formulation by solvent diffusion evaporation using ethyl cellulose and other excipients. The organic phase containing 0.2µL of lemongrass oil, 100mg voriconazole, varying ratios of E.C., and 10-20 mL of organic solvent dichloromethane was probe sonicated for 10 minutes. The organic phase was slowly dispersed into an aqueous phase containing 500mg polyvinyl alcohol in 100 mL distilled water to form a blueish tinch nanodispersion and then stirred for 2 hours on a magnetic stirrer between 1000-1500 rpm. After that, the nanodispersion of Chinese lemon grass oil was filtered, the solvent was vacuum dried, and nanosponges were dried in an oven at 40ºC and stored in a desiccator using an aluminum wrapper for further analysis (Table 2).

2.3. Characterization of Nanopsonges [15-18]

2.3.1. Particle size, P.D.I. and Zeta potential Determination

Particle size, zeta potential, and polydispersity index were determined using Malvern zeta sizer, U.K. The particle size distribution was determined using the Dynamic Light Scattering (D.L.S.) technique using a Malvern zeta sizer analyzer. Using this technique, nanosponges batch particle size was determined. Further, the mean particle size and polydispersity index were calculated using standard deviation for nanosponges. P.D.I. measures the variation in particle sizes of nanosponges formulation. The zeta potential measures the surface charge (Malvern zeta sizer, U.K.) of dispersed particles in colloidal dispersion. It determines the physical stability of prepared nanosponges [15,16] (Table 3), Figure 1.

2.3.2. % age Entrapment efficiency

100 mg dried and weighed Chinese lemon grass and voriconazole nanosponges were collected and dissolved in 10 ml ethanol, ultra centrifuged at 15000rpm. The solution was filtered, and the free drug was collected from the supernatant layer and analyzed spectroscopically at 283nm and 238nm, respectively, for Voriconazole and lemon grass oil in triplicates to obtain % E.E. for Voriconazole and lemon grass oil using the formula (Table 3):

%EE= amount (wt.) total drug-free drug (wt.) X dilution factor X100/ amount (wt.) total drug

2.3.3. Viscosity

The viscosity of the prepared nanodispersion was determined using a T-spindle shape and an LV-II Pro Brookfield viscometer (Table 3).

2.3.4. Drug content

The drug content of prepared nanosponges of Voriconazole and lemon grass oil was determined by adding 100mg dried nanosponges in 10 ml of phosphate buffer at 7.4 pH and spectroscopic analysis at 283nm and 238nm, respectively, using the U.V. method, data of drug content mentioned in (Table 4, and Table 5), Figure 2.

2.3.5. *Invitro* drug release

A modified Franz diffusion cell was used for these studies. Cellophane membrane mounted on Franz diffusion cell. A known quantity of 100 mg of nanosponges was spread uniformly on the cellophane membrane on the donor side. The 7.4 pH phosphate buffer solution was used as the receptor medium. 1 mL samples were collected every hour and the same fresh medium was replaced to maintain sink conditions. The study was carried out for 12 hours. Samples were analyzed at 283nm and 238nm, respectively, for voriconazole and lemongrass oil against the corresponding blank solution using a UV-visible spectrophotometer [19] (Table 5)—figures 3, 4, 5, and 6 present the release kinetics graphs.

2.3.6. Surface morphology of Optimized formulation

2.3.6.1. Scanning Electron Microscopy

Scanning electron microscopy (S.E.M.) was used to determine nanosponges morphology and surface topography. The prepared samples were lightly sprinkled on double adhesive tape, stuck to an aluminum stub, and then coated with platinum. Further, they have a thickness of 10 A°. The coated samples were then placed in the S.E.M. chamber, and respective photographs were taken. S.E.M. analysis of an optimized formulation was performed to study the surface morphology (JSM 6100 JEOL, Tokyo, Japan) [20] (Figure 7).

2.3.6.2. T.E.M. Studies

Nanosponges transmission electron microscopy was performed to determine morphological studies. The prepared samples were placed on copper grids, then dried and stained using 2%w/v phosphotungstic acid. Then, using digital software, images were captured for particle size analysis. The transmission electron microscopic image of the optimized formulation was measured to determine the shape of the particles [20] (Figure 8).

3. Formulation of Gel base

Carbopol-934 was dissolved by slowly adding it to 70mL of distilled water, allowing it to swell for 3 hours. The polymeric solution of Carbopol-934 was then stirred on a magnetic stirrer for 3 hours to form a viscous, transparent, gel-based film. Sodium benzoate (S.B.) 0.3mL was added to the formulated herbal gel base as a preservative, and the pH was adjusted to 7.4 with triethanolamine (T.E., 0.6mL). The resultant polymeric solution was again stirred for another 10 minutes after adding T.E. and S.B. Permeation enhancer propylene glycol (2 mL) was then added to the resultant solution, characterized for further parameters [21,22], Table 6.

4. Evaluation parameters of herbal gels [23-33]

4.1. Physical inspection and homogeneity

All the prepared gels were visually inspected for physical appearance and homogeneity for their appearance (Table 7).

4.2. pH measurement

A digital pH meter determined the pH of the prepared gels (Table 7).

4.3. Viscosity, Extrudability and Spreadability Measurement

The viscosity measurement of the gel was determined using a Brookfield Viscometer (LVDV-E) with spindle TS6 at 25°C with 50rpm. The prepared nano gels were filled in a capillary tube and sealed. The tube was gently pressed to extrude the gel. Spreadability was measured by

placing herbal gel between two slides; one end was tied with thread, and the other side weight was placed. The time taken by the two slides to slip from the prepared gel determines the spreadability, calculated using the formula:

Spreadability (S)= WXL/ T, W: Weight (g) on slide (upper), L: Length (cm) on slide (upper), T: Time (min) taken to separate slides (upper and lower) (Table 7).

4.4. Measurement of Drug Content

The drug content of herbal gel was determined by soaking 1g (gel) in 10 mL of phosphate buffer at 7.4 pH and spectroscopic analysis at 283nm and 238nm for Voriconazole and lemon grass oil against blank using the U.V. method [34, 35] (Table 7).

4.5. *Ex vivo* permeation

Goat animal abdominal skin was collected from the slaughter house and hydrated with phosphate buffer 7.4 pH for an hour. Hairs were removed using a razor, subcutaneous fat tissues were exposed, and around 2.5cm^2 area was cut. Then, the skin was mounted on a Franz diffusion cell, and around 2mL nanodispersion was applied to the stratum corneum of abdominal skin facing the donor compartment. 30 mL buffer was filled in the receptor compartment, and the temperature was kept at 32±0.5ºC, agitated at 100rpm on a magnetic stirrer. The drug content was evaluated at regular intervals. An aliquot sample of 2mL was withdrawn and replaced with a fresh buffer. Using a UV-visible spectrophotometer at 283nm and 238nm wavelength, the drug content of samples was estimated (Table 8) [36].

4.6. Confocal Laser Scanning Microscopy (CLSM)

An *ex vivo* CLSM study was performed to check the permeation for the optimized formulation, presented in Figure 8**.** For performing a CLSM penetration study, each optimized formulation was labeled with Rhodamine 123 (0.5 mg/mL) probe dye. The goat abdominal skin dorsal portion was then selected, and hairs were removed without causing any cut or injury to the animal. The optimized formulation F4 penetration potential containing probe (Rh123) was studied and compared against blank nanosponges (applied topically at 1 cm^2) and dye solution. Selected formulations were then applied to the marked area, and animals were observed for 24 hours. After 24 hours, the animal was sacrificed, and excised skin was obtained. It was followed by thrice washing of the excised skin surface with ethanol. Further, the excised area from the skin surface was then sliced into thin slices of 1 mm^2 size respectively for determination of probe penetration using the CLSM by fluorescent labeling using an argon laser beam having emission at 590nm and excitation at 488 nm [36, 37] (Figure 9).

4.7. Washability Test

Small amounts of the prepared herbal gel formulations were placed on the skin, rubbed, and washed with warm water. All the prepared herbal gel formulations showed good washability.

4.8. Measurement of Zone of Inhibition for Antifungal and antibacterial activity

Antifungal and antibacterial studies were determined by employing the agar well diffusion method using different Petri dishes having a cork bore size of 6mm. The microbial strains, such as *S. aureus* (MTCC96), *E. coli* (MTCC1430, MTCC1573), and fungal strains, such as *C. albicans*, were inoculated per the standard protocols for cultivating microbial cultures. 0.5mL of cultures (*S. aureus*, *E. coli*, and *C. albicans*) was placed in the centre of a sterile Petri plate using a sterile graduated pipette and placed in a laminar flow chamber. The agar media was allowed to solidify. After the media was solidified, 6 wells of cork borer 6mm in size were placed in the center of Petri plates for different cultures. The first well consists of 100mg Carbopol gel containing drug-free nanosponges, which acts as a negative control (labeled as A). The second well contains the marketed cream of Voriconazole (labeled as B). The third well contains Carbopol gel containing drug encapsulated (1:1), equivalent to 100mg voriconazole nanosponges (labeled as C). The fourth well was filled with 1% citral marker as a marketed formulation for lemon grass oil (labeled as D). The fifth well was filled with Carbopol gel encapsulated with lemon grass oil nanosponges (labeled as E). The sixth well was incorporated with a combination of Carbopol encapsulated gel of lemon grass oil and Voriconazole (labeled as F). All the petri plates were incubated at 37ºC for 48 hours. After 48 hours, the zone of inhibition was measured, and results were reported, depicted in Table. The method was repeated for all the different cultures, such as *S. aureus* (MTCC96), *E. coli* (MTCC1430, MTCC1573), and fungal strains such as *C. albicans* [38] (Table 9), (Figures 10-12).

5. Results

The mean particle size, P.D.I., and zeta potential of the prepared nanosponges suggested the particles are good enough to maintain physical stability. The zeta potential indicated that the physical stability of nanosponges was excellent (Table 2, Figure 1). The % drug content represented in Figure 2 showed satisfactory results. The in-vitro release kinetics data were constructed for zero order, first order, Higuchi, and Korsmeyer-peppas (Figures 3-6). The results suggested that R^2 value 0.9931 for zero order shows linearity and provided controlled release up to 12h. Korsmeyer-Peppas release showed an R^2 value of 0.9861, n=0.6693, more than 0.5 (non-fickian diffusion) following the super case-II transport mechanism**.** The S.E.M. photographs suggested a spherical, spongy, and good structural composition of prepared nanosponges with a definite boundary (Figure 7). Furthermore, the T.E.M. analysis showed the spongy internal structure of the nanosponges (Figure 8). The nano gel met all physical

characteristics, showing good texture, appearance, spreadability, and homogeneity without clogging and lumps. The pH of the formulated nano gels ranges between 6.4±0.47, 6.1±0.11, 6.8±0.17, 7.2±0.54, 7.00±0.19 and 7.10±0.15 for formulation codes A, B, C, D, E, and F. The pH was effective for topical delivery without skin toxicity. The viscosity ranges between (1011.5±0.11-1314.4±0.47 m.pas) and spreadability was good, making it convenient to apply and improving patient compliance, as presented in Table 7. The antifungal and antibacterial results suggested that the optimized formulation (F4) of voriconazole and lemongrass oil-loaded Carbopol gel showed better potency than the marketed formulation against different strains is presented in Table 9, and the comparative zone of inhibition is presented in Figures 10-12. It showed promising antifungal effects against *C. albicans* and antibacterial effects against *E. coli* and *S. aureus.*

6. Discussion

Table 3 represents particle size, P.D.I., and zeta potential for all the formulations, and Figure 1 shows the P.D.I. value for the optimized formulation (F4), offering a narrow and uniform particle size distribution. Zeta potential ranges from (19.62) - (32.3 mV), suggesting the physical stability of prepared nanosponges. Further, particle size ranges from 129.7-178.3 nm, indicating that particle size increases as the ratio of polymer increases; further, because at lower drug concentrations and high polymer ratios, less polymer is available to encapsulate the drug, and the thickness of the polymer membrane decreases. The increased stirring speed suggested reduced particle size and improved % yield. The study further revealed that the polymer adheres to the stirrer surface at high speed and decreases % E.E. (entrapment efficiency). F8 formulation showed a decreased % E.E. for voriconazole and lemongrass oil compared to F4 by varying the speed. Another critical factor determined during the formulation of nanosponges was with increased D.C.M. volume, the viscosity of formulated dispersion was reduced, ranging from (31.11±0.12-39.52±0.44 m. Pas). This low viscosity is not suitable for topical application. So, L.G.O. nanosponges were added to the gel matrix using different combinations of polymers to improve the viscosity. Based on permeation studies, F4 was chosen as an optimized formulation—the *ex vivo* permeation data of formulations presented in Table 8 shows penetration into deeper tissue. Figures 7 and 8 showed S.E.M. and T.E.M. images of optimized formulation F4 showing spherical, uniform, smooth surface pores of lemongrass and Voriconazole. CLSM photographs labeled with rhodamine dye (Figure 9) for the marketed formulation and optimized formulation (F4) of Voriconazole and lemongrass suggested penetration in deeper tissues. Further, the formulation was effective for topical delivery. Stability studies showed no changes in the stored formulations after three months (t-test, $P<0.05$), suggesting more stability at refrigerated temperature. Table 9 and Figures 10-12 show

the antifungal and antibacterial activity with a zone of inhibitions for all prepared gels, which were found effective against fungal and bacterial strains in a dose-dependent manner. Formulation code A containing Carbopol gel drug-free nanosponges showed a zone of inhibition (2.8±1mm, 3±1mm, and 2.9±3mm), respectively, against *C. albicans*, *S. aureus*, and *E. coli*. Formulation B containing the marketed cream of Voriconazole showed higher ZOI 19±3mm, 4±3mm, and 4.2±2mm against *C. albicans*, *S. aureus*, and *E. coli.* C formulation (voriconazole nanosponges) showed ZOI (21±2mm, 6±1mm and 2.7±5mm. Formulation D (citral marker) and E (lemon grass nanosponges) showed ZOI 1.8±1mm, 2.2±3, 6.3±1mm and 23±2mm, 26±1mm, 8.3±2mm, respectively. Synergistic formulation F containing voriconazole and lemongrass nanosponges loaded Carbopol gel showed ZOI 25±3mm, 29±2mm, and 34±3mm against *C. albicans, S. aureus*, and *E. coli*. These results suggested that gram-negative bacteria *E. coli* showed the highest zone of inhibition, followed by S. aureus and C. albicans. Thus, the bacteria and fungi can inhibit bacterial and fungal cell walls. Results of the zone of inhibition suggested potent antifungal efficacy against *C. albicans and excellent antibacterial effect against S. aureus and E. Coli*. The antifungal and antibacterial study for all formulations requires exploring future data for *invivo* activity and broader research on other fungal and bacterial strains. Table 10 represents calibration data of Voriconazole and lemongrass oil. Figure 13 shows the absorption maxima of Voriconazole and lemongrass oil. Figure 14 and Figure 15 represent the linearity curves of voriconazole (283nm) and lemongrass oil (238nm), respectively.

Limitations

1. Few strains were selected for antifungal and antibacterial activity.
2. Research on exploration for future findings in *invivo* activity.
3. Only one oil was chosen for the study.
4. Small research is conducted and requires broader research to explore other therapeutic potential to frame the conclusion better.

Conclusions

The findings conclude that the prepared nano gels have potent antifungal and antibacterial effects that need exploration for research in future studies in clinical trials. Formulation E showed the best results in ZOI (34±3mm) against *E. coli*, suggesting a potent antibacterial effect for lemon grass oil. Furthermore, the formulation E, measured ZOI (25±3mm) showed promising antifungal effect against *C. albicans.* All the prepared herbal gels are in good condition till today (November 2024). Carbopol-934 gelling agent showed promising results when combined with paraben-free preservatives to avoid side effects like skin and eye irritation and environmental hazards to aquatic animals. Prepared nanogel reported no toxicity in the absence of parabens. It can be used for topical application. CLSM photographs (Figure 9) for

the *ex vivo* permeation study suggested that the formulation could penetrate deeper tissues and was effective for topical delivery.

Conflict of Interest

None

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Table 1: Phytochemical screening of lemon grass oil

Herbal Oil	Saponins	Terpenoids	Flavonoids	Steroids	Coumarins	Tannins	Alkaloids	Phenols
CM	+	+	+	+	+	+	-	-

+: Presence; -: Absence; CM: *Cymbopogan mekongensis*

Table 2: Composition of Nanosponges formulation

Formulation codes	L.G.O (µl)	EC (mg)	PVA (mg)	Drug (VOR) mg	DCM (ml)	Stirring speed (RPM)	DW (ml)
F1	0.2	50	500	100	10	1000	100
F2	0.2	100	500	100	10	1000	100
F3	0.2	150	500	100	10	1000	100
F4	0.2	200	500	100	10	1000	100
F5	0.2	50	500	100	20	1500	100
F6	0.2	100	500	100	20	1500	100
F7	0.2	150	500	100	20	1500	100
F8	0.2	200	500	100	20	1500	100

LGO: Lemon grass oil; EC: Ethyl cellulose; PVA: Poly vinyl alcohol; DCM: Dichloromethane; VOR: Voriconazole; DW: Distilled water; ml: millilitre; mg: milligram; µl: microlitre.

Table 3: Characterization Parameters of Nanosponges

Formulation codes	Particle size (nm)	PDI	Z.P (mV)	%age entrapment efficiency (EE) (Lemon grass oil)	%age entrapment efficiency (EE) (Voriconazole)	Viscosity (m. pas)
F1	129.6	0.347	11.62	67.61±0.47	65.31±0.17	34.15±0.01
F2	146.8	0.714	21.73	71.13±0.58	69.53±0.22	35.21±0.31
F3	164.8	0.501	18.6	78.23±0.59	76.11±0.19	38.12±0.14
F4	179.6	0.326	32.3	79.87±0.13	77.54±0.11	39.52±0.44
F5	120.7	0.264	11.4	64.12±0.45	62.32±0.24	31.11±0.12
F6	131.2	0.647	14.15	68.57±0.15	66.32±0.71	33.48±0.44
F7	160.2	0.329	13.47	73.92±0.42	71.13±0.33	34.10±0.25
F8	178.3	0.204	19.62	76.12±0.81	74.65±0.23	35.12±0.35

Table 4: *Invitro* Permeation of Nanosponges using lemon grass oil and Voriconazole

Time	Drug permeated (cumulative), mcg/sq.cm
Lemon grass oil	

Hours	F1	F2	F3	F4	F5	F6	F7	F8
0	0	0	0	0	0	0	0	0
0.5	8.84	6.21	5.79	9.11	4.11	5.14	3.41	4.62
1	11.14	9.01	9.18	14.12	8.12	7.85	8.15	8.49
2	20.14	15.11	13.12	23.14	10.14	11.01	13.12	11.18
3	26.88	17.3	16.87	31.35	13.85	14.33	15.61	17.14
4	37.41	26.12	24.31	37.47	27.47	20.14	22.41	24.35
5	41.12	37.15	33.12	49.21	38.65	28.67	29.45	31.41
6	55.11	49.9	47.21	61.23	46.89	37.65	38.11	47.14
7	64.31	61.08	59.02	70.41	58.72	44.34	53.12	59.01
8	73.54	71.54	68.21	80.92	62.12	58.16	62.37	64.14
9	80.11	78.93	74.13	86.21	67.13	65.31	68.47	68.19
10	83.21	81.12	79.41	88.54	69.54	71.11	73.21	71.25
11	87.93	84.32	82.12	91.34	73.34	74.12	79.54	76.54
12	89.24	88.85	85.41	92.11	78.11	80.12	84.54	80.27
Voriconazole								
Hours	F1	F2	F3	F4	F5	F6	F7	F8
0	0	0	0	0	0	0	0	0
0.5	6.34	5.34	4.92	7.56	3.18	4.98	2.98	3.86
1	9.54	7.64	7.65	12.45	7.14	6.21	7.14	6.94
2	18.12	13.65	11.21	20.32	9.16	10.45	12.48	9.12
3	24.14	14.54	13.54	29.12	11.54	12.54	13.25	15.25
4	34.21	24.65	22.14	34.65	25.65	18.24	20.36	21.69
5	39.25	35.68	31.21	47.54	35.59	26.17	27.21	28.15
6	53.65	46.67	44.87	58.65	43.57	34.98	36.98	44.69
7	62.14	59.36	55.62	68.45	55.98	42.78	52.21	57.68
8	70.25	69.12	64.21	78.21	60.24	56.14	60.28	62.17
9	78.12	76.54	71.25	84.12	64.32	63.85	65.93	66.58
10	80.26	79.65	77.62	86.32	66.95	69.65	71.24	68.21
11	84.65	82.32	79.34	89.45	71.65	72.47	77.65	74.63
12	86.59	86.11	83.21	91.56	76.52	78.52	82.12	78.68

Table 5: Optimized formulation (F4): *Invitro* Drug Release and Drug content of Lemon grass oil and Voriconazole Nanosponges

Time (h)	*Invitro* drug release		Formulations	% Drug Content	
	Lemon grass Oil	Voriconazole		Lemon grass Oil	Voriconazole
0	0	0	F1	93.41	91.14
0.5	10.04	8.11	F2	94.35	92.14
1	14.14	11.65	F3	97.65	95.24
2	20.3	17.25	F4	98.45	97.35
3	27.14	24.12	F5	93.25	91.14
4	37.65	33.12	F6	94.51	94.54
5	48.41	46.45	F7	97.54	96.21
6	55.65	51.12	F8	95.66	94.65
7	63.12	58.12	Marketed formulation	91.24	90.48
8	69.14	64.23			
9	73.14	69.15			
10	80.14	76.24			
11	86.12	82.14			
12	92.64	88.23			

Marketed formulation for lemon grass: citral marker, and for voriconazole: voriconazole 1% cream

Table 6: Composition of nanogels

S. No	Formulation ingredients	Quantity
1	A: Carbopol gel-934 (mg) free nanosponges	100mg
2	B: Voriconazole nanosponges (mg)	100mg
3	C: Voriconazole cream (mg) (Marketed formulation)	100mg
4	D: Citral marker (mL) (marketed)	1mL
5	E: Lemon grass nanosponges (mg)	100mg
6	F: Carbopol-lemongrass oil and voriconazole nanosponges (mg)	100mg

mL: millilitre; mg: milligram

Table 7: Physical Characteristics of nanogels

S. No	Gel Codes	Extrudibility (g. sec)	Appearance	Odour	Homogeneity	Washibility	Viscosity	pH	Skin Toxicity	% Drug content (LGO)	% Drug content (VOR)
1	A	0.94	Smooth, transparent, White	Characteristic, lemon like, aromatic	Excellent	Easily removed	1314.4±0.47	6.4± 0.47	No	90.28	90.05
2	B	0.81	Smooth, transparent, White	Characteristic, lemon like, Aromatic	Excellent	Easily removed	1011.5± 0.11	6.1± 0.11	No	91.24	91.11
3	C	1.1	Smooth, transparent, White	Characteristic, lemon like, aromatic	Excellent	Easily removed	1050.2± 0.34	6.8± 0.17	No	90.24	91.24
4	D	0.75	Smooth, transparent, White	Characteristic, lemon like, Aromatic	Excellent	Easily removed	1171.3± 0.24	7.2± 0.54	No	92.35	91.54
5	E	0.93	Smooth, transparent, White	Characteristic, lemon like, aromatic	Excellent	Easily removed	1203.4± 0.06	7.00 ±0.19	No	93.11	923.65
6	F	0.85	Smooth, transparent, White	Characteristic, lemon like, aromatic	Excellent	Easily removed	1106.4± 0.02	7.1± 0.15	No	93.48	93.14

Table 8. *Ex vivo* drug permeation Data containing lemon grass oil and voriconazole Carbopol gel

Lemon grass oil									
Time (h)	F1	F2	F3	F4	F5	F6	F7	F8	MF
0	0	0	0	0	0	0	0	0	0
2	38.5	20.3	31.5	31.4	23.4	40.4	38.9	15.3	21.2
4	44.6	30.1	41.5	45.3	33.2	46.2	41.8	23.6	30.6
6	50.7	34.5	50.3	60.4	38.9	51.8	54.5	30.5	37.8
8	55.5	40.3	61.8	68.3	42.4	58.4	60.4	45.4	40.3
10	66.7	51.8	73.1	80.2	49.8	68.4	76.7	53.6	47.4
12	72.9	63.4	81.2	92.3	60.2	76.7	85.8	69.4	56.9
Voriconazole									
0	0	0	0	0	0	0	0	0	0
2	36.4	17.1	27.2	27.4	20.1	37.4	35.4	11.2	18.5
4	41.2	26.4	37.4	41.2	29.4	41.5	38.7	19.4	27.4
6	47.2	30.4	46.3	56.7	24.1	47.4	50.1	27.1	33.6
8	53.1	36.1	56.1	63.4	39.4	54.2	56.4	41.2	36.1
10	63.1	47.4	70.3	78.1	46.1	65.1	72.5	50.1	42.8
12	69.2	59.1	76.4	90.5	57.4	72.4	82.3	64.3	52.7

MF: Marketed formulation

Table 9: Comparative Zone of inhibition of optimized formulation (F4) against *C. albicans*, *S. aureus, E. Coli*

S. No	Codes	Zone of inhibition (mm), n=3		
		C. albicans	*S. aureus*	*E. Coli*
1.	A	2.8±1	3±1	2.9±3
2.	B	19±3	4±3	4.2±2
3.	C	21±2	6±1	2.7±5
4.	D	1.8±1	2.2±3	6.3±1
5.	E	23±2	26±1	8.3±2
6.	F (Combined formulation of LG, VOR loaded Carbopol gel)	25±3	29±2	34±3

A: Carbopol gel drug free nanosponges, B: Voriconazole marketed cream, C: Voriconazole nanosponges, D: Citral marker, E: Lemongrass oil nanosponges, F: Combination of voriconazole and lemon grass nanosponges loaded gel

Table 10: Calibration Curve Data of voriconazole (283nm) and lemon grass oil (238nm-citral) in ethanol at 283nm.

Sr. no.	Concentration (µg/ml), Phosphate buffer, 7.4, 238nm (lemon grass oil)	Absorbance	Concentration (µg/ml), Phosphate buffer, 7.4, 283nm (voriconazole)	Absorbance
1	2	0.45	2	0.008
2	4	0.80	4	0.017
3	6	1.21	6	0.025
4	8	1.68	8	0.034
5	10	1.90	10	0.043

Size Distribution Report by Intensity

v2.2

Malvern

Sample Details

Sample Name: F 4
SOP Name: mansettings.nano
General Notes:

File Name: STU FINAL.dts
Record Number: 1484
Material RI: 1.59
Material Absorbtion: 0.010
Dispersant Name: Water
Dispersant RI: 1.330
Viscosity (cP): 0.8872
Measurement Date and Time: Thursday, December 7, 2023 10:5...

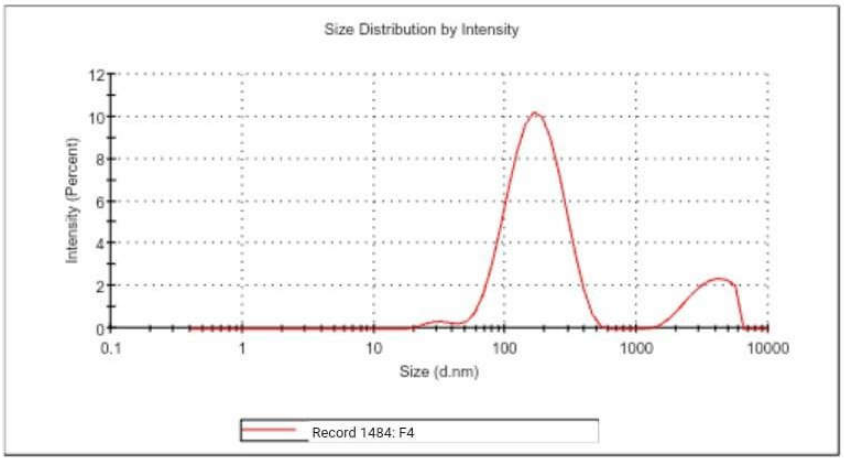
System

Temperature (°C): 25.0
Count Rate (kcps): 404.8
Cell Description: Disposable sizing cuvette
Duration Used (s): 10
Measurement Position (mm): 1.25
Attenuator: 9

Results

			Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	179.6	**Peak 1:**	182.7	83.1	82.06
PdI:	0.326	**Peak 2:**	3673	12.5	1152
Intercept:	0.947	**Peak 3:**	32.50	1.5	6.859

Result quality : Good



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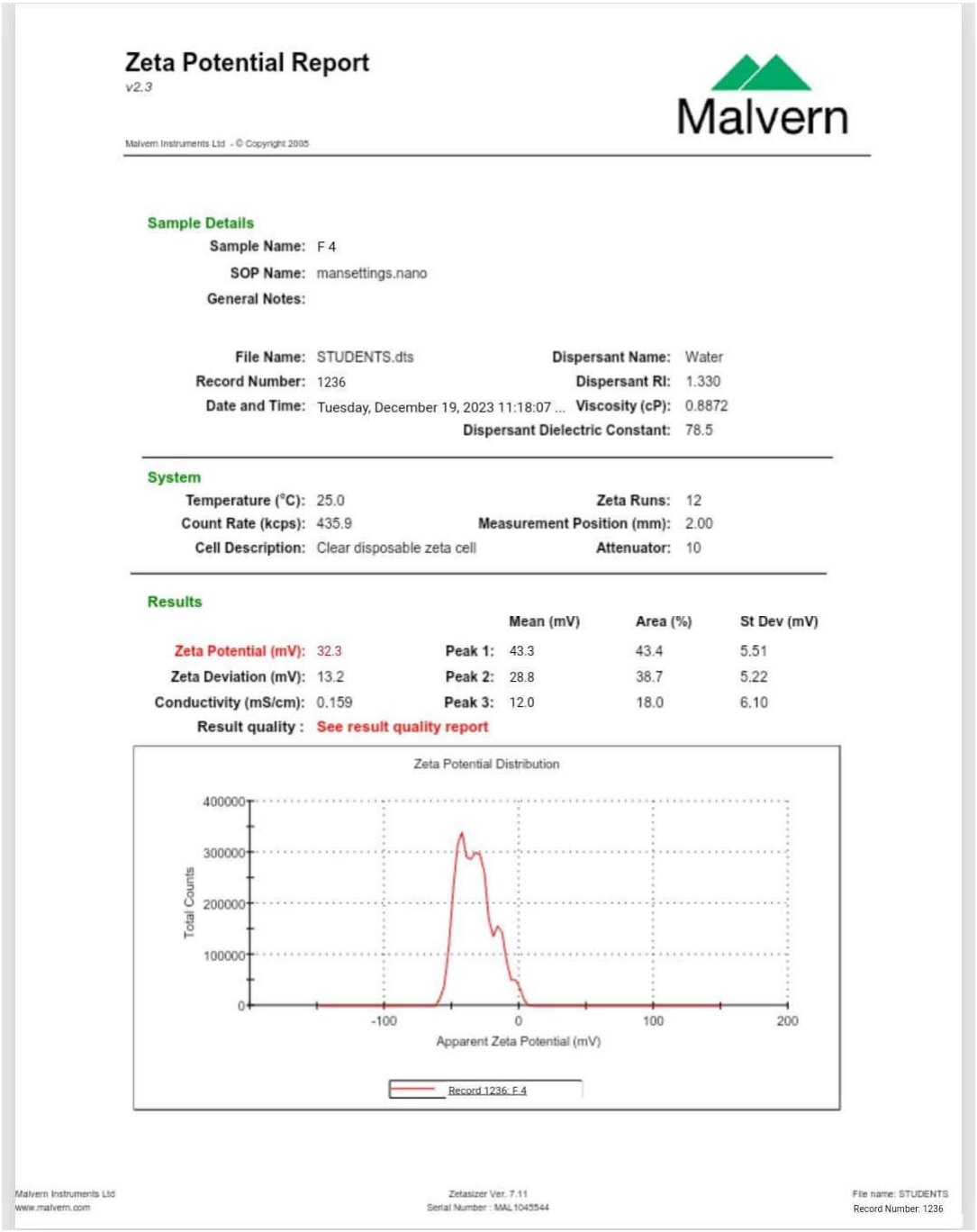
Zetasizer Ver. 7.11
Serial Number : MAL1045544

File name: STU FINAL
Record Number: 1484



Zeta Potential Report
v2.3

Malvern

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Sample Details

Sample Name: F 4
SOP Name: mansettings.nano
General Notes:

File Name: STUDENTS.dts
Record Number: 1236
Date and Time: Tuesday, December 19, 2023 11:18:07 ...
Dispersant Name: Water
Dispersant RI: 1.330
Viscosity (cP): 0.8872
Dispersant Dielectric Constant: 78.5

System

Temperature (°C): 25.0
Count Rate (kcps): 435.9
Cell Description: Clear disposable zeta cell
Zeta Runs: 12
Measurement Position (mm): 2.00
Attenuator: 10

Results

			Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	32.3	Peak 1:	43.3	43.4	5.51
Zeta Deviation (mV):	13.2	Peak 2:	28.8	38.7	5.22
Conductivity (mS/cm):	0.159	Peak 3:	12.0	18.0	6.10

Result quality : See result quality report

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Zetasizer Ver. 7.11
Serial Number : MAL1045544
File name: STUDENTS
Record Number: 1236

Figure 1: Optimized formulation F4: Particle size, PDI and Zeta Potential

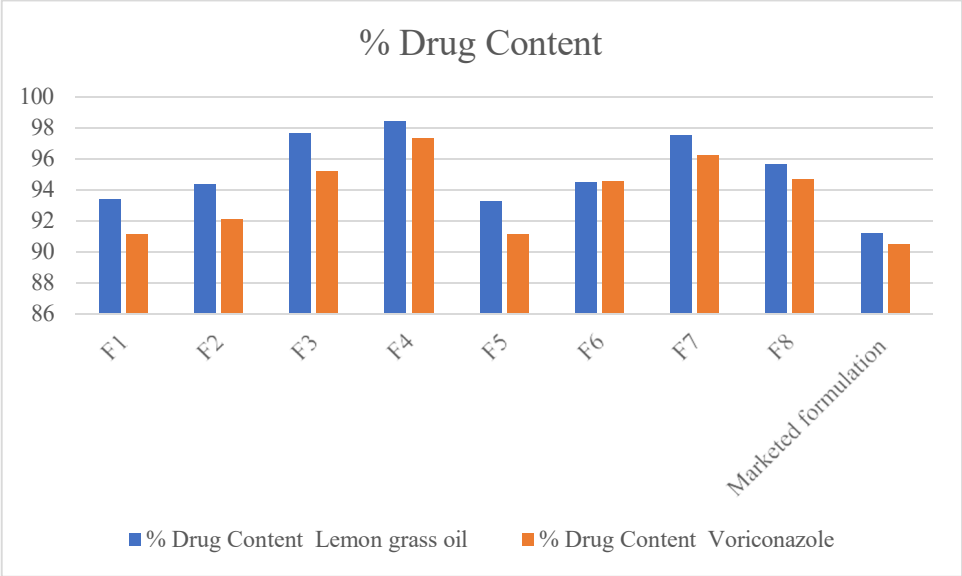


Figure 2: % Drug content of lemon grass and voriconazole nanosponges

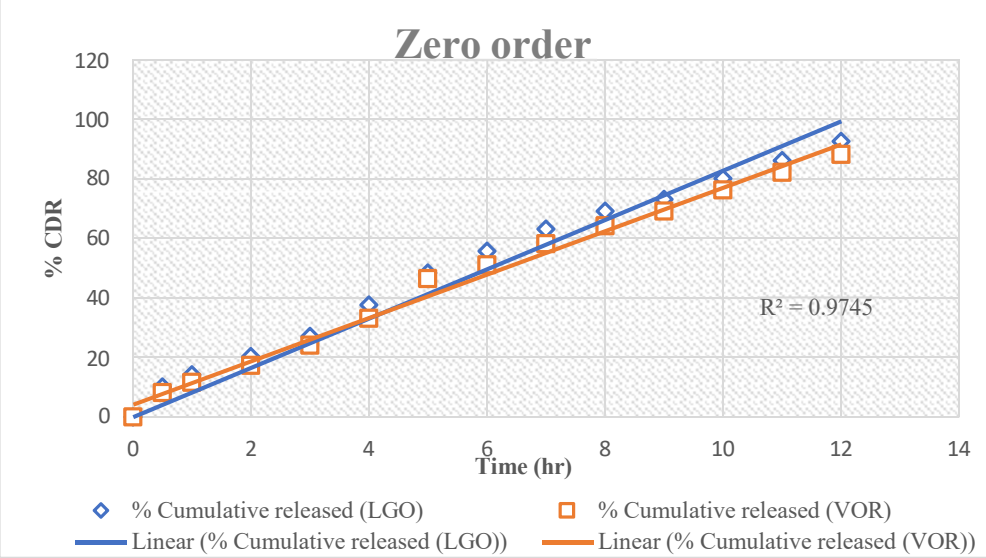


Figure 3: Comparative zero order release kinetics for lemon grass oil (LGO) and Voriconazole (VOR) nanosponges

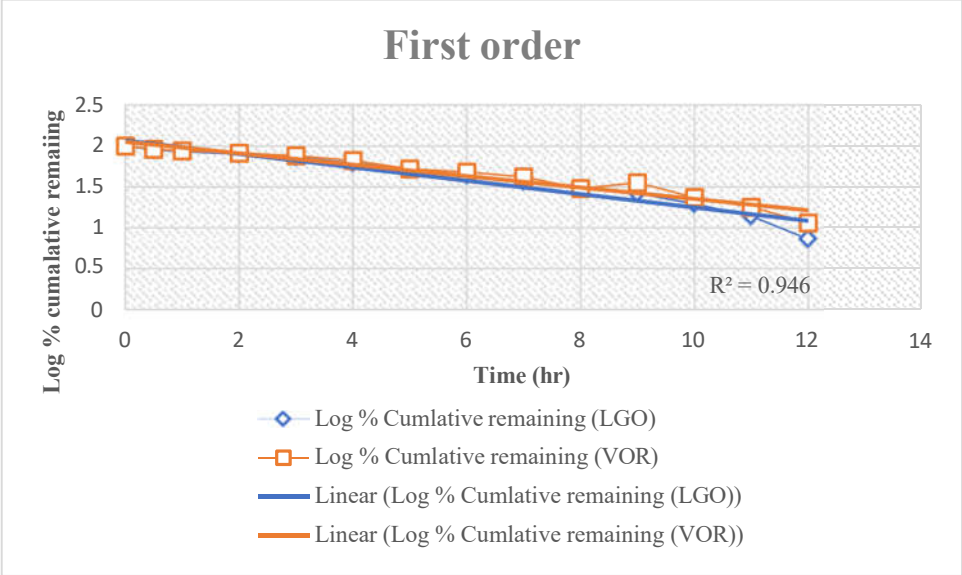


Figure 4: Comparative first order release kinetics for lemon grass oil (LGO) and Voriconazole (VOR) nanosponges

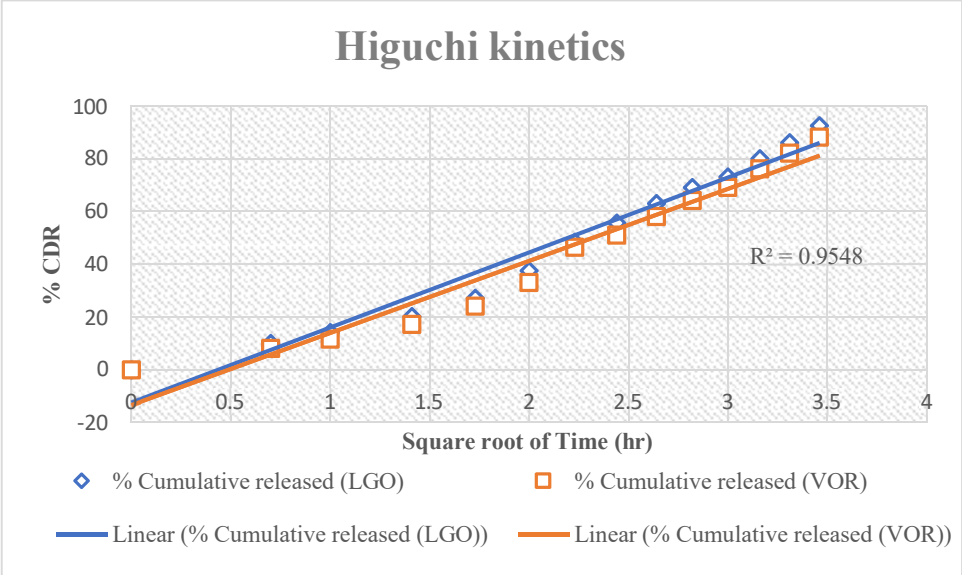


Figure 5: Comparative higuchi release kinetics for lemon grass oil (LGO) and Voriconazole (VOR) nanosponges

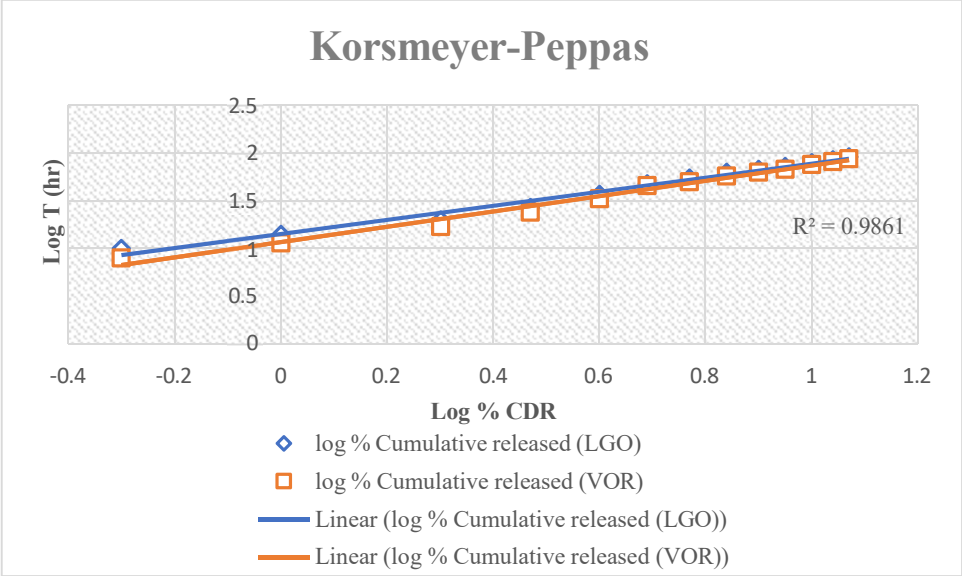


Figure 6: Comparative korsmeyer-peppas release kinetics for lemon grass oil (LGO) and Voriconazole (VOR) nanosponges

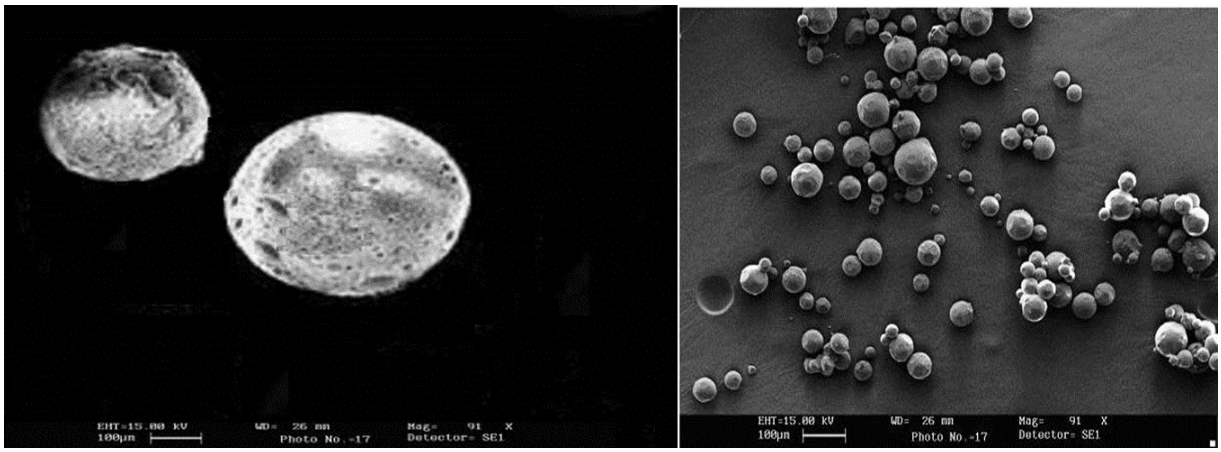


Figure 7: SEM Photograph of optimized formulation (F4)

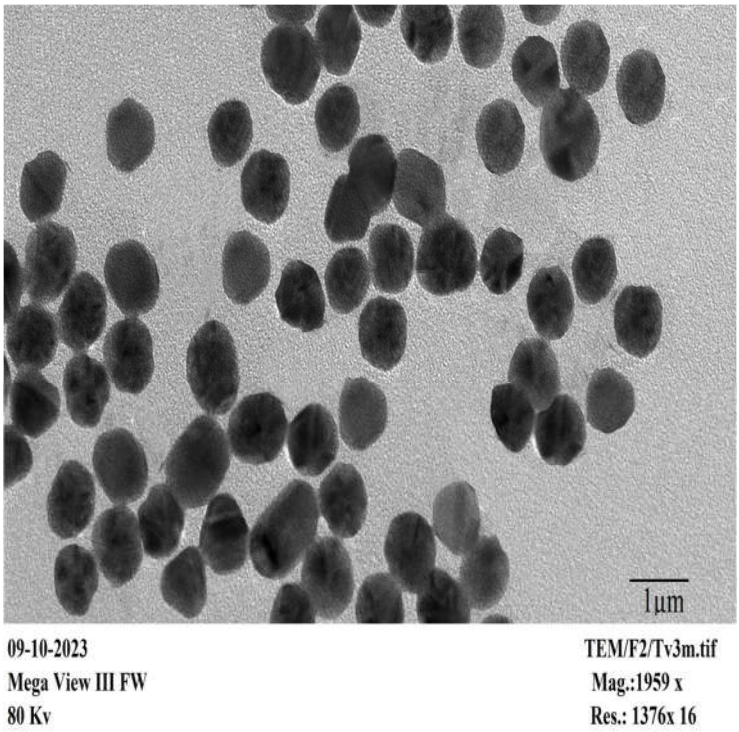


Figure 8: TEM Photograph of Optimized formulation (F4)

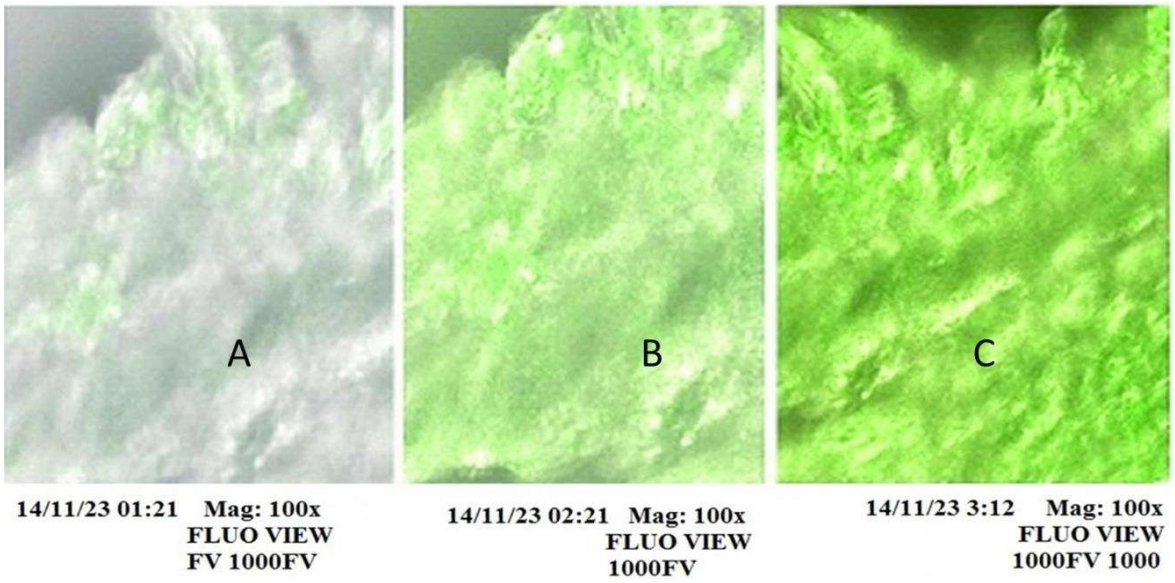


Figure 9: CLSM Images of optimized formulation F4 and Marketed formulation

Where A: Marketed formulation, B: Optimized formulation F4 (Lemon grass oil), C: Optimized formulation F4 (Voriconazole).

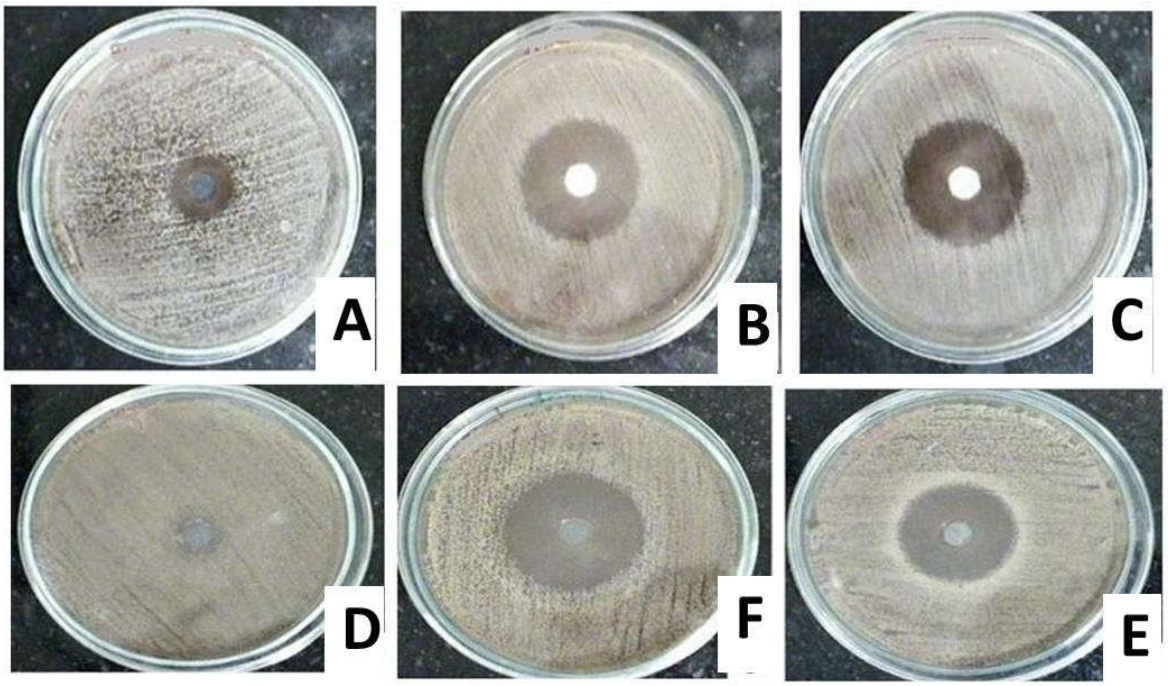


Figure 10: Comparative analysis of *in vitro* antifungal activity of optimized lemon grass oil and voriconazole nanosponges loaded Carbopol gel against *C. albicans*

Where A: Carbopol gel drug free nanosponges, B: Voriconazole marketed cream, C: Voriconazole nanosponges, D: Citral marker, E: Lemongrass oil nanosponges, F: Combination of voriconazole and lemon grass nanosponges loaded gel

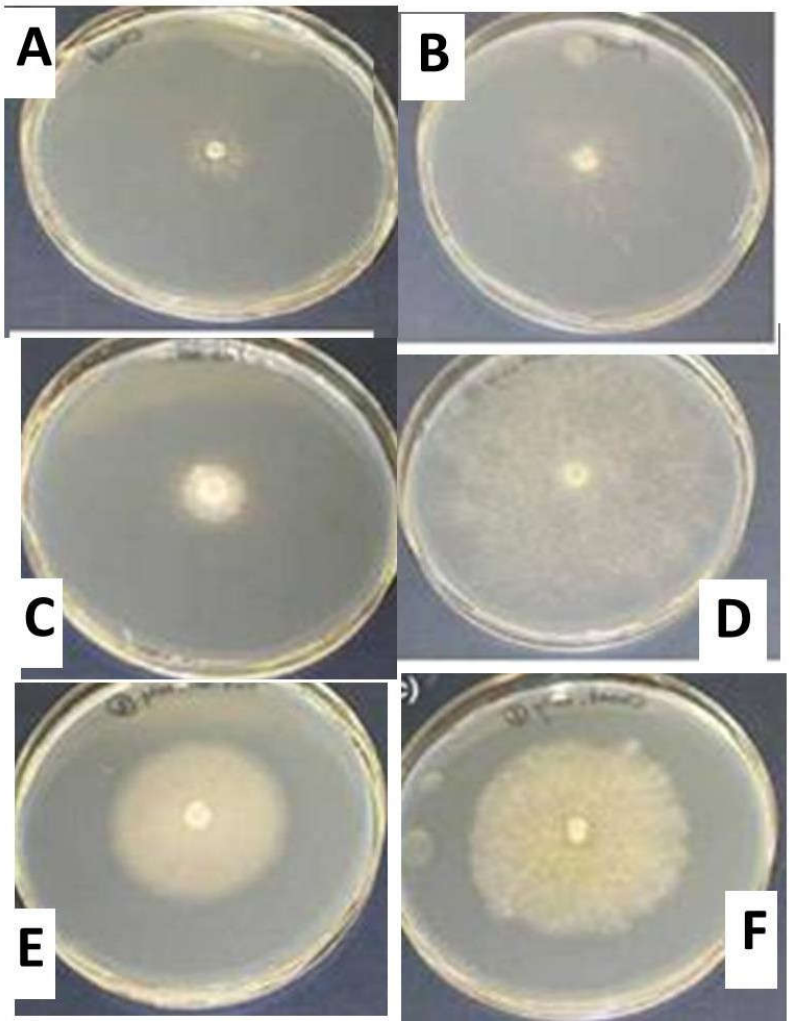


Figure 11: Comparative analysis of *in vitro* antifungal activity of optimized lemon grass oil and voriconazole nanosponges loaded Carbopol gel against *S. aureus*

Where A: Carbopol gel drug free nanosponges, B: Voriconazole marketed cream, C: Voriconazole nanosponges, D: Citral marker, E: Lemongrass oil nanosponges, F: Combination of voriconazole and lemon grass nanosponges loaded gel

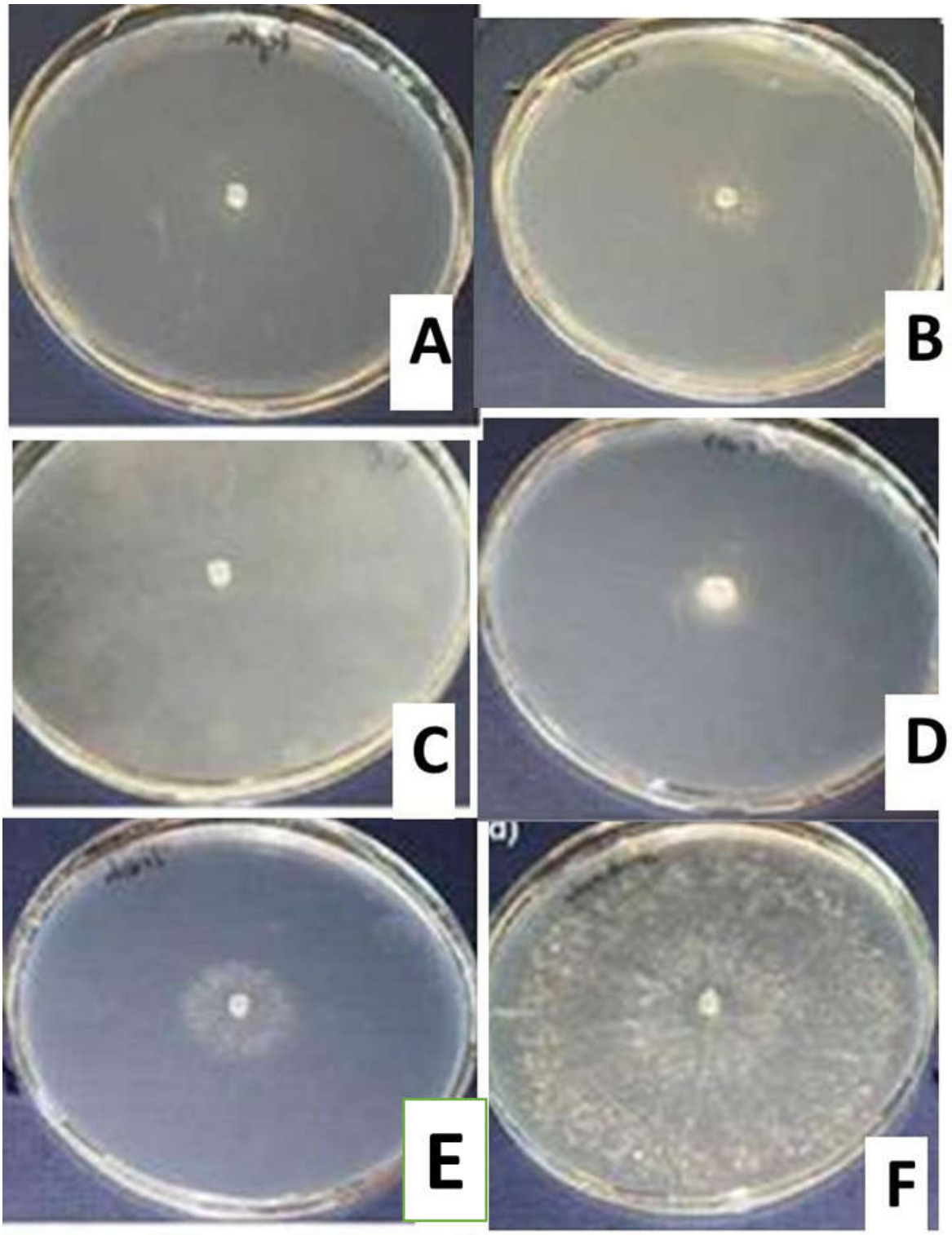


Figure 12: Comparative analysis of *in vitro* antifungal activity of optimized lemon grass oil and voriconazole nanosponges loaded Carbopol gel against *E. Coli*

Where A: Carbopol gel drug free nanosponges, B: Voriconazole marketed cream, C: Voriconazole nanosponges, D: Citral marker, E: Lemongrass oil nanosponges, F: Combination of voriconazole and lemon grass nanosponges loaded gel

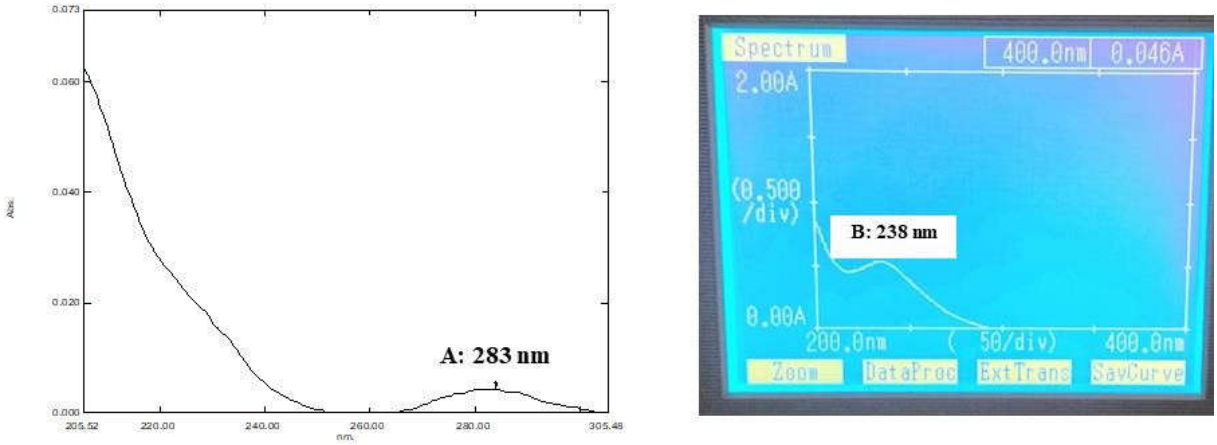


Figure 13: Calibration Curve, A: λ max (lamda max) of voriconazole (283nm), B: lemon grass oil (238nm) in ethanol representing linearity.

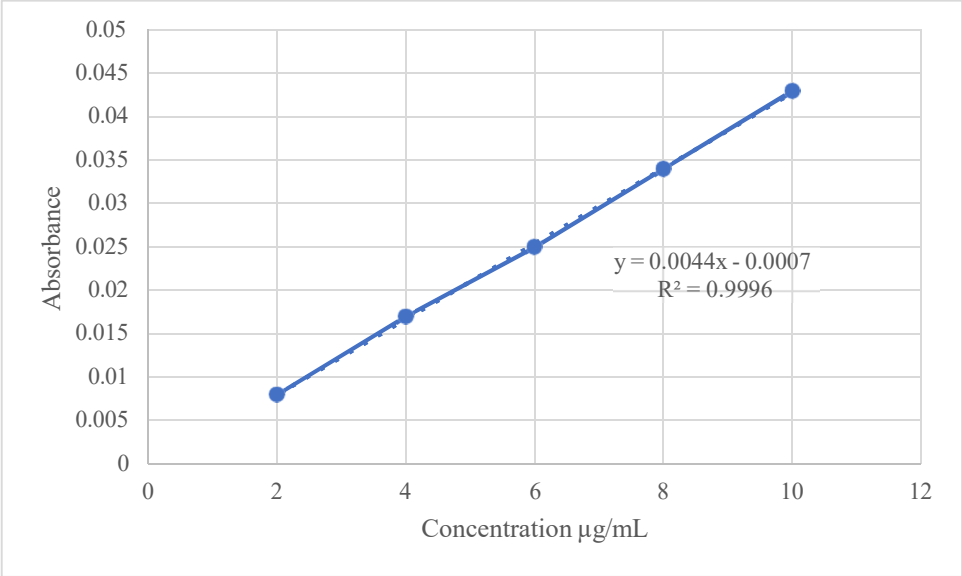


Figure 14: Linear curve of Voriconazole (283 nm)

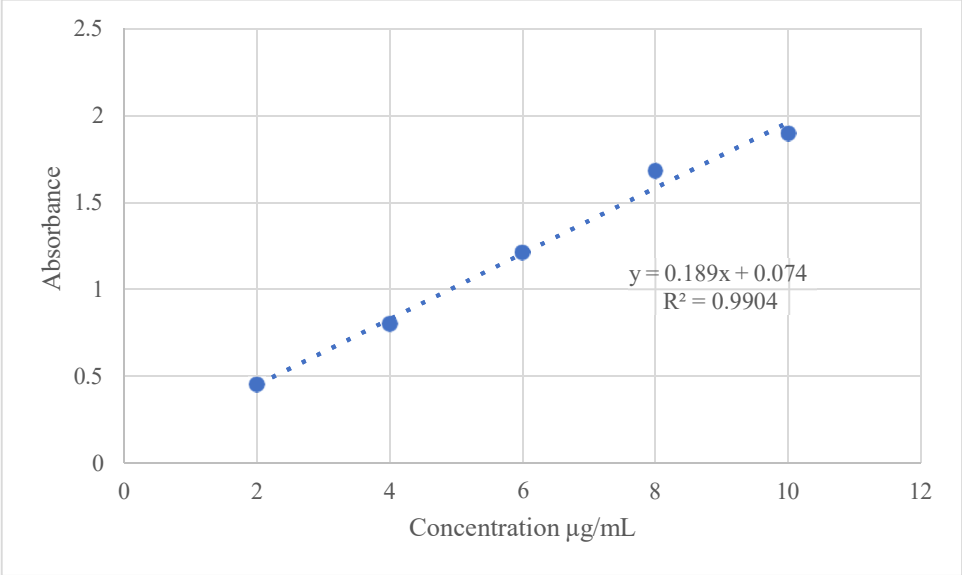
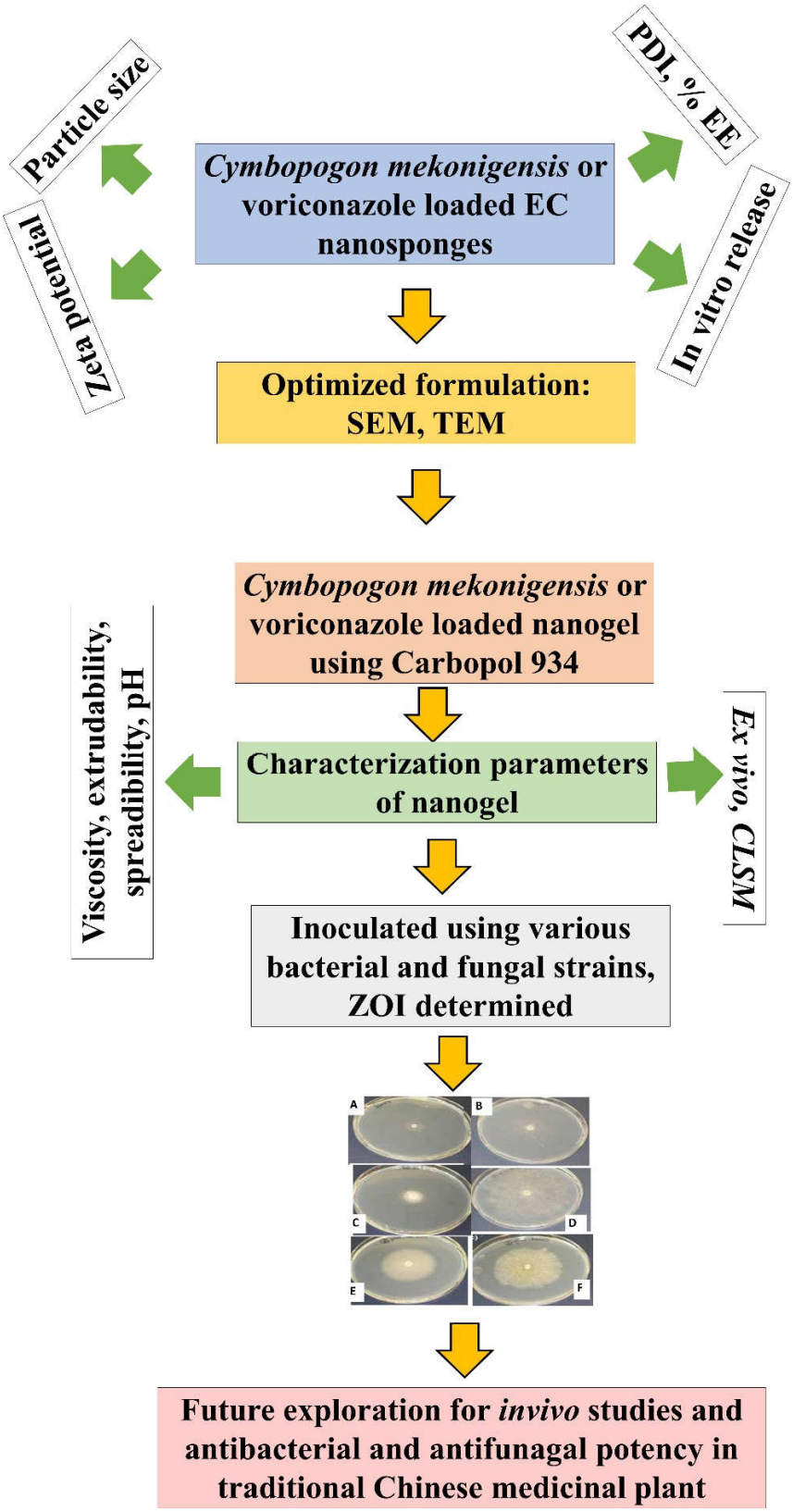


Figure 15: Liner curve of Lemon grass oil (238nm)

Graphical Abstract



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