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FORMULATION AND EVALUATION OF CLARITHROMYCIN POORLY SOLUBLE DRUG AS MICROEMULSION

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ABSTRACT
Clarithromycin is well absorbed from the gastro intestinal tract, but its systemic bioavailability is 55% due to first pass metabolism. It undergoes rapid biodegradation to produce the microbiologically active 14-hydroxy metabolite. The design of the novel o/w microemulsion formulation, which enhances the oral bioavailability by raising the solubility of poorly water soluble clarithromycin was studied. Microemulsion were prepared by using oil phase (ethyl oleate and olive oil), diglyceryl monooleate (DGMO-C) lipophilic surfactant, polyoxyethylene 40 hydrogenated castor oil (HCO-40) hydrophilic surfactant, ethanol (co-surfactant), phosphate buffer solution (PBS) pH 6.8 as a aqueous phase and clarithromycin. Prepared formulations were evaluated for size, viscosity, zeta potential, drug content, *in vitro* release studies & *in vivo* studies. Average particle size of clarithromycin o/w microemulsion for F1 and F2 was 350-450 nm and 800-900 nm. Results of viscosity, exhibited a simple newtonian flow. Zeta potential of F1 indicates plateau of stability and no agglomerates & F2 o/w microemulsions which indicates plateau of slight stability and few agglomerates. Drug content of the F1 was more than F2 this may be because of increased solubility of drug in the oil (ethyl oleate). The release profile revealed that formulations F1 followed peppas model and F2 higuchi matrix model. The plasma concentration was more in formulation (F1) 47.03 µg /mL then (F2) 31.10 µg /mL. Hence F1 o/w microemulsion can be a useful formulation, which enhances the oral bioavailability by increasing the solubility of poorly water soluble drug clarithromycin.
Key words: Clarithromycin; Diglyceryl Monooleate (DGMO-C), Polyoxyethylene 40 Hydrogenated Castor oil (HCO-40); Ethyl Oleate; Olive oil; O/W Microemulsions.

INTRODUCTION

Clarithromycin is a broad spectrum macrolide antibacterial agent that is effective both in vitro and in vivo against major pathogens responsible for peptic ulcer by *Helicobacter pylori* and other respiratory tract infections of *Clamydia pneumoniae, Mycoplasma pneumonia, Streptococcus pneumonia* and *Haemophilus influenzae*. It is well absorbed from the gastro intestinal tract, but its systemic bioavailability is 55% due to first pass metabolism. It undergoes rapid biodegradation to produce the microbiologically active 14-hydroxy metabolite[1]. Peak plasma concentration is 2 to 3mg/ml after 2 hours from a regimen of 500mg every 12 hours or 2 to 4 hours after two 500mg extended release tablets given orally.

Hence the present study was planned to prepare microemulsions which helps for better absorption in stomach. So that the degradation due to first pass effect can be minimized.

Two microemulsions were prepared by using oils (ethyl oleate, olive oil), surfactants [diglyceryl monooleate (DGMO-C) lipophilic surfactant, polyoxyethylene 40 hydrogenated Castor oil (HCO-40) hydrophilic surfactant], cosurfactant (anhydrous ethanol), drug (clarithromycin) and Phosphate buffer solution (pH 6.8). The present study includes the screening of surfactants and cosurfactants, to prepare a stable microemulsion. Further the prepared microemulsions were evaluated for drug content, shape, viscosity, *in vitro* dissolution studies, *in vivo* bioavailability study and stability studies.

MATERIALS AND METHODS

Clarithromycin was given as gift sample by aurbindo pharma and surfactants DGMO-C, HCO-40 was gifted by Nikko chemicals co. ltd. Tokyo Japan and other chemicals used were of analytical grade. Albino wistar rats weighing around 2.8-3.0 kg were used. They were maintained in a clean room. Animal ethical committee of K.L.E.S's college of pharmacy Belgaum approved the protocol of this animal study.

Screening of oil and surfactants

To evaluate which oils and surfactants are capable of forming microemulsions, two types of oils (ethyl oleate and olive oil), two types of surfactants (DGMO-C, HCO-40) and two types of cosurfactants (alcohol and L- alanine) were tested by ocular inspection to evaluate suitability of oil and surfactants for microemulsion . First, 2ml of 10% w/w surfactant solution was prepared and 20µl of oil was added with vigorous vortexing. Phosphate buffer solution pH 6.8 was used to prepare the surfactant solutions. The obtained solution was immersed into a bath and kept at 37± 0.2^{0}C. If a one-phase clear solution was obtained, the addition of the oil was repeated until the solution became cloudy. Therefore, the surfactant concentration of the final solution was lower than 10% w/w. When cosurfactants were added, the summation of surfactant and cosurfactant concentration was 10% w/w. Microemulsions were subjected to the physical stability test and all of them were confirmed to be stable for a few weeks[2].

Phase diagram

Phase diagrams were obtained by weighing appropriate amounts of each component into glass tubes and investigating the phase state after incubation for several hours in the thermobath. The temperature was controlled at 25 or 37 ± 0.2^{0}C. The phase state was classified into three, which is clear one-phase of low viscosity, clear one-phase gel and multiple phases. The one-phase of low viscosity was separated further into water-in-oil or oil-in-water micro emulsions phase by simply considering the composition that is whether it was oil-rich or water-rich[2].

Preparation of compound entrapped formulations for administration

An oil (ethyl oleate and olive oil), a lipophilic surfactant (DGMO-C) and a hydrophilic surfactant (HCO-40) warmed and dissolved at 50^{0}C, and the anhydrous ethanol solution containing Clarithromycin (concentration: 25 mg/mL) was accurately weighed in a glass vial at the mixture ratio given in table no 1. The components were mixed by agitating at room temperature for 10 min. Then, 80% (w/w) of PBS (pH 6.8) was added to each vial and it was agitated at room temperature for 2 h, and 125 mg/5mLof clarithromycin entrapped o/w microemulsions were prepared[3].

EVALUATION OF PREPARED O/W MICROEMULSIONS

Drug content estimation

Microemulsions containing 125mg of drug was dissolved in 100ml of 0.1N HCL taken in volumetric flask. The drug was allowed to dissolve in the solvent. The solution was filtered, 1ml was taken in

50ml of volumetric flask and diluted up to mark with 0.1N HCL and analyzed spectrophotometrically at 760nm. The concentration of clarithromycin in mg/ml was obtained by using standard calibration curve of the drug. Drug content studies were carried out in triplicate for each formulation batch.

Rheological studies

The flow properties and the viscosity of the systems were determined at 32 ± 1 °C. Viscosity determinations employed a DV III cone and plate Brookfield viscometer (Brookfield Engineering Laboratories Inc., Stoughton, MA, USA), fitted with CP-40 spindle[4]. The system was calibrated using Brookfield viscosity standard fluids.

Determination of shape and surface morphology

The shape and surface morphology of the o/w microemulsions was studied by using scanning electron microscope[1].

Zeta potential measurement

The stability of the microemulsions (F1, F2) was evaluated by measuring the zeta potential of the particles by Zeta meter ± 3M [5,6].

***In vitro* dissolution studies**

Drug release from formulated formulations F1and F2 was studied in pH 1.2 buffer from 0 to 2 hr, pH 6.0 buffer from 2 to 3 hr, and in phosphate buffer of pH 7.2 from 3 to 8 hr. Appropriate volume of o/w microemulsions (125mg) was taken in dialysis tube against 100 ml of media, continuously stirred with magnetic stirrer at 37±0.5^{0}C and with 75 rpm. After appropriate time intervals 1ml sample was withdrawn and analyzed for clarithromycin content at 760nm, in a uv/ vis spectrometer[1,7]. Equal volume of fresh media preheated to 37^{0}C was added to replace the withdrawn sample.

***In vivo* study**

Dose of clarithromycin to be administered to rats was calculated according to body surface area ratio of rats to human being. Six week-old male Albino wistar rats which were cannulated with a silicone-polyethylene tube in juglar vein 2 days before the experiment were used for the oral administration study. Nine rats of 280-300g body weight were used for experiment. They were grouped into three the first group was used to evaluate pharmacokinetic parameters of clarithromycin and second group for pharmacokinetic evaluation of F1 the third group for evaluation of F2 formulation. The absorption studies were used for one set of experiment. The absorption studies were performed on fasted rats. In the fasted study, the rats were fasted over-night (18 hr) but accessible to water. After light anesthetization with ether, formulations were administered to rats orally (22.5 mg/Kg of body weight) F1, F2 and pure drug using a syringe, followed by administration of 2ml of water. Blood samples (0.5ml for each) were collected via cannulated tubes after 0.5, 1, 2, 4, and 8 hrs after the administration; the rats were sacrificed[8].

Quantification of plasma concentration

The collected blood samples was treated with 20µl of 1M NaOH and 2.5ml of n-hexane/1-butanol (98:2 v/v) to 1ml of plasma in 4ml polypropylene tube (10mm x 70mm) and shaking for 2 min. After centrifugation at 12000 x g for 3 min, the whole organic layer was separated and transferred into another 4ml tube. Then 50 µl of 0.1% acetic acid was added. The mixture (about 1ml) was transferred into a 1.5ml microcentrifuge tube. After centrifugation at 11300 x g for 2 min, the upper organic phase was discarded completely. Finally a volume of 40 µl of aqueous phase was injected into the chromatograph. The analyses were performed on a shimadzu chromatographic system (Japan) equipped with an LC-6A solvent delivery pump, SPD-10AVP ultraviolet detector (operated at 205nm) and C-R8A integrator. The samples were applied by a Rheodyne 7725 loop injector with an effective volume of 100 µl. A shim pack CLCODS (M) columns (150mm x 4.6 mm i.d: 5µm particle) was used for the chromatographic separation[8,9]. The mobile phase comprised of acetonitrile-50mM aqueous sodium dihydrogen phosphate (32:68) adjusted to pH 4.5 with concentrated phosphoric acid and 4 M sodium hydroxide. Analyses were run at flow rate of 1ml/min at 40^0 C.

Stability studies

Two formulations F1 and F2 of clarithromycin o/w microemulsion were tested for stability according ICH guidelines. The preparations were divided into 3 sets and were stored at 4°C (refrigerator), room temperature and 40°C (thermostatic oven)[10,11]. After 15, 30 days drug content of all the formulations was determined by the method discussed previously in *in vitro* drug release and percentage drug content is determined.

RESULTS AND DISCUSSION

Screening of oils

The solubilization behaviors of the employed oils into two types of surfactants and two types of cosurfactant solutions which are capable of solubilizing relatively large amount of oil as shown in table no 2. Ethyl oleate is better oil for microemulsion than olive oil as it is solubilized in small quantity of surfactant solutions.

Screening of surfactants and cosurfactants

Table no 3. shows the solubilization behaviors of oils (ethyl oleate and olive oil) into various surfactant solutions. Two individual surfactants (DGMO-C, HCO-40) and one mixture of two surfactants were selected as microemulsion forming surfactants. The HLB values of these surfactant is 12.5 and 5.5, the shape of surfactants is also important factor to form microemulsions. The solubility of DGMO-C and HCO-40 is more compared to the individual surfactant. It was also confirmed that the addition of cosurfactants alcohol and L-alanine enhanced the solubilization of oils in most surfactant systems. But the cosurfactant alcohol showed better solubilization of oil compared to L-alanine.

Phase diagram

Screening study succeeded in finding various types of o/w microemulsion preparation. Fig 1, 2 shows several types of phase diagrams obtained at 37 ^{0} C. That can be seen, surfactants (DGMO-C, HCO-40) are miscible with oils (ethyl oleate, and olive oil) (table no.4, 5). Therefore the formulation for oral administration can be prepared as an oil/ surfactant mixture which contains drugs. Very small o/w microemulsions zones have found for DGMO-C/HCO-40/ Olive oil/ PBS pH 6.8 compared with that of DGMO-C/HCO-40/ Ethyl Oleate/ PBS pH 6.8.

Evaluation of prepared o/w Microemulsions

Drug content

The percentage drug content was evaluated in all the two formulation (F1 and F2) of clarithromycin o/w microemulsions. The drug present in 125mg/5ml of the clarithromycin o/w microemulsion was determined and presented in table no 6. Percentage drug content for F1 and F2 formulation was found to be 81.60% and 61.36% respectively. It was observed that the drug content of F1 was more than the F2 microemulsion which may be due of increased solubility of drug in the oil (ethyl oleate).

Viscosity

Viscosity of o/w microemulsions was studied on Brookefield viscometer by using CP-40 spindle 50 revolution per minute (RPM at constant temperature). The viscosity values in mPa are shown in table no 6. The flow properties and the viscosity of the prepared formulations depended on the water content. The microemulsion systems exhibited a simple newtonian flow. The viscosity was increased with increasing water concentration in the microemulsion formulation systems. The presence of clarithromycin did not change the flow behavior. The formulation F1 (122 mPa) was less viscous compared to F2 (180 mPa)

Determination of shape and surface morphology

Scanning electron photomicrographs of the two formulations are shown in plate's no. 1 and 2. Magnifications of 7,500 X were used while taking these photomicrographs. Average particle size of clarithromycin o/w microemulsion for F1 and F2 was 350-450 nm and 800-900 nm. Particles of formulation of F1 were smooth, oval and discrete whereas particles of F2 were slightly rough surfaced and non discrete.

Zeta potential measurement

The stability of the microemulsions was evaluated by measuring the zeta potential of the particles by zeta meter ± 3M. The results are evaluated in table no 6. Zeta Potential of formulation of F1 o/w microemulsions were in the range of –31.0 to –32.0 milli volts, which indicates moderate stability and no agglomerates F2 o/w microemulsions were in the range of –26.0 to –28.0 milli volts, which indicates plateau of slight stability and few agglomerates.

***In-vitro* release studies**

The overall cumulative % drug release for pure drug and formulations (F1, F2) were found to be 50.04, 87.41% and 65.38% shown in table no 7. The in-vitro release of two formulations of o/w microemulsions showed an interesting burst effect. In the first 3 hour, drug released was 69.37%, 44.14% for F1 and F2, respectively. Afterwards the drug release followed a diffusion release. The 'n' values for F1 and F2 were 0.406 and 0.6469 respectively. F1, which is less than 0.5 indicates that the release approximates fickian diffusion mechanism and F2 which is less than 1 and greater than 0.5 follows non fickian diffusion mechanism. Peppa's was found to be best fit model for F1 formulation, Higuchi's was found to be best fit model for F2.The percentage of drug released at different pH and time is showed in fig No 3.

***In vivo* study**

The plasma concentration of pure drug (clarithromycin) and formulations (F1 and F2), after oral administration was determined. The absorption of drug from microemulsion increased rapidly and remarkably compared with that of pure drug. The pharmacokinetic parameters of the sample are given in table no 8. $AUC_{(0\text{-}8h)}$ of F1 microemulsion increased 1.8 fold compared with that of clarithromycin pure drug [PD (162.72 µg. h/mL) Vs F1 (289.74 µg. h/mL)] and F2 microemulsion showed some enhanced $AUC_{(0\text{-}8h)}$ compared with that of clarithromycin pure drug [PD (162.72 µg. h/mL) Vs. F2 (199.60 µg. h/mL)]. The F1 microemulsion showed enhanced C_{max} of two fold compared with clarithromycin [PD (26.5 µg/ml) Vs F1 (47.03µg/ml)] and F2 microemulsion showed some enhanced C_{max} compared with clarithromycin [PD (26.5µg/ml) Vs F2 (31.10 µg/ml)] table no 13. However T_{max} was not significantly different between the two formulations and pure drug (table no 13). The results indicated that the bioavailability of o/w microemulsion of F1 was more than F2 and clarithromycin (pure drug). But F2 has greater bioavailability compared to clarithromycin (pure drug). The enhanced bioavailability is probably due to the increase in solubility and immediate dispersion of the drug in the GI tract. Furthermore the presence of a surfactant (DGMO-C, HCO-40) in microemulsion system in the GI tract caused changes in membrane permeability by the inhibition of an apocally polarized efflux system, which could lead to enhancement of the oral absorption of drug. The plasma concentration profiles were statistically analyzed. The parameters of *in vivo* studies were expressed as the mean ± for 3 rats. The difference for each parameter was assessed using one-way annova. $p < 0.0158$ was defined as stastically significant.

Stability Studies

The studies revealed that there is slight reduction in the drug content of o/w microemulsions after storage for 30 days. Table no 9 shows the data for in vitro release studies of the formulations (F1 & F2) stored at 4°C has shown 85.09 & 62.38% releases, the one which was stored at 40°C has shown 86.02% & 60.38% and at room temperature has shown 86.41% & 65.78% release after 8 hours. The results indicates that the drug release from the formulation stored at 40°C was highest followed by formulation stored at room temperature and 4°C.

CONCLUSION

From the results of the present study ethyl oleate can form better o/w microemulsion then Olive oil. It was confirmed that the addition of cosurfactants ethanol and L-Alanine enhanced the solubilization of oils in surfactant systems. But the cosurfactant ethanol showed better solubilization of oil compared to L-alanine. It was also observed that the drug content of the F1 microemulsion was more than the F2 microemulsion this may be because of increased solubility of drug in the oil (ethyl oleate).The microemulsion systems exhibited a simple Newtonian flow. Particles of formulations F1 were smooth, oval and discrete where as particles of F2 slightly rough surfaced and non discrete. Zeta Potential of F1 o/w microemulsions which indicates moderate stability and no agglomerates & F2 o/w microemulsions which indicates plateau of slight stability and few agglomerates. The enhanced bioavailability is probably due to the increase in solubility and immediate dispersion of the drug in the GI tract. Furthermore the presence of a surfactant (DGMO-C, HCO-40) in microemulsion system in the GI tract might caused changes in membrane permeability by the inhibition of a apocaly polarized efflux system, which could lead to enhancement of the oral absorption of drug. It can be concluded that o/w type of microemulsion prepared by using surfactants (DGMO-C and HCO-40) and oil ethyl oleate is suitable for increasing the bioavailability of poorly soluble clarithromycin in GI tract.

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Table No.1: Formula for different batches of microemulsions

Batch Code	Drug	Oil	Oil: DGMO-C: HCO-40: Anhydrous alcohol: Phosphate buffer 6.8 (w/w %)
F1	Clarithromycin (25mg/ml)	Ethyl Oleate	5:1:9:5:80
F2	Clarithromycin (25mg/ml)	Olive Oil	5:5:5:5:80

Table No. 2: Screening of oils for microemulsions formulations

Surfactants	Wt % Solubilised Oil	
	Ethyl oleate	Olive oil
DGMO-C	10%	4%
HCO-40	12%	5%
DGMO-C/ Alcohol (8:2)	16%	6%
HCO-40/Alcohol (7:3)	18%	5%
DGMO-C/HCO-40 /Alcohol (1:9:5)	22%	4%

Table No. 3: Screening of surfactants/cosurfactants for microemulsions formulations

Surfactant	CoSurfactant	Oil	Cosurfactant/ Surfactant + Cosurfactant Wt %									
			5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0
HCO-40	Ethanol	Ethyloleate	5	9	10	12	14	18	12	10	9	6
	L-Alanine	Ethyloleate	2	3	6	6	9	8	6	4	3	2
	Ethanol	Olive Oil	2	3	3	3	4	6	3	3	1	1
	L-Alanine	Olive Oil	1	1	1	1	1	2	1	1	1	1
DGMO-C	Ethanol	Ethyloleate	5	5	7	8	9	13	7	4	4	3
	L-Alanine	Ethyloleate	2	3	3	4	5	3	2	2	1	1
	Ethanol	Olive Oil	2	3	5	6	4	4	3	3	2	2
	L-Alanine	Olive Oil	1	1	3	2	1	1	1	1	1	1
DGMO-C : HCO-40	Ethanol	Ethyloleate	7	10	12	14	18	20	14	12	9	7
	L-Alanine	Ethyloleate	3	4	8	8	11	6	4	3	2	2
	Ethanol	Olive Oil	3	3	4	5	6	8	3	3	1	1
	L-Alanine	Olive Oil	0	1	1	1	1	2	1	1	1	1

Table No. 4: Phase mixing of oil – Ethyl oleate (EOO)

Oil: Surfactant: Cosurfactant: Water (EOO: DGMO-C: HCO-40: Ethanol: PBS pH 6.8). (w/w %)	Emulsification
5%:1%:9%:5%:80%	Emulsified
5%:2%:8%:5%:80%	Emulsified
5%:3%:7%:5%:80%	Emulsified
5%:4%:6%:5%:80%	Emulsified
5%:5%:5%:5%:80%	Emulsified

Table No. 5: Phase mixing of oil – Olive Oil

Oil: Surfactant: Cosurfactant: Water (Olive Oil: DGMO-C: HCO-40: Ethanol: PBS pH 6.8). (w/w %)	Emulsification
5%:1%:9%:5%:80%	Not Emulsified
5%:2%:8%:5%:80%	Not Emulsified
5%:3%:7%:5%:80%	Not Emulsified
5%:4%:6%:5%:80%	Not Emulsified
5%:5%:5%:5%:80%	Emulsified

Table No. 6: Measurement of viscosity, zeta potential and drug content of o/w microemulsions

Formulation	Viscosity mPa s 37° C n=3	Zeta Potential (mV) n=3	% Drug Content n=3
F1	122 ± 0.0013	-32.0 ± 1.2	81.60±
F2	180 ± 0.0005	-26.1 ± 1.2	61.36±

Table No. 7: *In vitro* release profile of clarithromycin pure drug (PD), F1 & F2 formulation

Time in hrs	Cum. %Drug Released Pure drug n=3	Cum. %Drug Released F1 n=3	Cum. %Drug Released F2 n=3
0.5	02.73 ± 0.19	27.97 ± 0.46	10.33 ± 0.55
1	08.36 ± 0.18	44.50 ± 0.17	23.32 ± 1.50
2	19.00 ± 0.70	51.70 ± 1.15	32.10 ± 1.00
3	27.21 ± 0.07	69.37 ± 0.58	44.14 ± 2.65
4	34.24 ± 1.31	77.50 ± 0.47	53.23 ± 1.67
5	41.01 ± 0.84	81.53 ± 0.24	60.51 ± 1.80
6	45.96 ± 1.38	83.06 ± 0.43	62.37 ± 1.34
7	48.72 ± 0.34	85.65 ± 0.73	63.71 ± 0.87
8	50.04 ± 1.38	87.41 ± 1.40	65.38 ± 1.18

Table No. 8: Pharmacokinetic parameters of clarithromycin in plasma after oral administration of clarithromycin o/w microemulsion formulation to rats

Formulation	C_{max} (µg /mL), n=3		T_{max} (hours), n=3		$AUC_{(0-8h)}$ (µg. h/mL), n=3		$T_{1/2}$ (hours), n=3	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
PD	26.50	± 0.544	2	± 0.0	162.72	± 2.121	1.585	± 0.045
F1	47.03	± 1.365	2	± 0.0	289.74	± 6.435	1.760	± 0.057
F2	31.10	± 0.573	2	± 0.0	199.60	± 3.394	1.425	± 0.035

n = 3 rats

p < 0.0158 is defined as stastically significant.

Table No. 9: Stability studies – In vitro release studies of optimized formulation F1 (Stored At 4°C & 45% RH, Room temperature And 40°C & 65% RH±5%)

Cum % Drug Release at 4°C				Cum % Drug Release at room temperature				Cum % Drug Release at 40°C			
F1 n=3		F2 n=3		F1 n=3		F2 n=3		F1 n=3		F2 n=3	
15 days	30 days	15 days	30 days	15 days	30 Days	15 days	30 days	15 days	30 days	15 days	30 days
27.39 ± 0.08	26.74 ± 0.09	10.40 ±1.10	10.01 ±0.05	27.22 ±0.22	26.94 ±0.08	10.33 ±0.04	10.00 ±0.07	27.34 ±0.03	27.09 ±0.67	12.33 ±0.09	13.47 ±0.07
44.12 ± 0.05	43.87 ± 0.02	23.22 ±0.89	22.32 ±0.08	44.18 ±0.45	44.03 ±0.07	23.32 ±0.09	22.79 ±0.04	44.22 ±0.34	44.12 ±0.76	24.32 ±0.03	24.67 ±0.09
51.28 ±0.05	49.65 ± 0.05	32.10 ±0.14	31.10 ±0.15	51.35 ±0.05	51.08 ±0.03	31.10 ±0.04	30.10 ±0.08	51.38 ±0.12	51.01 ±0.10	33.10 ±0.17	35.16 ±0.19
69.18 ± 0.08	68.35 ± 0.12	44.14 ±0.34	42.14 ±0.06	69.03 ±0.08	68.63 ±0.80	44.51 ±0.12	44.40 ±0.17	69.02 ±0.22	68.74 ±0.08	45.11 ±0.45	46.51 ±0.07
77.10 ± 0.09	76.43 ± 0.99	53.23 ±0.78	51.23 ±0.08	77.24 ±0.05	76.69 ±0.07	53.89 ±0.23	53.00 ±0.04	77.27 ±0.18	77.01 ±0.17	54.89 ±0.23	57.89 ±0.03
81.36 ± 0.06	81.02 ± 0.12	60.51 ±0.39	58.51 ±0.76	81.42 ±0.07	81.13 ±0.06	60.76 ±0.09	59.76 ±0.89	81.48 ±0.12	81.11 ±0.16	59.76 ±0.21	60.76 ±0.08
82.70 ± 0.15	82.08 ± 0.89	62.37 ±0.93	60.37 ±0.09	82.98 ±0.08	82.47 ±0.07	63.37 ±0.06	63.37 ±0.02	82.96 ±0.04	82.53 ±0.06	62.37 ±0.01	62.90 ±0.17
85.49 ± 0.08	84.45 ± 0.45	63.71 ±0.04	61.71 ±0.06	85.32 ±0.98	85.02 ±0.36	64.71 ±0.04	64.71 ±0.27	85.45 ±0.23	85.14 ±0.13	63.71 ±0.07	64.45 ±0.24
87.07 ± 0.05	85.09 ± 0.78	64.38 ±0.08	62.38 ±0.07	87.18 ±0.07	86.02 ±0.23	65.38 ±0.30	60.38 ±0.27	87.12 ±0.15	86.41 ±0.17	64.38 ±0.12	65.78 ±0.18

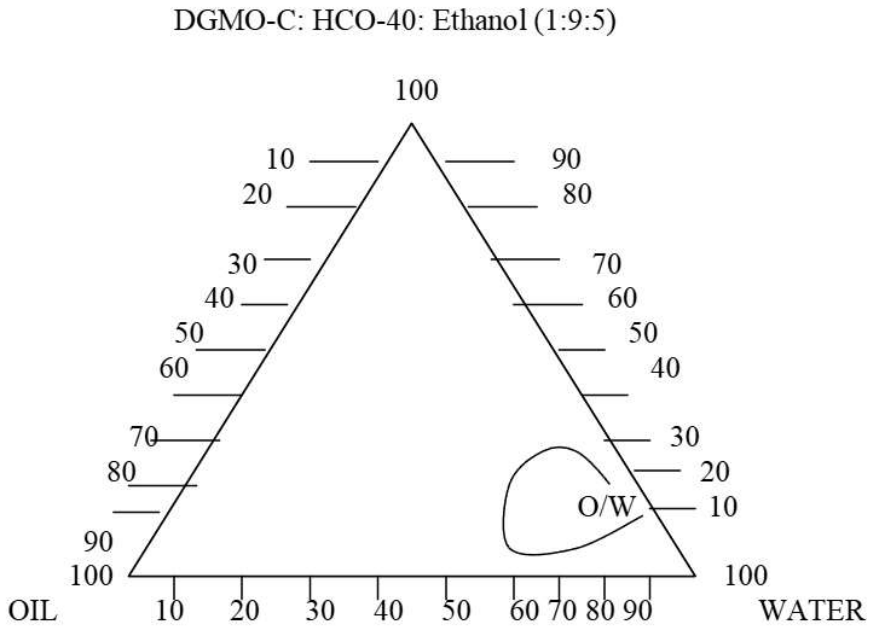

Fig. No 1: Pseudotarnary Phase Diagram for the mixture Ethyl Oleate/ DGMO-C/ HCO-40/ PBS pH 6.8

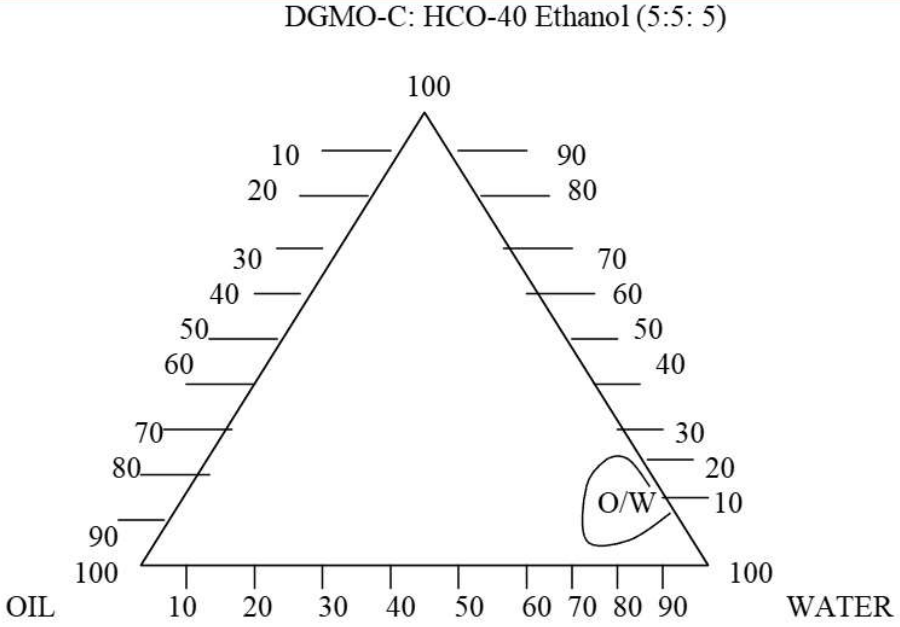

Fig. No 2: Pseudotarnary Phase Diagram for the mixture Olive Oil/ DGMO-C/ HCO-40/ PBS pH 6.8

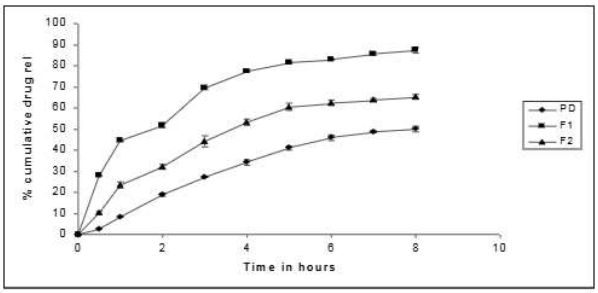

Fig no 3: plot of Cum. % drug release Vs. Time of formulation (F1, F2) and Clarithomycin (PD)

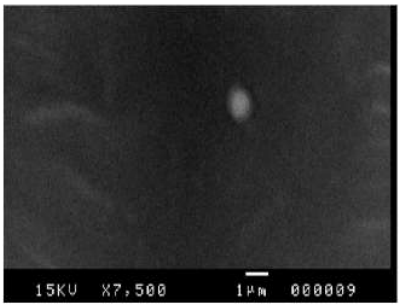

Plate No. 1: SEM of F1 O/W Microemulsion Formulation

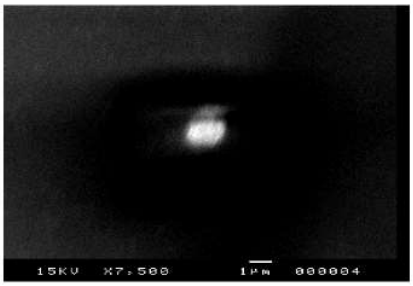

Plate No. 2: SEM of F2 O/W Microemulsion Formulation

Source of support: Nil, Conflict of interest: None Declared