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## FORMULATION AND *IN-VITRO* EVALUATION OF DRY EMULSION OF MEFENAMIC ACID IN HARD VEGETARIAN CAPSULES

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### Keywords:

HPMC, Mefenamic Acid, Dry Emulsion, Vegetarian environment, Capryol, mild to moderate pain

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**ABSTRACT:** The dry emulsion formulation aims to improve the solubility and dissolution of mefenamic acid. Dry emulsions are attractive because they are physically and micro biologically stable formulations. They represent a potential oral drug delivery system for lipophilic and low soluble drug substances. Dry emulsions can be prepared by spray drying, lyophilisation and rotary evaporation. Dry emulsion prepared here was by lyophilisation using capryol as the lipid phase and HPMC as the carrier. Mefenamic acid is the drug of choice which belongs to non-steroidal anti-inflammatory drug category. It is used as anti-pyretic, analgesic and anti-rheumatic and is used in the treatment of mild to moderate pain, including menstrual pain. The dry emulsion is formed by freeze drying method and incorporated in HPMC (hydroxyl propyl methyl cellulose) capsules, which provides a vegetarian environment and is a good alternative to gelatin. The formed dry emulsion formulation has been evaluated for the respective parameters in comparison with the marketed variant and the results are obtained.

**INTRODUCTION:** Mefenamic acid is considered as a competitive inhibitor of cox-1 and cox-2. It is a member of fenamate group of nonsteroidal Anti-Inflammatory drugs. It is used to treat moderate pain and menstrual pain. It is used in the treatment of primary dysmenorrhea, soft tissue and dental pain and also has analgesic, anti-inflammatory, anti-rheumatic and antipyretic properties. The mechanism of action of mefenamic acid involves inhibition of cyclooxygenase (COX-1 and COX-2) inhibiting the action of prostaglandins <sup>1</sup>. Mefenamic acid is a potent inhibitor of prostaglandin synthesis. Mefenamic acid is rapidly absorbed after oral administration.

Capryol, contains liquid caprylic acid which is extracted from coconut oil and oleic acids in safflower oil <sup>2</sup>. Caprylic acid, a fatty acid, and it is considered as broad-spectrum anti-fungal agent which is effective against *Candida albicans* and other fungi. Capryol has a coating action on the intestinal wall. Conversion of the yeast form of *Candida* to the more injurious mycelial form is hindered by oleic acid. Capryol gets dispersed in bentonite gel and releases caprylic acid throughout the intestinal tract.

Gelatin is a protein made from animal products like tendons, ligaments. It is a protein made from the skins and bones of pigs and cows. It's a common ingredient in a number of products, including:

- ✓ Makeup products
- ✓ Medicine formulations, vitamins
- ✓ Food products, drugs
- ✓ Vaccines preparations

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**Problems with Gelatin:**<sup>1</sup>

- ✓ Allergy, Dietary Restrictions, toxins exposure
- ✓ Cannot incorporate highly moisture sensitive drugs and deliquescent materials
- ✓ Cannot incorporate efflorescent material
- ✓ Difficult to incorporate water soluble materials.

Search for gelatin replacement lead to absence of bovine spongiform encephalopathy (BSE), cross linking of gelatin, abnormal liver and kidney function, strict regulations regarding the use of animal derived gelatin, drug incompatibilities. Hence HPMC, which is one of the alternatives to gelatin in pharmaceutical preparations, has been chosen for the study. Another reported advantage of HPMC capsules over gelatin capsules is related to the difference in moisture content of the shells. Because HPMC shells contain significantly less moisture compared to hard gelatin capsules by almost one third, it is compatible with hygroscopic materials<sup>2</sup>.

Oral Emulsions are liquids with one or more active pharmaceutical ingredients and are stable oil-in-water dispersions, where one or both phases may contain dissolved solids. Liquid emulsions have distinct advantages as they improve the bio availability and reduce the side effects of drug but lack physical, chemical and compliance problems<sup>3</sup>. To overcome these problems dry emulsions are prepared. Dry emulsions are lipid based powder formulations from which an O/W emulsion can be reconstituted *in-vivo* or *in-vitro*. They are prepared by drying liquid O/W emulsions containing a solid carrier in the aqueous phase. A solid carrier provides them with bulk and mass.

General method of preparation of dry emulsion follows the steps given below<sup>4</sup>:

1. Drug is dissolved in lipophilic solvent.
2. Aqueous phase is added containing bulking agents
3. Emulsion is formed
4. Water is removed (Lyophilization, spray drying)
5. Powder is filled into capsules or tablets are compressed

Dry emulsions are prepared by using:

- ✓ Spray drying
- ✓ Freeze drying/Lyophilization

- ✓ Rotary evaporation

The method chosen to prepare the mefenamic acid dry emulsion was lyophilization or Freeze drying. The lyophilization procedure is used to preserve a liquid, creamy or a solid product by withdrawing the water through sublimation under vacuum, the water changes directly into the gaseous state (steam). The cooling coil releases the steam which is caught up in and removed.

**The different Phases of Lyophilization:**

**The Process of Freezing:** The products should be frozen, the water is turned into ice.

**The Primary Drying:** The intracellular water is sublimed and then water gets evaporated, which is caught up and re-solidified on cold condenser plates at - 60 to - 70 °C.

**The Secondary Drying:** The products are heated up. The maximum temperature is + 50 °C. Products with residual moisture of about 1 - 5% are obtained.

**Two Phases are extremely important during the Lyophilization:**

**The Freezing:** Water gets transformed into ice very fast in this phase and not in form of small ice crystals.

**The Vacuum:** The most important step for the lyophilization is the quality of vacuum. The freeze-dryer has to be absolutely leak proof (structure, valves, etc.). The vacuum pumps must have enough power to allow a perfect vacuum.

**MATERIALS AND METHODS:****TABLE 2: MATERIALS USED**

S. no.	Material	Specifications
1	Mefenamic Acid	Gattefosse Mumbai, India
2	Span 80	Gattefosse Mumbai, India
3	Propylene Glycol	Gattefosse Mumbai, India
4	Tween 80	Gattefosse Mumbai, India
5	Capryol	Gattefosse Mumbai, India
6	HPMC 5 cps	JNTU Hyderabad, India
7	Hydrochloric acid	JNTU Hyderabad, India
8	UV Spectrophotometer	India
	Shimadzu UV- 1800	
9	IR Spectrophotometer:	United states
	Bruker Alpha	
10	Dissolution Apparatus	India
	DS8000 lab India	
11	Zeta sizer: Malvern	United Kingdom
12	Scanvac Coolsafe	-
	Lyophilizer	

### Method Development and Validation of UV-Spectroscopic Method for the Determination of Mefenamic Acid:

#### Preparation of Standard Stock Solution:

Standard solution of mefenamic acid was prepared by taking 10 mg of the drug and dissolving it in 10 ml of 0.1 N HCl (1000 ppm).

#### Preparation of Concentrations of Analytical

**Range:** The working standard solution of the drug is prepared by pipetting out 1ml of the solution into 10 ml of the volumetric flask and made up to the volume with 0.1 N HCl (100 ppm). Then, respective volumes of solutions were pipetted out from the 100 ppm solution into 10ml volume flasks to obtain 10, 20, 30, 40, 50 ppm of mefenamic acid.

#### Determination of $\lambda_{\max}$ of Mefenamic Acid in 0.1N HCl:

- 10, 20, 30, 40, 50 ppm of mefenamic acid solutions were prepared in 0.1N HCl.
- UV scan of the above solution was taken between 400-200 nm.
- The spectrum shows a maximum absorption at 285 nm

#### Standard graph of Mefenamic Acid in 0.1 N HCl:

- A Standard stock solution of mefenamic acid was prepared by dissolving 100mg of drug in 100 ml of 0.1 N HCl
- From the standard stock solution, conc. of 10, 20, 30, 40, 50 ppm were prepared using 0.1 N HCl
- Each solution was analyzed by UV spectrometer at 285 nm. The absorption values were noted.

#### Compatibility Studies:

- FT-IR spectrum of pure drug and drug-excipient were obtained by FT-IR spectrophotometer
- FT-IR spectrum of pure drug and drug-excipient were almost similar because of same functional groups. It indicates there was no interaction between mefenamic acid and excipients used in formulation.

**FTIR Procedure:** FT-IR spectrum of pure drug, mefenamic acid and formulation were obtained by FT-IR spectrophotometer. The spectrums were taken with the accumulations 24 scans and a resolution of 4  $\text{cm}^{-1}$  over the range of 400 - 4000  $\text{cm}^{-1}$ . The spectrum of formulation so obtained was compared with the spectrum of pure drug for any interactions.

**Solubility Studies:** Solubility of drug in oil (capryol) was carried by placing an excess amount of mefenamic acid in vial containing 1 ml of capryol and heated on a water bath at 35 °C to facilitate the solubilization using vortex mixer. The vial was then continuously agitated on a rotary shaking incubator for 48 h at ambient temperature. After reaching equilibrium the sample was centrifuged at 3000 rpm for 10 min and the supernatant was taken by pipette. The sample were suitably diluted with 0.1N HCl and analyzed spectrophotometrically for the dissolved drug at 285 nm. 0.1N HCl was used as blank.

Solubility studies were carried by placing an excess amount of mefenamic acid in vials containing 1 g of excipients (oil, surfactants and co-surfactants). The rest of the solubilizing procedure was same as that done for the oil. After reaching equilibrium the samples were centrifuged at 3000 rpm for 10 min and the supernatant was taken by pipette. The samples were suitably diluted with 0.1N HCl and analyzed spectrophotometrically for the dissolved drug at 285 nm. Blank was prepared by dissolving respective vehicles in 0.1 N HCl with same dilution as for the samples.



**FIG. 1: SOLUBILITY OF OIL IN DIFFERENT EXCIPIENTS**

**Droplet Size and Zeta Potential:** 1 ml of samples of products with drug, oil and surfactant combinations were taken in 10 ml volumetric flask and were diluted with milli Q water up to the mark. These samples were then analyzed for particle size and zeta potential using Malvern instrument (Zeta sizer).

**Lyophilization Process:** Two 10 ml vials were dried and each filled with Span 80 & Propylene Glycol in the ratios 1:4 respectively with oil in the ratio 1:9 and 77 mg drug. 1 gram 5% HPMC of grade 5cps in milli Q water was mixed in Vial 1 and labelled as F1, 3 grams of 5% HPMC of grade 5cps in milli Q water was mixed in Vial 2 and labelled as F2 and are partially stoppered and transported to the lyophilizer.

Each formulation was lyophilized separately to minimize possible interactions. After completion of lyophilization, vacuum was released and vials were automatically stoppered by the lyophilizer which were then sealed and stored for further evaluation studies.



FIG. 2: (A) = F2 VIAL AFTER LYOPHILIZATION. (B) = F1 VIAL AFTER LYOPHILIZATION

Formulation in F2 vial has been dried successfully and the formulation in F1 vial still was in liquid state. Hence the formulation F2 was selected for further studies.

TABLE 3: STANDARD GRAPH OF MEFENAMIC ACID

	Sample ID	Type	Concentration	Wave Length 272.0	Wgt.Factor
1	Mef 10ppm	Standard	10.000	0.158	1.000
2	Mef 20ppm	Standard	20.000	0.273	1.000
3	Mef 30ppm	Standard	30.000	0.358	1.000
4	Mef 40ppm	Standard	40.000	0.457	1.000
5	Mef 50ppm	Standard	50.000	0.525	1.000
6	Mef 60ppm	Standard	60.000	0.613	1.000

### **In-vitro Dissolution:**

**Apparatus used:** Paddle (USP Type II)

**Speed:** 50 RPM

**Medium:** 0.1N HCl

**Temperature:** 37 +/- 1 °C

**Drug:** 100 mg

**Time intervals:** 5, 10, 20, 30, 45, 60 min

Dissolution rate of pure mefenamic acid and dry emulsion of mefenamic acid which are filled in HPMC capsules were carried out using the basket apparatus (USP Type I) at 37 °C in 900 ml of 0.1 N HCl at 50 rpm. Samples equivalent to 75 mg of mefenamic acid were subjected to the testing. At the specific time intervals 5 ml samples of dissolution medium were withdrawn, filtered and analyzed at 285 nm using UV spectrophotometer. At each time of withdrawal, 5 ml of fresh 0.1 N HCl was added in the dissolution tank.

**Accelerated Stability Studies:** Accelerated stability studies of formulation were carried out as per ICH guidelines by storing the formulation at 40°C ± 2 °C and RH 75% ± 5% for 1 month in stability chamber and later after 1 month formulation was evaluated for parameters such as particle size, PDI and *In-vitro* drug release.

### **RESULTS:**

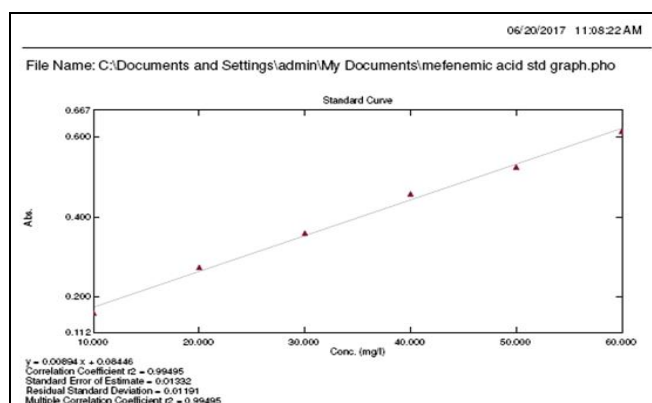
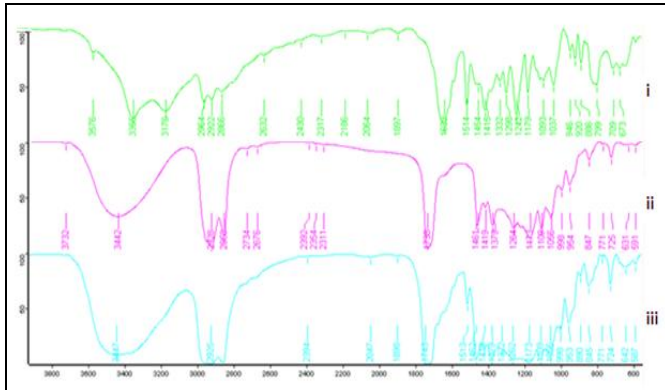
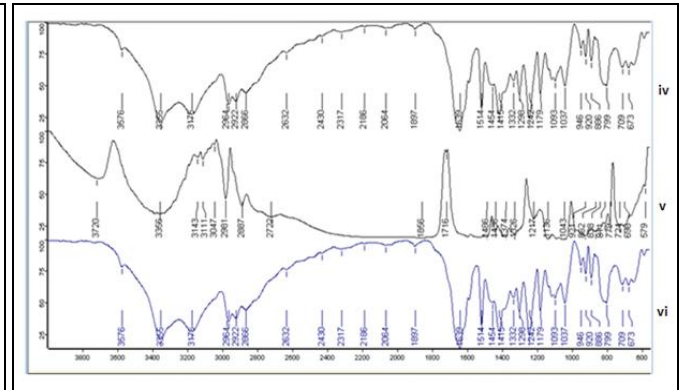


FIG. 3: STANDARD GRAPH OF MEFENAMIC ACID

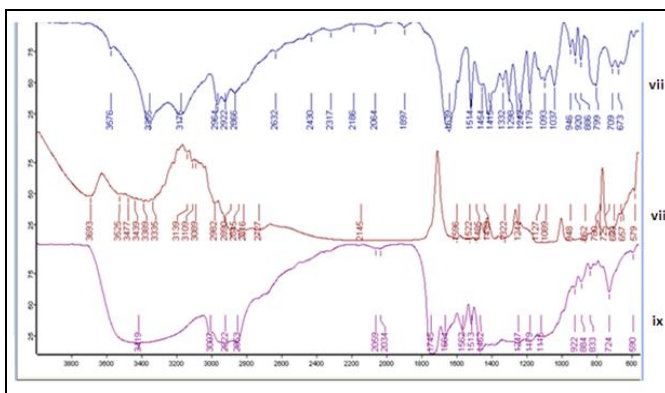
**Results of FT-IR studies:**



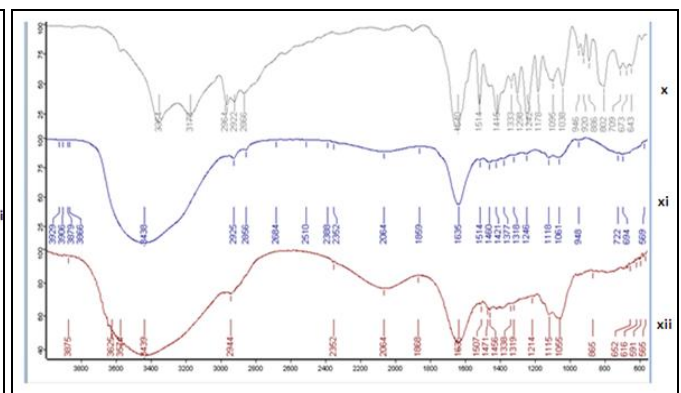
**FIG. 3: (I) = MEFENAMIC ACID, (II) = CAPRYOL, (III) = CAPRYOL+MEFENAMIC ACID**



**FIG. 4: (IV) = MEFENAMIC ACID, (V) = PROPYLENE GLYCOL, (VI) = MEFENAMIC ACID+PROPYLENE GLYCOL**



**FIG. 5: (VII) = MEFENAMIC ACID, (VIII) = SPAN 80, (IX) = SPAN 80 + MEFENAMIC ACID**



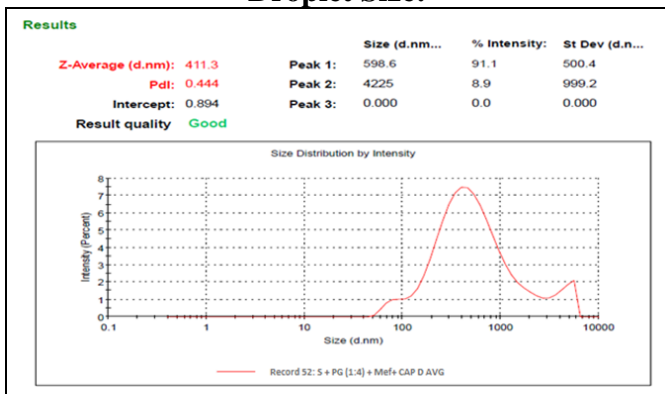
**FIG. 6: (X) = MEFENAMIC ACID, (XI) = HPMC, (XII) = MEFENAMIC ACID+HPMC**

**Solubility Profile:**

**TABLE 4: SOLUBILITY OF DRUG IN VARIOUS EXCIPIENT COMBINATION RATIOS**

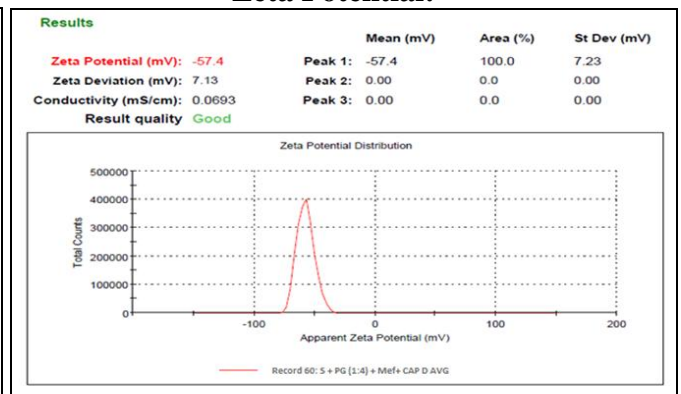
S <sub>MIX</sub>	S <sub>MIX</sub> ratio	OIL	S <sub>MIX</sub> & OIL ratio	Concentration
Span 80+Tween 80	1:4	Capryol	1:9	43mg/ml+/-0.0099
Span 80+Tween 80	1:4	Capryol	2:8	60mg/ml+/-0.0152
Span80+Propylene Glycol	1:4	Capryol	1:9	77mg/ml+/-0.0100
Tween 80+Propylene Glycol	1:4	Capryol	3:7	67mg/ml+/-0.0208
Tween 80+Propylene Glycol	1:4	Capryol	1:9	61mg/ml+/-0.0099

**Droplet Size:**



**FIG. 7: SPAN 80+ PROPYLENE GLYCOL (1:4) + MEFENAMIC ACID + CAPRYOL (1:9)**

**Zeta Potential:**

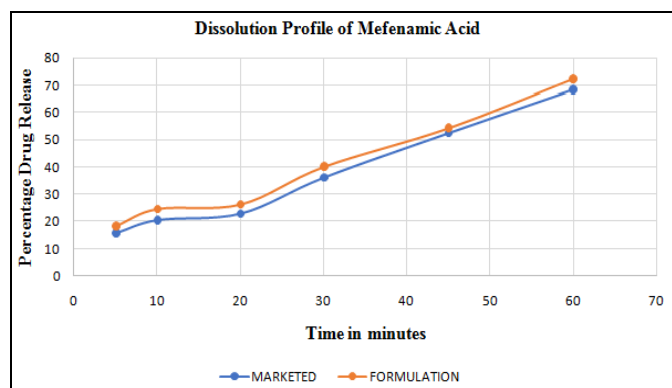


**FIG. 8: SPAN 80+PROPYLENE GLYCOL (1:4) + MEFENAMIC ACID + CAPRYOL (1:9)**

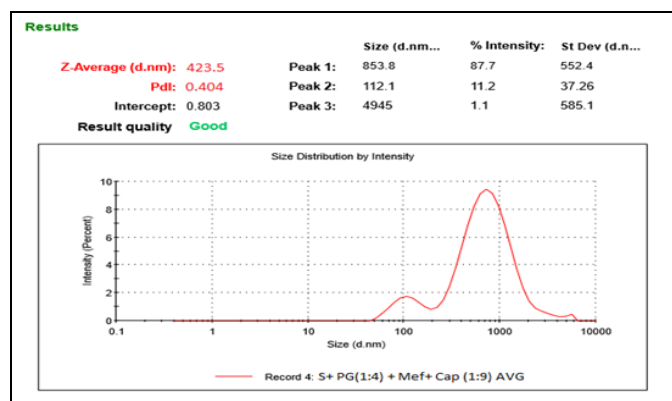
**In-vitro Dissolution:****TABLE 5: CUMULATIVE % DRUG RELEASE OF MARKETED DRUG AND PREPARED FORMULATION**

Time (in minutes)	% Drug Release of Marketed Product	% Standard Deviation	% Drug Release of Prepared Formulation	% Standard Deviation
5	15.61	0.0099	18.24	0.0099
10	20.43	0.02	24.53	0.026
20	22.82	0.0152	26.24	0.0099
30	36.1	0.1	40.15	0.0099
45	52.66	0.02	54.45	0.025
60	68.53	0.0152	72.44	0.0152

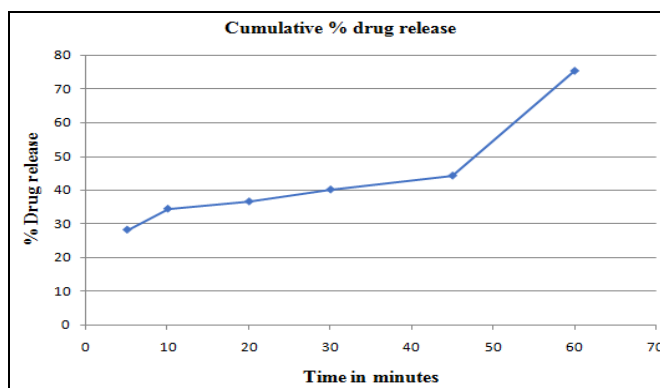
Where n = 3

**FIG. 9: COMPARISON OF % DRUG RELEASE****Accelerated Stability Studies:****TABLE 6: RESULTS OF DROPLET SIZE, PDI, % DRUG RELEASE**

Formulation	Droplet size (d. nm)	PDI	% Drug Release
F2	423.5	0.404	75.32 +/- 0.0099

**FIG. 10: DROPLET SIZE DISTRIBUTION OF DRY EMULSION FORMULATION****TABLE 7: CUMULATIVE % DRUG RELEASE FROM DRY EMULSION OF MEFENAMIC ACID IN 0.1 N HCl AT THE END OF 1 MONTH ACCELERATED STABILITY STUDY**

Time in minutes	% drug release of Dry emulsion
5	28.11 +/- 0.0099
10	34.32 +/- 0.0208
20	36.51 +/- 0.0100
30	40.03 +/- 0.0152
45	44.16 +/- 0.0152
60	75.32 +/- 0.0200

**FIG. 12: IN-VITRO DISSOLUTION PROFILE OF MEFENAMIC ACID DRY EMULSION AT THE END OF 1 MONTH ACCELERATED STABILITY STUDY****DISCUSSION:****Interpretation of Peaks:****TABLE 8: COMPARISON OF IR SPECTRUM OF PURE MEFENAMIC ACID AND DRUG WITH EXCIPIENTS**

Group	MEF	CAP+MEF	MEF+SPAN80	MEF+PG
C-N	1242	1262	1247	1214
C-O	1037	1056	1145	1055
C=C	1738	1743	1745	1635

Mefenamic acid drug showed a peak in the region 1242, and Capryol with mefenamic acid showed a peak in region 1262, mefenamic acid with Span 80 showed a peak in the region 1247, mefenamic acid with Propylene glycol showed a peak in the region 1214 which all indicate the presence of C-N functional group in them.

Mefenamic acid drug showed a peak in the region 1037, and Capryol with mefenamic acid showed a peak in region 1056, mefenamic acid with Span 80 showed a peak in the region 1145, mefenamic acid with Propylene glycol showed a peak in the region 1055 which all indicate the presence of C-O functional group in them.

Mefenamic acid drug showed a peak in the region 1738, and Capryol with mefenamic acid showed a

peak in region 1743, mefenamic acid with Span 80 showed a peak in the region 1745, mefenamic acid with Propylene glycol showed a peak in the region 1635 which all indicate the presence of C=C functional group in them.

There were no major changes seen in the FT-IR spectra of the pure drug in comparison to the drug and excipient combinations.

**Solubility Profile:** The solubility of the drug in various excipient ratios and combinations were seen and it was found that the following combination showed the highest solubility among the rest.

**TABLE 9: SOLUBILITY OF MEFENAMIC ACID IN SELECTED COMBINATION**

S <sub>MIX</sub>	S <sub>MIX</sub> ratio	OIL	S <sub>MIX</sub> & OIL ratio	Conc.
Span 80+	1:4	Capryol	1:9	77 mg/ml
Propylene Glycol				

The S<sub>mix</sub> combination *i.e.*, (Span 80 + Propylene glycol) in the ratio of 1:4 with capryol in the ratio of 1:9 has the concentration of 77 mg/ml.

**Droplet Size and Zeta Potential:** The zeta potential and the droplet size analysis of the drug along with the oil and surfactant mix was done and the desired combination of surfactant mix and oil was found to be Span 80 and propylene glycol in the ratios 1:4 respectively with oil in the ratio 1:9 having droplet size 411.3 d.nm and PDI of 0.444 and Zeta potential of -57.4 mV.

**Lyophilization:** Mefenamic acid dry emulsion was prepared by lyophilization using capryol as the lipid phase and Span 80, Propylene Glycol as the surfactant and co-surfactant and HPMC as the carrier. The dry emulsion obtained was filled in HPMC capsules and was compared with the marketed formulation.

**Dissolution:** Dissolution profile of the prepared dry emulsion was done in comparison with the marketed variant and it was found that the prepared formulation showed a slightly greater dissolution profile than the marketed variant.

**Accelerated Stability Studies:** Accelerated stability studies at 40 °C and 75% RH for dry emulsion of mefenamic acid was performed and the droplet size is 423.5 d.nm and PDI was found to be

0.404. Cumulative % drug release of mefenamic acid was found to be 75.32% at the end of 1 month indicating no change in % drug release after stability study for 1 month.

**CONCLUSION:** The drug mefenamic acid which is a BCS Class-2 drug and was chosen for formulation of dry emulsion because of its poor water solvency. The formulation was developed on the basis to improve the effect of the drug in combination with the lipophilic solvent *i.e.*, the drug mefenamic acid was used in combination with the lipophilic solvent - capryol oil. Solubility of mefenamic acid was determined in various surfactants, co-surfactants by UV-spectrophotometric method. Mefenamic acid has shown maximum solubility in surfactants span 80, propylene glycol.

According to FTIR, There were no major changes seen in the FT-IR spectra of the pure drug in comparison to the drug and excipient combinations.

According to solubility profile, the solubility of the drug in various excipient ratios and combinations were seen and it was found that the following combination showed the highest solubility *i.e.*, span and propylene glycol in ratio of 1:4 and capryol oil in ratio of 1:9 of 77 mg/ml concentration.

According to droplet size and zeta potential, the zeta potential and the droplet size analysis of the drug along with the oil and surfactant mix was done and the desired combination of surfactant mix and oil was found to be Span 80 & Propylene Glycol in the ratios 1:4 respectively with oil in the ratio 1:9 having droplet size 411.3 d.nm and PDI of 0.444 and Zeta potential of -57.4 mV.

According to lyophilisation, mefenamic acid dry emulsion was prepared by lyophilization using Capryol as the lipid phase and Span 80, Propylene Glycol as the surfactant and co-surfactant and HPMC as the carrier. The dry emulsion obtained was filled in HPMC capsules and was compared with the marketed formulation

According to dissolution profile, the prepared dry emulsion was done in comparison with the marketed variant and it was found that the prepared formulation showed a slightly greater dissolution profile than the marketed variant.

According to accelerated stability studies, Accelerated stability studies at 40 °C and 75% RH for dry emulsion of mefenamic acid was performed and the droplet size is 423.5d.nm and PDI was found to be 0.404. Cumulative % drug release of mefenamic acid was found to be 75.32% at the end of 1 month indicating no change in % drug release after stability study for 1 month

The dry emulsion formulation showed improved and increased drug dissolution for poorly soluble drug. This helps to keep the drug in soluble state in GIT, so the prepared dry emulsion has capability for delivering poorly water soluble drug mefenamic acid in soluble state in GIT.

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