RESEARCH ARTICLE

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Preparation, Characterization and Evaluation of Tenoxicam Gels and Microemulsion Gels for Topical Drug Delivery

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Abstract: Gels and microemulsions are classes of dosage forms that have emerged as promising drug delivery system for the delivery of various drugs. The objective of this study is to prepare tenoxicam, a non-steroidal anti-inflammatory drug (NSAID), topical gel formulae using cellulose derivatives (Sodium carboxymethyl cellulose (NaCMC), Methyl cellulose (MC), Hydroxypropyl methyl cellulose (HPMC), Hydroxy ethyl cellulose (HEC), and Hydroxy propyl cellulose (HPC) as gelling agents and to prepare topical microemulsions, microemulsion-gel formulae using (abrasol, tween 80 and tween 20 mainly as surfactants, Oleic acid and isopropylmyristate (IPM) as oils. Fifteen gel formulae were prepared (F1-F15) and evaluated for physical properties (color, clarity, homogeneity), rheological properties, pH measurements, *in-vitro* drug permeation through cellulose dialysis membranes and *in-vitro* drug permeation studies through natural rat skin. The gel formulae which showed best physical properties and highest drug permeation were chosen in the preparation of microemulsion gel formulae. Ten microemulsion formulae were prepared (M1-M10) and those with the broadest microemulsion region as shown by the phase diagrams and those with the maximum loading capacity of tenoxicam were chosen to be formulated into microemulsion gel formulae. Nine microemulsion gel formulae were prepared (S1-S9) and evaluated for physical properties (color, clarity, homogeneity), rheological properties, pH measurements, *in-vitro* drug permeation through cellulose dialysis membranes and *in-vitro* permeation studies through natural rat skin. *In-vivo* rat-paw edema tests were carried out to evaluate the activity of gel formulae and microemulsion-gel formulae which showed maximum tenoxicam permeation through rat-skin, and compared them with market product Feldene® gel (Piroxicam 0.5%) The obtained results showed that S3 microemulsion gel formula possesses the maximum drug permeation as well as anti-inflammatory activity which proved to be even greater than Feldene® gel. Therefore, S3 is effectively promising as topical anti-inflammatory dosage forms, due to the permeation enhancement caused by using microemulsions together with the gel base.

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used as analgesic, anti-inflammatory, and antipyretic. [1] Their primary use is as anti-inflammatory agents for the treatment of musculoskeletal disorders including rheumatoid arthritis, osteoarthritis and ankylosing spondylitis. [2]

Oral therapy of NSAIDs is very effective, but clinically it is usually limited because of their potential adverse effects such as irritation, ulceration of gastrointestinal mucosa, [3] bleeding, or other less frequent forms such as broncho-spasms, renal failure and other hypersensitivities. [4]

Administration of these agents through the skin can overcome the disadvantages of the oral route and may maintain relatively consistent plasma levels for long term therapy from even a single dose. [5]

Tenoxicam belongs to the well known group of Cox-II inhibitors, oxicams. It is a well-established, potent non-steroidal anti-inflammatory agent with analgesic actions achieved by inhibiting prostaglandin synthesis. [5]

Tenoxicam has been found to be approximately 99% protein bound with a mean elimination half life of 67 h, which allows the administration of a daily single oral dose of 20 mg. [6] It is widely used in various musculoskeletal disorders, arthritis, toothaches, dysmenorrheal and symptomatic relief of pain and inflammation.

The drug undergoes substantial hepatic first-pass metabolism. This creates a need for an alternative route of administration, which can bypass the hepatic first pass metabolism. [7]

Administration of tenoxicam through transdermal route offers many advantages over oral dosage forms [8-10] as it avoids problems associated with other routes of administration, [11] such as improving patient compliance in long term therapy, bypassing first pass metabolism, sustaining drug delivery, maintaining a constant and prolonged level in plasma, minimizing inter and intra-patient variability and making it possible to terminate treatment when necessary. [12-16]

Gels are a class of dosage forms created by entrapment of large amounts of aqueous or hydro-alcoholic liquid in a network of colloidal solid particles, which may consist of inorganic substances or organic polymers of natural or synthetic origin. [17]

Gels are easily prepared, highly biocompatible with a lower risk of inflammation or adverse reactions, easily applied and don't need to be removed. [18] Gels often provide a faster release of drug substance, independent of the water solubility of the drug, as compared to creams and ointments. [19] Different polymers have different mechanisms of gelation. There is not one single mechanism that can generally be applied for all polymers.

In this study five semisynthetic polymers (cellulose derivatives) were used to prepare tenoxicam topical gels. These polymers are (Sodium carboxymethyl cellulose (NaCMC), Methyl cellulose (MC), Hydroxypropyl methyl cellulose (HPMC), Hydroxy ethyl cellulose (HEC) and Hydroxy propyl cellulose (HPC).

Microemulsions improve the transdermal delivery of several drugs over the conventional topical preparations such as emulsions [20, 21] and gels. [22, 23] Mobility of drugs in microemulsions is more facile. [21, 23, 24] In microemulsions,

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the added co-surfactant lowers the interfacial tension of the surfactant film, resulting in a more flexible and dynamic layer. [21, 25, 26] In microemulsion systems, the drug can diffuse across the flexible interfacial surfactant film between the phases, which increases partitioning and diffusion into the stratum corneum.

The main transdermal flux from microemulsions has been shown to be mainly due to their high solubilization potential for both lipophilic and hydrophilic drugs. This generates an increased thermodynamic activity towards the skin. [21, 24, 27] Microemulsions may affect the permeability of drug into the skin. In this case, the components of microemulsions serve as permeation enhancers. Several compounds used in microemulsions were reported to enhance transdermal permeation by altering the structure of the stratum corneum.

MATERIALS AND METHODS

Materials

1. Chemicals

Sodium Carboxymethyl Cellulose (NaCMC) (kindly supplied by Biochemica, Switzerland), Methyl cellulose (MC), Hydroxypropyl methyl cellulose (HPMC) and Hydroxy propyl cellulose (HPC)(kindly supplied by Sedico, Cairo, Egypt), Hydroxy ethyl cellulose (HEC), and Tenoxicam (kindly supplied by E. P. I. C. O., Cairo, Egypt), Feldene® gel (Pfizer, Egypt), Sodium hydroxide (Al Nasr Company, Cairo, Egypt), Dialysis membrane tubing (MWCO 12000-14000, diameter 22mm, Servae Electrophoresis, Germany), Labrasol, Transcutol, Plurol Oleique (kindly donated by Gattefosse', France), Tween 20, Tween 80, Glycerol, Span 20, Oleic acid, Light liquid paraffin, ethanol, propylene Glycol (kindly supplied by El-Gomhoreya Chemicals Company, Egypt), Isopropyl myristate (IPM) and carageenan (kindly supplied by Sigma, USA). All other ingredients including buffer components are of analytical grade.

2. Animals

Male webmaster rats weighing 150-200 gm intact and their skin.

Preparation of Tenoxicam Gel Formulae

Cellulose derivatives were used in different concentrations to prepare fifteen gel formulae (F1-F15). Each was loaded with 1% tenoxicam. Composition of gel formulae are shown in Table 1. Gel formulae were prepared by gradually adding the polymer to (distilled water + least amount of 0.1 N Sodium Hydroxide in which tenoxicam is dissolved) while stirring on a magnetic stirrer (PYRO-Magnestir, USA). The prepared gels were stored overnight in a refrigerator.

Evaluation of Tenoxicam Gels

1. Visual Inspection

The appearance and other physical properties including color, clarity, turbidity, and precipitation were inspected. [28, 29] Results are shown Table 2.

2. Rheological Measurements and Data Analysis

The rheological properties of all the prepared gel formulae were performed at 25±0.1°C (to examine performance at room temperature) using spindle CP 40 of the Brookfield cone and plate viscometer (Brookfield DVIII, programmable rheometer of cone and plate type, thermostatic water bath, USA), with the shear rate ranging from 2 to 400 sec^{-1} corresponding to 1-200 rpm with 10 seconds between each 2 successive speeds then in a descending order. [30]

3. Spreadability

Aliquot of 1 gram of each gel formula was placed between 2 flat glass surfaces, under a pressure of 100 grams weight for one minute. The diameters of the circles created were measured before and after the addition of the weight, and then the difference was recorded for each formula. [31, 32] The experiment was run in triplicates and the average was calculated.

4. pH Measurements

pH of the fifteen gel formulae was measures before and after loading with tenoxicam 1%. One gm of each gel was diluted to 10 gm using distilled water, and the pH was determined by pH meter (Model 3510 Jenway, UK). [33] Results are shown in Table 3.

5. *In-vitro* Permeation of Tenoxicam from the Prepared Gel Formulae through Synthetic Cellulose Dialysis Membrane

Tenoxicam permeation was determined from all of the gel formulae using a dissolution apparatus (Pharma test, type PTW 2, Germany) according to the USP method type II (paddle). Two gm of different gel formulae loaded with 1 % Tenoxicam were packed into cellulose membrane tubing (length = 15 cm, diameter = 22 mm) which were tied into bags, then fixed onto the paddle. Phosphate buffer (PH= 7.4) was used as a release medium in a volume of 900 ml. The permeation study was carried out at 37°C, and the paddles were rotated at 100 rpm. Aliquots of 3 ml were withdrawn during the six hours time interval, and equal volumes of the release media were added after each aliquot to keep the volume constant throughout the experiment. Samples withdrawn were measured spectrophotometrically (UV-1800(Shimadzu), Japan) at λ_{max} 372 nm after proper dilution. The experiment was done in triplicate and the average was calculated. Results are shown in Figure 1.

6. *In-vitro* Permeation Studies of Tenoxicam Released from the Prepared Gel Formulae through Natural Skin (Rat Skin) Membranes

Skin was obtained from male webmaster rats weighing 150-200 gm. After removing hair with a depilatory, the skin was excised from the dorsal region of every sacrificed rat. The dermal surface was carefully cleaned to remove subcutaneous fats without damaging the epidermal surface. Skins were washed and examined for integrity, then stored at 2°C and used within 24 hours after the skin harvest. The protocol of the study was reviewed and approved by the institutional review board of the Drug Research Center, Cairo, Egypt. Gel formulae (F1, F7 and F10) with highest *in-vitro* permeation through cellulose membranes were

Table 1: Composition of the Prepared Gel Formulae

Formula Number	Polymer Concentration
F1	Na CMC 1 %
F2	Na CMC 2 %
F3	Na CMC 3 %
F4	MC 2 %
F5	MC 3 %
F6	MC 4 %
F7	HPMC 8 %
F8	HPMC 9 %
F9	HPMC 10 %
F10	HEC 2 %
F11	HEC 3 %
F12	HEC 4 %
F13	HPC 10 %
F14	HPC 12 %
F15	HPC 14 %

Table 2: Physical Properties of the Prepared Gel and Microemulsion Gel Formulae

Formula Number	Clarity	Color	Homogeneity	Phase Separation
F1	Clear	Light Yellow	Homogenous	No
F2	Clear	Light Yellow	Homogenous	No
F3	Clear	Light Yellow	Homogenous	No
F4	Clear	Light Yellow	Homogenous	No
F5	Clear	Light Yellow	Homogenous	No
F6	Slightly turbid	Light Yellow	Clumpy	No
F7	Clear	Light Yellow	Homogenous	No
F8	Clear	Light Yellow	Homogenous	No
F9	Slightly turbid	Light Yellow	Homogenous	No
F10	Clear	Light Yellow	Homogenous	No
F11	Clear	Light Yellow	Homogenous	No
F12	Clear	Light Yellow	Homogenous	No
F13	Clear	Light Yellow	Homogenous	No
F14	Clear	Light Yellow	Homogenous	No
F15	Clear	Light Yellow	Clumpy	No
S1	Translucent	Light Yellow	Homogenous	No
S2	Translucent	Light Yellow	Homogenous	No
S3	Translucent	Light Yellow	Clumpy	No
S4	Translucent	Light Yellow	Homogenous	No
S5	Translucent	Light Yellow	Homogenous	No
S6	Translucent	Light Yellow	Homogenous	No
S7	Translucent	Light Yellow	Homogenous	No
S8	Translucent	Light Yellow	Homogenous	No
S9	Translucent	Light Yellow	Clumpy	No

Table 3: pH of the Prepared Gels, Microemulsions and Microemulsion Gels (Medicated)

Formula	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
pH*	6.3±0.1	6.7±0.2	6.8±0.1	6.9±0.3	7±0.4	6.9±0.2	6±0.1	5.9±0.2	5.8±0.4
Formula	**F10**	**F11**	**F12**	**F13**	**F14**	**F15**	**M2**	**M3**	**M9**
pH*	6.7±0.3	6.5±0.3	6.6±0.1	6.9±0.1	6.8±0.1	6.5±0.3	5.7±0.4	6.1±0.2	5.9±0.3
Formula	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**
pH*	6.2±0.5	6.4±0.1	6.4±0.3	5.8±0.6	5.7±0.4	5.8±0.2	6.3±0.1	6±0.3	5.9±0.4

*Results are mean of 3 reading (n=3) ± standard deviation

subjected to *in-vitro* percutaneous permeation experiments through natural rat skin. Aliquots of 0.1 gm of gel containing Tenoxicam 1 % w/w were added onto prepared skin membranes (2.5 $cm^{2)}$ that were fitted onto diffusion vessels of Franz cell apparatus (Shimadzu), Japan). [34] The diffusion medium was phosphate buffer (pH = 7.4), kept at 37°C and rotated at 100 rpm.

Aliquots of 0.5 ml were withdrawn during the six hours and equal volumes of the release media were added after each aliquot to keep the volume constant throughout the experiment. Samples withdrawn were measured spectrophotometrically at λ_{max} 372 nm after proper dilution. The experiment was done in triplicate and the average was calculated. Results are shown in Figure 2a.

Table 4: Composition of the Microemulsion Systems

Formula Name	Oil	Surfactant	Co-surfactant	Water
M1	Oleic acid	Tween 80	Ethanol	Water
M2	IPM	Tween 80	Ethanol	Water
M3	Oleic acid	Tween 20	Propylene Glycol	Water
M4	IPM	Tween 20	Propylene Glycol	Water
M5	Oleic acid	Labrasol	Plurol Oleique	Water
M6	IPM	Labrasol	Plurol Oleique	Water
M7	Oleic acid	Labrasol	Transcutol	Water
M8	IPM	Labrasol	Transcutol	Water
M9	Oleic acid	Labrasol	Ethanol	Water
M10	IPM	Labrasol	Ethanol	Water

Table 5: Composition of Selected Microemulsion Systems Loaded with 2% Tenoxicam and Results of their Visual Inspection

System Number	Composition of the Midpoint Formula	Color and Clarity	Phase Separation
M2	IPM 10% - (Tween 80/Ethanol) 50% - Water 35%	Yellow, Clear	No Phase Separation
M3	Oleic acid 10% - (Tween 20/PG) 65% - Water 25%	Yellow, Clear	
M9	Oleic acid 10% - (Labrasol/Ethanol) 60% - Water 30%	Yellow, Clear	

Table 6: Composition of Various Microemulsion Gel Formulae

Formula Number	Composition of the Microemulsion Gel
S1	IPM/ Tween 80/ Ethanol/ Water- HPMC 8%
S2	IPM/ Tween 80/ Ethanol/ Water- HEC 3%
S3	IPM/ Tween 80/ Ethanol/ Water- NaCMC 1%
S4	Oleic acid/ Tween 20/ PG/ Water- HPMC 8%
S5	Oleic acid/ Tween 20/ PG/ Water- HEC 3%
S6	Oleic acid/ Tween 20/ PG/ Water- NaCMC 1%
S7	Oleic acid/ Labrasol/ Ethanol/ Water-HPMC 8 %
S8	Oleic acid/ Labrasol/ Ethanol/ Water- HEC 3%
S9	Oleic acid/ Labrasol/ Ethanol/ Water- NaCMC 1%

Table 7: Distribution of Rat Groups and Results of Rat Paw Edema Test

Formula	Name of Rat Group	Composition	Percent Inhibition of Rat Paw Edema*
Standard	A	Feldene Gel (0.5% Piroxicam)	49.6 ± 1.1%
S1	B	IPM/ Tween 80/ Ethanol/ Water- HPMC 8% (0.5% Tenoxicam)	51.73±0.9 %
S3	C	IPM/ Tween 80/ Ethanol/ Water- NaCMC 1% (0.5% Tenoxicam)	70.21±2.1 %
S9	D	Oleic acid/ Labrasol/ Ethanol/ Water- NaCMC 1% (0.5% Tenoxicam)	45.35±1.2 %
F1	E	Na CMC 1 %(0.5% Tenoxicam)	10.23±0.3 %
F7	F	HPMC 8 %(0.5% Tenoxicam)	11.84±0.7 %
F10	G	HEC 2 %(0.5% Tenoxicam)	15.79±0.9 %
-	H	Control Group	-

*Results are mean of 5 rats (n=5) ± standard deviation

Preparation of Microemulsion Systems and Construction of Pseudo Ternary Phase Diagrams

The solubility of tenoxicam in many surfactant was determined (data not shown) and it showed maximum solubility in labrasol, tween 20 and tween 80, which were used to formulate the microemulsion systems together with oleic acid and isopropylmyristate (IPM) oils.

Pseudo-ternary phase diagrams were constructed to obtain the components and their concentration ranges that result in large existence areas of plain microemulsions.

For preparation of microemulsions, blends of surfactants and cosurfactants were mixed in the desired S/COS ratio (K_m ratio) for each microemulsion system. Each of those blends was stirred using a magnetic stirrer at high speed for one hour and stored overnight. Aliquots of each blend were then mixed with each oil to give S/COS:Oil weight ratios of 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9 and their in-betweens. These S/COS/Oil mixtures were stirred using a vortex at 3000 rpm for 15 minutes until a homogenous dispersion was obtained. Water was then added drop wise by the titration method with gentle stirring until a clear microemulsion system was obtained. The prepared microemulsion systems were stored for 48 hours before any experiments or observations were made. After being stabilized, they were examined visually and determined as being liquid microemulsion or gel. [35]

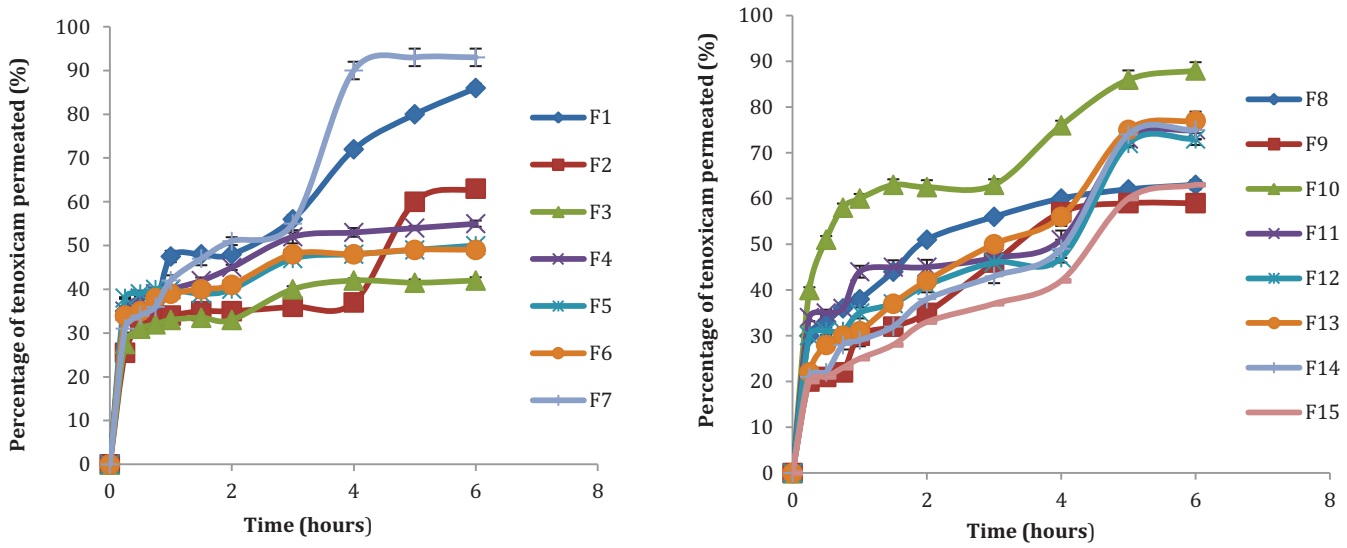

Figure 1: Percentage of tenoxicam permeated from different gel formulae through synthetic cellulose membrane

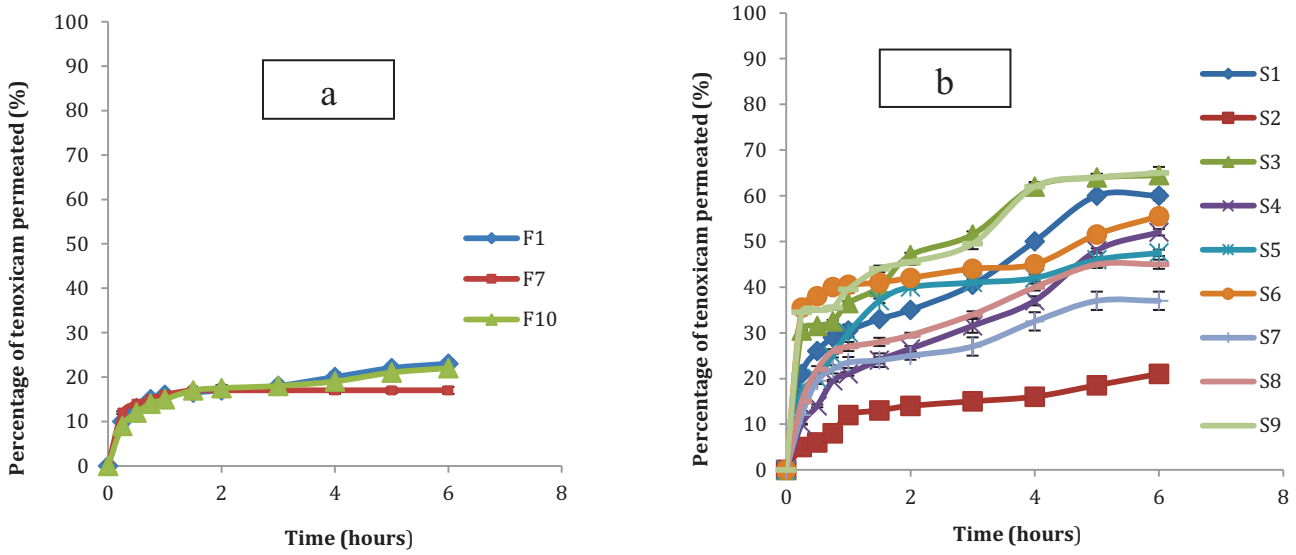

Figure 2: Percentage of tenoxicam permeated from different gel formulae (a) and different microemulsion gel formulae (b) through rat skin

Based on the pseudo-ternary phase diagrams, appropriate concentration of materials were selected and used for the preparation of microemulsion systems containing tenoxicam.

Selection of Microemulsion Bases and their Evaluation after Loading with Tenoxicam

1. Selection of Microemulsions

The prepared plain microemulsion systems (M1-M10), their composition are shown in Table 4, were examined visually for clarity, color, fluidity and phase separation. For further studies, from the constructed phase diagrams of the 10 systems, 6 were chosen according to the broadest microemulsion region. Six formulae were prepared by mixing the chosen concentrations of S/COS/Oil (w/w), mixing them using magnetic stirrer at high speed for one hour then stored overnight. Water was then added in the desired amount then the whole mixtures were vortexed for 15 minutes at 3000 rpm. The 6 systems were then loaded with increasing amounts of tenoxicam to check its solubility in them. Only 3 of them could be loaded with up to 2% tenoxicam (w/w), so those were the microemulsion systems used for further studies (M2, M3, and M9). The ternary phase diagrams of M2, M3, M9 are shown in Figure 3-5 and their composition in Table 5.

2. Evaluation of Microemulsion Systems after Loading with Tenoxicam

a. Visual Inspection

The three microemulsion systems (M2, M3 and M9), each loaded with 2% Tenoxicam (w/w) were examined visually for clarity, color, fluidity and phase separation. Table 5 shows results of visual inspection.

b. Examination under Cross-polarized Microscope

The 3 microemulsion systems (M2, M3, and M9), each loaded with 2% tenoxicam (w/w) were examined under cross polarized microscope (Olympus, Japan) to check for

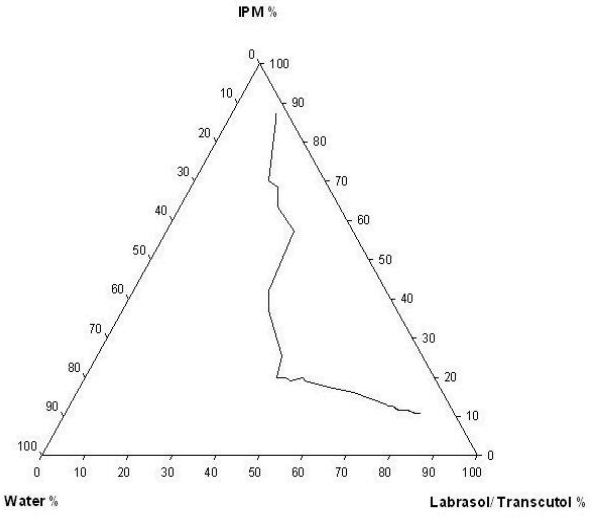

Figure 3: Showing the pseudo-ternary phase diagram of M2 at K_m = 1:1

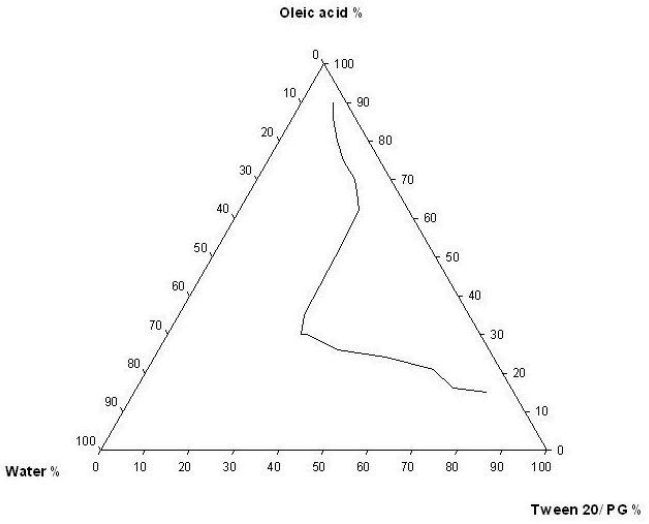

Figure 4: Showing the pseudo-ternary phase diagram of M3 at K_m = 2:1

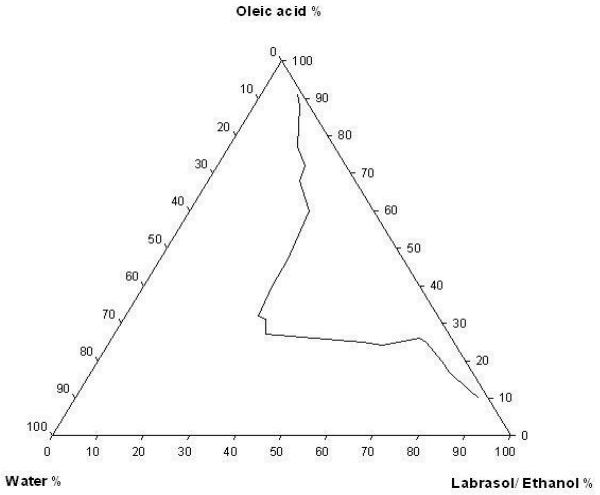

Figure 5: Showing the pseudo-ternary phase diagram of M9 at K_m = 5:1

the absence of birefringence thus to exclude liquid crystalline systems, and to verify the isotropic behavior of the prepared microemulsion systems. A drop of sample was added between a cover slip and a glass slide and then examined under polarized light.

c. pH Measurements

The pH of 10% aqueous solution of each formula in distilled water was measured by pH meter. Results are shown in Table 3.

Preparation of Microemulsion Gel Formulae

Each of the chosen microemulsion system (M2, M3, M9) were used with each of the previously chosen gel formulae (F1, F7, F10) to prepare new nine microemulsion gel formulae (S1 to S9).

The 2% tenoxicam microemulsion systems were prepared as previously mentioned.

Microemulsion gel formulae were prepared by mixing each of the prepared tenoxicam loaded microemulsion systems together with NaCMC in concentrations of (1%) w/w, HEC in concentrations of (2%) w/w; HPMC in concentrations of (8%) equal amount of distilled water (1:1) was added while stirring on a magnetic stirrer. The prepared microemulsion gel formulae (S1 to S9) were stored overnight in a refrigerator. Any air bubbles were removed by centrifugation. [36, 37] Table 6 shows the composition of various microemulsion gel formulae.

Evaluation of the Prepared Microemulsion Gel Formulae

1. Visual Inspection

Done as mentioned in the previous section. Table 2 shows physical properties of the microemulsion gel formulae.

2. Rheological Measurements, Spreadability and pH Measurements

Done as mentioned in the previous section. pH results are shown in Table 3.

3. *In-vitro* Permeation of Tenoxicam from the Prepared Microemulsion Gel Formulae through Cellulose Dialysis Membrane

Tenoxicam permeation was determined from all of the micro-

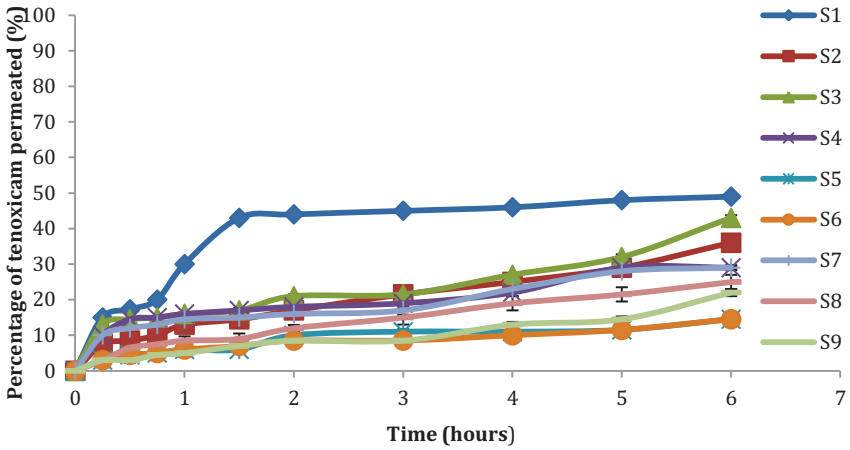

Figure 6: Percentage of tenoxicam permeated from different microemulsion gel formulae through synthetic cellulose membrane

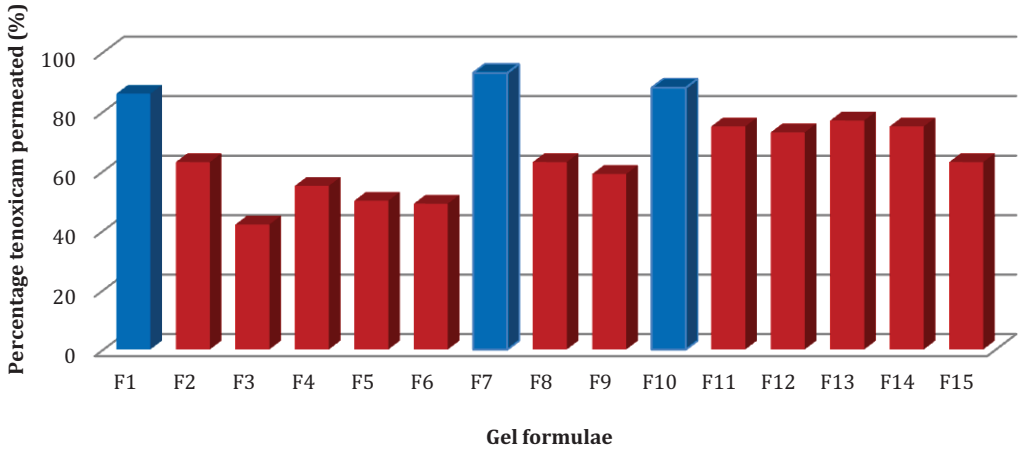

Figure 7: Percentage of tenoxicam permeated from different gel formulae through synthetic cellulose membrane after 6 hours

emulsion gel formulae (S1-S9) using a dissolution apparatus according to the USP method type II (paddle) as done in previous section. Results are shown in Figure 6.

4. *In-vitro* Permeation Studies of Tenoxicam Released from the Prepared Microemulsion Gel Formulae through Natural Skin (Rat Skin) Membranes

Skin was treated as previously stated. (S1-S9) were subjected to *in-vitro* percutaneous permeation experiments through natural rat skin as done for gel in previous section. Results are shown in Figure 2b.

Kinetic Analysis of Permeation Data of Tenoxicam Released from the Prepared Gel and Microemulsion Gel Formulae through Synthetic Cellulose Membrane and Rat Skin

Analysis of the permeation date was done according to zero order, first order, second order and diffusion kinetics.

Statistical Analysis of Permeation Data of Tenoxicam Released from the Prepared Gel and Microemulsion-Gel Formulae through Synthetic Cellulose Membranes

One Way ANOVA statistical analysis of tenoxicam permeation from the 15 gel formulae and 9 microemulsion gel formulae was done followed by multiple comparison test (post-Tukey).

Evaluation of the Anti-Inflammatory Effect of the Chosen Gel Formulae and Microemulsion Gel Formulae (Rat Paw Oedema Test)

1. Animals Used

White male webmaster rats of weight (120-150) grams were used in this study. The animals were acclimatized to the environment for one week, where they were housed under controlled environment at 25°C with a 12 hours light/dark cycle. All animals had free access to standard rodent pellet food. The protocol of the study was reviewed and approved by the institutional review board of the Drug Research Center, Cairo, Egypt.

2. Methodology

The best gel formulae (F1, F7 and F10) and best microemulsion gel formulae (S1, S3 and S9) were used. Animals were divided into 8 groups each consists of 5 animals. A group tested with Feldene gel as a standard, a control group, 3 groups for 3 gel formulae, and 3 groups for 3 microemulsion gel formulae. Table 7 shows the distribution of the 8 rat groups.

The animals were first injected with 0.1 ml of carrageenan 1% solution in saline in the planar region of the right hind paw, then the paw volume was measured after 1 hour using micrometer caliper. One gram of each formula (containing 0.5 % Tenoxicam) was applied to the

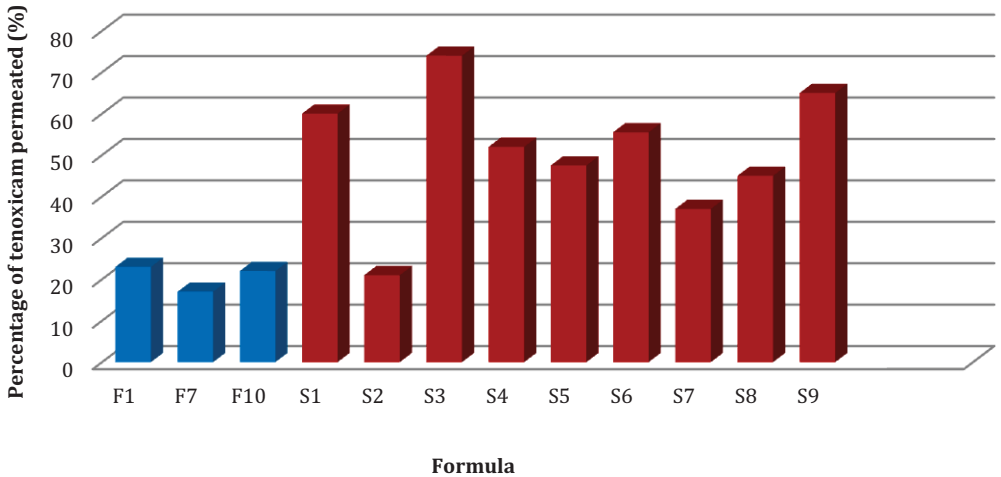

Figure 8: Percentage of tenoxicam permeated from different gel and microemulsion gel formulae through rat skin after 6 hours

right hind paw of rats. The area of application was left in place for 3 hours, after which the remaining formulae were wiped off the hind paws and then the paw volume was measured again using micrometer caliper. Feldene® gel (0.5%) was used as a reference for comparison.

The protocol of the study was reviewed and approved by the institutional review board of the Drug Research Center, Cairo, Egypt.

RESULTS AND DISCUSSION

Visual Inspection

The obtained results showed that microemulsion systems (M2, M3 and M9) were clear, yellow with no phase separation as shown in Table 5. All of the 3 systems appeared dark when viewed between the cross polarizer, and confirmed to be microemulsions showing no liquid crystals and indicating isotropic properties of the system.

All tenoxicam gel and microemulsion gel formulae are clear, homogenous, light yellow gels with no phase separation except F6 and F9 which are slightly turbid and F15, F6, S3 and S9 are clumpy as shown in Table 2.

Rheological Measurements and Data Analysis

The flow behavior of different gel, microemulsion gel formulae was studied according to power law (equation 1 and 2). [38- 40]

$$\gamma = K \sigma^n \quad (1)$$

$$\text{Log } \gamma = \text{Log } K + n \text{ Log } \sigma \quad (2)$$

Where, γ is the shear rate, σ is the shear stress, K and n are two constants.

A plot of log shear rate versus log shear stress yields a straight line of slope *n* (Farrow's constant) which is an index of deviation from the Newtonian flow behavior [38] (Data not shown).

All of the formulae had *n*>1 except F9, which means that all the prepared gel and microemulsion gel formulae possess non-Newtonian flow, shear thinning, pseudoplastic and thixotropic flow behavior which are desirable properties in topical semisolid preparations as they allow thinning of the product during application and thicker otherwise, [41,42] except F9 which is shear thickening. There's a decrease in viscosity by increasing the shear rate. The causes of pseudoplastic flow maybe due to progressive rapture of the internal structure of the formulations (by increasing shear) and its later reconstruction by means of Brownian movement. [41]

Spreadability of the Prepared Gel and Microemulsion Gel Formulae

The measured diameters give an indication of the spreadability of the gel and microemulsion gel formulae. As the diameter increases, the spreadability increases and as the viscosity increases, the spreadability decreases. The experiment was run in triplicates and the average was calculated. The measured diameters of the prepared formulae ranged from (1.9 - 3.2) cm.

pH Measurements

All the gel, microemulsion and microemulsion gel formulae have pH (5.7 - 7) which is within pH range desired for skin as shown in Table 3.

In-vitro Permeation of Tenoxicam Released from the Prepared Gel and Microemulsion Gel Formulae through Synthetic Cellulose Membrane

Figure 1 shows the permeation rate of tenoxicam released from the prepared gel formulae. Figure 7 represents a bar chart showing the percentage of tenoxicam permeated from gel formulae after 6 hours. Ranking the permeation data in a descending order for each polymer according to their ability to release the drug was as follows: (NaCMC 1% > NaCMC 2% > NaCMC 3%), (MC 2% > MC 3% > MC 4%), (HPMC 8% > HPMC 9% > HPMC 10%), (HEC 2% > HEC 3% > HEC 4%) and (HPC 10% > HPC 12% > HPC 14%).

This inversely proportional relationship between the polymer concentration and the percentage of tenoxicam permeated from a one type polymer may be attributed to the possibility that at higher polymer concentrations the active substance is trapped in smaller polymers and it is structured by its close proximity to that polymer molecule. This increases the diffusional resistance by more than

expected. [43, 44] Also it may be attributed to the increase in viscosity of the formulations which is associated with increasing the polymer concentrations. This is in accordance with Jones *et al.* [45]

The difference in permeation of drug from different polymers with different concentrations is likely to be due to chemical factors such as molecular weight, nature of the polymer, and its chain lengths. Also the viscosities of the formed gels affect the rates of permeation. [46]

MC gels exhibited lower tenoxicam permeation when compared with the other cellulose derivatives. This can be explained by the drug polymer interactions which may change the tenoxicam partition to diffusion barrier thus changing the permeation of the drug, and this is in accordance with F.P. Bonina and Montenegro. [47]

ANOVA test revealed that there is a significant difference between all of the formulae and one another except between the following which showed no significant differences at the p=0.05 level between F2, F8, F15, between F5, F6, between F10, F1, between F11, F12, F14 and between F13, F14.

Figure 6 shows the permeation of tenoxicam released from the prepared microemulsion gel formulae. Microemulsion gel formulae showed overall poor permeation of tenoxicam after 6 hours, where S1 showed the maximum percentage permeated (49%) and S5, S6 showed the least percentage permeated (14.5 %). The overall poor permeation of tenoxicam through cellulose membrane may be attributed to its relatively large solubility in the microemulsion portion due to the solubilizing capacity of microemulsions for hydrophobic drugs, consequently leading to its poor partitioning to the receptor medium. This is in accordance with the findings of Sintov and Botner. [48]

For microemulsion gel formulae, ANOVA test showed a significant difference between all of the formulae and one another.

Analysis of permeation data of tenoxicam released from the prepared gel and microemulsion-gel proved that they follow zero and diffusion kinetics.

In-vitro Permeation Studies of Tenoxicam Gel, Microemulsion Gel Formulae through Natural Skin Membranes (Rat Skin)

F1, F7and F10 possessed the highest percentage of drug permeation through synthetic cellulose membrane after six hours which were 86%, 93% and 88% respectively while they had relatively poor permeation through rat skin which is 23%, 17%, 22% respectively as shown in Figures 7, 8. This may be attributed to the poor partitioning of tenoxicam from gel bases to the skin layers, and this is in accordance with Tas *et al.* [49] The kinetics shows that the three formulae exhibited passive diffusion through the skin.

Although S1, S3, S9 possessed low percentage of drug permeation through cellulose membrane after six hours which were 49%, 43%, 22% respectively, drastic increased in permeation through rat skin to 60%, 74%, 64.5% respectively occurred as shown in Figure 8. This may be attributed to increased diffusion coefficient of drug and this is in accordance with Maghraby. [50] Moreover, the small globule size of microemulsion provides larger area for drug permeation into skin and high drug concentration in the affected area which results in a larger concentration gradient, which is essential for efficient dermal drug delivery. [51]

Analysis of permeation data of tenoxicam released from the prepared gel and microemulsion gel proved that they follow zero and diffusion kinetics.

Evaluation of the Anti-Inflammatory Effect of the Chosen Gel Formulae and Microemulsion Gel Formulae

Table 7 shows the classification of the 8 groups. Formulae S1 and S3 showed highest percent of edema inhibition after 3 hours in comparison with Feldene® gel which were 51.73%, 70.21% and 49,6 % respectively. The gel formulae F1, F7, F10 showed very low percent of inhibition of edema 10.23 %, 11.84 %, and 15.79 % respectively. S1 and S3 are the most effective formulae as shown by the rat paw edema inhibition test; they proved to be more effective than Feldene® gel.

One Way ANOVA was used for statistical analysis of percentage inhibition of rat paw edema, comparing the efficacy of the chosen gel /microemulsion gel formulae and Feldene® gel (the marketed topical oxicam product). The results showed that there was a significant difference between all groups and one another except between the following which showed no significant difference at the p value 0.05 level (between groups A, B and between groups A, D and between groups E, F, G. Therefore, S3 was the formula promising as topical anti-inflammatory dosage forms.

CONCLUSION

Tenoxicam gels and microemulsion gels showed very good physical and rheological properties. F1, F7 and F10 gel formulae possessed highest percent of drug permeation through synthetic cellulose membrane. When it comes to permeation of the drug through the skin, tenoxicam showed poor permeation from gel formulae through the skin. Consequently, when formulated into microemulsion gels, drug permeation through rat skin was greatly enhanced up to 74% in formula S3 with enhanced anti-inflammatory effect proved by rat paw edema test (70.21% inhibition)

S3 is effectively promising as topical anti-inflammatory dosage forms, due to the permeation enhancement caused by using microemulsions together with the gel base.

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Conflict of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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