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Development and Evaluation of Amphotericin B Nanoemulsion for Treatment of Cutaneous Leishmaniasis

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ABSTRACT

Objective: This study aims to develop and evaluate Amphotericin B nanoemulsion for the treatment of cutaneous leishmaniasis. **Methods**: Amphotericin B nanoemulsion was prepared by phase titration method followed by high-pressure homogenization. **Results:** The Amphotericin B nanoemulsion gel showed a drug release of 98.75% from the NFA2 batch at the end of 24 hours. This was followed by NFE2>NFC2>NFB2>NFD2.The marketed preparation showed drug release of about 60% at the end of 24 hours. The ex vivo permeation was about NFA2 batch with 73.75% while this was followed by NFD2>NFB2>NFC2>NFE2.The marketed preparation showed a drug permeation of about 66.85%. The antifungal studies results showed that the antifungal activity of the tested formulations was in the following order Marketed Gel>Nanoemulsion>Nanoemulsion Gel>Plain drug gel. **Conclusion**: Amphotericin B nanoemulsion was prepared by mixing suitable proportions of oil, surfactant, and drug and by utilizing the water phase titration method. The solubility studies were also carried out along with thermodynamic stability tests. Optimized formulations were selected based on these tests. The optimized formulations showed good viscosity and high drug content. The scanning electron microscopy image showed white-colored structures in presence of nanoemulsion droplets. The nanoemulsion was incorporated into the gel base. The gel batches were evaluated for ex vivo permeation where they showed good permeation and also for drug release. The drug release was high at the end of 24 hours. The batches were also evaluated for their viscosity as well as satisfactory drug content. The antifungal studies showed that the nanoemulsion showed the highest activity against the fungi followed by nanoemulsion gel. Hence Amphotericin B nanoemulsion can be a good candidate or treatment of Cutaneous Leishmaniasis.

INTRODUCTION

Leishmaniasis is a disease with a broad spectrum of clinical symptoms caused by distinct species of flagellae protozoa falling under the Leishmania genus [1]. There are two important clinical symptoms of leishmaniasis: cutaneous and visceral. Cutaneous leishmaniasis (CL) first shows up as a localized papule, which then transforms into an ulcer upon the shedding of the epidermis, leading to a great hindrance of the skin barrier. Parenteral administration of pentavalent antimony organic compounds is still considered as the first-line treatment for all leishmaniasis manifestations. But, resistance and higher incidences of side effects (anorexy, myalgias, arthralgias, chemical pancreatitis, leucopenia, cardiotoxicity, etc.) are still applicable issues that are present with this treatment [2,3]. Over the past decades, more importance has been given to the growth of other therapies, including the development of formulations for both oral and topical treatment of CL [2,4]. Topical treatment shows a promising alternative, giving many advantages in comparison with the parenteral administration such as hassle-free administration, lesser adverse reaction chances, and an attractive cost-benefit ratio [5]. Amphotericin B is, a high water-insoluble antifungal drug with high molecular weight is a useful second-line treatment for CL. The drug shows good activity, but its clinical utility is hindered by its recurrent toxicity mostly connected to the parenteral administration of Fungizone®, the traditional formulation made of mixed micelles of AmB and sodium deoxycholate [6]. The topical administration of AmB shows a promising alternative for bypassing such problems. However, the main drawback is unearthing an acceptable nanocarrier to enhance the topical delivery of AmB into the dermal layer of the skin. Many studies have concentrated on the development of new AmB formulations, mainly a lipid carrier, such as liposomes, emulsions [7]. Conjunctions with cholesterol sulfate [8]. And in not much time ago, microemulsions [9]. Nearly all these systems initiate smaller toxicity of AmB when compared with Fungizone®, but they show many technological limitations that slow the progress of a marketable product. Nanoemulsion (NE) was chosen as a drug carrier since this system has been vastly utilized for enhancing skin delivery of hydrophobic drugs [10].

MATERIALS AND METHODS

1.1 MATERIALS

Amphotericin B was received as a gifted sample from Piramal Enterprises Ltd.Linseed oil, PEG 400, Sesame oil, Neem oil, Castor oil, Ethyl oleate, Glycerine, Span 80, Carbitol, Transcutol p Eucalyptus oil, Silicon oil, Sunflower oil, Tween 40, Peppermint oil, Soybean oil PEG 200 were all obtained from KJ Enterprises.

1.2 Formulation of Nanoemulsions

1.3 Solubility Studies

The solubility studies have to be done to detect the oil that solubilizes the maximum amount of drug. The solubility of the amphotericin B was determined in various oils by adding an excess amount of drug to 1 ml of selected oils (soyabean oil, sesame oil, sunflower oil) in stopper vials. The vials were kept 25± 0.5˚C in a Wrist action shaker for 72 hours to reach equilibrium. The equilibrated samples were removed from the shaker and centrifuged at 3000rpm for 15 min. The supernatant was taken and through a 0.45L membrane filter and concentration of amphotericin B was determined in the oils after dilution using a UV-Visible spectrophotometer at 408nm.

1.3.1 Pseudo-ternary phase diagram (TPD)

For the making, the TPD water phase titration method was used [11]. For this method, oil and Smix in a proportion in the test tube were taken and water was added stepwise to the mixture. The mixture was then mixed at 25 ºC with the help of a vortex mixer. With the help of visual observation, the formulations, which were showing clear and transparent regions, were identified as NE formulations. From the screening of parts mix of Isopropyl myristate as the oil phase was taken, Tween 80 and Transcutol - P was chosen as SA and co-SA, individually. The Co-SA and SA (Smix) were taken and mixed in a ratio of (1:0, 1:1, 1:2, 2:1,1:3, and 3:1) by weight proportion. Smix ratios were chosen to study the phase diagram for NE formulation. Concerning Co-SA the SA concentration was increased and vice versa. 16 unique blends in distinctive proportions by weight of oil and Smix 1:8, 1:9, 1:7, 1:6, 2:8 (1:4), 1:5, 1:3.5, 1:3 ,1:2, 4:6 (1:1.5), 5:5 (1:1), 6:4 (1:0.7), 7:3 (1:0.43), 8:2 (1:0.25), 9:1 were taken. For distinguishing proof of NE district, the formulation was checked on a pseudo-3- component TPD. Where one hub was for the fluid phase, the second pivot was for

the oil phase and the third hub speak to a mix of SA and co-SA; SA at altered weight proportions (Smix proportion) respectively.

1.4 Preparation of Nanoemulsion gel

The nanoemulsion-based gel was prepared by dispersing 1gm of carbopol 934 in a sufficient quantity of distilled water. After complete dispersion, the carbopol solution was kept in the dark for 24 hours for complete swelling. Then the Amphotericin B-loaded nanoemulsion was added slowly to the viscous solution of carbopol 934 under magnetic stirring [12]. The pH values were maintained at 6-7 with triethanolamine was added to the obtaining a homogenous dispersion of gel. The final concentration of Amphotericin B in the nanoemulsion-based gel was 0.02%w/w.

2.0 EVALUATION OF NANOEMULSION

2.1 Thermodynamic stability tests

Selected formulations were subjected to different thermodynamic stability tests.

2.2 Heating cooling cycle

Between refrigerator temperature, 40˚C and 45˚C of six cycles with storage at each temperature of not less than 48 h were studied. Those formulations, which were stable at these temperatures, were subjected to centrifugation.

2.3 Centrifugation

Those formulations that passed were centrifuged at 3500 rpm for 30 min by using a centrifuge. The formulations that did not show any phase separation were taken to further tests.

2.4 Freeze thaw cycle

Between –21˚C and +25˚C three freeze-thaw cycles with storage at each temperature for not less than 48 h were done for the formulations.

2.5 Drug content

The drug content was calculated by UV visible spectrophotometer. The formulation was diluted to the required concentration using methanol as solvent and the absorbance was measured at 408nmagainst a solvent blank. The drug content was calculated as:

$$\text{Drug content} = \frac{\text{Analyzed content} \times 100}{\text{Theoretical content}}$$

2.6 Scanning Electron Microscopy

The optimized Nanoemulsion was evaluated using scanning electron microscopy (Joel jsm-6490la analytical SE). One drop of the Nanoemulsion was mounted on an aluminum stub with double-sided adhesive carbon tape. The drop of the Nanoemulsion was then sputter-coated with gold using a vacuum evaporator and examined with the scanning electron microscope.

3.0 EVALUATION OF NANOEMULSION GEL

3.1.1 *Ex-vivo* permeation studies

Using a Franz Diffusion Cell these studies were performed on the abdominal skin of goats. Epidermal skin samples on the area of the sample were 0.598 cm^2 were affixed into the diffusion cells. 2gm equivalent to 0.4mg of Amphotericin B of gel was kept on the dorsal side of the skin. The skin sample was equilibrated with release media at 37± 0.2^o C for 0 to 24 hrs employing a water jacket on the Franz diffusion cell which is kept on a magnetic stirrer whose RPM was kept at 240. At different time intervals, aliquots (2ml) from the receptor compartment were withdrawn and examined by UV method for drug content. Immediately same fresh quantity of solvent mixture was replenished to the receiver compartment [13].

3.1.2 *In-vitro* drug release

In-vitro drug release studies are most important to study the release pattern of the drug from the delivery system and help in characterization of drug delivery profile In-vitro profile of drug release study of Amphotericin B loaded nanoemulsion were obtained by performing a dissolution test in a release medium [Phosphate buffer pH 7.4]. Regenerated cellulose membrane known as a dialysis membrane with a molecular weight of 12-14 kDa, with a diameter of 24.96 mm was used. The amount of nanoemulsion equivalent to a daily dose of 2

mg was filled in a dialysis bag to experiment. Dialysis bag containing a daily dose submerged into 250 ml of buffer solution which is maintained at 37°C under an agitation condition of 150 RPM/min on a magnetic stirrer. After a predetermined interval of time, an aliquot of 5 ml was withdrawn from the release medium and the same volume of the buffer has been added to the medium to maintain the sink conditions. The withdrawn sample is assayed for the drug content determination using the UV method at 408 nm and calculated on a pre-generated calibration curve.

3.1.3 Viscosity determination.

To determine the viscosity of the formulation Brookfield viscometer LV DV-E (USA) was used with spindle no. (62).

3.1.4 Homogeneity:

To check the consistency and homogeneity a small quantity of gel between the thumb and the index finger is pressed (whether homogeneous or not) and if there is any coarse particle appeared then the formulation was discarded.

3.1.5 Viscosity determination

The viscosity was computed utilizing Brookfield viscometer LV DV-E (USA) utilizing shaft No. 2 (62) at 25 ± 0.5°C (triplicate).

3.1.6 Measurement of gel strength and Homogeneity

After 48h once the gel was ready the strength was computed for the required weight measurement for upper plate movement by 3cm, between two 20cm×20cm plates when1g of each gel was placed by using the formula the gel strength was calculated:

$$S = \frac{M \times L}{T}$$

S = gel strength and M=weight fixing to the upper slide, L = length glass part voyaged, and T = time. The homogenous properties of the gel formulation were examined visually.

3.1.7 pH determination

The pH meter manufactured by Equip-Tronics, India is used to measuring the pH at 25 ± 1ºC (triplicate).

3.1.8. Antifungal Activity

Candida albicans slant was obtained from the microbiology department of Patkar-varde college, Mumbai.5ml of Sabouraud Dextrose Broth (SDB) was added to the slant and incubated for the growth of Candida albicans.2.6gm of Sabouraud Dextrose agar (SDA) was added in 40ml Distilled water in a conical flask and stoppered with a cotton plug and autoclaved for 15 mins at 15lbs pressure and then cooled at room temperature. Pour 20ml into the sterile Petri plate between two flames, swirl the plate to remove air bubbles, and let it solidify at room temperature near sterile condition. Addition of CA fungi through cotton swab and streaked in all directions in the solidified agar, bore four wells to add Marketed gel, Nanoemulsion, Nanoemulsion gel, and plain drug gel. Incubate at 25-30˚C for 72 hrs for inhibition of fungi and observe MIC(zone of inhibition) and measure diameter in mm.

3.1.9. Stability Studies

The NE gel formulation was stored for 3 months at different temperatures. For the estimation of drug content, the HPLC method was used.

Stability studies as per ICH guidelines

Three packs of NE were subjected to 40 ± 2ºC and RH of 75 ± 5 %. At an interval of zero, thirty, sixty- and ninety-days samples were taken, diluted with mobile phase, and analyzed using UV method at 310 nm. The logarithmic remaining % age of drug in the NE was plotted against time. Each line slope for each temperature was obtained and degradation rate constants (K) were calculated using the standard slop equation.

RESULTS AND DISCUSSIONS

Figure No. 1: Solubility instrument

Evaluation of Nanoemulsion

1.1 Solubility Studies

The solubility of the drug in different excipients vehicles is as follows as shown in Table 1.

Table No. 1: Results of Solubility Studies

S. No.	Ingredients	Solubility (mg/ml)
1	Linseed oil	18.23
2	PEG 400	28.94
3	Sesame oil	94.53
4	Neem oil	145.65
5	Castor oil	45.87
6	Ethyl oleate	9.98
7	Glycerin	211.87
8	Span 80	43.34
9	Carbitol	168.7
10	Transcutol p	180.43
11	Eucalyptus oil	13.76
12	Silicon oil	52.98
13	Sunflower oil	83.54
14	Tween 40	95.94
15	Permint oil	12.65
16	Soyaben oil	103.3
17	PEG 200	53.67

1.2 Pseudo Ternary Phase Diagram

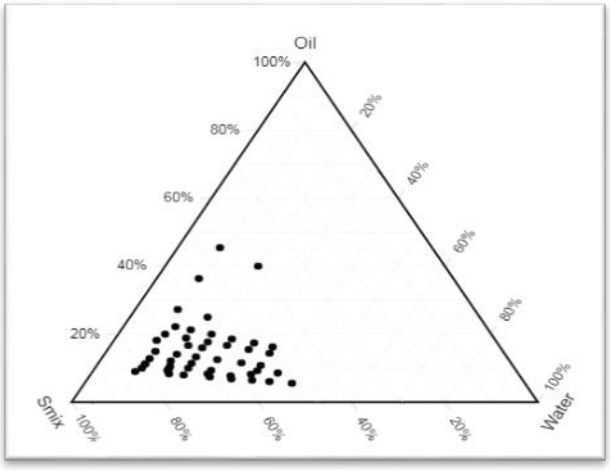

Figure 2: Pseudo-ternary phase diagrams showing the o/w Nanoemulsion (shaded area) regions of castor oil and triacetin (1:1) Tween 80 (Surfactant) and Transcutol P (Co-surfactant) at Smixratios: 1:1

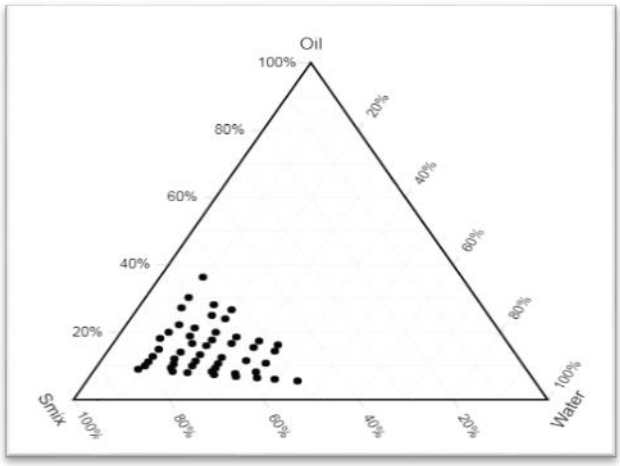

Figure 4: Pseudo-ternary phase diagrams showing the o/w Nanoemulsion (shaded area) regions of castor oil and triacetin (1:1) (Oil), Tween 80 (Surfactant) and Transcutol P (Co-surfactant) at Smixratios: 2:1

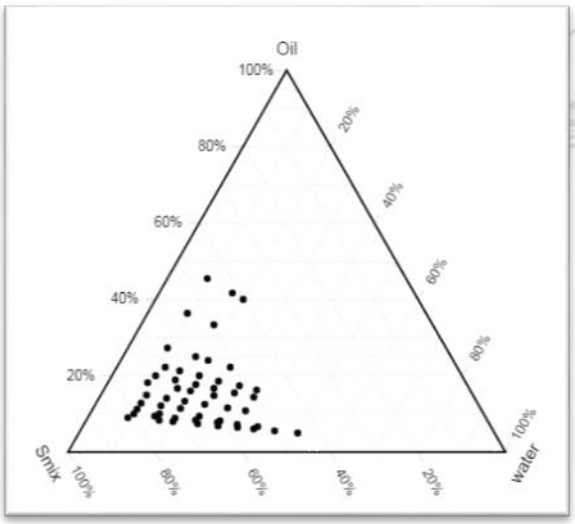

Figure 3: Pseudo-ternary phase diagrams showing the o/w Nanoemulsion (shaded area) regions of castor oil and triacetin (1:1) Tween 80 (Surfactant) and Transcutol P (Co-surfactant) at Smixratios: 1:2

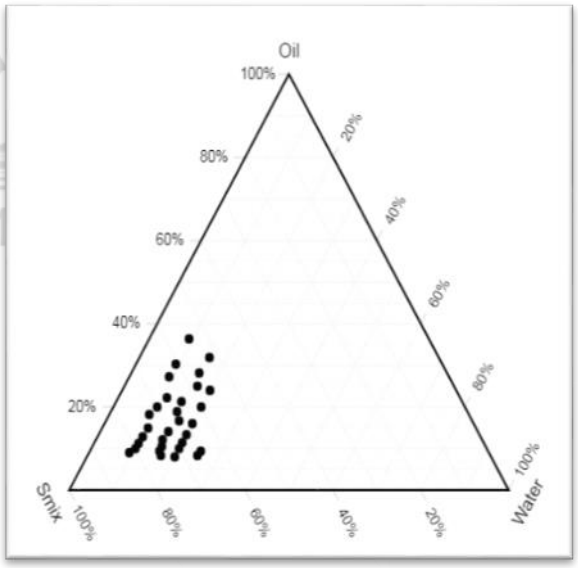

Figure 5: Pseudo-ternary phase diagrams showing the o/w Nanoemulsion (shaded area) regions of castor oil and triacetin (1:1) Tween 80 (Surfactant) and Transcutol P (Co-surfactant) at Smixratios: 1:3

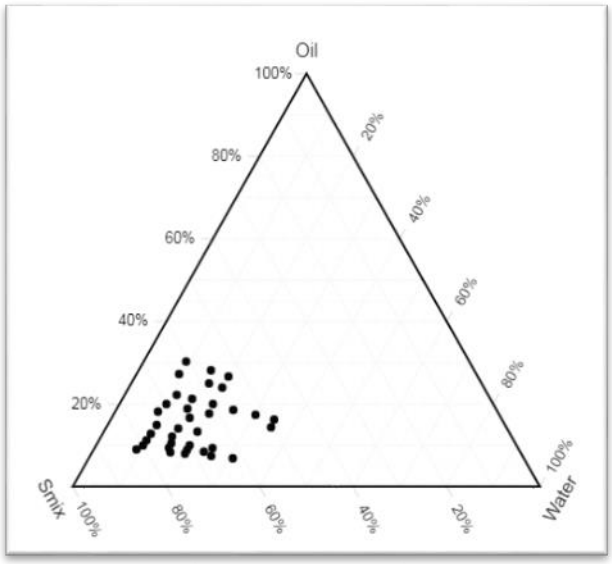

Figure 6: Pseudo-ternary phase diagrams showing the o/w Nanoemulsion (shaded area) regions of castor oil and triacetin (1:1) Tween 80 (Surfactant) and Transcutol P (Co-surfactant) at Smixratios: 3:1

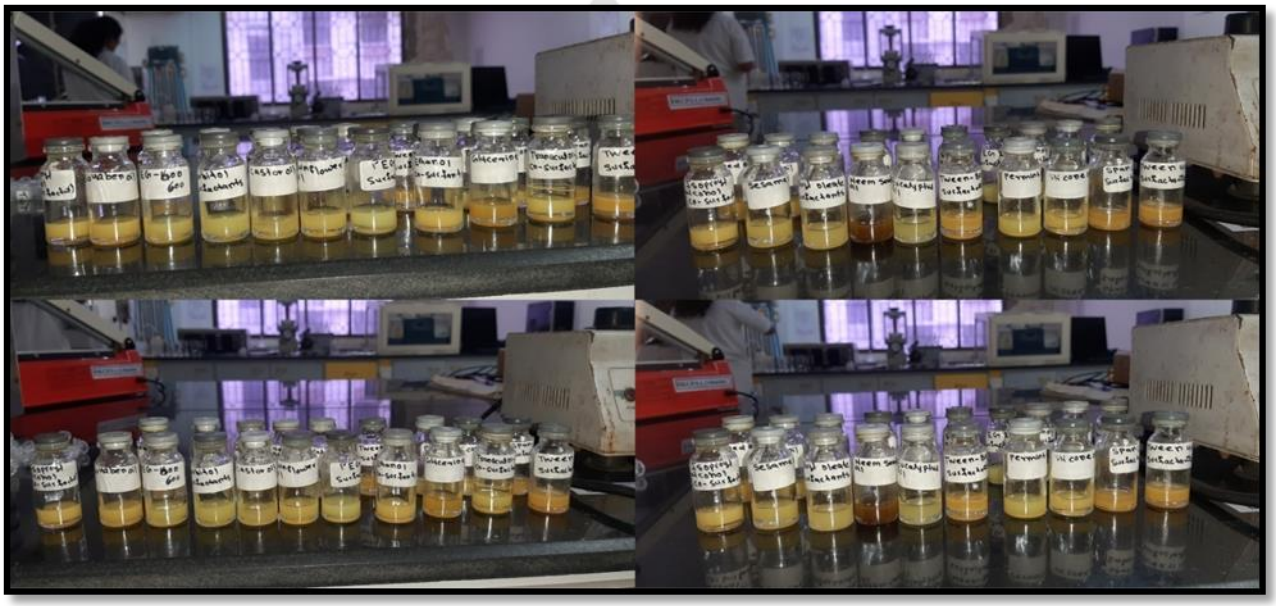

Photo 1: solubility in deferent excipients

Table No. 2: Thermodynamic stability and dispersibility tests of different formulations selected from phase diagrams.

SMIX RATIO	OIL(%)	SMIX(%)	AQUEOUS(%)	H/C	CENT	Freeze Thaw	RESULT
1:1	5.56	50	44.44	X	✓	X	FAIL
	18.18	72.73	9.09	X	X	X	FAIL
	25	58.33	16.67	✓	✓	✓	PASS
	20	60	20	X	X	X	FAIL
	18.69	56.7	25.23	✓	X	✓	FAIL
	17.7	61.95	20.35	X	X	X	FAIL
	24	56	20	✓	✓	✓	PASS
1:2	20	60	20	✓	✓	✓	PASS
	17.39	52.17	30.43	X	X	X	FAIL
	15.5	54.26	30.23	✓	✓	✓	PASS
	9.43	75.47	15.09	X	✓	X	FAIL
	8.89	71.11	20	X	X	X	FAIL
	25	58.33	16.67	✓	✓	✓	PASS
	36.36	54.55	9.09	✓	X	X	FAIL
2:1	5.56	50	44.44	X	X	X	FAIL
	26.67	53.33	20	X	✓	✓	FAIL
	18.69	56.7	25.23	✓	✓	✓	PASS
	25	58.33	16.67	✓	✓	✓	PASS
	36.36	54.55	9.09	X	X	X	FAIL
	25	58.33	16.67	✓	✓	✓	FAIL
1:3	30.3	60.61	9.09	X	X	X	FAIL
	20	60	20	✓	✓	✓	PASS
	18.87	66.04	15.09	X	X	X	FAIL
	14.93	74.63	10.45	X	✓	X	FAIL
	25	58.33	16.67	✓	✓	✓	PASS
	12.82	76.92	10.26	X	X	X	FAIL
	12.12	72.73	15.15	X	X	X	FAIL
	24	56	20	✓	✓	✓	PASS
3:1	9	81.82	9.09	X	✓	X	FAIL
	8.33	75	16.67	✓	X	X	FAIL
	25	50	20	✓	✓	✓	PASS
	6.9	62.7	31.03	X	X	X	FAIL
	18.69	56.7	25.23	✓	✓	✓	PASS
	17.39	52.17	30.43	✓	✓	✓	PASS
	14.08	70.42	15.49	X	✓	X	FAIL

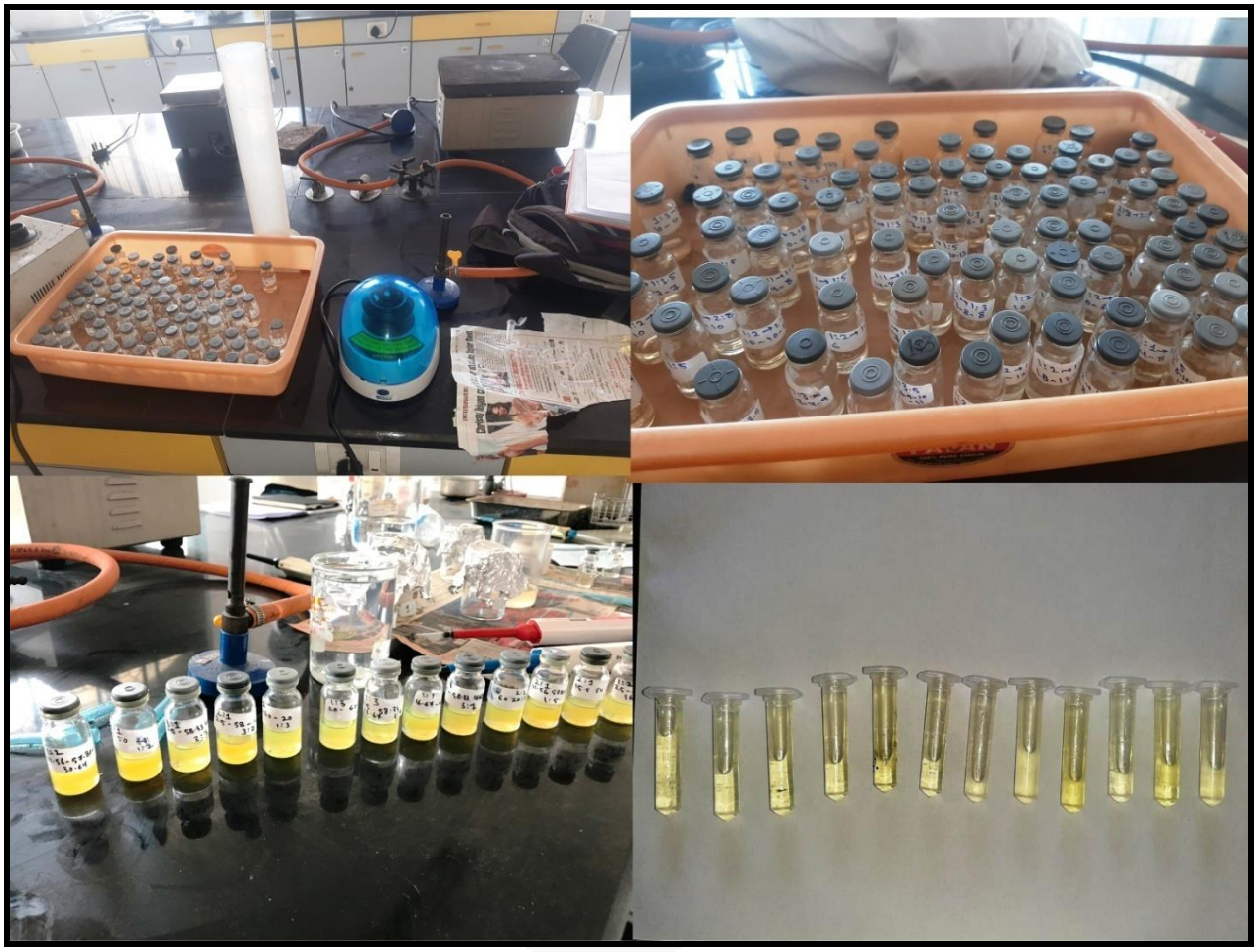

Photo 2: Heating Cooling Cycle, Centrifugation, Freeze-Thaw Cycle, Drug Content.

Table No. 3: Composition of Nanoemulsion selected from phase diagram with S_{mix} (1:1)

NA	Oil phase (%v/v)	Smix (%v/v)	Tween 80 (%v/v)	Transcutol -P (%v/v)	Dwater (%v/v)	Drug (mg)
NFA1	16.67	58.33	29.16	29.16	25	2
NFA2	25.00	58.33	29.16	29.16	16.67	2
NFA3	24.00	56.00	28	28	20.00	2

Table No. 4: Composition of Nanoemulsion selected from phase diagram with S_{mix} (1:2)

NA	Oil phase (%v/v)	Smix (%v/v)	Tween 80 (%v/v)	Transcutol -P (%v/v)	Dwater (%v/v)	Drug (mg)
NFB1	20	60	15	45	20	2
NFB2	15.50	54.26	13.56	40.96	30.23	2
NFB3	25	58.33	14.08	42.24	16.67	2

Table No. 5: Composition of Nanoemulsion selected from phase diagram with S_{mix} (2:1)

NA	Oil phase (%v/v)	Smix (%v/v)	Tween 80 (%v/v)	Transcutol -P (%v/v)	Dwater (%v/v)	Drug (mg)
NFC1	25	50	37.5	12.5	25	2
NFC2	18.69	56.07	42.52	14.17	25	2
NFC3	25	58.33	43.66	14.55	16.67	2

Table No. 6: Composition of Nanoemulsion selected from phase diagram with S_{mix} (1:3)

NA	Oil phase (%v/v)	Smix (%v/v)	Tween 80 (%v/v)	Transcutol -P (%v/v)	Dwater (%v/v)	Drug (mg)
NFD1	20	60	45	15	20	2
NFD2	25	56.33	44.4	14.08	16.67	2
NFD3	25	58.33	32.74	10.91	20	2

Table No. 7: Composition of Nanoemulsion selected from phase diagram with S_{mix} (3:1)

NA	Oil phase (%v/v)	Smix (%v/v)	Tween 80 (%v/v)	Transcutol -P (%v/v)	Dwater (%v/v)	Drug (mg)
NFE1	25	50	37.5	12.5	25	2
NFE2	18.69	56.07	42.52	14.17	25	2
NFE3	17.39	52.17	39.12	13.4	30.43	2

Table No. 8: Optimized formulations selected from the Phase diagram at the difference of 0.02% w/w of oil having the least Smix concentration

CODE	Smix ratio(ml)	Oil phase (%v/v)	Smix (%v/v)	Tween 80 (%v/v)	Dwater (%v/v)	Oil:Smix Ratio
NFA2	1:1	25.00	58.33	29.16	16.67	3:7
NFB2	1:2	15.50	54.26	13.56	30.23	1:3.5
NFC2	2:1	18.69	56.07	42.52	25	1:3
NFD2	1:3	25	56.33	44.4	16.67	3:7
NFE2	3:1	18.69	56.07	42.52	25	1:3

Table No. 9: Characterization of optimized formulations (NFA2 to NFE2)

CODE	Drug content (%)	Viscosity (cP)
NFA2	99.10	8.29
NFB2	98.86	7.96
NFC2	96.69	7.13
NFD2	97.34	8.10
NFE2	98.54	7.82

1.3 Scanning electron Microscopy

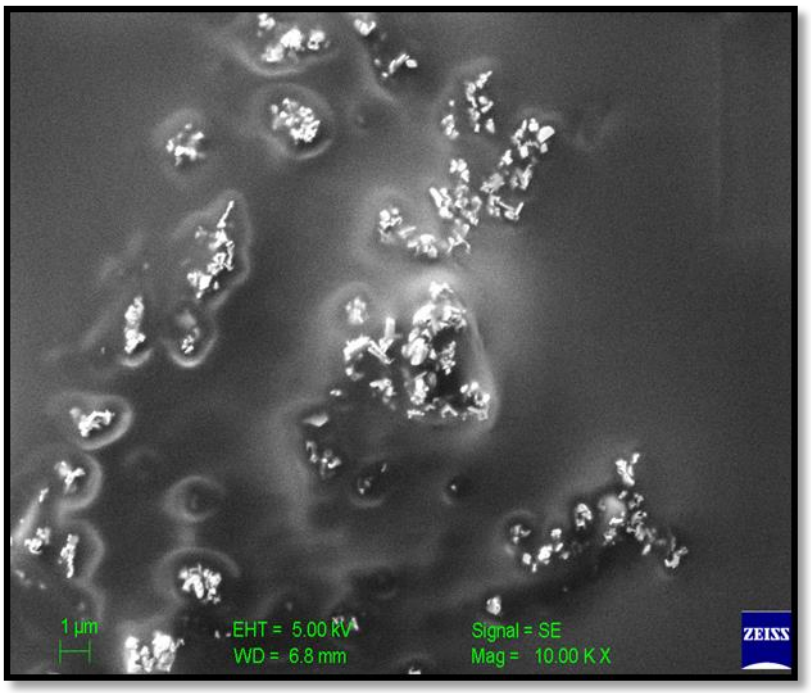

Figure 7: SEM result of optimized formulation NFA2

Evaluation of Nanoemulsion Gel

Table No. 10: *Ex-vivo* study results

Time (hr)	cumulative% of drug permeated					
	NFA2	NFB2	NFC2	NFD2	NFE2	marketed
0.25	13.75	6.25	6.875	9.375	10.625	16.875
0.5	17.5	13.75	11.25	14.375	15.625	20
0.75	23.75	20	18.125	18.75	21.875	24.375
1	32.5	22.5	22.5	23.125	28.75	30
2	37.5	30	28.75	31.25	36.875	33.125
4	45.625	34.375	36.875	38.75	42.5	48.75
6	51.25	44.375	43.125	42.5	47.5	55
8	58.125	48.75	48.75	50.625	52.5	57.5
12	70.625	60	59.375	55	58.125	63.125
24	73.75	63.125	63.75	66.25	62.5	66.875

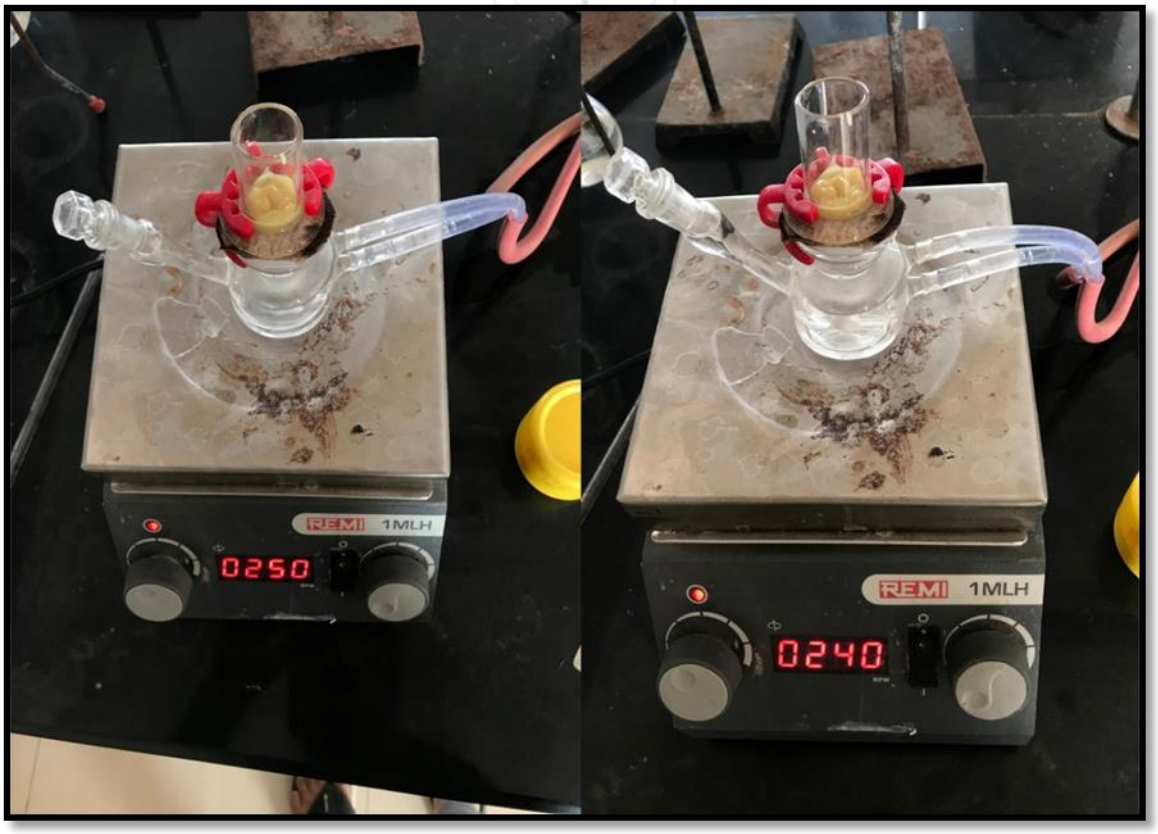

Photo 3: Franz diffusion apparatus

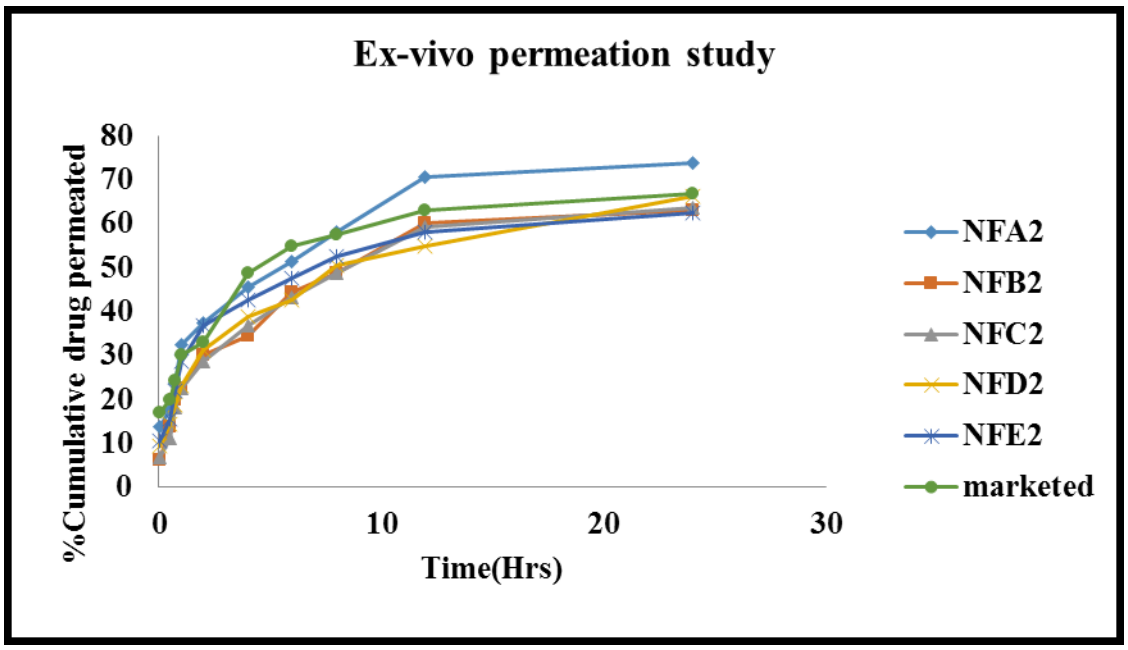

Figure 8: *Ex-vivo* permeation study chart

The ex vivo studies showed that drug permeation was highest for the batch NFA2 with 73.75% while this was followed by NFD2>NFB2>NFC2>NFE2.The marketed preparation showed drug permeation of about 66.85%.

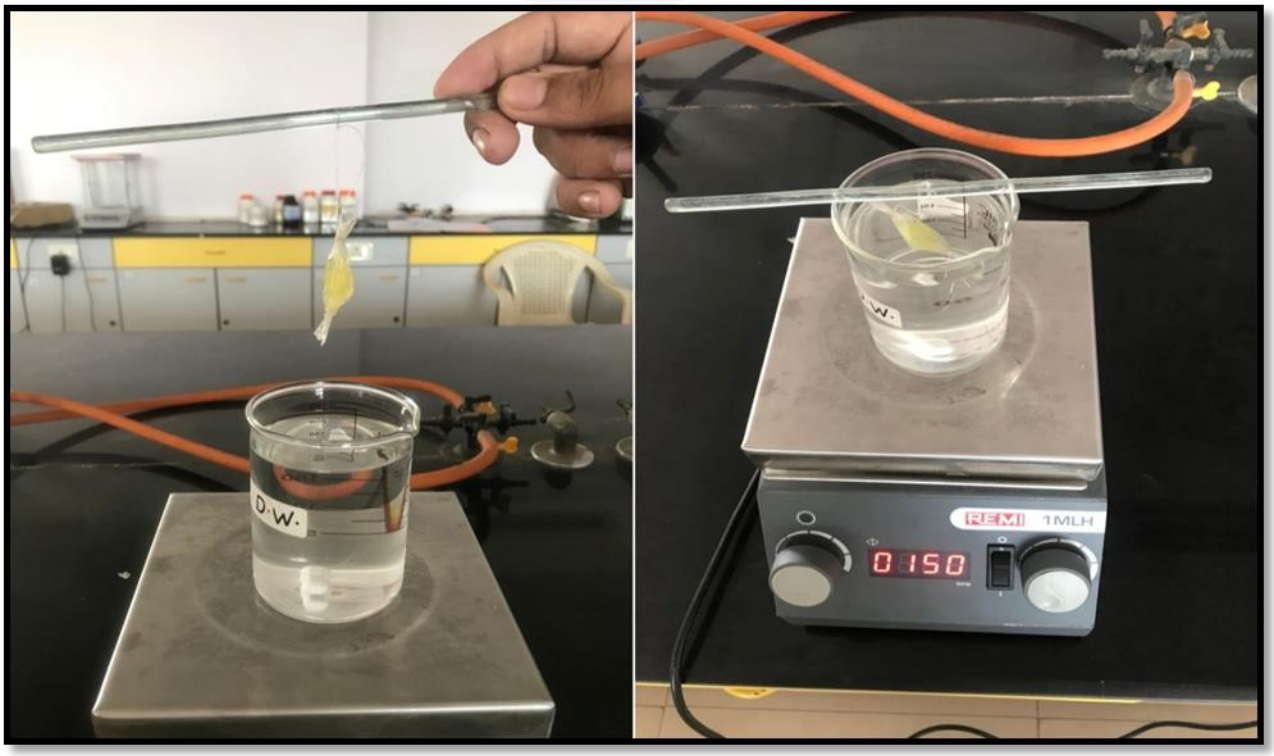

Photo 4: Dialysis membrane

Table No. 11: Drug Release study results

Time (hr)	% Drug release					
	NFA2	NFB2	NFC2	NFD2	NFE2	marketed
0.25	2.203125	1.09375	1.984375	1.984375	1.71875	1.25
0.5	7.84375	5.9375	6.9375	7.25	7.5	2.8125
0.75	18.95313	13.28125	17.04688	17.35938	18.125	5.3125
1	32.71875	25.9375	30.59375	31.0625	31.40625	8.4375
2	49.92188	40.9375	46.64063	47.10938	48.28125	12.03125
4	67.75	57.8125	65.96875	63.9375	67.65625	16.5625
6	81.20313	69.6875	81.07813	77.64063	81.875	23.125
8	90.75	78.125	88.6875	84.78125	89.84375	32.8125
12	95.60938	80.3125	92.54688	88.01563	93.4375	45.3125
24	98.75	81.71875	94.0625	90	95	60

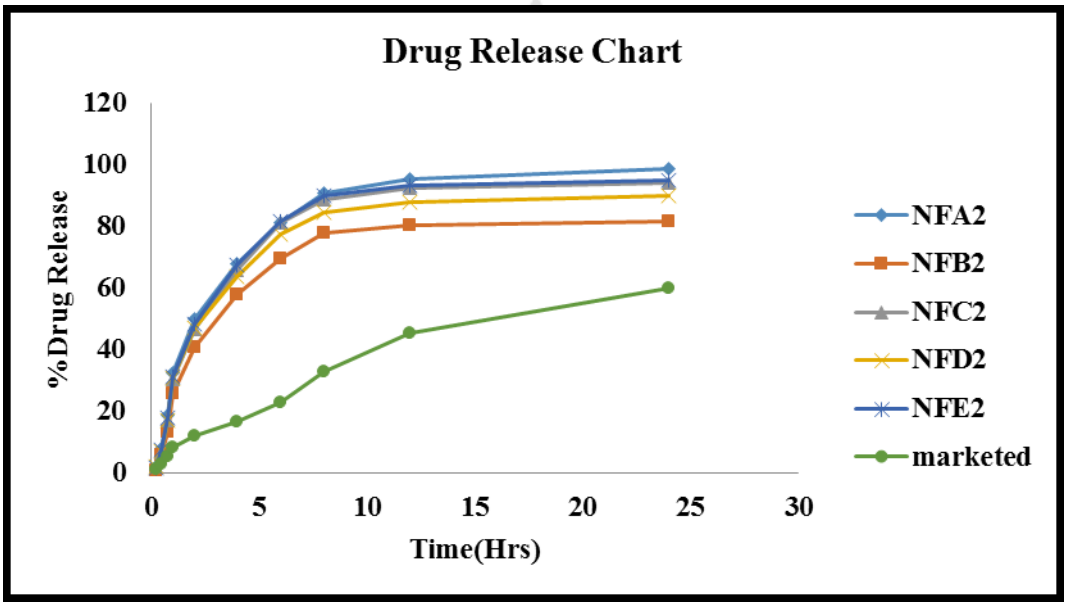

Figure 9: *In-vitro* drug release

The drug release study shows that the drug release was 98.75% from NFA2 at the end of 24 hours. This was followed by NFE2>NFC2>NFB2>NFD2.The marketed preparation showed drug release of about 60% at the end of 24 hours.

Table No. 12: Drug Release Kinetics

Time	%Drug Release	log% Drug Release
0	0	0
0.25	2.203125	0.343039139
0.5	7.84375	0.894523743
0.75	18.95313	1.277680941
1	32.71875	1.514796703
2	49.92188	1.698290932
4	67.75	1.8309093
6	81.20313	1.90957277
8	90.75	1.957846634
12	95.60938	1.980500502
24	98.75	1.994537104

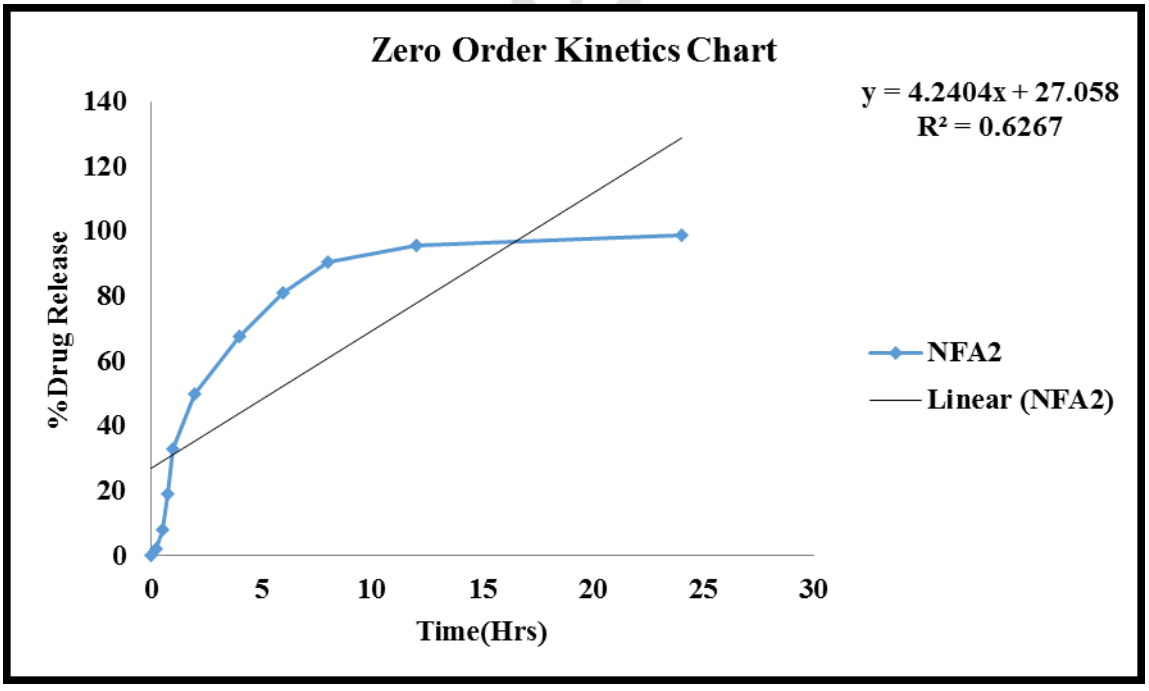

Figure 10: Zero-order kinetics

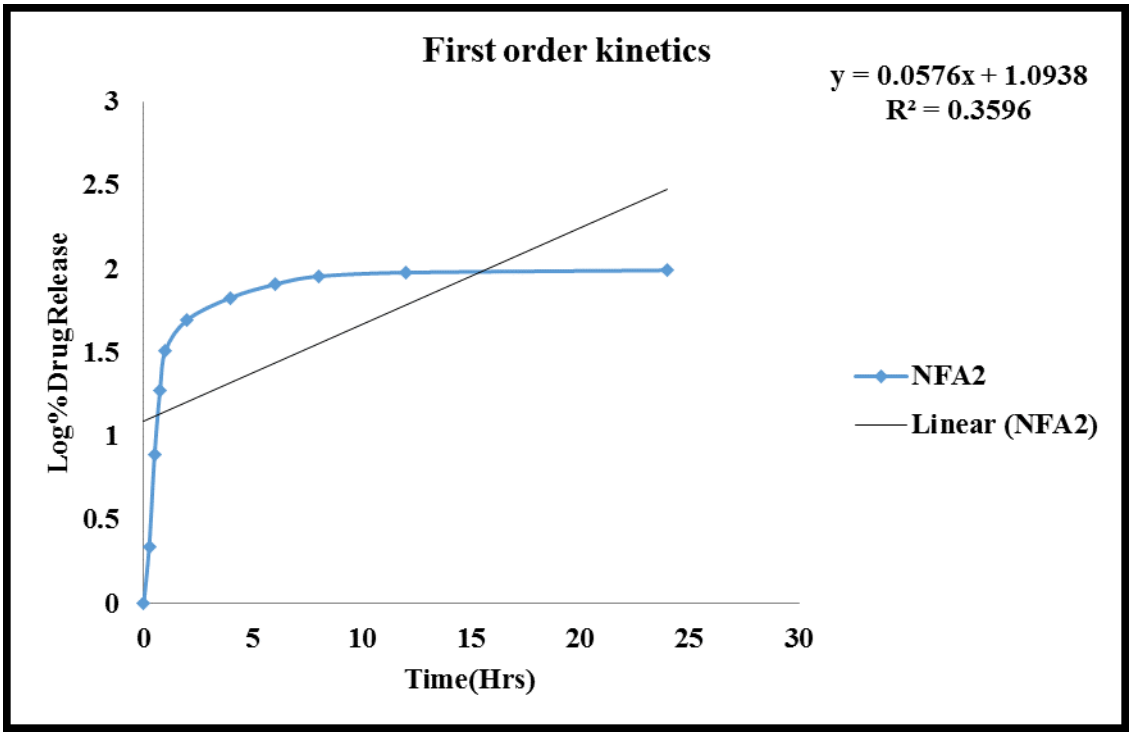

Figure 11: First Order Kinetics

Table No. 13: Kinetics Model Table

Sr. No.	Kinetic model	Regression coefficient
1	Zero order	0.626
2	First order	0.359

Based on the above data it can be concluded that the amphotericin B nanoemulsion follows Zero-order kinetics.

Selection of Final Formulation

Based on Particle size, Ex-vivo drug permeation, as well as Drug release out of the 5 batches NFA2, showed the best results. Hence this batch was chosen as the final batch for further evaluations.

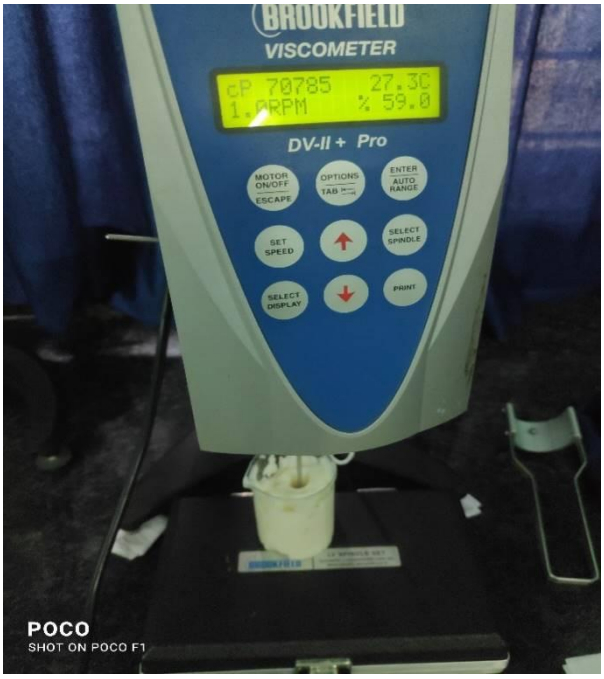

Photo 5. Brookfield Viscometer

Table No. 14: optimization of NFA2 for Viscosity, pH, spreadability, Drug Content

Code	Viscosity	pH	Spread ability	Drug content
NFA2	70785	6.8	68.79	97.49

Antifungal studies

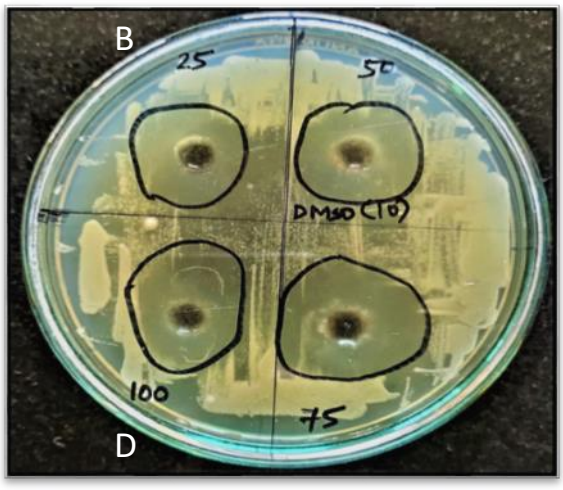

Figure 12: Antifungal activity of Amphotericin B against Candida Albicans.A= Plain gel, B= Nanoemulsion gel NFA2, C= Nanoemulsion NFA2, D= Marketed gel

Nanoemulsion NFA2Marketed gel

Table No. 15: Results of Antifungal Studies

Sr No	Zone of inhibition (mm)
A	25
B	50
C	75
D	100

The results showed that the antifungal activity of the tested formulations was in the following order. Marketed Gel>Nanoemulsion>Nanoemulsion Gel>Plain drug gel.

Stability studies as per ICH guidelines

Three samples of the same batch of nanoemulsions were subjected to these studies. The samples were subjected to 40 ± 2°C and 75 ± 5 % RH. At the end of 0, 30, 60, and 90 days, the sample aliquot was withdrawn, diluted with ethanol, and analyzed using a UV spectrophotometer. A graph was plotted between log percent drug remaining v/s Time. The slope of the straight line from the graph was determined and the degradation rate constant (K) was calculated by using the equation:

Table No. 16: Stability Study Results

Time (days)	Color	Texture	Drug content (mg)	% drug Remaining	Log % drug Remaining	Phase Separation	Skin Irritation
0	light yellow	Smooth	2	100	2	Not observed	No irritation
30	light yellow	Smooth	1.90	95	1.9930	Not observed	No irritation
60	light yellow	Smooth	1.81	90	1.9874	Not observed	No irritation
90	light yellow	Smooth	1.75	87.5	1.9831	Not observed	No irritation

CONCLUSION

Amphotericin B nanoemulsion was prepared by mixing suitable proportions of oil, surfactant, and drug and by utilizing the water phase titration method. The solubility studies were also carried out along with thermodynamic stability tests. Optimized formulations were selected based on these tests. The optimized formulations showed good viscosity and high drug content. The scanning electron microscopy image showed white-colored structures in the presence of nanoemulsion droplets. The nanoemulsion was incorporated into the gel base. The gel batches were evaluated for ex vivo permeation where they showed good permeation and also for drug release. The drug release was high at the end of 24 hours. The batches were also evaluated for their viscosity as well as satisfactory drug content. The antifungal studies showed that the nanoemulsion showed the highest activity against the fungi followed by nanoemulsion gel. Hence Amphotericin B nanoemulsion can be a good candidate or treatment of Cutaneous Leishmaniasis.

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