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Formulation, Evaluation and Characterization of Periodontal Microemulsion Gel

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ABSTRACT
The aim of the present study was to develop microemulsion gel of Satranidazole for the treatment of periodontitis. The objective was to increase the solubility of Satranidazole, a lipophilic drug and to enhance depth of penetration of the drug into the periodontal pocket for efficacious treatment of periodontitis. Pseudo ternary phase diagrams were constructed to determine the region of existence of microemulsions prepared using cosurfactant titration method. The formulations were developed using isopropyl myristate, tween 80, ethanol; oleic acid, tween 80, propylene glycol; oleic acid, cremophor RH 40, ethanol. Optimization of formulations was done based on in vitro diffusion studies. The microemulsion was gelled using carbopol 940 as the gelling agent. The formulations were evaluated for pH, viscosity, percent transmittance, centrifugation (phase separation), and characterized by scanning electron microscopy, particle size, zeta potential and polydispersity index. The formulation inhibited the growth of micro organism, Salmonella typhimurium which indicates that the formulation could be used to treat periodontal infection.

Keywords: Microemulsion gel, Satranidazole, Periodontitis, Phase diagrams.

INTRODUCTION

Periodontal disease could be defined as a disorder of supporting structures of teeth, including the gingival, periodontal ligament and alveolar bone. [1] Periodontal disease results in loss of connective tissue and bone support and is a major cause of tooth loss in adults. [2]

The current practice for the treatment of periodontitis involves scaling and root planing followed by administration of systemic antibiotics or application of local antibiotics like metronidazole gel directly on the gums several times as adjuncts to conventional mechanical therapy. [3-4] Although systemic administration of antibiotics has advantages of easy and simple administration, this route of administration poses some disadvantages like uncertain patient compliance, inability of drugs to achieve adequate concentration at the site of infection, increased risk of adverse drug reactions, development of resistance by various micro organisms and effects of systemic antibiotics on extraoral bacteria. [4] These disadvantages have led to the development of local application of antibiotics which are intended to exclusively affect bacteria within the periodontal pocket. [5]

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Metronidazole gel is a very bitter formulation and, thus, reduces patient compliance. Satranidazole is a 5-nitroimidazole derivative that has been found to be more active against aerobic, microaerophilic, and anaerobic bacteria than Metronidazole. The MIC_{90} of Satranidazole was found to be fourfold lower than Metronidazole against 50 clinical isolates of anaerobes. [3] The literature survey indicates that Satranidazole, inspite of its therapeutic efficacy, is not explored effectively for the treatment of periodontal disease. Therefore, we focussed on the microemulsion gels of Satranidazole in our present study.

Microemulsions are clear, thermodynamically stable, isotropic mixtures of oil, water and surfactant, frequently in combination with a cosurfactant. [6] In case of periodontal delivery, microemulsion can overcome the problem with the existing topical products (jelly, ointment or spray) such as lack of efficacy due to inadequate depth of penetration, too short duration and difficulties in administration due to spread, taste etc. Microemulsion alone or in conjunction with in situ gelling system is a promising tool for drug delivery in periodontitis. [7] Microemulsion gel offers advantage of long contact time with the periodontal pocket due to the mucoadhesive polymer used as a gelling agent in the formulation of microemulsion gel.

MATERIALS AND METHODS

Satranidazole was obtained as a kind gift sample from Alkem Laboratories Ltd., Mumbai; Oleic acid, Tween 80,

Cremophor RH 40, Ethanol, Carbopol 940 were obtained from SD Fine Chemicals Ltd.; Isopropyl myristate was obtained from NR Chem and Propylene glycol was obtained from Nice Chemicals Pvt. Ltd.

Solubility Studies

The components of microemulsion were selected based on solubility studies. Solubility studies were conducted by adding excess amount of Satranidazole to oils (oleic acid, isopropyl myristate, olive oil and light liquid paraffin), surfactants (tween 80, tween 20, tween 60, cremophor RH 40, span 80), and cosurfactants (propylene glycol, ethanol, butanol, sorbitol) taken in vials. The vials were shaken on a rotary shaker (Table top orbital shaker, Eltek®) for 48 hours. The solutions were then centrifuged at 3000 rpm for 15 minutes and filtered through Whatman filter paper. The concentration of drug in each of the components was determined using UV-Visible double beam spectrophotometer (Lab India®UV 3000+) at a wavelength of 318 nm.

Construction of phase diagram

Pseudo ternary phase diagrams were constructed to determine the area of microemulsion existence. This region was determined by cosurfactant titration method. Oil phase, Satranidazole, water and surfactant were mixed to form an emulsion. To this mixture, cosurfactant was added drop wise under continuous mechanical stirring. The contents of oil and water were varied from 9:1 to 1:9 ratio. The mixture was visually examined for transparency following the addition of cosurfactant. Transparent, single phase, low viscous mixtures were designated as microemulsions.

Preparation of microemulsion

Cosurfactant titration method was employed for the preparation of microemulsion. The concentrations of oil, water, surfactant and cosurfactant were varied in each case keeping the concentration of drug constant. Predetermined amount of drug was accurately weighed and dissolved in oil. Water and surfactant were added to oily solution of the drug and mechanically stirred (Magnetic stirrer, Remi Equipments Pvt. Ltd.) to form an emulsion. Cosurfactant was added drop wise to the emulsion till the formation of a transparent mixture. Formation of transparent solution indicates formation of microemulsion. [8] The details of the formulations are shown in Table 1.

Preparation of microemulsion gel

Microemulsion gel was prepared using carbopol 940 as the gelling agent. Carbopol was hydrated by soaking in water for a period of 24 hours. Triethanolamine was then added to the swollen polymer to form a gel. Microemulsion was gelled by adding the aqueous portion (gelling agent) to the non-aqueous portion (microemulsion) with continuous mechanical stirring. [9-10]

Evaluation and Characterization of Microemulsion/Microemulsion Gel

Percent transmittance

Percent transmittance of the microemulsions was measured using UV-Visible double beam spectrophotometer at a wavelength of 560 nm. Keeping distilled water as blank.

Centrifugation

The microemulsions were centrifuged (Research Centrifuge, R 24, Remi Equipments Pvt. Ltd.) at 9000 rpm for 30 minutes in order to eliminate metastable systems.

pH

pH of the formulations was measured using digital pH meter (Digital pH meter, Model-112).

Viscosity

Viscosity of microemulsions was measured using Brookfield digital viscometer (Brookfield viscometer, DV-II+ Pro) fitted with S-34 spindle at 5, 10, 20, 50 and 100 rpm.

Viscosity of microemulsion gel was measured using Brookfield digital viscometer fitted with S-64 spindle at 0.5, 1, 2, 5, 10 and 12 rpm. [8]

Morphology

Morphology of the microemulsion was studied using Scanning Electron Microscopy (Hitachi S - 3700N). [11]

Particle size and Zeta potential

Particle size and zeta potential was measured using Malvern Nano (ZS) zeta sizer Ver. 6.20.

FTIR Studies

Fourier Transform Infra Red analysis was conducted to verify the possibility of interaction of chemical bonds between drug and other excipients of the formulation. FTIR analysis was performed using Shimadzu 8400 S FTIR spectrophotometer. The samples were scanned in the spectral region of 4000-500 cm^{-1}. Solid samples were crushed, mixed with potassium bromide and pressed at 15000 psig using hydraulic press to make a disc. Gel samples were sandwiched between two IR transparent plates made up of KBr.

***In-vitro* drug release studies**

Drug release studies were performed using Franz diffusion cell employing a dialysis membrane (dialysis membrane-135). Dialysis membrane was initially soaked in pH 6.8

Table 1: Formulation details of microemulsions showing concentration of each component in % w/w

Formulation code	Isopropyl myristate	Oleic acid	Water	Tween 80	Cremophor RH 40	Ethanol	Propylene glycol
F1	44.73	-	4.97	15.80	-	34.50	-
F2	50.97	-	12.74	33.77	-	2.51	-
F3	25.02	-	10.72	21.97	-	42.29	-
F4	21.97	-	14.65	27.71	-	35.68	-
F5	17.53	-	17.53	31.89	-	33.06	-
F6	12.91	-	19.36	35.91	-	31.82	-
F7	9.86	-	23.02	13.94	-	53.18	-
F8	7.48	-	29.92	19.82	-	42.78	-
F9	7.48	-	40.28	19.93	-	35.31	-
F10	-	72.04	8.01	2.55	-	-	17.41
F11	-	14.26	3.56	1.89	-	-	80.29
F12	-	14.07	14.07	32.81	-	-	39.06
F13	-	13.94	20.91	22.17	-	-	42.97
F14	-	4.27	9.96	12.07	-	-	73.71
F15	-	6.95	27.79	36.83	-	-	28.43
F16	-	4.36	39.24	27.73	-	-	28.68
F17	-	62.19	6.91	-	21.35	9.54	-
F18	-	46.61	11.65	-	30.01	11.72	-
F19	-	30.69	13.15	-	31.61	24.56	-

phosphate buffer solution for 24 hours. It was then clamped between donor and receptor compartments of Franz diffusion cell. The receptor compartment was filled with pH 6.8 phosphate buffer solution and was magnetically stirred throughout the experiment. The donor compartment contained appropriate amount of the formulation.

Aliquots (5 ml.) of sample were withdrawn from the receptor compartment at specified time intervals for 8 hours and were replaced with fresh buffer solution to maintain sink conditions. The samples were analyzed for drug concentration using UV-Visible double beam spectrophotometer. The drug concentration was calculated using standard calibration curve.

Stability studies

Stability studies of the developed microemulsions were carried out by storing the formulations at three different temperatures for 3 months. The optimized formulations were stored at refrigerated condition (2-8ºC), room temperature (25±2ºC) and elevated temperature (50±2ºC). Stability of the stored formulations was evaluated by visually inspecting the formulations for phase separation or turbidity. [8]

***In-vitro* Microbiological Evaluation of Microemulsion**

Formulation F13 was selected as the optimized formulation based on in vitro diffusion studies. F13 was evaluated for anti-microbial activity. The micro organism selected for the study was *Salmonella typhimurium*. The bacterium was subcultured in Luria Bertia broth (LB broth) and then transferred onto LB agar medium to carry out microbiological evaluation of the formulation. Disc diffusion method was employed for the study. Formulation F13 was employed as test and placebo was employed as control in the study. [12]

RESULTS AND DISCUSSION

Solubility studies

Solubility studies were conducted to determine solubility of the drug in various oils, surfactants and cosurfactants. Satranidazole showed high solubility in oleic acid and isopropyl myristate among oils, in tween 80 and cremophor RH 40 among surfactants, in propylene glycol and ethanol among cosurfactants. Therefore, oleic acid and isopropyl myristate were selected as oil phase, tween 80 and cremophor RH 40 were selected as surfactants, and propylene glycol and ethanol were selected as cosurfactants for the formulation of microemulsions. The solubility of Satranidazole in various oils, surfactants and cosurfactants is shown in Table 2.

Construction of phase diagram

Pseudo ternary phase diagrams were constructed using Chemix School Software Ver. 3.60 (2012). Microemulsion existence region was then determined. Fig. 1 describes pseudo ternary phase diagrams of the microemulsions containing various weight ratios of (a) isopropyl myristate, water, tween 80 and ethanol (b) oleic acid, water, tween 80, propylene glycol (c) oleic acid, water, cremophor RH 40, ethanol. The shaded region indicates microemulsion existence region. The rest of the region represents turbid and conventional emulsions based on visual inspection. Phase diagram of isopropyl myristate, water, tween 80 and ethanol shows highest microemulsion existence region. This could probably be due to greater solubility of the drug in tween 80.

Percent transmittance

Percent transmittance of the microemulsions was measured using UV-Visible double beam spectrophotometer at a wavelength of 560 nm; keeping distilled water as blank. The results are shown in Table 3. The percent transmittance results revealed that microemulsions were transparent like that of water.

Centrifugation

The microemulsions were centrifuged at 9000 rpm for 30 minutes in order to eliminate metastable systems. No phase separation was observed after centrifugation which indicates stability of the formulations.

Viscosity

Viscosity of microemulsions was measured using Brookfield digital viscometer fitted with S-34 spindle at 5, 10, 20, 50 and 100 rpm. Viscosity of microemulsion gel was measured using Brookfield digital viscometer fitted with S-64 spindle at 0.5, 1, 2, 5, 10 and 12 rpm. The results are shown in Tables 4 and 5. The results indicate that microemulsions have low viscosity and are Newtonian liquids.

pH

pH was determined using digital pH meter. Average pH of the formulations was found to be 6.7. The results are shown in Table 6. The results indicate that pH of the formulations are similar to that of buccal cavity. Thus, the formulation may not cause irritation.

Particle size, Zeta potential and Polydispersity index

Fig. 2 and 3 show particle size distribution and zeta potential of the formulation F13. Particle size of the microemulsion was found to be 369 nm and zeta potential was found to be -0.0529 mV which indicates that the particles of microemulsion are negatively charged which provide electrostatic stabilization. The polydispersity index was found to be 0.317 which indicates narrow particle size distribution.

FTIR Studies

FTIR analysis was conducted to verify the possibility of interaction of chemical bonds between drug and other excipients of the formulation. The IR spectrum of drug, formulations F13 and F19 recorded by FTIR spectrometer are shown in fig. 4, 5 and 6. The spectra of the formulations were

Table 2: Solubility of Satranidazole in various components

Component	Solubility (mg/ml)
Isopropyl myristate	0.2230 ± 0.011
Oleic acid	0.3750 ± 0.032
Olive oil	0.1919 ± 0.036
Light liquid paraffin	0.1714 ± 0.035
Cremophor RH 40	0.7624 ± 0.038
Tween 80	0.9671 ± 0.027
Tween 20	0.3521 ± 0.039
Tween 60	0.5329 ± 0.017
Span 80	0.4384 ± 0.038
Ethanol	1.3023 ± 0.077
Butanol	0.8451 ± 0.088
Propylene glycol	1.1368 ± 0.066
Sorbitol	0.5675 ± 0.044

Table 3: Percent transmittance values of F13 and F19

Formulation	Percent transmittance
F13	98.6
F19	99.4

Table 4: Viscosity of F13 and F19 microemulsions

RPM	Viscosity (cps)	
	F13	F19
5	263.9	168
10	144	72
20	138	48
50	132	43.2
100	118.8	40.8

Table 5: Viscosity of F13 and F19 microemulsion gel

RPM	Viscosity (cps)	
	F13	F19
0.5	88301	33593
1	53988	18596
2	36232	15657
5	19532	10798
10	11566	9370
12	10048	8658

Table 6: pH of microemulsion and microemulsion gel

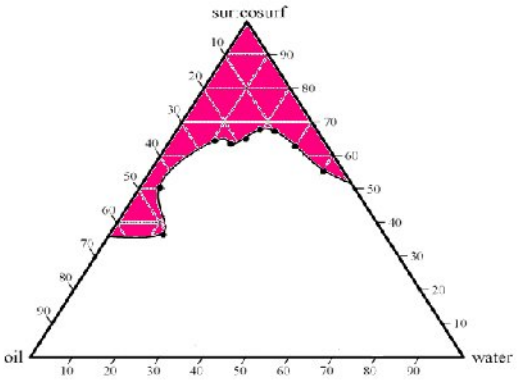
Formulation	pH	
	Microemulsion	Microemulsion gel
F13	6.7	6.8
F19	6.6	6.6

Table 7: Stability studies of F13 and F19

Temperature	Phase separation/Turbidity	
	F13	F19
2-8ºC	No	Slight turbidity
Room temperature	No	No
Elevated temperature (50±2ºC)	No	No

Table 8: Cumulative percent drug release of optimized formulations

Time (min)	Microemulsions		Microemulsion gel	
	F13	F19	F13	F19
0	0	0	0	0
15	3.3 ± 1.60	2.42 ± 0.81	2.42 ± 1.44	1.72 ± 0.57
30	7.64 ± 3.58	5.89 ± 1.81	6.02 ± 3.39	4.52 ± 1.20
45	11.75 ± 5.85	8.66 ± 2.61	8.09 ± 2.09	7.47 ± 1.43
60	15.91 ± 8.10	11.83 ± 3.36	12.28 ± 3.90	10.85 ± 1.59
120	20.94 ± 9.88	17.23 ± 2.90	17.11 ± 3.75	16.05 ± 2.68
180	27.84 ± 10.37	24.50 ± 3.59	24.34 ± 1.77	23.03 ± 4.24
240	36.58 ± 9.79	33.27 ± 4.37	32.43 ± 0.77	30.63 ± 4.72
300	47.43 ± 7.42	45.05 ± 3.65	42.13 ± 1.43	39.41 ± 4.57
360	56.31 ± 8.73	58.47 ± 4.27	52.64 ± 2.98	49.19 ± 4.14
420	76.03 ± 4.56	72.92 ± 3.97	65.30 ± 1.73	60.50 ± 4.51
480	93.72 ± 3.34	89.65 ± 3.52	79.64 ± 0.65	73.26 ± 3.26



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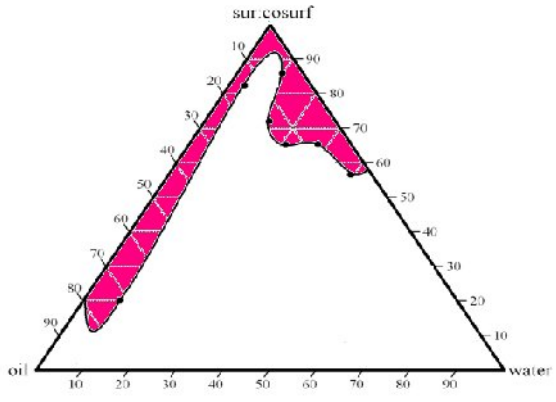

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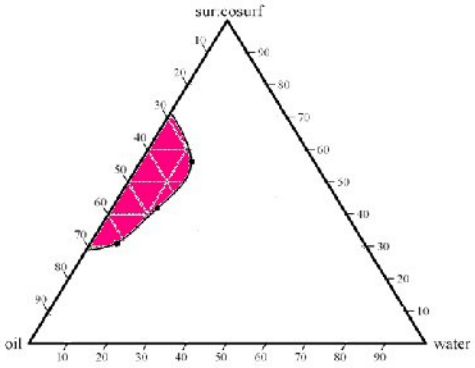

(1c)

Fig. 1: Pseudoternary phase diagrams of (a) isopropyl myristate, water, tween 80, ethanol (b) oleic acid, water, tween 80, propylene glycol (c) oleic acid, water, cremophor RH 40, ethanol

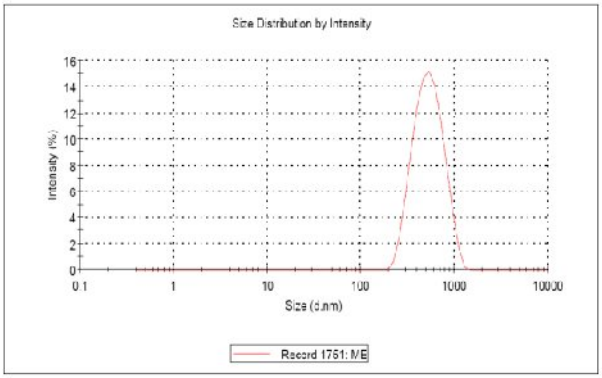

Fig. 2: Particle size distribution of F13

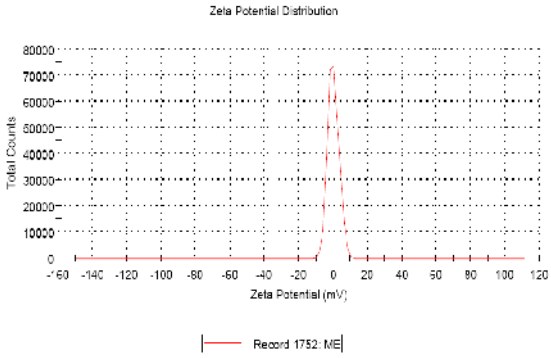

Fig. 3: Zeta potential of F13

compared with that of Satranidazole. The characteristic peaks of optimized formulations followed the same trajectory as that of drug alone with minor differences. Thus there may be no drug-excipient interactions.

***In-vitro* drug release studies**

Drug release studies were performed using Franz diffusion cell employing a dialysis membrane. pH 6.8 phosphate buffer was used as the release medium. Drug release was found to be highest for the formulations F13 (93.72%) containing 13.94% oleic acid, 20.91% water, 22.17% tween 80 and 42.97% propylene glycol and F19 (89.65%) containing 62.19% oleic acid, 6.91% water, 21.35% cremophor RH 40 and 9.54% ethanol. Hence, these formulations were gelled using carbopol 940 and drug release from the gels was less (79.64% (F13) and 73.26% (F19)) when compared to that of microemulsions (Table 8).

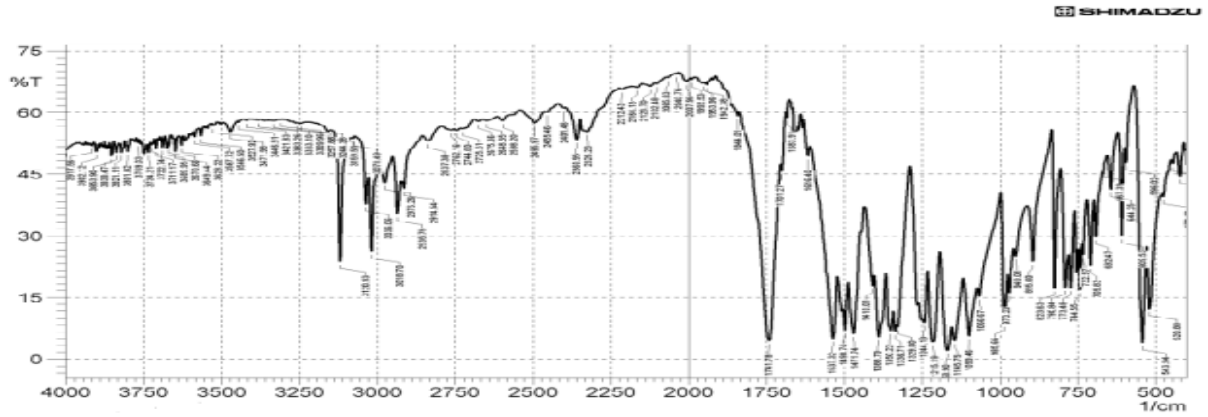

Fig. 4: FTIR image of Satranidazole

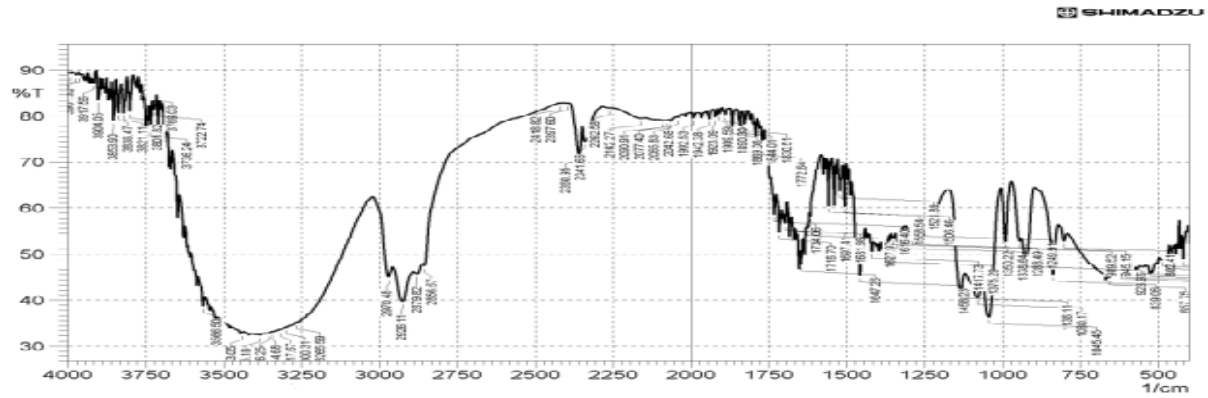

Fig. 5: FTIR image of F13

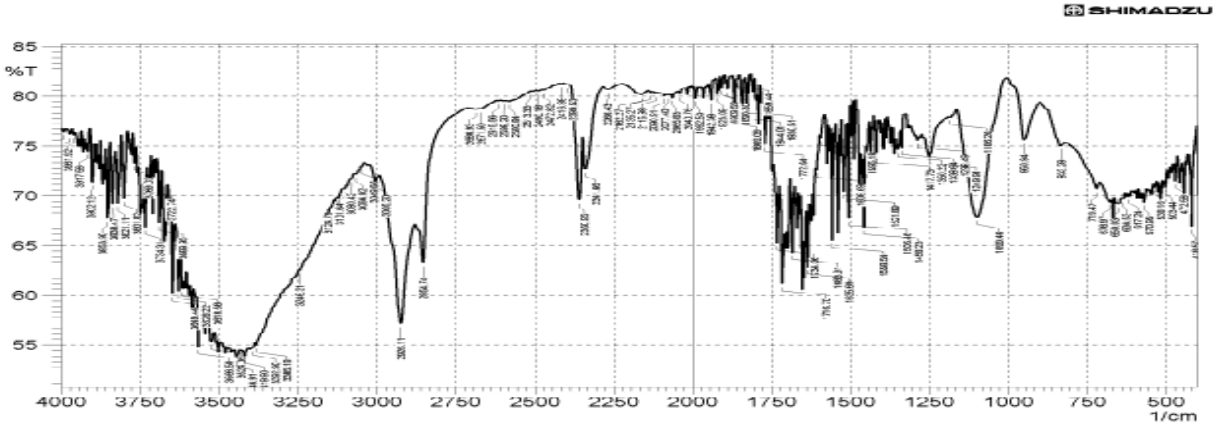

Fig. 6: FTIR image of F19

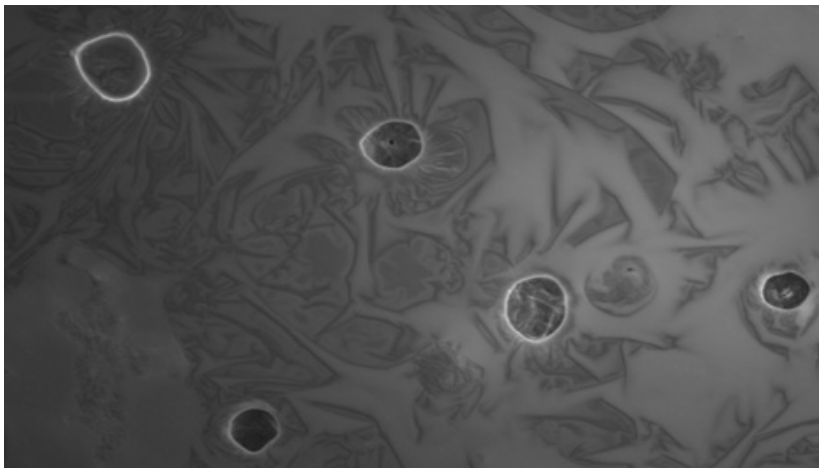

Fig. 7: SEM photograph of F13

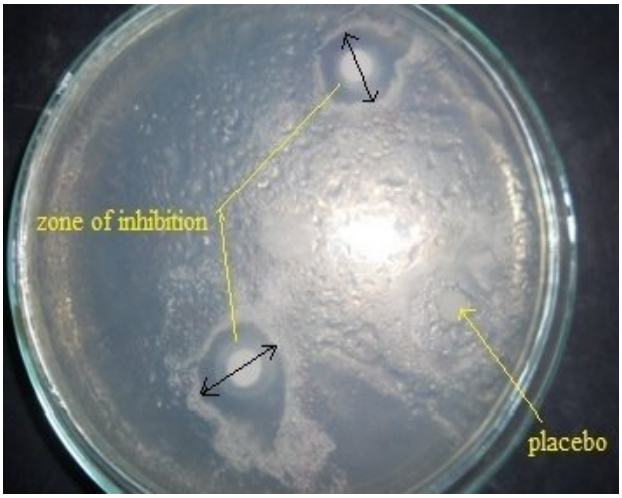

Fig. 8: Microbiological evaluation of formulation F13 showing zone of inhibition

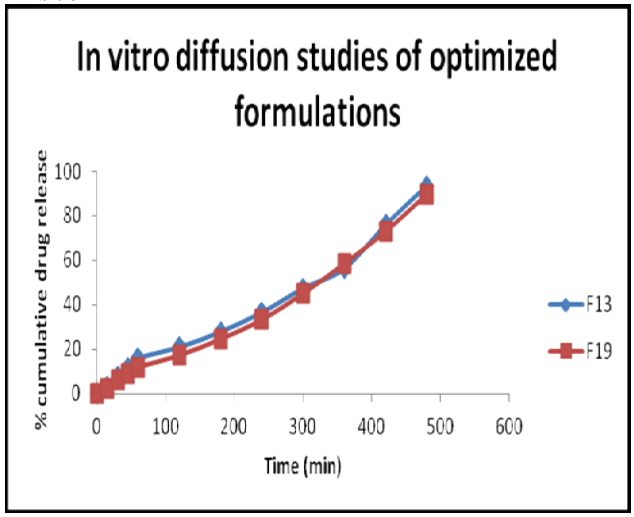

Fig. 9: Cumulative percent drug release of optimized formulations

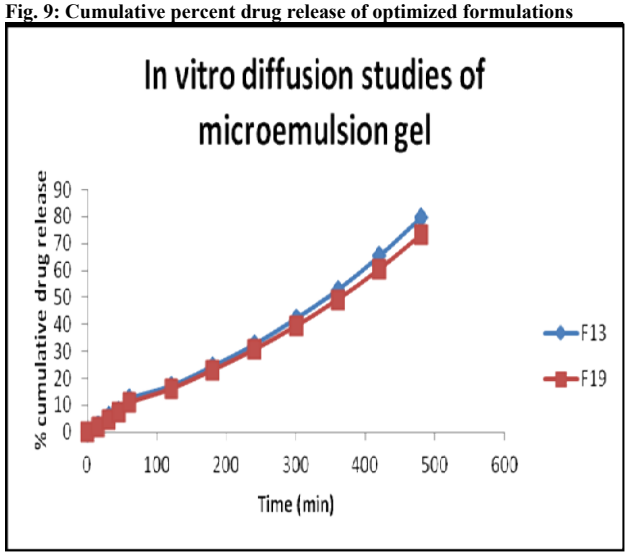

Fig. 10: Cumulative percent drug release of microemulsion gel

Morphology

Morphology of the microemulsions was studied using Scanning Electron Microscopy. Fig. 7 shows SEM photograph of formulation F13. The SEM photograph indicates that the microemulsion contains spherical globules and no agglomerates were seen.

Stability studies

Stability studies were conducted to evaluate the microemulsions for phase separation or turbidity. The optimized formulations were stored at refrigerated condition (2-8ºC), room temperature (25±2ºC) and elevated temperature (50±2ºC) for 3 months (Table 7). The formulations were found to be stable as no phase separation or turbidity was observed in the formulations.

***In-vitro* microbiological evaluation of microemulsion**

The optimized formulation F13 was evaluated for anti-microbial activity. The activity was tested against the bacterium *Salmonella typhimurium*. The formulation F13 inhibited growth of the bacterium and zone of inhibition was observed. The diameter of the zone of inhibition was found to be 1.5 cm. The formulation inhibited the growth of *Salmonella typhimurium* which indicates that the formulation could be used to treat periodontal infection.

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