Invitro and Invivostudies: Ketoconazole Nanosponges Loaded Gel for Topical Delivery and Antifungal Application

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Abstract

Background: Ketoconazole is an imidazole derivative employed for topical delivery and treating fungal infections and acne. Ketoconazole (KET) prevents ergosterol synthesis, increases membrane fluidity, and prevents fungal growth. Conventional antifungal medication has side effects for topical delivery, including itching, burning, reduced permeation, stinging, and poor bioavailability. The drawbacks and side effects of conventional topical formulations shifted the focus to formulating a nanosponges-based gel of Ketoconazole. The present research aimed to develop a Ketoconazole nanosponges-based gel for topical delivery. The formulated preparation will help improve side effects, retention time, solubility, bioavailability, and permeability. **Methodology:** KET nanosponges were fabricated using the emulsion solvent evaporation method. The prepared formulations were characterized for particle size, zeta potential, PDI, % DEE (drug entrapment efficiency), viscosity, and in vitro drug diffusion studies. Optimized formulation F6 containing Ketoconazole (100mg): E.C. (300mg), having stirring speed 1000rpm, internal phase volume 10ml (D.C.M.) showed promising results. Further optimized formulation F6 was incorporated into the gel base matrix of Carbopol 934 with different excipients. Fungal strains of Candida albicans were selected for the study using the agar plate method. In vivo studies of optimized formulation (F6) showed better results compared to the marketed formulation against C. albicans, and toxicity was reduced.

Results: The prepared gel of Carbopol containing KET nanosponges met all physical characteristics and showed promising antifungal effects by inhibiting fungal growth. The zone of inhibition was measured and showed promising effects against C. albicans compared to the marketed formulation. **Conclusion:** The gel was highly effective against fungal strains and showed promising in vivo antifungal activity against C. albicans (ZOI: 11±0.89mm) in a dose-dependent manner compared to the marketed formulation (ZOI: 9±1.41mm). It further requires more research for topical delivery and to be involved in clinical trials.

Keywords: Ketoconazole, Carbopol gel, nanosponges, antifungal, topical delivery, C. albicans

Introduction

Nanosponges are tiny, porous voids and spongy virus-like structures and solid-based nanoparticles (N.P.s) having diameters less than 1µm. They can entrap hydrophilic and lipophilic drugs and are employed for topical, oral, and parenteral routes. The tiny and spongy nature makes solid base N.P.s promising for improving solubility, in vitro permeability, bioavailability, skin retention, and patient compliance [1-3]. They become futuristic nanotechnology-based carriers for the delivery of antifungal agents and topicals, improve the solubility of poorly water-soluble drugs, and provide target-based delivery in a controlled manner[4].

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 [4-6].

Ketoconazole is a promising imidazole derivative that is approved for the treatment of psoriasis, acne, skin burn, hyperpigmentation, and vaginal candidiasis. It has low aqueous solubility, poor bioavailability, and other side effects. To avoid side effects and improve solubility and bioavailability, ketoconazole nanocarriers are prepared to provide the reasonably consistent drugs for prolonged periods within the skin and incorporate them into the gel matrix to improve retention time [7].

Material and Methods

Ketoconazole were procured from Chemland Ind, Vapi, Gujrat. Ethyl cellulose (E.C.), Carbopol 934, dichloromethane (D.C.M.), and polyvinyl alcohol (P.V.A.) were obtained from S.D. Fine Chem Limited. All the reagents used during fabrication were of analytical grade.C. albicans was procured in Chandigarh. Animals used as per CPSEA guidelines (approval number (IAEC/VII/8/GLA/2024).

Methodology

Formulation of nanosponges

The emulsion solvent evaporation method formulated KET-loaded ethyl cellulose nanosponges using different combinations of excipients and polymers. Ketoconazole (100mg) with varying concentrations of E.C. (50-300mg) was dissolved into an organic phase containing dichloromethane (10-15mL). The internal phase containing E.C., D.C.M., and Ketoconazole was probe sonicated (Sonics & Materials Inc.) for 5 min intervals two times to reduce particle size. In the internal phase, P.V.A. (500mg) was dissolved in 100mL double distilled water and kept on a magnetic stirrer for 2 hours at

1000-1500 rpm. The organic phase was slowly-slowly added to the internal phase to formulate nanosponges. Prepared nanosponges were filtered through Whatman's filter paper 0.45µm, dried in an oven at 40ºC, and stored in a desiccator to remove residual solvent [8-10] (Table 1).

Characterization parameters of Nanosponges[11-16]

- **Particle size determination**

The particle size of prepared KET-NSs (Ketoconazole Nanopsonges) was determined using a Malvern zeta sizer; U.K. Particle size of less than 1µm-300nm is favorable for topical delivery (Table 2), Figure 1.

- **Zeta potential measurement**

Samples were analyzed in disposable zeta cells using the Malvern instrument, U.K. zeta sizer, to determine colloidal dispersion stability and measure the electric charge on the particle surface (Table 2), Figure 1.

- **P.D.I. values**

The polydispersity index was measured to ensure uniform particle size distribution of prepared NSs (Nanopsonges)(Table 2), Figure 1.

- **Percent entrapment efficiency (% E.E.)**

Prepared N.S.s were dried, weighed (100mg), dissolved in 10mL ethanol., and ultracentrifuged at 15000 rpm. The resultant mixture was filtered, and a supernatant layer containing free drug was collected, diluted, and analyzed spectrophotometrically (Shimadzu) at 278nm by U.V. analysis[17]. The percentage entrapment efficiency was calculated using the formula (Table 2).

% EE= Total drug-free amount of drug/ total drug X 100

- **Invitro diffusion studies**

Franz diffusion cell was selected for the study. The cellophane membrane was mounted on the diffusion cell, and 100mg of prepared nanosponge was uniformly placed on the cellophane membrane facing the donor compartment. The receptor or receiver compartment was filled with PBS (30mL) (pH 4.5) and kept on a stirrer for agitation at 32±0.5°C temp, 50 rpm. An aliquot (2 ml) was withdrawn at different intervals, and an equal volume was replaced with a fresh buffer. The aliquot samples were analyzed spectroscopically by the U.V. method at 278nm [17] (Table 3) and Figure 2.

- **Drug release kinetics**

The release studies were conducted by fitting the data of in vitro diffusion studies into various kinetic models, such as zero-order, first-order, Higuchi, and Korsmeyer Peppas, to evaluate the mechanism of drug release (Tables 4, 5, and Figure 3).

Surface morphology (optimized formulation)

- **Scanning electron microscopy (S.E.M.)**

S.E.M. analysis was performed to study surface morphology (JSM 6100 JEOL, Tokyo, Japan), and photographs were taken at suitable magnification [18] (Figure 4A).

- **Transmission electron microscopy (T.E.M.)**

TEM analysis was performed to study the morphology and check the discrete behavior of optimized nanosponges [18] (Figure 4B).

Formulation of nanosponges gel matrix

Overnight Carbopol 934 was soaked for 24 hours, and the pH value was adjusted to 4-6 for topical delivery by incorporating triethanolamine. Further, dried nanosponges were added and stirred on a magnetic stirrer to form a dense gel base, and nanopsonges-based gel matrix characterization parameters were determined [19-20].

Evaluation parameters of nanogels [19-20]

- **Physical inspection**

All the prepared herbal gel was visually inspected for physical appearance (Table 6).

- **pH measurement**

pH of herbal gel was determined using a digital pH meter(Table 6).

- **Viscosity measurement**

The viscosity measurement of the gel was determined using a Brookfield Viscometer (LVDV-E) with spindle S6(Table 6).

- **Measurement of Drug Content**

The drug content of prepared nanospongesbased gel was determined by dissolving 100mg of gel in 10 ml of phosphate buffer 4.5 pH and analyzing it spectroscopically at 278nm using the U.V. method(Table 6).

- **Measurement of extrudability**

The extrudability ofprepared herbal nano gel was determined by filling the gel into the collapsible tube. The tube was suitably pressed using fingers to extrude the gel(Table 6).

- **Spreadability**

Prepared gel was placed between two slides, one end was tied with thread, and weight was placed. The time taken by the two slides to slip determines the spreadability(Table 6), calculated using the formula:

Spreadability (S)= WXL/ T, where W denotes weight applied on the upper slide, L represents the length of the upper slide, and T indicates the time taken by both sides to separate.

- **Drugdiffusion studies**

Goat skin was collected from the slaughterhouse, and epidermal skin was carefully removed and washed with normal saline. It was then cut into smaller pieces of 2cm² and used for drug diffusion study. Franz diffusion cell was selected for the study. Goat skin was mounted on the donor compartment with the help of adhesive. The gel (1g gel containing 100mg of nanosponge) gel was spread uniformly on goat skin facing the

donor compartment. The receptor or receiver compartment was filled with PBS (30mL) (pH 4.5) and kept on a stirrer for agitation at 32±0.5°C temp, 50 rpm. An aliquot (2 ml) was withdrawn at regular intervals for 12 hours, and an equal volume was replaced with a fresh buffer. The aliquot samples were analyzed spectroscopically by the U.V. method at 278nm (Table 7 and Figure 5) [19, 22, 23].

- **Stability studies**

ICH guidelines were followed for studying the accelerated stability of nanosponges and gel in a stability chamber at temperature (40 ± 2°C) and R.H (75% ± 5) for three months. Prepared nanosponges based gel were sealed in glass vials and kept in humidity chamber. After three months, the effect on storage condition, drug diffusion, drug content, and pH was studied (Table 8) [23].

- **Antifungal studies**

Antifungal studies of optimized formulation F6 were done using the agar-well diffusion method against Candida albicans sp. Inoculated Petri plates were taken, poured with agar medium, and left in a B.O.D. incubator for 24 hours. The diameter of the ZOI was measured in millimeters and compared with the marketed gel formulation (Table 9), Figure 6A and 6B. [19-23].

- **Invivo Studies**

All the animals were acclimatized under standard animal house conditions for 7 days before starting the in vivo studies. The animals were randomly divided into two groups containing three Wistar albino healthy male rats. Group I and II were treated with the preparations as a positive control. Group III was considered a negative control and treated with a blank preparation. Group IV is injected with the marketed formulation. After inoculation with a fungal infection, all the animals were treated once a day for 7 days, respectively [18, 19, 23] (IAEC/VII/8/GLA/2024) (Table 10, 11, Figure 7).

Results

The Carbopol gel loaded with KET nanosponges met all physical characteristics, showing good texture without clogging and lumps. It showed promising antifungal effects against C. albicans. The pH of the formulated gel was found to be 4.4±0.47, and it reported no significant skin toxicity, making it effective for topical delivery. The viscosity (4731.3± 0.24cps) and spreadibility were good, making it convenient to apply and improving patient compliance (Table 6). Table 7 showed 99.81% (NSs-CP gel), 99.97% (NSs), and 99.42 % (Marketed formulation) at the end of 12, 11, and 8 hours, respectively, pH 4.5 (Phosphate buffer). The study revealed drug diffusion for the nanosponges-loaded gel (NSs-CP) showed controlled release over 12 hours compared to the marketed formulation. (Table 8) stability studies showed no significant changes in drug diffusion, pH, physical appearance, and drug content after three months (t-test, $P<0.05$), suggesting stability at varying temperatures. Table 9 and Figure 6A (antifungal activity) and zone of inhibition show promising effects against Candida

albicans compared to the marketed formulation. Table 11 and Figure 7 represent in vivo studies showing better results compared to marketed formulations.

Discussion

Table 1 represents the effect of variables like drug-to-polymer ratio, stirring speed, and internal phase volume on % entrapment efficiency and particle size of prepared nanosponges. Table 2 represents particle size, PDI, and zeta potential for all the formulations. Figures 1A and 1B showed PDI values ranging from (0.246-0.478), low PDI values suggested narrow and uniform particle size distribution. Zeta potential ranges from (-16.74) - (+22.5 mV), suggesting the physical stability of colloidal dispersion. Further, the effect of the drug-to-polymer ratio on particle size (109.1-143.8 nm), indicates that particle size increases with increased polymer concentration; 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 and forms small size nanosponges. When the effect of stirring speed changed from 1000-1500rpm, the particle size and % entrapment efficiency were reduced. The results suggested that the polymer adheres to the stirrer surface at high stirring speed and decreases yield. F12 formulation showed a decreased % E.E. compared to F6 by varying the speed at the same polymer concentration. Another critical factor determined was with increased D.C.M. volume (internal phase), the viscosity of formulated dispersion was reduced, ranging from (22.42±0.74-41.48±0.44 m. Pas). This low viscosity is not suitable for topical application. So, nanosponges were added to the gel matrix to improve the viscosity and retention time.Invitrodrug diffusion study (Table 3, Figure 2) for nanosponges showed drug diffusion for up to 11 hours using a cellophane membrane. Nanosponges loaded with Carbopol gel showed drug diffusion across goat skin for up to 12 hours (Table 7). The correlation coefficient values (r) are shown in Table 5, and Figure 3 revealed diffusion profiles followed zero order kinetics, and the release data followed Peppa's model, having n values 0.645. The mechanism of drug release is non Fickian diffusion model. In Figures 4A and 4B, SEM and TEM images showed spongy and discrete nanosponges. Table 8 Stability studies showed no significant changes in drug diffusion, pH, physical appearance, and drug content of the stored formulations after three months (t-test, $P<0.05$), suggesting stability at varying temperatures. Table 9 and Figure 6A represent (antifungal activity) and zone of inhibition, showing promising effects against Candida albicans compared to marketed formulation. Table 11 and Figure 7 represent in vivo studies showing better results than marketed formulations.

Table 1: Formulations of Ketoconazole loaded EC Nanosponges (NSs)

Formulation codes	EC (mg)	PVA (mg)	Drug (mg)	DCM (ml)	Stirring speed (RPM)	Distilled water (ml)
F1	50	500	100	10	1000	100
F2	100	500	100	10	1000	100
F3	150	500	100	10	1000	100
F4	200	500	100	10	1000	100
F5	250	500	100	10	1000	100
F6	300	500	100	10	1000	100
F7	50	500	100	20	1500	100
F8	100	500	100	20	1500	100
F9	150	500	100	20	1500	100
F10	200	500	100	20	1500	100
F11	250	500	100	20	1500	100
F12	300	500	100	20	1500	100

*PVA: Polyvinyl alcohol, E.C: Ethyl cellulose, DCM: Dichloromethane, µl: microlitre, mg: milli gram, ml: milli litre, RPM: Rotations per minute.

Table 2: Characterization parameters of Nanosponges

Formulation codes	Particle size (nm)	PDI	Z.P (mV)	%age entrapment efficiency (EE)	Viscosity (m. pas)
F1	109.1	0.246	-19.31	69.64±0.22	25.06±0.11
F2	122.5	0.508	-26.14	73.48±0.38	26.54±0.34
F3	143.8	0.398	15.4	78.23±0.59	29.71±0.14
F4	158.7	0.322	-15.5	79.87±0.13	33.12±0.39
F5	179.6	0.379	18.4	82.46±0.45	38.34±0.32
F6	186.4	0.620	-32.3	88.74±0.24	41.48±0.44
F7	103.2	0.278	13.47	63.92±0.39	22.42±0.74
F8	120.6	0.250	-17.62	70.47±0.11	23.36±0.49
F9	136.3	0.357	22.5	71.93±0.31	24.26±0.53
F10	143.8	0.478	11.62	74.34±0.42	30.30±0.41
F11	164.8	0.347	-16.74	79.89±0.13	31.48±0.05
F12	182.9	0.409	19.5	85.83±0.39	36.12±0.43

*n=3

Table 3: InvitroDrug Diffusion Studies Data of nanosponges formulations (F1-F12)

Hours	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
0	0	0	0	0	0	0	0	0	0	0	0	0
0.5	8.94	6.41	5.59	5.15	5.58	2.47	4.75	3.81	5.51	4.85	4.21	3.81
1	13.45	14.65	11.24	9.35	13.14	8.12	8.12	5.91	13.74	11.27	10.85	5.91
1.5	21.47	20.54	17.59	15.42	17.58	13.58	13.24	11.14	17.78	18.23	15.17	11.14
2	28.49	25.95	25.89	19.58	20.87	17.21	17.12	15.27	23.64	21.14	19.84	15.27
2.5	34.58	33.81	30.21	26.21	26.78	22.93	22.21	18.09	29.87	27.19	26.37	18.09
3	43.06	37.56	36.17	31.2	33.84	27.28	27.24	24.87	35.65	33.21	30.71	24.87
3.5	49.87	45.65	42.54	36.47	39.35	31.59	33.87	27.71	41.54	38.47	35.47	27.71
4	56.24	52.54	48.12	41.52	46.74	36.89	38.12	33.64	47.21	41.28	42.57	33.64
4.5	63.78	59.09	54.35	45.87	54.28	41.39	45.41	39.04	52.87	48.64	46.12	39.04
5	70.65	65.42	61.17	51.37	67.57	45.58	49.87	44.7	57.82	54.21	52.15	44.67
5.5	76.87	72.24	66.47	57.25	75.47	49.98	54.53	49.8	64.71	59.42	57.78	49.78
6	84.21	77.14	73.59	61.84	80.12	54.42	61.71	53.8	70.56	64.76	62.98	53.78
6.5	92.65	83.41	79.02	66.74	88.09	58.69	66.24	58.7	75.87	69.21	67.57	58.74
7	99.47	90.54	84.36	71.81	90.57	63.79	71.51	64.6	81.92	77.52	73.61	64.57
7.5		99.28	92.98	77.07	93.76	67.52	77.52	70.6	85.43	82.23	77.02	70.56
8			99.32	83.12	99.26	72.89	84.68	75.2	93.28	88.97	83.21	75.24
8.5				86.29		76.34	93.06	81.1	99.22	93.05	87.25	81.14
9				92.51		80.27	99.09	87.4		99.12	94.45	87.41

9.5				99.48		85.67		94.5			99.09	94.54
10						91.38		99.2				99.52
10.5						95.98						
11						99.97						
11.5												
12												

Table 4: Release Kinetics for Optimized formulation (F6)

Hours	% CDR	Log % CDR	Log T	√T (Hrs)	% CDR Remaining	Log Remaining (% CDR)
0	0	0	0	0	100	2
0.5	2.47	0.39	-0.3	0.7	97.53	1.98
1	8.12	0.9	0	1	91.88	1.96
1.5	13.58	0.55	0.17	1.22	86.42	1.94
2	17.21	1.23	0.3	1.41	82.79	1.91
2.5	22.93	1.36	0.39	1.58	77.07	1.88
3	27.28	1.43	0.47	1.73	72.72	1.86
3.5	31.59	1.49	0.54	1.87	68.41	1.83
4	36.89	1.56	0.6	2	63.11	1.8
4.5	41.39	1.61	0.65	2.12	58.61	1.76
5	45.58	1.65	0.69	2.23	54.42	1.73
5.5	49.98	1.69	0.74	2.34	50.02	1.69
6	54.42	1.73	0.77	2.44	45.58	1.65
6.5	58.69	1.76	0.81	2.54	41.32	1.61
7	63.79	1.8	0.84	2.64	36.21	1.55
7.5	67.52	1.82	0.87	2.73	32.48	1.51
8	72.89	1.86	0.9	2.82	27.11	1.43
8.5	76.34	1.88	0.92	2.91	23.66	1.37
9	80.27	1.9	0.95	3	19.73	1.29
9.5	85.67	1.93	0.97	3.08	14.33	1.15
10	91.38	1.96	1	3.16	8.62	0.93
10.5	95.98	1.98	1.02	3.24	4.02	0.6
11	99.97	1.99	1.04	3.31	0.03	-1.52
11.5						
12						

Table 5: Release Kinetic Study Data of Optimized formulation (F_4)

Kinetics Model	Regression Coefficient (R^2)	n (slope)
Zero order	0.9995	
First order	0.5215	
Higuchi release	0.9365	
Korsmeyer-Peppas	0.941	0.645

Table 6: Characterization parameters of gel

pH	(%) Drug Content	Extrudability	Viscosity (cps)	Spreadibility
4.4±0.47	99.41%±0.87	93.54%	4731.3± 0.24	34.56 g.cm/sec

Table 7: Comparative % Drug diffusion across goat skin

% Drug diffused across goat skin			
Time (h)	NSs	NSs-CP	MF
0	0	0	6.29
0.5	2.47	3.86	14.32
1	8.12	7.07	20.52
1.5	13.58	11.64	25.95
2	17.21	16.82	30.62
2.5	22.93	19.81	35.21
3	27.28	24.94	42.47
3.5	31.59	27.75	46.24
4	36.89	32.87	52.47
4.5	41.39	37.52	58.87
5	45.58	41.51	65.14
5.5	49.98	45.62	70.54
6	54.42	49.21	75.68
6.5	58.69	54.32	82.32
7	63.79	57.08	86.24

7.5	67.52	62.34	92.45
8	72.89	65.71	99.42
8.5	76.34	70.61	
9	80.27	75.62	
9.5	85.67	79.84	
10	91.38	83.29	
10.5	95.98	86.52	
11	99.97	91.74	
11.5		97.52	
12		99.81	

Table 8: Stability Studies of formulated optimized gel

S.No	Time (h)	% Drug diffusion (initial)	% Drug diffused (NSs-Carbopol gel)					
			25°C/ 60% RH			40°C/ 75% RH		
			30 days	60 days	90 days	30 days	60 days	90 days
1	0	0	0	0	0	0	0	0
2	0.5	3.86	3.8	3.71	3.62	3.66	3.52	3.47
3	1	7.07	7.02	6.96	6.81	6.85	6.73	6.65
4	1.5	11.64	11.6	11.52	11.35	11.68	11.56	11.49
5	2	16.82	16.24	16.21	16.17	16.21	16.05	15.98
6	2.5	19.81	19.75	19.7	19.62	19.66	19.58	19.49
7	3	24.94	24.81	24.73	24.63	24.74	24.61	24.54
8	3.5	27.75	27.6	27.54	27.47	27.53	27.39	27.34
9	4	32.87	32.72	32.61	32.52	32.63	32.57	32.41
10	4.5	37.52	37.49	37.41	37.36	37.39	37.22	37.13
11	5	41.51	41.47	41.13	41.09	41.72	41.61	41.56
12	5.5	45.62	45.52	45.49	45.42	45.46	45.36	45.25
13	6	49.21	49.14	49.05	48.57	49.11	49.02	48.96
14	6.5	54.32	54.27	54.11	53.95	53.98	53.8	53.73
15	7	57.08	57.06	56.92	56.82	56.95	56.25	56.14
16	7.5	62.34	62.28	62.12	62.06	62.27	62.18	61.78
17	8	65.71	65.66	65.53	65.43	65.57	65.21	65.05
18	8.5	70.61	70.59	70.51	70.22	70.38	70.3	70.19
19	9	75.62	75.52	75.45	75.25	75.46	75.19	75.12
20	9.5	79.84	79.75	79.69	79.51	79.65	79.49	79.46
21	10	83.29	83.22	83.17	83.01	83.15	82.89	82.85
22	10.5	86.52	86.31	86.19	86.09	86.24	86.08	85.98
23	11	91.74	91.69	91.62	91.47	91.67	91.36	91.2
24	11.5	97.52	97.5	97.45	97.4	97.46	97.33	97.23

25	12	99.81	99.62	99.52	99.38	99.44	99.35	99.28

Table 9: Measured (Zone of Inhibition) against C. albicans

Specification	Measured Inhibition zones (mm)±SD
Negative control	0
Carbopol gel (free nanosponges) (C)	29±0.11
KET NSs (D)	35±0.24
Marketed NSs (M)	28±0.12
NSs loaded gel (G)	32±0.15
Marketed gel (T)	30±0.17

*SD: Standard deviation, n=3

Table 10: In vivo Study Protocol design

S.No	Parameters	Requirements
1	Drug	Ketoconazole
2	Formulation	Nanosponges gel
3	Animal model	Healthy male wistar albino rats
4	Animal weight	230-250gm each
5	No. of animals	6
6	No. of groups	2
7	No. of animals per group	03 (n=3)
8	Control formulation	Pure drug solution
9	Test formulation	Nanosponges
10	Dose	2.5mg/cm²
11	Route of administration	Topical route

Table 11: In vivo Animal activity Measured ZOI (mm) and colony count

S.No	Formulations	Colony count 8^{th}day
1	Optimized formulation	11±0.89
2	Marketed	9±1.41
3	Drug free gel	56.03±3.3

(± SD, n=3

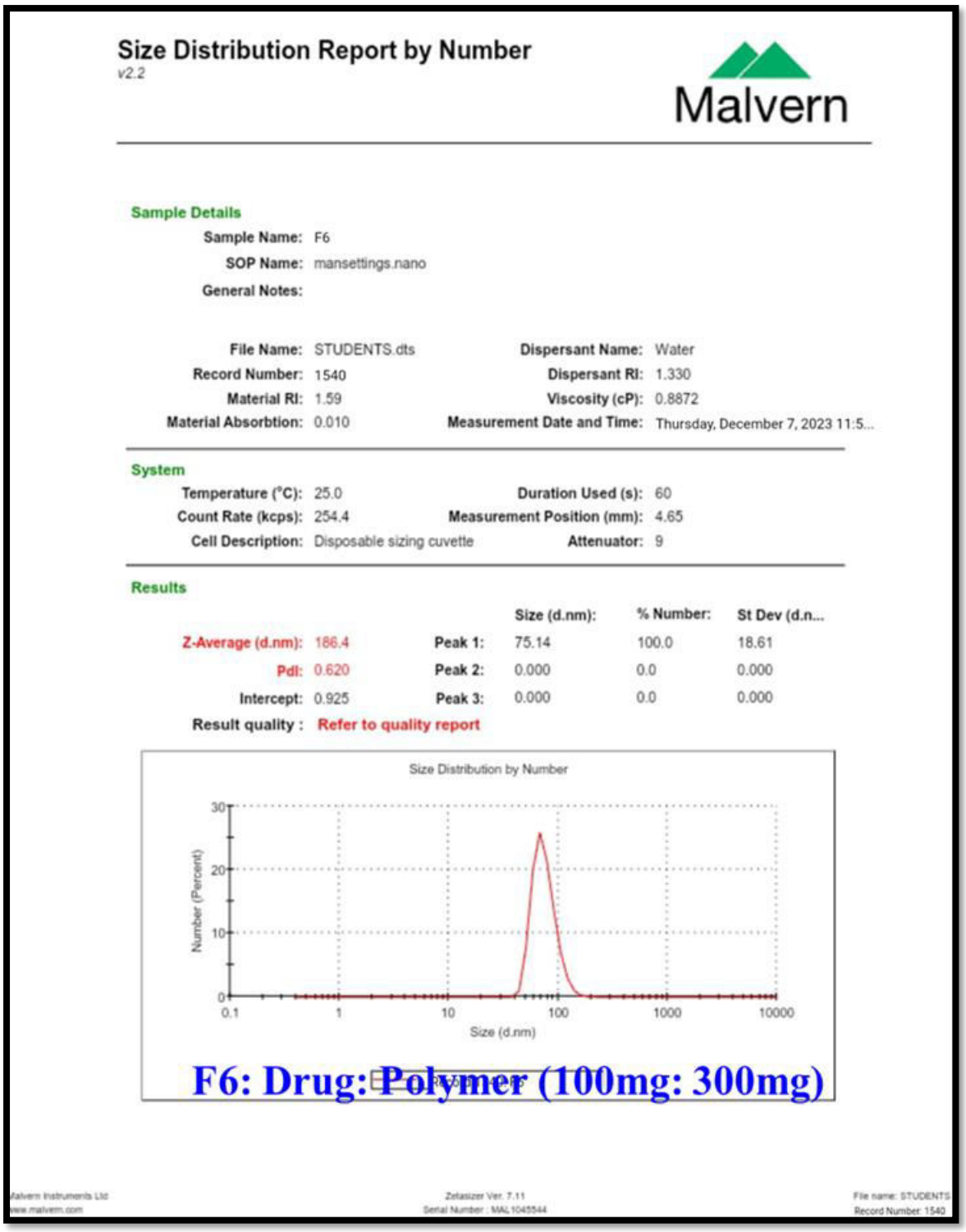


Figure 1 A: Optimized formulation particle size (F4)

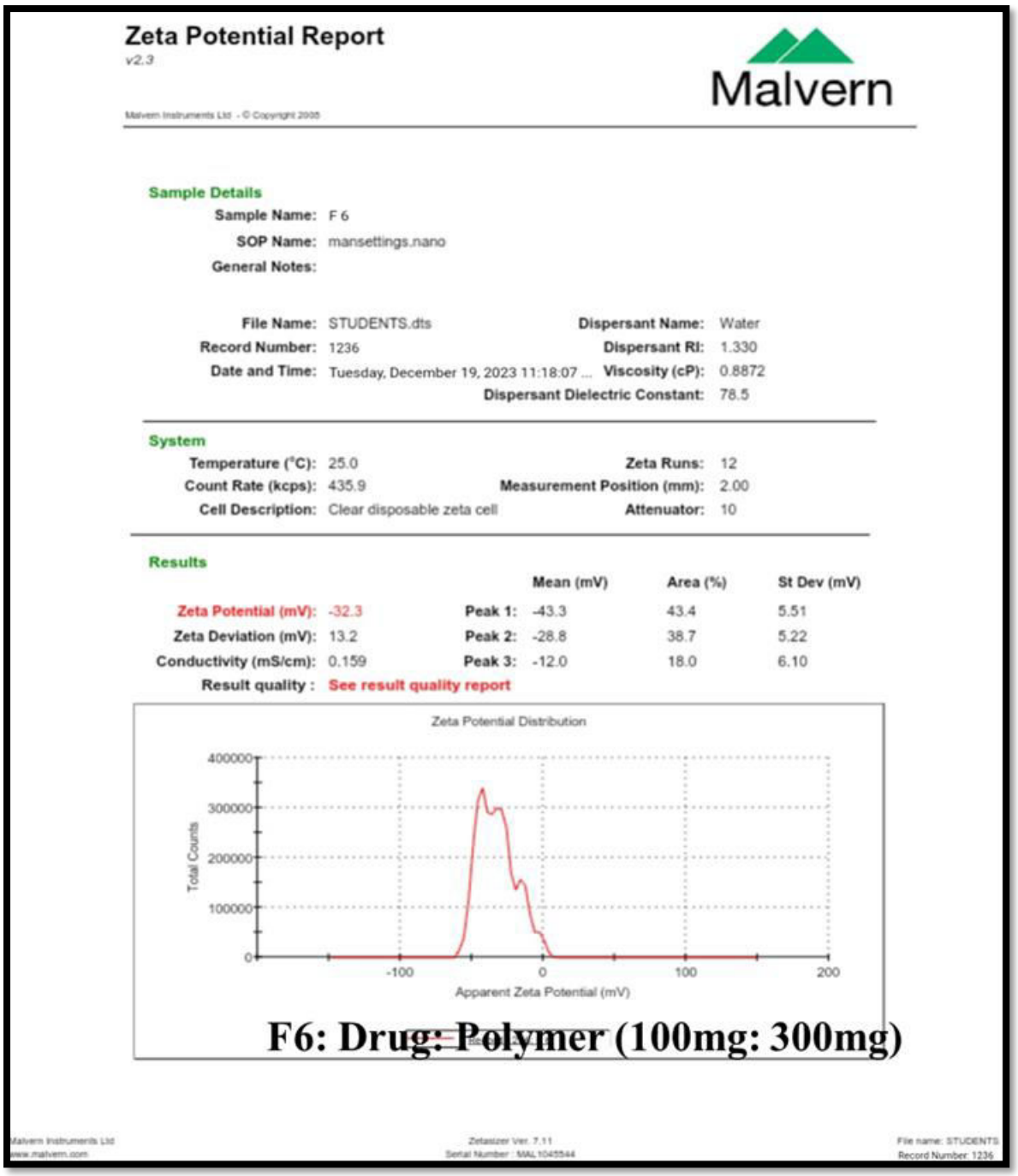


Figure 1 B: Optimized formulation zeta potential (F4)

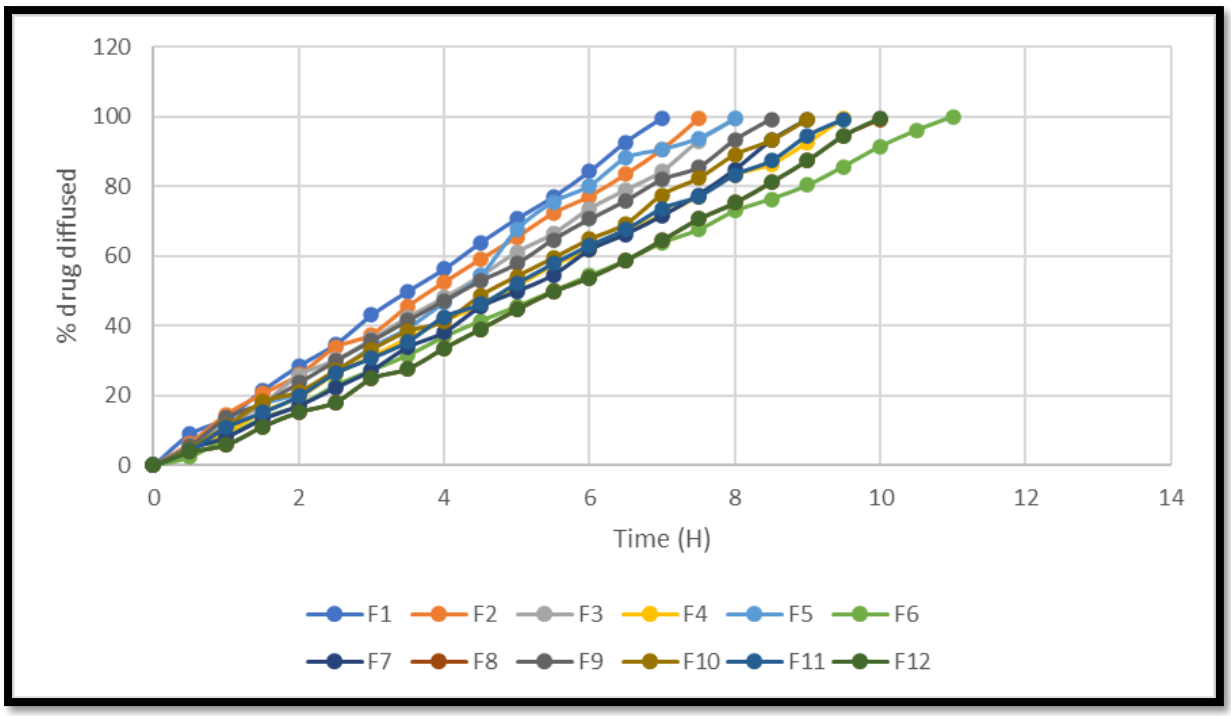
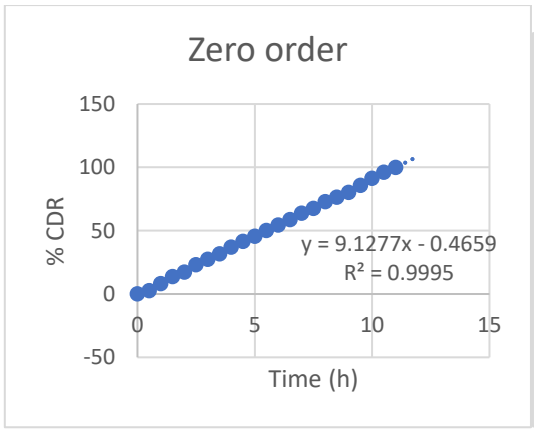
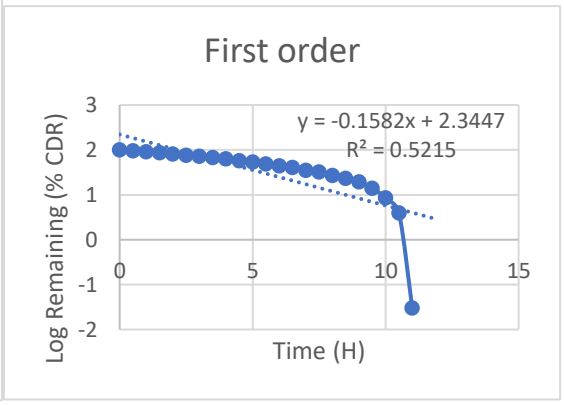


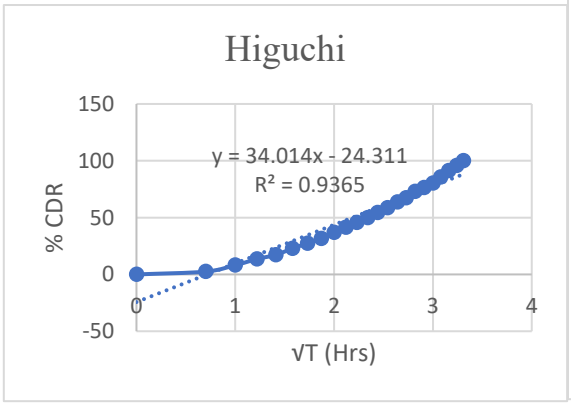
Figure 2: Comparative invitrodrug diffusion for formulations F1-F12



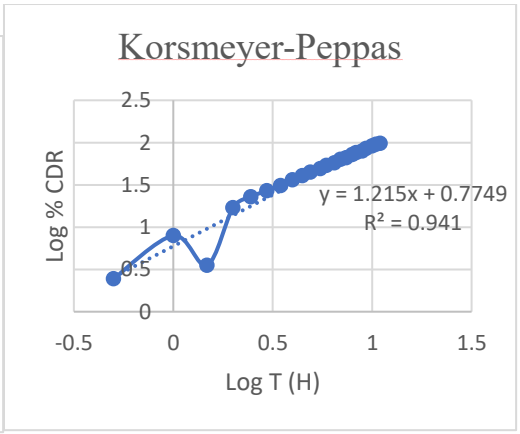
Zero order release



First order release



Higuchi release kinetics



Korsmeyer-Peppas release

Figure 3: Release kinetic graphs of F6 optimized formulation

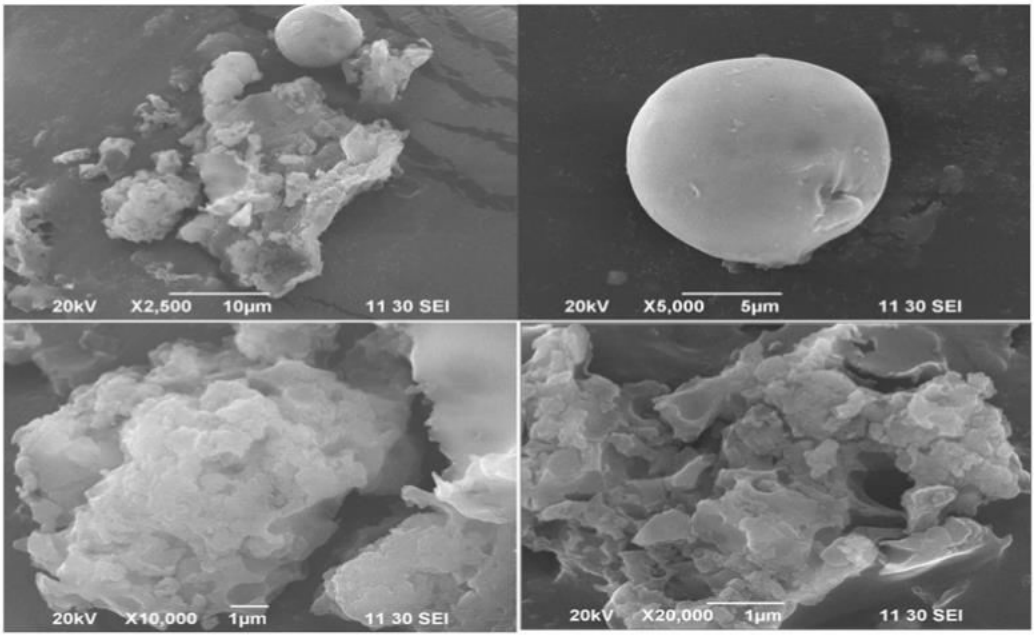


Figure 4A: SEM of Optimized formulation F6

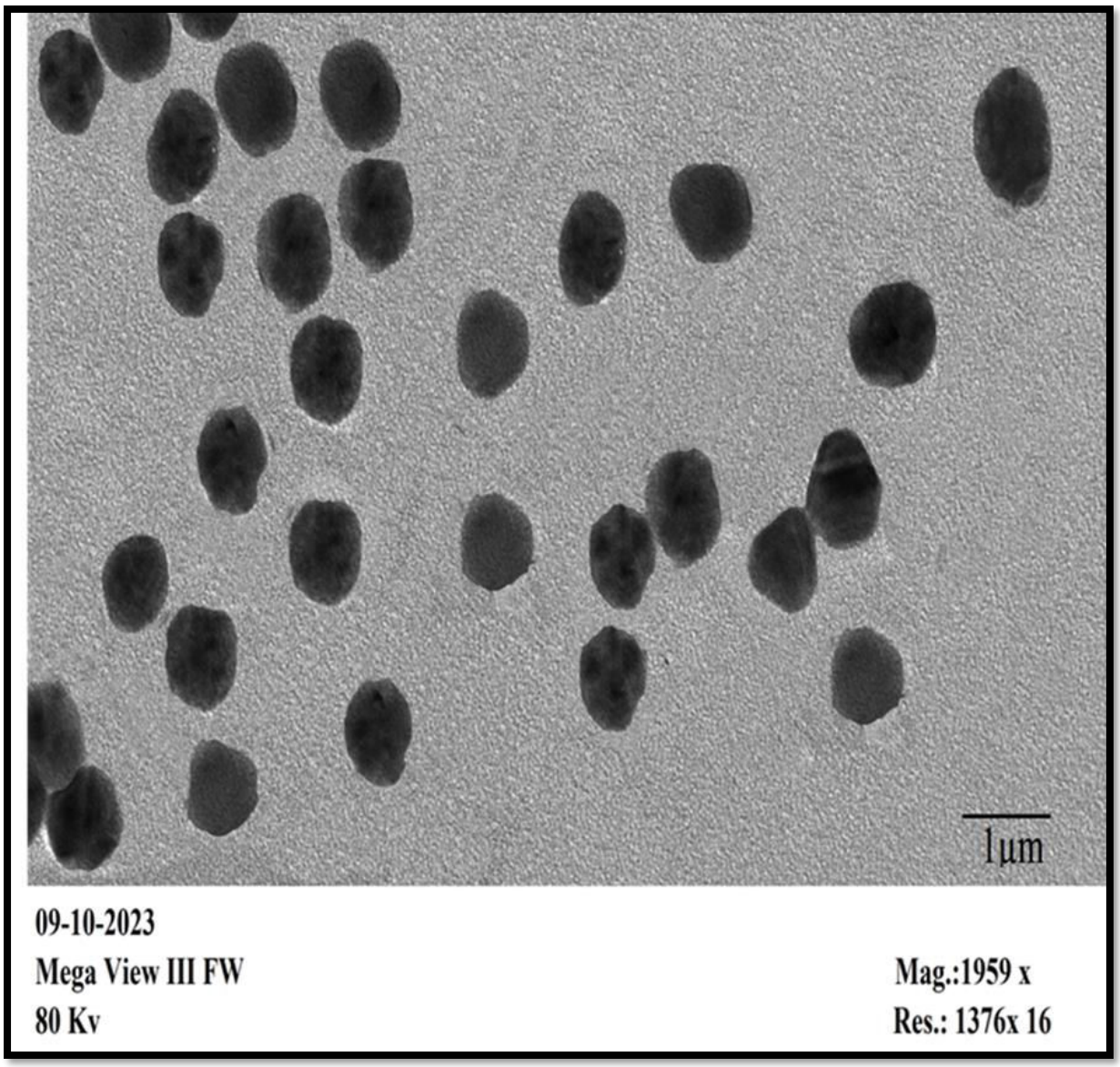


Figure 4B: TEM of Optimized formulation F6

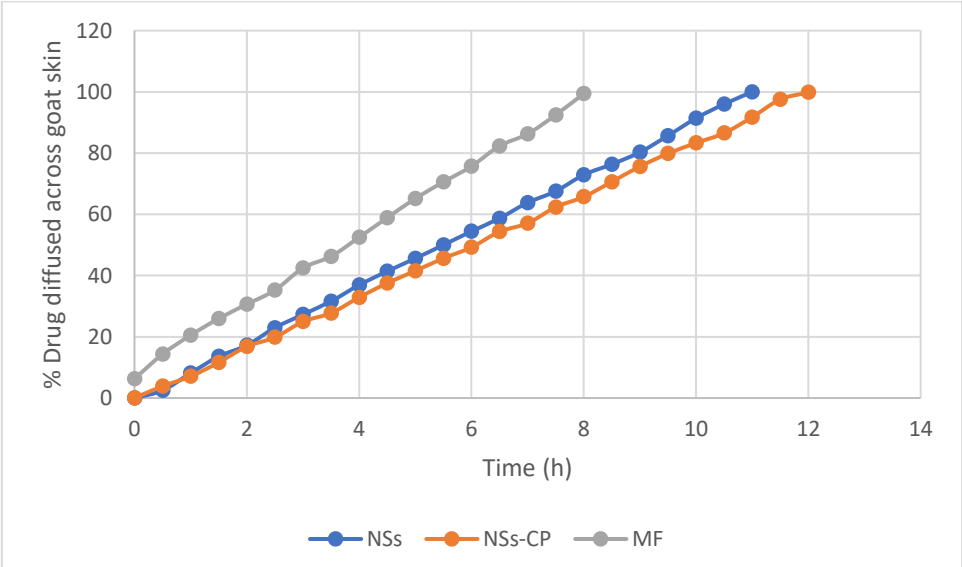
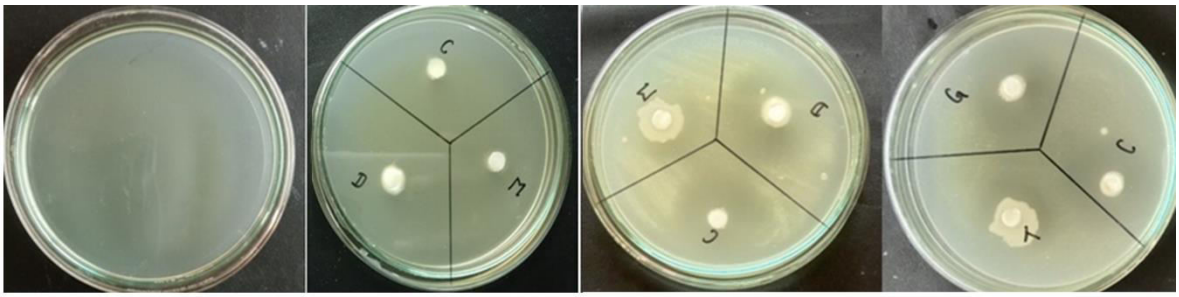


Figure 5: Comparative drug diffusion study (goat skin surface)



Stage 1: Control | Stage 2: addition of sample | Stage 3: ZOI (mm) of NSs | Stage 4: ZOI (mm) of NSs loaded gel

D: KET NSs
M: Marketed NSs
T: Marketed gel (KET)
G: KET-NSs gel
C: Carbopol gel (free drug)

Figure 6 A: Antifungal activity against C. albicans

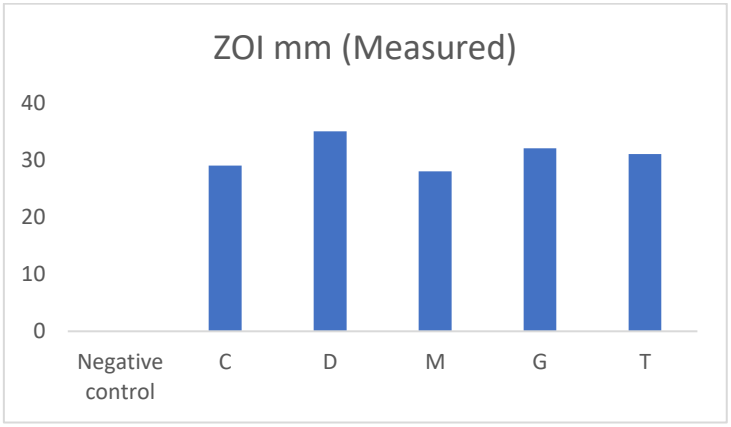
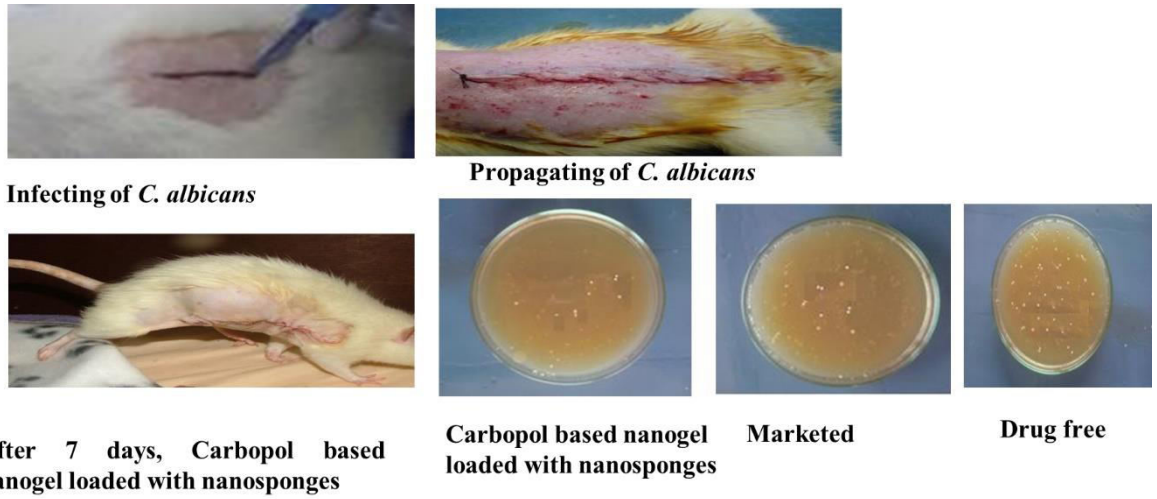


Figure 6B. Bar Graph representing ZOI (mm)against C. albicans



Infecting of *C. albicans*

Propagating of *C. albicans*

After 7 days, Carbopol based nanogel loaded with nanosponges

Carbopol based nanogel loaded with nanosponges

Marketed

Drug free

Figure 7: In vivo animal activity against C. albicansand ZOI (mm)

Limitations

1. Selection of only one fungal strain for study.
2. More studies can be done to frame the conclusion better.

Conclusion

The prepared herbal gel showed improved antifungal activity compared to the marketed formulation. In the future, many antifungal properties can be discovered for prepared gel. F6 formulation showed promising antifungal effects against Candida albicans, both in vitro and in vivo. The gels are in good condition and can be explored for further clinical trials. Formulation F6 showed promising results in % E.E., particle size, zeta potential, and in vitro drug diffusion release. The different kinetic studies predicted an R2 value of 0.995, showing drug diffusion followed the zero-order kinetics compared to Korsmeyer-Peppas release and first-order. Further, the Korsmeyer-Peppas release showed an R2 value of 0.941 with n= 0.645, revealing drug release followed the super case-II transport mechanism (non-fickian diffusion). Stability results suggested that the formulated preparations were stable after storage and showed no significant changes in drug content, pH, or drug diffusion studies. The formulated gels produced sustained effects for up to 12 hours and were effective for topical delivery. The gel preparation opened newer doors for scientists and researchers and can be successfully used to prepare selected Indian monographs of medicinal plants.

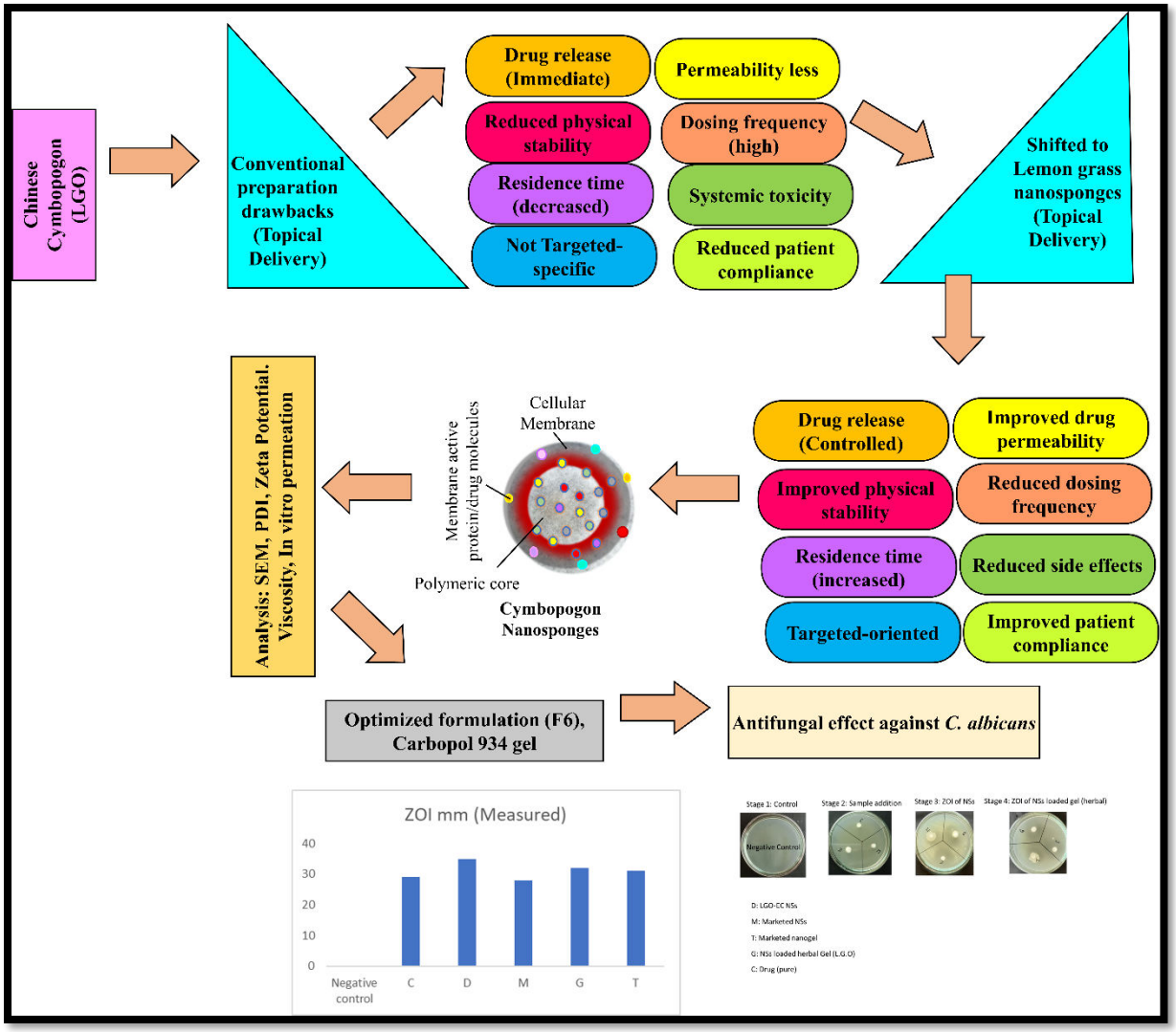
Conflicting of Interest

None

Acknowledgment

The authors appreciated the efforts of GLA University, which provided space for conducting research, and Chemland Ind, which provided chemicals for the project.

Graphical Abstract



Antifungal potential of KET nanogel against C. albicans

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