FORMULATION AND *IN VITRO* EVALUATION OF A NOVEL MEFENAMIC ACID TOPICAL CREAM

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ABSTRACT

Mefenamic acid (MA) is a Non-Steroidal Anti-Inflammatory Drug (NSAID), which has been established in pharmacologic management of musculoskeletal disorders. This study was designed to formulate and conduct an *in vitro* evaluation of a novel MA topical cream, which would reduce the gastrointestinal associated toxicities related to oral administration. A concentration of 1-2% of MA was prepared by using a different combination of the excipients. Either ethanol or dimethyl sulfoxide (DMSO) are employed as vehicles for hydrophobic compounds, which are commonly used as solvents. The creams were evaluated by their physical properties, stability studies and addition of an *in vitro* anti-inflammatory test for mefenamic acid creams. The physical studies include organoleptic test, pH test, spreadability test and dye test while for the stability studies, the creams were tested with accelerated stability studies and microbial growth. MA in DMSO showed better anti-inflammatory effect compared to MA in ethanol in the correlation of results with that of marketed products. In conclusion, this study proved that MA cream can also be a good choice of semi-solid formulation and further studies would be suggested to enhance this cream as a novel choice of formulation to be introduced and marketed by the pharmaceutical industry.

Keywords: Mefenamic acid, cream, *in vitro*

INTRODUCTION

Mefenamic acid is an anthranilic acid in the class of non-steroidal anti-inflammatory drugs (NSAIDs). It has anti-inflammatory and analgesic activities and useful for the treatment of rheumatoid arthritis, musculoskeletal, menstrual symptoms and headache. Furthermore, mefenamic acid has been shown to have therapeutic effects in neurodegenerative diseases (e.g. Alzheimer's disease) and is emerging as new chemopreventive agents against cancer[1]. It inhibits the activity of the enzymes cyclo-oxygenase I and II, resulting in persistent gastric bleeding and peptic ulceration. It can also cause serious cardio-vascular undesirable effects via inhibition of COX II receptors[2]. Conventionally, mefenamic acid is only available in the form of tablets, capsule and suspensions[3]. There is no marketed topical formulation of mefenamic acid available until now. The systemic half-life of mefenamic acid is 2 to 4 h. Repeated dosing is required to achieve the steady state level due to short half-life. Mefenamic acid is classified as class II on the basis of biopharmaceutical classification system, due to its poor solubility over the pH range of 1.2-7.5 but higher permeability[4]. This made the use of mefenamic acid limited. Mefenamic acid has shown *ex vivo* drug release of 56.23% in 240 min in Franz diffusion cell using Wistar male rat skin. It has also shown *in vivo* anti-inflammatory and analgesic effects in rat[4]. Therefore, an attempt is made here to deliver mefenamic acid through the skin, which has many advantages over the conventional oral route such as better bioavailability due to ability to bypass first pass effect, avoiding irritation of GI mucosa, controlled rate of drug release, keeping balanced maintained plasma concentration of drug, decreased dose rate, decreased side effects with minimum discomfort and improved patient compliance[2]. Hence, this study was conducted to prove the anti-inflammatory effect of the mefenamic acid when formulated as a transdermal cream which have almost the same anti-inflammatory activity as the available topical NSAIDs in the market like Voren® gel, Uniren® gel, Voltaren® emulgel, Ketofen® gel, Sanbeflam® gel, Kenofen® gel and Fastum® gel[3].

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MATERIALS AND METHODS

Chemicals

Mefenamic acid was received as a gift from IKOP Pharma Sdn. Bhd. in Kuantan, Pahang. Stearic acid, cetyl alcohol and glycerol (R&M Chemicals, Malaysia), paraffin liquid, triethanolamine, Tween 80 and phosphate buffer solution pH 7.0 ± 0.05 (Systerm®, Malaysia), glycerine (HmbG® Chemicals, Malaysia), methyl paraban (Sigma-Aldrich, USA), almond oil (ChemSoln, Malaysia), gram stain safranine (BML OXOID), Muller Hinton Agar (MHA), Dimethylsulfoxide (DMSO) and distilled water were used.

Apparatus and Instruments

Glass beaker 10 mL, 50 mL and 100 mL, mortar and pestle, spatula, glove (Lattice Synergy, Malaysia), measuring cylinder 10 mL and 50 mL, glass rod, evaporating basin, dropper, weighing boat, glass plate (10 x 10 cm), 1kg of load, China dish and magnetic stirrer from UoC Lab, portable weighing balance (Scout® Pro, Malaysia), pH meter (Mettler Toledo, US), Oven and water bath (Memmert, Germany), Microscope (Nuevue, Malaysia), Cream container (MYLAB SCIENTIFIC, Malaysia), cuvette and small glass bottle (Thomas Scientific, USA) and Spectrophotometer (AHS Laboratory Supplies, Malaysia) were employed.

Preparation of blank cream

The preparation of the blank creams F1 and F2 was formulated by the method of Muthukumarasamy et al[5]., while blank cream F3, F4 and F5 were formulated by the method of Gupta et al.[6]. Then, some modifications were made. In this study, O/W emulsion was prepared. The formulation of the blank cream is given in Table I.

Blank cream was prepared by taking separately the oily and aqueous phases into beakers and heating to 70 ± 1˚C over a water bath. After heating, aqueous phase was added drops wise to the oily phase with constant stirring. The balance distilled water was poured in and mixed continuously unit a homogenous cream was formed.

Preparation of mefenamic acid creams

Among all the blank creams prepared, one of the best blank cream formulations was selected for the preparation of mefenamic acid cream. Before that, 1% or 2% of mefenamic acid powder needs to be dissolved in a suitable solvent to make sure it dissolved completely and avoid grittiness particles when being incorporated into the blank cream. The formulation of mefenamic acid cream and the solvent used is as shown in Table II before being added into the best blank cream.

Table I: Formulation of the blank creams

Sr. No.	Ingredient	Amount (%w/w)				
		F1	F2	F3	F4	F5
OILY PHASE						
1	Stearic acid	7	7	11	13	16
2	Cetyl alcohol	2	2	4	2	4
3	Almond oil	-	-	4	4	4
4	Liquid paraffin	20	20	-	-	-
AQUEOUS PHASE						
5	Glycerin	10	10	-	-	-
6	Glycerol	-	-	3	3	3
7	Triethanolamine	-	2	4	4	4
8	Tween 80	2	-	-	-	-
9	Methyl paraben	0.05	0.05	0.05	0.05	0.05
10	Distilled water	qs	qs	qs	qs	qs

Table II: Formulation of mefenamic acid creams

Sr. No.	Ingredient	Amount (%w/w)			
		F6	F7	F8	F9
1	Mefenamic acid	1	2	1	2
2	DMSO	-	-	5	5
3	Ethanol	5	5	-	-

Evaluation of creams

Organoleptic evaluations

The organoleptic evaluations of the formulated creams were conducted by visual appearance and touch after the formulations were set in containers[7]. The formulated creams were assessed for their organoleptic properties such as color, odor and state. The appearance of the cream was judged by its texture and homogeneity of any phase separation[8]. Homogeneity test of cream was done to assure the absence of gritty particles in the preparation upon its application to the skin[9].

pH determination

The pH of the creams was determined by using digital pH meter. The pH of the cream was measured by dissolving 0.5 g of the cream into 50 mL of distilled water[6]. Then, the pH electrode was in contact with the solution until the fixed reading was obtained. The determinations were conducted three times and average was calculated[7].

Spreadability test

The spreadability of each cream formulation was determined using parallel-plate method. Circles of different radii were drawn on graph paper and a 10 cm^2 glass plate was put on it. A sample of 1 g cream was placed at the center of the glass plate. Another 10 cm^2 glass plate then was placed on the cream like a sandwich and a weight of 1 kg load was allowed to be put on the upper glass plate for 3 min. The increase in the diameter due to spreading of the formulation was recorded[7]. The experiment was repeated three times and the average diameter was calculated[9]. The spreadability value was calculated by using the following formula:

$$S = \frac{M \times L}{T} \qquad (1)$$

where S = Spreadability, M = Weight of the upper plate and standard weight rested on it (g), L = Diameter of the spreading cream (cm), T = Time taken (in min)

Dye test

The gram stain safranin was added to the cream. A drop of cream was dispersed on a microscopic slide and observed under a microscope. The cream is regarded as oil in water (O/W) type if the dispersed globules appears colorless and the ground is red. In the opposite situation where the disperse globules appear red in the colorless ground, the cream was determined as water in oil (W/O) type cream[10].

Accelerated stability study

Accelerated stability test of creams firstly were conducted at room temperature, studied for 7 days and then were kept at 40 ˚C ± 1 ˚C for 20 days. Both creams at room and high temperature were observed and recorded on 0th, 5th, 10th, 15th and 20th day for the phase separation[11].

Microbial growth

The formulated creams were inoculated on the plates of Muller Hinton agar media by streak plate method. The plates were then, placed in the incubator and incubated at 37 °C for 24 h. A control of Muller Hilton agar without any cream was prepared. The following day, the plates were taken out and compared with the control[5].

In vitro anti-inflammatory activity

In vitro protein denaturation was prepared by the method of Sangeetha et al.[12], and slight modifications have been made. The anti-inflammatory activity of mefenamic acid cream was studied by using inhibition of protein denaturation method using egg albumin as protein.

Control solution (5 mL); Phosphate buffer saline (2.8 mL) of pH 7.0 ± 0.05 was transferred to freshly prepared egg albumin (0.2 mL) and blank cream (2 mL) was added to this, to prepared control solution.

Standard solution (5 mL); Phosphate buffer saline (2.8 mL) of pH 7.0 ± 0.05 was transferred to freshly prepared egg albumin (0.2 mL) and (2 mL) solution of diclofenac sodium of varying concentrations was added to this, to prepared standard solution.

Test solution (5 mL); Phosphate buffer saline (2.8 mL) of pH 7.0 ± 0.05 was transferred to freshly prepared egg albumin (0.2 mL) and (2 mL) solution of cream formulation of varying concentrations was added to this, to prepared test solution.

The incubation condition was 37 ± 2˚C for 15 minutes for all samples. After the incubation period, the samples were heated at 70˚C on a water bath for 5 minutes. Then the samples were allowed to cool to room temperature. The samples were checked using UV-Visible spectrophotometer at 660 nm for its absorbance using vehicle as blank. The percentage inhibition of protein denaturation was calculated from the control using the following formula:

$$\% \text{ Inhibition of denaturation} = 100 \times \frac{\text{Abs control} - \text{Abs test}}{\text{Abs control}}$$

where Abs is the absorbance (2)

Statistical analysis

All experiments were carried out in triplicate. Data collected was analyzed using One-way ANOVA. Statistical Package for Social Sciences (SPSS) version 20.0 and Microsoft Excel was used. The results were expressed as the Mean ± SEM (standard error of mean) and a value of $p<0.05$ was considered statistically significant.

RESULTS

Evaluation of blank creams

Organoleptic evaluation

The photos of cream samples were presented in Fig. 1. The appearance of all of the blank cream formulations were opaque and the color of the blank creams was white. The results of the appearance, color, texture and homogeneity are tabulated in Table III.

Table III: Results of the organoleptic evaluation of the blank creams on day 1

Day 1 at 30 ± 2 °C/ 65 ± 5% RH				
Blank cream formulation	Color	Texture (Smooth/ Rough)	Physical appearance (Opaque/ transparent)	Homogeneity after compounding
F1	White	Smooth	Opaque	Homogeneous
F2	White	Smooth	Opaque	Homogeneous
F3	White	Smooth	Opaque	Homogeneous
F4	White	Smooth	Opaque	Homogeneous
F5	White	Rough	Opaque	Homogeneous

Fig. 1: Physical appearance blank cream on day 1

pH determination

The pH of the blank cream depends on the formulations. Different formulation will give different pH. The results of the pH of the blank creams are tabulated in Table IV.

Table IV: Results of pH of the blank cream (n=3)

Blank cream formulation	pH (Mean ± SD)
	Day 1
F1	5.45 ± 0.15
F2	7.96 ± 0.06
F3	8.29 ± 0.10
F4	8.14 ± 0.08
F5	8.32 ± 0.09

Spreadability test

The value of spreadability indicates that the cream is easily spreadable by small amount of shear. The results are tabulated in Table V.

Table V: Results of spreadability test of the blank cream (n=3)

Blank cream formulation	Spreadability (g.cm min^{-1}) (Mean ± SD)
F1	3702 ± 228
F2	4561 ± 198
F3	4098 ± 229
F4	3900 ± 114
F5	3636 ± 114

Dye test

All the cream formulations showed that the continuously phase appeared red and the globules appeared colorless. The results of dye test are recorded in Table VI.

Table VI: Results of dye test of the blank cream formulations at day 1

Blank cream formulation	O/W, W/O (Day 1)
F1	O/W
F2	O/W
F3	O/W
F4	O/W
F5	O/W

Accelerated stability study

Effect of temperature on organoleptic properties

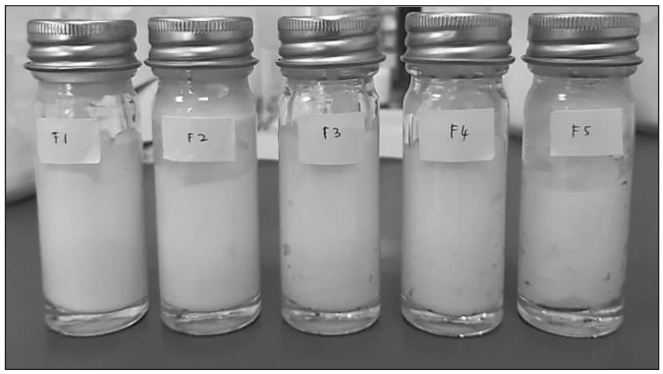
There were no changes in organoleptic properties of all blank creams after 1 month at room temperature and at high temperature, except for blank cream F1, as shown in Fig. 2. Result of organoleptic evaluations are shown in Table VII and Table VIII.

Table VII: Result of the organoleptic evaluation of the blank creams after 1 month at room temperature

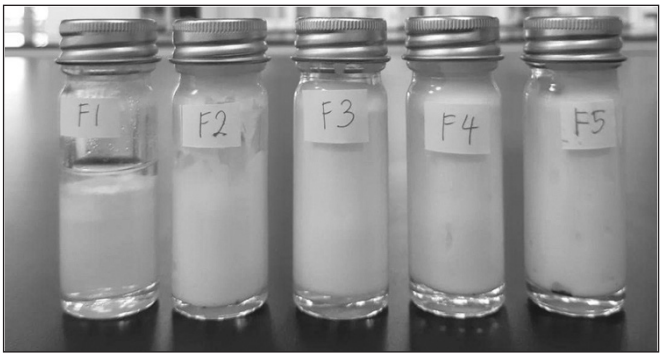
After 1 month at 30 ± 2 °C/ 65 ± 5% RH				
Blank cream formulation	Color	Texture (Smooth/ rough)	Physical appearance (Opaque/ transparent)	Homogeneity after compounding
F1	White	Smooth	Opaque	Homogeneous
F2	White	Smooth	Opaque	Homogeneous
F3	White	Smooth	Opaque	Homogeneous
F4	White	Smooth	Opaque	Homogeneous
F5	White	Rough	Opaque	Homogeneous

Table VIII: Results of the organoleptic evaluation of the blank creams after 1 month at high temperature

After 1 month at 40 ± 1 °C / 75 ± 5% RH				
Blank cream formulation	Color	Texture (Smooth/ Rough)	Physical appearance (Opaque/ transparent)	Homogeneity after compounding
F1	White	Smooth	Opaque	Heterogenous
F2	White	Smooth	Opaque	Homogeneous
F3	White	Smooth	Opaque	Homogeneous
F4	White	Smooth	Opaque	Homogeneous
F5	White	Rough	Opaque	Homogeneous



(a)



(b)

Fig. 2: (a) Physical appearance of blank creams after 1 month at room temperature. (b) Physical appearance of blank creams after 1 month at high temperature

Effect of temperature on pH

pH values were measured for all creams after 1 month at room temperature and elevated temperature. The pH vlaues are recoreded in Table IX.

Table IX: Results of pH of the blank cream during accelerated stability study (n=3)

Blank cream formulation	pH (Mean ± SD)	
	After 1 month at 30 ± 2°C/ 65 ± 5%)	After 1 month at 40 ± 1°C / 75 ± 5% RH
F1	5.31 ± 0.14	6.11 ± 0.26
F2	8.37 ± 0.01	8.31 ± 0.03
F3	8.43 ± 0.10	8.35 ± 0.01
F4	8.48 ± 0.06	8.43 ± 0.00
F5	8.48 ± 0.02	8.42 ± 0.02

Effect of temperature on dye test

All cream formulations still appear in O/W state after being left for 1 month at room and elevated temperatures. The results of the dye test are shown in Table X.

Table X: Results of dye test of the blank cream formulations after 1 month at room temperature and high temperature

Blank cream formulation	O/W, W/O (After 1 Month at 30 ± 2°C/ 65 ± 5% RH)	O/W, W/O (After 1 Month at 40 ± 1°C / 75 ± 5% RH)
F1	O/W	O/W
F2	O/W	O/W
F3	O/W	O/W
F4	O/W	O/W
F5	O/W	O/W

Microbial growth test

There was no presence of micoribial growth for all blank cream formulations on the Muller Hinton plate as compared to the control. The reuslts are tabulated in Table XI.

Table XI: Results of microbial growth of the blank cream formulations on day 1 and after 1 months at room temperature and high temperature

Blank cream formulation	Growth Day 1	Growth after 1 month at 30 ± 2 °C/ 65 ± 5% RH)	Growth after 1 month at 40 ± 2 °C / 75 ± 5% RH)
1	NO	NO	NO
2	NO	NO	NO
3	NO	NO	NO
4	NO	NO	NO
5	NO	NO	NO

Evaluation of mefenamic acid creams

F2 was chosen as the best blank cream and was used to be incorporated with mefenamic acid because it fulfilled all the parameters of the evaluations.

Organoleptic evaluation

The appearance of mefenamic acid cream formulations was white and opaque, as shown in Fig. 3. Other than that, the cream's texture was smooth and homogenous. Results of appearance, color, texture, homogeneity and grittiness of the mefenamic acid cream on day 1 are recorded in Table XII.

Table XII: Results of the organoleptic evaluation of mefenamic acid cream on day 1

DAY 1					
Mefenamic acid cream	Color	Texture (Smooth/ Rough)	Physical appearance (Opaque/ transparent)	Homogeneity after compounding	Grittiness (Yes/ No)
F6	White	Smooth	Opaque	Homogenous	Yes
F7	White	Smooth	Opaque	Homogenous	Yes
F8	White	Smooth	Opaque	Homogenous	No
F9	White	Smooth	Opaque	Homogenous	No

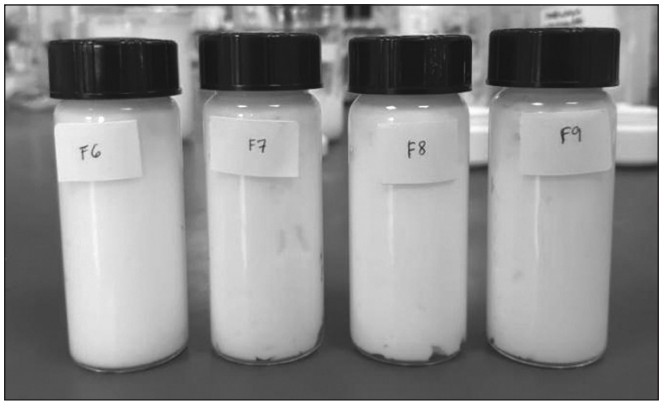


Fig. 3: Physical appearance of mefenamic acid creams on day 1

pH determination

When mefenamic acid was added into the blank cream, the pH of the cream decreased due to the acidic properties of mefenamic acid. Results of the pH of the mefenamic acid topical cream formulations on day 1 are shown in Table XIII.

Table XIII: Results of pH of the mefenamic acid cream on day 1 (n=3)

Mefenamic acid cream	pH (Mean ± SD)
	Day 1
F6	6.54 ± 0.10
F7	6.33 ± 0.80
F8	6.45 ± 0.12
F9	6.39 ± 0.16

Spreadability test

The value of spreadability indicates that the cream is easily spreadable by small amount of shear. The results are tabulated in Table XIV.

Table XIV : Results of spreadability test of the mefenamic acid cream on day 1 (n=3)

Mefenamic acid cream	Spreadability (g.cm min^{-1}) (Mean ± SD)
F6	3371 ± 198
F7	2578 ± 198
F8	3834 ± 229
F9	3768 ± 198

Dye test

All mefenamic acid cream formulations showed that the continuous phase appeared red and the globules appeared colorless. The results of the dye test are recorded in Table XV.

Table XV: Results of dye test of the mefenamic acid cream at day 1

Mefenamic acid cream	O/W, W/O (Day 1)
F6	O/W
F7	O/W
F8	O/W
F9	O/W

Accelerated stability study

Effect of temperature on organoleptic properties

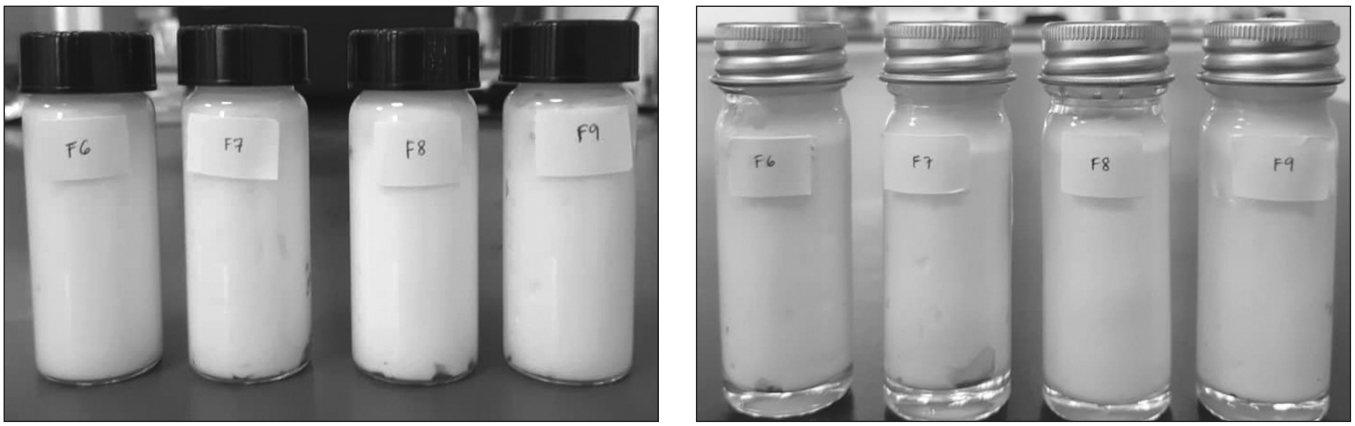
There were no changes in organoleptic properties of all mefenamic acid creams after 1 month at room temperature and at elevated temperature, as presented in Fig. 4. The results are tabulated in Table XVI and Table XVII.

Table XVI: Results of the organoleptic evaluation of the mefenamic acid creams after 1 month at room temperature

After 1 month at 30 ± 2 °C/ 65 ± 5% RH					
Mefenamic acid cream	**Color**	**Texture (Smooth/ rough)**	**Physical appearance (Opaque/ transparent)**	**Homogeneity after compounding**	**Grittiness (Yes/No)**
F6	White	Smooth	Opaque	Homogenous	Yes
F7	White	Smooth	Opaque	Homogenous	Yes
F8	White	Smooth	Opaque	Homogenous	No
F9	White	Smooth	Opaque	Homogenous	No

Table XVII: Results of the organoleptic evaluation of the mefenamic acid creams after 1 month at high temperature

After 1 month at 40 ± 1 °C / 75 ± 5% RH					
Mefenamic acid cream	**Color**	**Texture (Smooth/ rough)**	**Physical appearance (Opaque/ transparent)**	**Homogeneity after compounding**	**Grittiness (Yes/No)**
F6	White	Smooth	Opaque	Homogenous	Yes
F7	White	Smooth	Opaque	Homogenous	Yes
F8	White	Smooth	Opaque	Homogenous	No
F9	White	Smooth	Opaque	Homogenous	No



a b

Fig. 4: (a)Physical appearance of mefenamic acid after 1 month at room temperature. (b) Physical appearance of mefenamic acid after 1 month at high temperature

Effect of temperature on pH

pH of the mefenamic acid creams was taken again after 1 month at room and elevated temperature. The result of mefenamic acid creams pH is recorded in Table XVIII.

Effect of temperature on dye test

All mefenamic acid creams still appears in O/W state after being left for 1 month at room and elevated temperature. The results of the dye test are recorded in Table XIX.

Table XVIII: Results of pH of the mefenamic acid cream during accelerated stability study (n=3)

Mefenamic acid cream	**pH (Mean ± SD)**	
	After 1 month at 30 ± 2 °C/ 65 ± 5 % RH	**After 1 month at 40 ± 1 °C / 75 ± 5 % RH**
F6	6.67 ± 0.03	6.55 ± 0.16
F7	6.32 ± 0.10	6.39 ± 0.10
F8	6.55 ± 0.07	6.39 ± 0.07
F9	6.40 ± 0.10	6.28 ± 0.08

Table XIX: Results of dye test of the blank cream formulations after 1 month at room temperature and high temperature

Mefenamic acid cream	o/w, w/o (After 1 month at 30 ± 2 °C/ 65 ± 5% RH)	o/w, w/o (After 1 month at 40 ± 1 °C / 75 ± 5% RH)
F6	O/W	O/W
F7	O/W	O/W
F8	O/W	O/W
F9	O/W	O/W

Microbial growth test

As compared to control, there was no growth of microbe's presence on the Mueller Hinton agar plate of all the mefenamic acid cream formulations. Results of microbial growth of the mefenamic acid cream on day 1 and after 1 month at room temperature and high temperature are recorded in Table XX.

Table XX: Results of dye test of the blank cream formulations after 1 month at room temperature and high temperature

Mefenamic acid cream	Growth DAY 1	Growth after 1 month at 30 ± 2 °C/ 65 ± 5% RH)	Growth after 1 month at 40 ± 2 °C / 75 ± 5% RH)
F6	NO	NO	NO
F7	NO	NO	NO
F8	NO	NO	NO
F9	NO	NO	NO

Table XXI: Result of percentage inhibition and IC_{50} (n=3)

Concentration of cream (g mL^{-1})	Mean ± SD		
	Diclofenac sodium emulgel (% inhibition)	Mefenamic acid cream in ethanol (% inhibition)	Mefenamic acid cream in DMSO (% inhibition)
1	14.8 ± 3.02	7.6 ± 3.49	9.1 ± 4.53
2	16.7 ± 2.74	9.5 ± 2.41	11.3 ± 5.31
IC_{50}	19.52 g mL^{-1}	23.32 g mL^{-1}	19.59 g mL^{-1}

In vitro anti-inflammatory activity

Present findings revealed that mefenamic acid cream and diclofenac sodium Emulgel (reference drug) exhibited a concentration-dependent inhibition of protein denaturation at 1 and 2 g mL^{-1}. Inhibitory concentration (IC_{50}) value of mefenamic acid cream in ethanol was recorded as 23.32 g mL^{-1}, while mefenamic acid cream in DMSO was recorded as 19.59 g mL^{-1} and diclofenac sodium was 19.52 g mL^{-1}, as shown in Table XXI.

DISCUSSION

Blank cream formulations

In this study, five blank creams were prepared using different concentrations of excipients. Among all the blank creams prepared, F2 was chosen as the best blank cream with a suitable pH range, good spreadability, homogeneity, appearance, no microbial growth and during the accelerated stability studies, F2 showed homogeneity until the end of studies. In organoleptic studies, all blank cream formulations appeared white and opaque after inspection while most of the blank cream formulations showed smooth and homogenous texture. The mixing of aqueous phase and oily phase results in a creamy emulsion appearance, due to different refractive indices and optical dispersion powers of the two phases[13]. F5 has a rough texture due to the high amount of thickening agent stearic acid being used in the formulation. The pH of the cream preparation must be maintained in the range of 5.6 to 7.5, which is the normal range of the pH skin[14]. F1 has the most ideal pH value which is safe and suitable to be applied on the skin compared to other formulations. Since mefenamic acid is acidic is nature, to incorporate it into the blank cream, the pH of the blank cream formulation being used should not be too alkaline or acidic. In this case, F2 is suitable.

Formulations with higher spreadability values allow ease of application, by increasing the surface area for drug permeation. A good cream takes less time to spread and will have a high spreadability range[15]. F2 gave good spreadability value while F5 gave lowest spreadability value compared to the other formulations. This is also due to the higher amount of stearic acid being used in the formulation. The higher the concentration of cetyl alcohol, the greater the spreadability of cream. In contrast, as the concentration of stearic acid increased, the spreadability value of the cream decreased[16]. All the physicochemical parameters were consistent. However, F1 showed a dispersed phase where it became coalesced and showed phases separation between oil and water phases. This is due to the use of different emulsifying agents in formulation, which is Tween 80 instead of using triethanolamine. Based on the microbial result, all blank cream formulations showed no microbial growth on the

Muller Hinton agar plate. This is due to the use of effective concentration of preservative in the cream preparation, which is 0.05% of methyl paraben[17].

Preparation of mefenamic acid creams

Mefenamic acid was dissolved in ethanol before being incorporated into the cream base. 5% of ethanol has been used to dissolve mefenamic acid when preparing a topical product[18]. Since mefenamic acid is partially soluble in alcohol, after being incorporated into the cream base, the cream has some grittiness, when applied onto the skin. For this reason, 5% of dimethyl sulfoxide (DMSO) was used to dissolve mefenamic acid. Ethanol and DMSO are commonly used solvents and employed as vehicles for hydrophobic compounds[19]. DMSO is considered by many as universal solvent, where both polar and nonpolar compounds can be dissolved easily in it[20].

Mefenamic acid cream formulations

All formulated mefenamic acid creams appeared white, smooth and opaque after the inspection since mefenamic acid powder is in white color so it does not change the color of the blank cream. Most of the formulated mefenamic acid creams showed homogenous texture during the studies. The pH of all formulated mefenamic acid creams was within normal range of pH skin, so the creams are safe and suitable to be applied on the skin. F7 and F9 showed lower pH compared to F6 and F8. This is due to the different concentrations of mefenamic acid being used in the formulation. Since mefenamic acid is an acidic drug, the higher the concentration of mefenamic acid being used, the lower the pH of the cream. Based on the spreadability test, mefenamic acid cream in DMSO has better spreadability compared to mefenamic acid cream in ethanol. Galer, B. S.[21] reported that mean subjective responses to topical diclofenac solution with DMSO were more favorable for most items in the questionnaire including ease of application on the skin. This proves that cream containing DMSO has a good spreadability which is easier to be applied on the skin and more patient preferred. However, mefenamic acid cream in ethanol was still in a good range of spreadability value since this is a quantitative study and the other ingredients used were all the same as mefenamic acid cream in DMSO. F6 had higher spreadability effect compared to F7, and F8 had higher spreadability effect compared to F9. This was due to increasing concentrations of mefenamic acid used. The higher the concentration of mefenamic acid, the lower the spreadability of the cream.

The dye test conducted on all mefenamic acid cream formulations confirmed that there was oil in water (o/w) type of emulsion cream because the continuous phase appeared red and the globules appeared colorless. All the physicochemical parameters were well maintained during the period of accelerated stability studies for all mefenamic acid cream formulations. This shows that mefenamic acid and the solvent used do not interfere with the stability of the blank cream preparations. Based on the result of microbial growth, after the incorporation of mefenamic acid into the blank cream, all the mefenamic acid creams still showed absence of growth of microbes on the Mueller Hinton agar plate. This proves that mefenamic acid and the chemical solvent used do not interfere with the effectiveness of the methyl paraben as the preservative to prevent the growth of microbes.

In vitro anti-inflammatory activity

In this study, the protein denaturation bioassay was selected for anti-inflammatory activity of mefenamic acid cream formulations. In anti-denaturation assay, the denaturation of egg albumin is induced by heat treatment. Mefenamic acid had been well known for its anti-inflammatory effects[22]. This *in vitro* anti-inflammatory activity test was conducted to prove that the cream base formulated with the varieties of excipients does not interfere with the effectiveness of mefenamic acid when being prepared as cream. The ability of the mefenamic acid cream to inhibit albumin denaturation has been investigated for potential anti-inflammatory action mechanisms. Results reveal that mefenamic acid cream was effective in inhibiting thermally induced albumin denaturation at all tested concentrations, indicating their capability of controlling protein denaturation involved in the inflammatory process[23].

The results showed that mefenamic acid cream in DMSO had better anti-inflammatory activity compared to mefenamic acid cream in ethanol. According to Brien et al.[24], DMSO can reduce peripheral pain, inflammation and arthritis, and might inhibit the degenerative changes occurring in osteoarthritis. The higher anti-inflammatory effect of mefenamic acid cream in DMSO might be due to the contribution of anti-inflammatory effects of DMSO. From the IC50 values, it becomes evident that mefenamic acid cream in DMSO was active as diclofenac sodium emulgel. The lower the IC_{50} value, the stronger the anti-inflammatory activity of the drug[23]. 2% of mefenamic acid in DMSO proved that it has a comparable anti-inflammatory effect with that of marketed products.

The *in vitro* results appear as interesting, promising and effective as marketed topical anti-inflammatory drugs. It is proposed that anti-inflammatory effects need to be further assessed in other experimental models in

pursuit of newer topical medications against inflammatory diseases. Therefore, from the results of the present study, it can be concluded that mefenamic acid cream in DMSO possessed anti-inflammatory activity as marketed topical anti-inflammatory drugs, which is diclofenac sodium in emulgel.

CONCLUSION

The correct concentration of excipients used gives the best formulation. Every excipient added to the formulation such as emulsifier, emollient, thickening agent, penetration enhancer, lubricant and vehicle has its own role and functions to form a good cream. F2 has been selected as the best blank cream formulation as it fulfilled all the parameters of the cream evaluation. Accelerated stability study is the most important parameter in finding the best formulation as it shows the shelf-life and stability of the cream. If a blank cream is stable throughout the study, it will also be stable when 1% or 2% of mefenamic acid and its solvent are added. All the evaluations of mefenamic acid cream formulations have shown to be in a good range during the study. However, F6 and F7 showed some grittiness particles when being applied to the skin due to the solubility of mefenamic acid in ethanol. Apart from that, an *in vitro* anti-inflammatory test had proven that mefenamic acid was still able to have its anti-inflammatory activity even though it was incorporated into the blank cream. From this study, it can be concluded that 2% of mefenamic acid cream in DMSO is suitable to be prepared topically as it shows comparable anti-inflammatory effect with that of marketed product.

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