Original Article

Development of a microemulsion-based formulation to improve the availability of poorly water-soluble drug

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ABSTRACT: The objective of our investigation was to design a thermo-dynamically stable microemulsion formulation of the model drug piroxicam with minimum surfactant concentration in order to improve its solubility. The solubility of piroxicam in different oils was examined. Effects of the co-surfactant:surfactant ratio and water content on microemulsion formulation were evaluated. Phase studies were performed for systems composed of oleic acid as the oil phase, Tween-80 as surfactant, and propylene glycol as co-surfactant at a constant percentage of water to elucidate the effect of microemulsion components on the area of microemulsion formulation. The viscosity and conductivity of certain microemulsion formulations were examined as a function of water dilution. The results showed that oleic acid, Tween-80, and propylene glycol resulted in the highest solubilization of piroxicam. The amount of water that was successfully incorporated into a microemulsion system was directly proportional to the co-surfactant:surfactant ratio and inversely proportional to the percentage amount of the oil phase present in the system. Microemulsion systems displayed changes in their viscosity and conductivity upon water dilution. The pre-microemulsion systems could be used as solvents to provide enhanced solubilizing capacity and stabilization for the solubilized drug. These systems could be loaded with the drug and stored in their original form in order to produce a microemulsion containing the drug *in situ* upon aqueous dilution. The incorporation of piroxicam in microemulsion formulations led to enhancement of the piroxicam release profile by allowing constant and regular *in vitro* release as well as reducing piroxicam's particle size to that suited to a microemulsion. Thus, the usage of a microemulsion technique led to improvement in piroxicam availability, suggesting the potential for technique's use as a topical vehicle for piroxicam delivery.

Keywords: Microemulsion, availability, solubility, poorly water soluble, model drug, piroxicam

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1. Introduction

Microemulsions, which are optically isotropic and thermodynamically stable systems of water, oil, surfactant, and co-surfactant, have been studied as drug delivery systems because of their capacity to solubilize poorly water-soluble drugs as well as their enhancement of topical and systemic availability. For example, oral microemulsion formulations have been successfully developed for cyclosporine, a highly lipophilic and poorly aqueous soluble drug, in order to improve its oral absorption and reduce variations in its absorption (*1*,*2*). A study also reported that the microemulsion formulation *N*-4472(*N*-[2-(3,5-di-*tert*-butyl-4-hydroxyphenethyl)-4,6-difluorophenyl]-*N'*-[4-(*N*-benzylpiperidyl)]urea), a poor water-soluble drug, significantly improved oral absorption, irrespective of whether the subject was fed (*3*). The improved absorption from a microemulsion is presumably due to incorporation of the drug into microemulsion droplets. Smaller microemulsion droplets result in an increased specific surface area and increased membrane permeability of the drug *via* solubilization of certain membrane components and pore formation. All these factors lead to enhanced contact with the gastro-intestinal tract. Another important factor is the inner polarity of droplets, which is governed by the hydrophilic-lipophilic balance of surfactant used. A change in droplet polarity may affect the arrangement of the drug and surfactant on the droplet interface and alter drug release (*4*). Microemulsions have also been considered as topical (*5*), transdermal (*6*), and parenteral drug delivery systems (*7*), and several studies have reported the use of a microemulsion as a nasal drug delivery system (*8*). In the present

work, different pre-microemulsion and microemulsion formulations was prepared in order to improve piroxicam availability. This was done by selection of the most suitable microemulsion components that led to the highest solubilization of piroxicam. Then, the selected microemulsion formulations were characterized to help in selecting the most suitable formulation.

2. Materials and Methods

2.1. *Materials*

Piroxicam was purchased from El-Nasr Pharmaceutical Chemicals Co., Cairo, Egypt (Batch No. 20030202). Castor oil and dimethylsulfoxide (DMSO) were from El-Gomheria Pharmaceutical Co., Cairo, Egypt. Olive oil was from El-Fayrouz Pharmaceutical Co., Cairo, Egypt. Linseed oil, coconut oil, turpentine oil, oleic acid, and propylene glycol were from Morgan Pharmaceutical Co., Cairo, Egypt. Paraffin oil was from SMGO Pharmaceutical Co., Cairo, Egypt. Peanut oil was from Sigma-Aldrich, St Louis, MO, USA. Tween-80, Tween-60, Tween-40, Span-80, and *n*-butanol were from ADWIC Pharmaceutical Co., Cairo, Egypt. Tween-20 was from Merck KGaA, Darmstadt, Germany.

2.2. *Solubility studies of piroxicam in different oils, surfactants, and co-surfactants*

The solubility of piroxicam was examined in different oils, *i.e.,* castor oil, olive oil, linseed oil, paraffin oil, coconut oil, turpentine oil, oleic acid, and peanut oil; surfactants, *i.e.,* Tween-20, Tween-40, Tween-60, Tween-80, and span-80; and co-surfactants, *i.e., n*-butanol and propylene glycol. The equilibrium solubility method was performed as follows. Briefly, an excess amount of piroxicam was added to 10 mL of each solvent (the aforementioned oils, surfactants, and co-surfactants) in 30 mL screw-capped vials and the whole mixture was mixed by vortexing. The vials were then shaken at 37°C for 72 h at 100 rpm in a thermostatically controlled water bath shaker (Weiss Gallenkamp, Loughborough, UK). Then, the supernatant layer was separated and subjected to centrifugation at 3,000 rpm for 5 min in order to remove the undissolved drug. Samples of these solutions were then collected and the drug concentration was determined spectrophotometrically at 350 nm against a suitable blank of DMSO using an ultraviolet spectrophotometer SP6-550 (Pye Unicam, Cambridge, England). All experiments were performed in triplicate.

2.3. *Microemulsion formulation and phase diagram preparation*

The selected oil, surfactant, and co-surfactant from the aforementioned solubility studies were used to formulate microemulsions and prepare phase diagrams. The microemulsion domains were distinguished by the corresponding phase diagrams. The microemulsion phases were identified as the area in the phase diagram where a clear and transparent formulation was produced based on visual inspection of numerous samples. No attempts were made to distinguish among the true solutions, micelles, bicontinuous structures, w/o (water dispersed in oil) and o/w (oil dispersed in water) microemulsions, *etc.* The domains of existing transparent, isotropic systems were considered to correspond to the microemulsion phases. Phase diagrams were determined at room temperature (approximately 25°C) as outlined by Li *et al.* (*8*) with slight modifications. The ternary phase diagrams of surfactant, co-surfactant, and oil were prepared at a constant percentage of water from 0 to 400% of total initial weight of surfactant, co-surfactant, and oil mixtures, with mixtures containing 0% water referring to pre-microemulsion. For initial determination of microemulsion phase areas within the entire phase diagram, about 36 sample mixtures (based on 10% change in weight) of oil, surfactant, and co-surfactant were carefully weighed, mixed with the aid of a vortex, and visually inspected for phase clarity and flowability. For more exact determination of the areas corresponding to pre-microemulsions, additional mixtures of surfactant, co-surfactant, and oil were prepared with a concentration change rate of 5% for each component at the boundary obtained from the previous step. Samples were then titrated with water in a drop-wise manner and mixed thoroughly by vortexing until clear and transparent microemulsion phase regions could be identified. Once the microemulsion phase was identified, additional samples were prepared to determine the boundary regions. No heating was used during the preparation. The clear areas corresponding to either pre-microemulsions or diluted microemulsions were depicted in a triangular phase diagram using AutoCAD 2000 from Microsoft.

2.4. *Preparation of microemulsions containing piroxicam*

Piroxicam was accurately weighed and simply added to the selected pre-microemulsion bases from the prepared phase diagrams. Vortexing was required to dissolve piroxicam completely in microemulsion systems. The final piroxicam concentration was adjusted to 0.5% w/v.

2.5. *Characterization of the selected microemulsions*

The following methods were used to characterize microemulsions.

2.5.1. *Determination of the particle size of microemulsions*

The particle size was determined for both pre-microemulsion formulations (*i.e.,* piroxicam-free

formulations) and microemulsions containing piroxicam using a JEOL Transmission Electron Microscope (JTEM) model 1010 (JEOL, Tokyo, Japan).

2.5.2. *Drug solubility in microemulsion components*

An excess amount of piroxicam was added to each oil, Tween-80, propylene glycol, and water in 30 mL screw-capped vials and the whole mixture was mixed by vortexing. The vials then were shaken at 37°C for 72 h at 100 rpm in a thermostatically controlled water bath shaker. Then, the supernatant layer was separated and subjected to centrifugation at 3,000 rpm for 5 min in order to remove the undissolved drug. Samples of these solutions were then collected and the drug concentration was determined spectrophotometrically at 350 nm against a suitable blank of DMSO. Samples with the same composition (without the drug) were treated similarly and used as a control.

In order to examine the effect of the co-surfactant: surfactant ratio on the solubility of piroxicam, an excess amount of the drug was added to pre-microemulsion concentrate with weight ratios of co-surfactant (propylene glycol) to surfactant (Tween-80) ranging from 0.29:1 to 2:1. After equilibration of 72 h at ambient temperature, the equilibrated samples were centrifuged at 3,000 rpm for 5 min to remove the undissolved drug. Drug free samples with the same composition were treated similarly and used as a control.

To examine the effect of water content in microemulsion formulations on the solubility of the drug, an excess amount of the drug was added to each ample containing different percentages of water ranging from 0 to 200% of total pre-microemulsion weight and the procedure for drug separation and analysis was repeated as mentioned above.

2.5.3. *Viscosity of microemulsions*

The effect of water dilution on microemulsion viscosity was studied using a Brookfield DV viscometer (model DV-II+; Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA) with a number 0 spindle at 50 rpm at room temperature (25°C). Samples of pre-microemulsion and microemulsion formulations were prepared and viscosity measurements were carried out at different concentrations of water (from 0 to 200% of total pre-microemulsion weight) in order to determine the effect of water dilution on the microemulsion structure.

2.5.4. *Electrical conductivity*

Conductivity measurements were carried out to demonstrate the effect of water dilution on microemulsion structure. Microemulsion formulations were chosen at different percentages of water (from about 10 to 400% of total microemulsion weight). Measurements were done at 25°C. The adherence of surfactant on the electrode and the cell inner wall was avoided by pre-washing the cell twice with the sample to be measured before each measurement.

2.5.5. *Thermodynamic stability of microemulsions*

Thermodynamic stability was examined for both pre-microemulsion formulations (*i.e.*, piroxicam-free formulations) and microemulsions containing piroxicam through the following procedures: (*i*) *Heating-cooling cycle* – Six cycles were carried out between refrigerator temperature (4°C) and 45°C with storage at each temperature of no less than 48 h. The formulations that were stable at these temperatures were subjected to a centrifugation test. *(ii*) *Centrifugation test* – Passing formulations were centrifuged at 3,500 rpm for 30 min. Those formulations that had no phase separation were used in a freeze-thaw stress test. (*iii*) *Freeze-thaw cycle* – Three freeze-thaw cycles were carried out between –21°C and 25°C with storage of formulations at each temperature for no less than 48 h (*9,10*).

2.5.6. *Physical stability of microemulsions*

Pre-microemulsions and microemulsions with different water concentrations were visually inspected over 6 months for any signs of drug precipitation, phase separation and/or color change. Viscosity measurements and repeated centrifugation of the system were carried out for 30 min at 13,000 rpm at specified time intervals to ensure the stability of the system formed (*11*).

2.5.7. *In vitro release studies for piroxicam*

In vitro release studies of piroxicam from the microemulsion bases were carried out using PWIT11 USP dissolution test apparatus (Pharma Test, Hainburg, Germany) with the temperature of the water bath kept at 37 ± 2°C according to the manufacturer's instructions. The dissolution apparatus was adapted for semi-solid pharmaceutical drug dosage forms and was set up as follows: the dissolution medium, 300 mL phosphate buffer, pH 7.4; diffusion system with static cell, 120 rpm. A synthetic cellulose acetate membrane (7.54 cm, Fischer Scientific Co., London, UK) previously treated with distilled water at 100°C for 5 min and maintained at 4°C was fixed at the end of a glass tube of a diffusion cell that was manufactured at the Faculty of Science, Ain-Shams University, Cairo, Egypt. The experimental procedure was carried out using 2 mL of either pre-microemulsion or microemulsion. The analysis was preformed with 2 mL samples taken from the dissolution medium at 15 min intervals. The removed samples were replaced by equal volumes of phosphate buffer of the same pH to maintain a constant volume for the receiving medium. Control samples with the

same composition of oil, surfactant, and co-surfactant were treated as before in order to eliminate the effect of microemulsion components on the UV absorption of piroxicam. The amount of the drug released from the formulations was determined spectrophotometrically at 350 nm by measuring the test samples against blank samples. Experiments were performed in triplicate and mean results were reported (*12*).

3. Results and Discussion

3.1. *Solubility studies of piroxicam in various solvents including water, different oils, surfactants, and co-surfactants*

Identifying an appropriate solvent to dissolve piroxicam and then formulating microemulsion formulations is crucial because only the dissolved drug can penetrate the skin. In order to screen appropriate solvents for the preparation of microemulsions, the solubility of piroxicam in various solvents including oils, surfactants, and co-surfactants was measured and the obtained results were summarized in Table 1. The solubility of piroxicam in oleic acid was found to be 12.6 mg/mL. This value was the best among all the investigated oils, but it was still much lower than that of Tween-80, which dissolved piroxicam of up to 17.8 mg/mL (Table 1). In addition, propylene glycol had better piroxicam solubility than *n*-butanol (Table 1).

As mentioned above, piroxicam is known to be water-insoluble. This fact has been proven by the experimental work in this study as water had the lowest solubility with respect to piroxicam among the investigated solvents. Piroxicam solubility in water was 0.0836 mg/mL (Table 1), which equals 0.66%, 0.47%, and 1.3% of piroxicam solubility in oleic acid, Tween -80, and propylene glycol, respectively.

These results revealed that the solubility of piroxicam in oleic acid, Tween-80, and propylene glycol was 150.8, 212.4, and 76.78 times the aqueous solubility of piroxicam. Therefore, oleic acid was selected as the oil phase, Tween-80 as the surfactant, and propylene glycol as the co-surfactant in this study.

Table 1. Solubility of piroxicam in microemulsions with different components

Components of microemulsion	Piroxicam solubility (mg/mL)
Water	0.0836
Castor oil	2.72
Linseed oil	4.65
Coconut oil	4.37
Oleic oil	12.6
Olive oil	3.78
Paraffin oil	0.133
Turpentine oil	1.52
Peanut oil	2.98
Tween-80	17.8
Tween-60	17.2
Tween-40	13.6
Tween-20	17.6
Span-80	2.68
Propylene glycol	6.42
n-Butanol	4.33

3.2. *Microemulsion formulation and phase diagram preparation*

The method used to prepare the phase diagrams of microemulsions was slightly modified, as described before (*8*). All possible regions for microemulsion formation at all possible ratios of surfactant:co-surfactant:oil were represented. The microemulsion phases were identified as the area in the phase diagram where a clear and transparent formulation was produced based on visual inspection of numerous samples. No attempts were made to distinguish among the true solutions, micelles, bicontinuous structures, w/o and o/w microemulsions, *etc*. The domains of existing transparent, isotropic systems were considered to correspond to the microemulsion phases. The ternary phase diagrams of surfactant, co-surfactant, and oil were determined at a constant percentage of water from 0 to 200% of total initial weight of surfactant, co-surfactant, and oil mixtures, with mixtures containing 0% water referring to a pre-microemulsion (Figure 1). Based on visual identification, regions corresponding to clear isotropic systems were considered microemulsion areas (gray shaded areas in Figure 1) while clear, highly viscous systems were considered gel areas (black-shaded area in Figure 1). The rest of the phase diagrams consisted of regions corresponding to turbid and conventional emulsion systems. The effect of water concentration on the areas of isotropic regions was evident in the given phase diagrams.

3.2.1. *Effect of water dilution on the area of microemulsions*

Based on visual observations, there was a complete separation of the phases or turbidity for the formulations that did not contain either surfactant or co-surfactant, respectively, regardless of the percent of water added. As the percent of water added increased, the clear isotropic area decreased until the percent of water added was from 60% to 400% of the total initial weight. The clear isotropic area disappeared in all the formulations investigated (Figure 1). Upon addition of 50% water, an oleic acid/Tween-80/propylene glycol system started to form a translucent gel phase (Figure 1), the area of which decreased in size with further dilution and which completely disappeared at 100% dilution of the system. Whether or not the observed transformation of the clear pre-microemulsion areas to turbid phases was preceded by translucent gel phases upon dilution may be due to a conversion to macroemulsion phases (*13*). Upon dilution with small portions of water, the pre-microemulsion system may be converted into w/o

microemulsions, particularly at higher ratios of the oil phase. However, upon dilution with excess aqueous phase w/o microemulsions are inverted into o/w emulsions, microemulsions, and/or w/o/w emulsions; a number of liquid crystalline phases are considered to be possible intermediates during this phase inversion process (*14*). Systems that remained clear and fluid at higher dilutions with the aqueous phase are expected to be of the o/w type where the oil phase ratio is lower.

3.2.2. *Effect of oil properties on the area of microemulsions*

An oil's properties can affect the production of microemulsions. The formation of a microemulsion is favored when small molecular weight oils are present. Unfortunately, pharmaceutically acceptable oils tend to be of large molecular weight and semi-polar in nature. This fact, together with the oil's properties and concentration, is important in determining the drug loading capacity of any microemulsion. Thus, examining the effect of the oil on microemulsion formation is essential (*15*).

Oleic acid is an oil with a relatively large molecular volume (*15*). In an oleic acid system, the pre-microemulsion had a large, clear isotropic area at the beginning of phase determination. However, upon addition of 10% water a great reduction in the isotropic clear area was observed (Figure 1). The oleic acid system started to develop a clear viscous gel area at 50% dilution with water. This viscous gel area appeared at only 50% dilution with water and promptly disappeared when this dilution limit was exceeded. These results agreed with those of Mokhtar *et al.* (*16*), who studied the effect of different oils on the microemulsion area formed. An explanation of oleic acid behavior, the miscibility of oleic acid with propylene glycol, and the lipophilicity of oleic acid must be taken into consideration. Oleic acid is miscible with propylene glycol. This could account for the difference in the area of the pre-microemulsion systems when comparing the behavior of oleic acid to that of different oils (*16*). The formation of the gel area during dilution with an increasing amount of water (50%) could be due to conversion from a w/o to an o/w microemulsion system (*13*). The roughly constant clear isotropic region at dilutions from 10 to 30% in an oleic acid system is an indication of a solubilized system where the incorporated water is miscible with propylene glycol and can solubilize Tween-80 while propylene glycol could be considered a co-solvent for oleic acid. The system in this area could be considered to be saturated with respect to both oleic acid and water. Upon further dilution, a gel phase was generated at 50%, followed by a sharp reduction in the clear isotropic area at 100% dilution due to system conversion. This may be due to the dilution of the surfactant Tween-80 to levels below effective oil solubilization (*16,17*).

Thus, the most suitable formulations for preparation of microemulsion bases containing piroxicam were selected based on the data in Table 1 and the corresponding phase diagrams (Figure 1). These formulations were then studied to further characterize their microemulsion properties. Table 2 summarizes the composition of the selected microemulsion formulations that were chosen for further investigation.

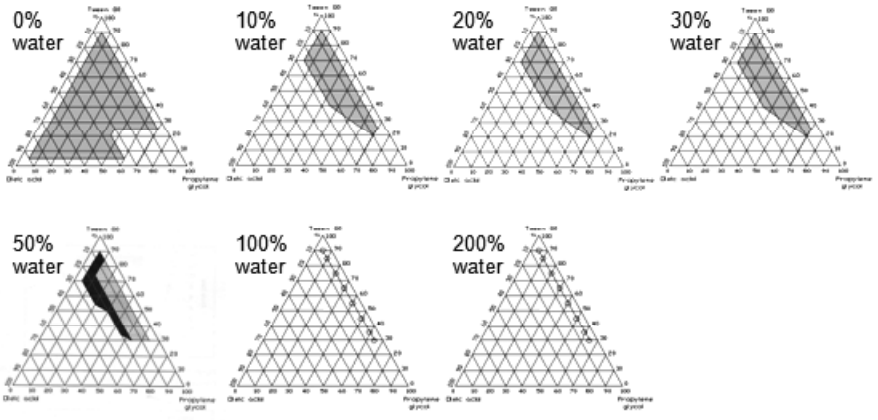

Figure 1. Triangular phase diagrams of different systems with varied water content. Grey and black shaded areas represent clear isotropic microemulsion and clear gel areas, respectively.

Table 2. Composition of the microemulsion formulations selected for further investigation

No.	Oil (%)	Surfactant (%)	Co-surfactant (%)	Water (%)	Drug (%)
F1	10	70	20	50	0.5
F2	10	60	30	50	0.5
F3	10	50	40	50	0.5
F4	10	40	50	50	0.5
F5	10	30	60	50	0.5

3.3. *Particle size of microemulsions*

Transmission electron microscopy is one of several techniques used to measure the size of microemulsion droplets. In this study, all the selected microemulsion samples, regardless of whether or not they contained piroxicam, had a particle size ranging from 100 nm to 500 nm. This falls within the range for the particle size of a microemulsion preparation (data not shown). Transmission electron microscopy revealed that pre-microemulsion formulation No. 2 and the corresponding formulation with the drug produced the best images among the investigated formulations (Figures 2 and 3).

Figure 2 shows TEM photos of the formulation No. 2 pre-microemulsion. In photos 2A and 2B (magnification, ×40,000 and ×50,000, respectively), the spherical shape of microemulsion droplets with a particle size of 500 nm is apparent.

Figure 3 shows TEM photos of formulation No. 2 microemulsion containing piroxicam. As is apparent from photos 3A and 3C (magnification, ×30,000 and ×40,000, respectively), piroxicam was incorporated in the spherical shape of the microemulsion droplets while retaining a particle size of 500 nm. This suggests that piroxicam's particle size was reduced to a size suited to a microemulsion in order for piroxicam to be incorporated in the microemulsion droplets. There was no change in the microemulsion particle size upon incorporation of piroxicam inside the microemulsion droplets since the photos indicate that the particle size remained 500 nm.

3.4. *Drug solubility in microemulsion components*

The development of a microemulsion system for the pharmaceutical delivery of both poorly soluble and slightly water-soluble drugs requires selection of a suitable surfactant, co-surfactant, and oil. The solubility of piroxicam was determined in each component of the microemulsion system. The solubility of piroxicam in the microemulsion components was determined by the equilibrium solubility method as described in the "Materials and Methods", and the results obtained are shown in Table 1.

3.5. *Effect of the co-surfactant:surfactant ratio on the solubility of piroxicam*

Since the pre-microemulsion system was used as a solvent to provide a better solubilization capacity and stabilization for the solubilized drug, the effect of the formulation parameters on drug solubility in such systems must be studied. These systems could be loaded with drugs and stored as-is in order to produce a microemulsion *in situ* upon aqueous dilution. As Figure 4 clearly shows, the solubility of piroxicam decreased from 5.874 to 3.294 mg/mL as the PG/T80 ratio increased from 0.29:1 to 1:1 when using a constant OA concentration (10%). This was followed by an increase to 3.7 mg/mL as the ratio increased to 1.25:1 and then again by a decrease to 3.1 mg/mL when the ratio reached 2:1. These results indicate that the highest solubility of piroxicam was found to occur at co-

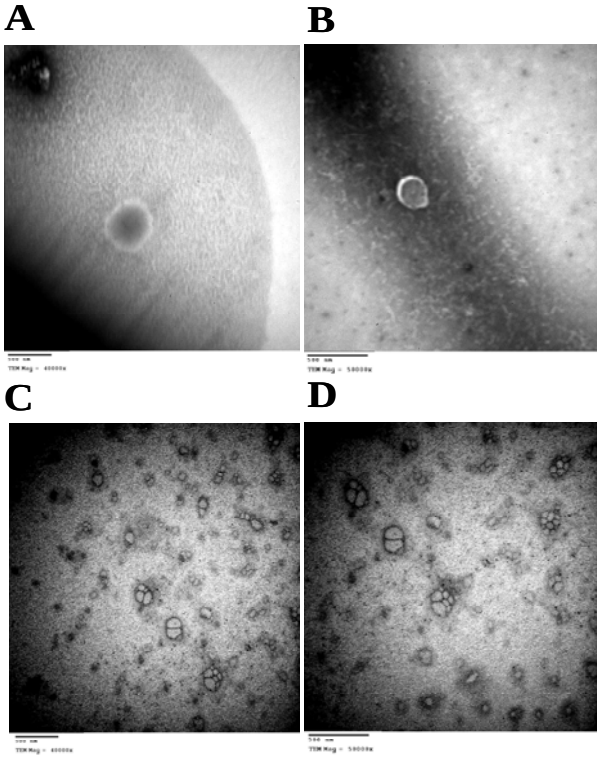

Figure 2. TEM photos of pre-microemulsion formulation 2.

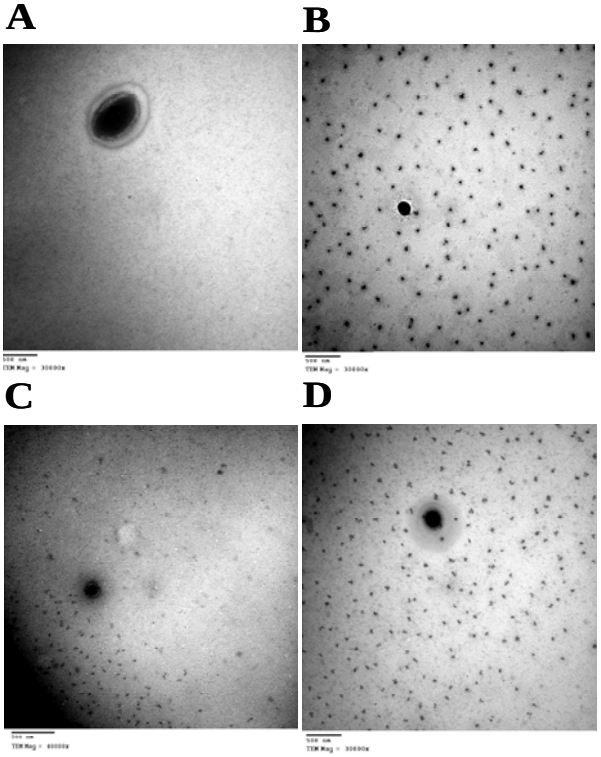

Figure 3. TEM photos of microemulsion formulation 2.

surfactant/surfactant ratios between 0.29:1 to 0.8:1 for all the systems studied

3.6. *Effect of water content in microemulsion formulations on the solubility of piroxicam*

Dilution of a microemulsion is very common both during mixing with other aqueous systems for reconstitution or in biological fluid after administration. Decreased drug solubility in microemulsion systems on dilution could be ascribed to the decreased concentrations mainly responsible for enhanced solubility (oil, surfactant, and co-surfactant).

Since orientation of microemulsion components is dependent on the enhanced solubility of drugs to a great extent, addition of components that can disturb this orientation (for example, addition of water to a microemulsion containing somewhat highly hydrophobic components such as oleic acid) could lead to decreased solubilizing capacity or even instability of the system itself (*16*).

In this study, water dilution of the microemulsion formulations greatly affected piroxicam solubility in all tested formulations. Solubility profiles are shown in Figure 5. The obtained results revealed a strange pattern of piroxicam solubility in each microemulsion formulation. To be more specific,

Microemulsion formulation 1 (*ME F1*): After 10% water was added, there was a decrease in solubility followed by an increase in solubility when the percent of water added reached 25%. A decrease in solubility occurred when the percent of water added reached 50%, followed by an increase in solubility when the percent of water added reached 100%. Then, a sharp decrease in solubility occurred when the percent of water added reached 200%.

Microemulsion formulation 2 (*ME F2*): There was a sharp decrease in solubility as the percent of water added increased from 0% to 25%. This was followed by an increase in solubility when the percent of water added reached from 50% to 100%. A decrease in solubility occurred again when the percent of water added reached 200%.

Microemulsion formulation 3 (*ME F3*): After 10% water was added, there was an increase in solubility followed by a sharp decrease in solubility when the percent of water added reached 25% to 50%. This was followed by an increase in solubility when the percent of water added reached 100%. A decrease in solubility occurred when the percent of water added reached 200%.

Microemulsion formulation 4 (*ME F4*): After 10% water was added, there was an increase in solubility followed by a small decrease in solubility when the percent of water added reached 25% to 50%. This was followed by a sharp decrease in solubility when the percent of water added reached 100%. A small increase in solubility occurred when the percent of water added reached 200%.

Microemulsion formulation 5 (*ME F5*): After 10% water was added, there was an increase in solubility followed by a decrease in solubility as the percent of water added reached 25%. This was followed by an increase in solubility as the percent of water added reached 50%. Then, there was a slight decrease in solubility as the percent water added reached 100%. A sharp decrease in solubility then occurred as the percent of water added reached 200%.

3.7. *Viscosity of microemulsions*

Interpretation of the viscosity of microemulsions is problematic not because of uncertainties about the role of the interfacial region but due to the difficulty in obtaining meaningful measurements. When microemulsion particles are spherical, they follow a Newtonian dispersion, and when the system undergoes a transition from spheres to cylinders or lamellae the viscosity changes abruptly and the flow is described as non-Newtonian (*14*). The extent of dilution could have

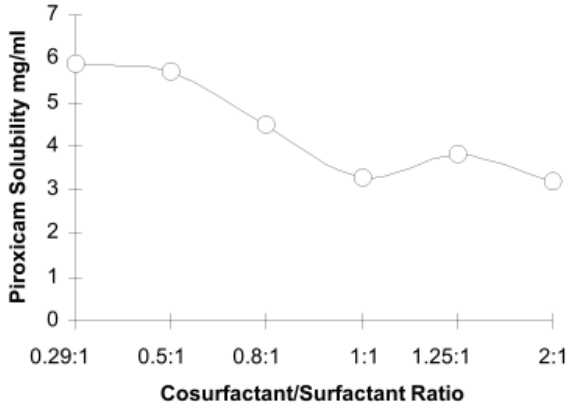

Figure 4. Effect of the co-surfactant-to-surfactant ratio on the solubility of piroxicam.

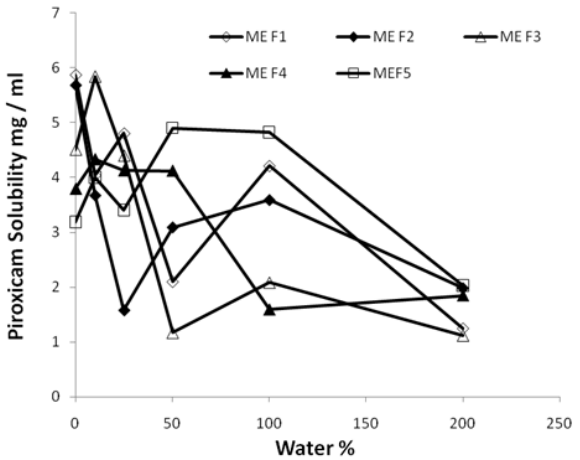

Figure 5. Summary of the effect of water content on the solubility of piroxicam.

a dramatic effect on the viscosity of a microemulsion. The viscosity of the tested microemulsion samples initially increased but then decreased upon dilution with water. The maximum increase in viscosity was observed at 50% aqueous dilution followed by a sharp decrease in viscosity afterwards. The increase in viscosity upon water dilution may be attributed to (*i*) an increase in the degree of hydration of the very hydrophilic polyoxyethylene oxide head groups of the surfactant and also to (*ii*) the presence of propylene glycol, which increases the hydrophilicity of the surfactant (*18*). The results obtained are shown in Figure 6.

3.8. *Electrical conductivity of microemulsions*

Electrical conductivity measurements can be used to analyze the microstructure of a microemulsion. An o/w microemulsion has a conductance in the same range as the conductance in the neat aqueous phase, and the conductance in a w/o microemulsion is typically four to five orders of magnitude lower. In a bicontinuous case, both water and oil self-diffusion coefficients are of the same order of magnitude as in the neat liquid (*16-19*).

The great changes in conductivity with water dilution can be attributed to phase inversion from reverse swollen micelles (w/o) to direct micelles (o/w). A constant correlation could exist between the specific structure and the electrical conductivity of the microemulsion, providing support for the use of electroconductivity measurements to localize bicontinuous media on the diagrams (*6*).

The concept of percolation transition proposed by de Gennes and Taupin (*20*) was used to interpret the conductivity of disordered media such as microemulsions (*21*). In such systems (microemulsions), conductivity is governed by a universal law independent of the physical properties of the medium such that

$$K = (Q_w - Q_p)t$$

where K is the conductivity in millivolts (mV), Q_w is the water volume fraction (dispersed volume fraction), Q_p is the dispersed volume fraction at the percolation threshold, and t depends on the system dimensionality (t = 1.5-1.6 for a three-dimensional system).

In the present work, the conductance values (K) in the selected microemulsions were examined to see if they followed this law or not (the data is shown in Figure 7). In order to determine Q_p (the percolation threshold), $K_{1/t}$ was plotted *versus* Q_w. Figure 8 represents the percolation threshold of the five formulations investigated. For t = 1.5, a linear correlation between $K_{1/t}$ and Q_w was noted. Similar results were obtained by Thevenin *et al*. (*6*), who posited that the intrinsic Q_p value depends on the droplet size and the interaction between them. Thevenin *et al*. also indicated that the low value of the threshold is the consequence of the attractive interactions of the conductive species in the system. An interesting finding from the current study is that the highest threshold

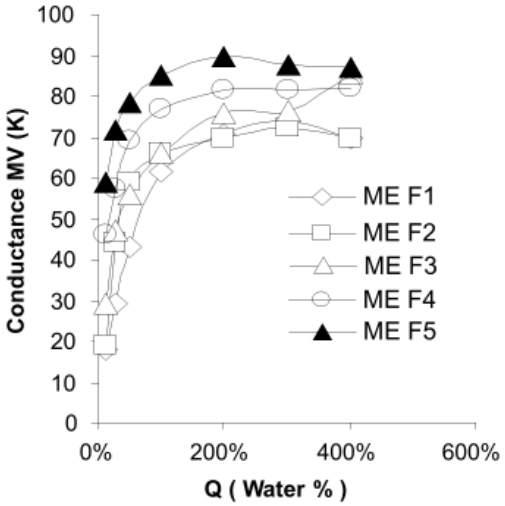

Figure 7. Electro-conductivity of selected microemulsion formulations with different water content.

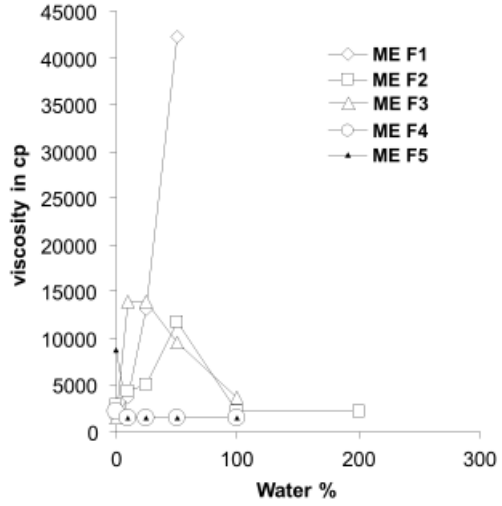

Figure 6. Effect of water content on the viscosity of microemulsion formulations.

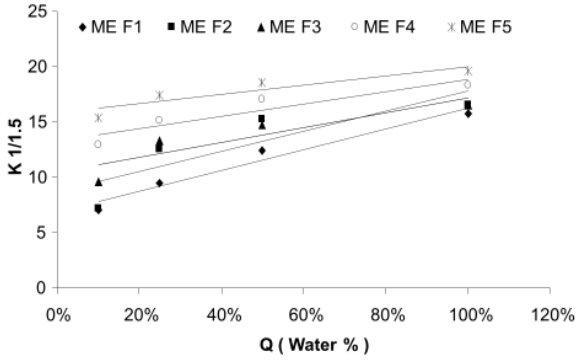

Figure 8. Percolation threshold determination.

value was obtained with a more lipophilic co-surfactant, which should require more dilution before transition to a bicontinuous system.

3.9. *Thermodynamic stability of microemulsions*

Thermo stability differentiates nano- or microemulsions from emulsions that have kinetic stability and will eventually display phase separation (*9,10*). All tested formulations succeeded in passing thermodynamic stability tests such as a heating-cooling cycle test, centrifugation test, and a freeze-thaw stress cycle test.

3.10. *Physical stability of microemulsions*

Pre-microemulsion and microemulsion formulations with different levels of water dilution were stored at 25°C and protected from light for about six months. The tested pre-microemulsion formulations remained in a single phase, were clear, and had no changes in color or viscosity (data not shown). In addition, the microemulsion formulations containing piroxicam were exposed to the same test conditions and the results clearly revealed that there was no drug precipitation and no color or viscosity changes (data not shown).

3.11. *In vitro release studies with piroxicam*

In vitro release of piroxicam was performed as described in the "Materials and Methods". The release profiles of piroxicam from each formulation were determined by plotting the percentage of piroxicam released over time in minutes (Figure 9). The results of piroxicam release were compared to those of a piroxicam solution in phosphate buffer, pH 7.4.

As shown in Figure 9, after 5 h about 74.2%, 62.4%, 52.2%, 44.9%, and 40.6% of piroxicam was released from ME F2, ME F1, ME F4, ME F3, and ME F5, respectively, whereas about 100% of the drug was released from the aqueous buffer solution of the drug over the same period of time. This suggests that free piroxicam is released very quickly from the buffer solution. Since the microemulsion formulations have higher viscosity than the reference solution, piroxicam was quickly released from this solution. The data clearly revealed that the release rates of piroxicam depended on the viscosity of the system. This agrees with data from Attwood and Florence (*14*), who stated that the rate of drug release from microemulsion formulations depended on the vehicle used, the viscosity of the system, and the existence of surfactant micelles. This conclusion also agrees with the results obtained by El-Badry (*22*).

The effect of vehicle viscosity on the amount of drug released was studied, and the amount of drug released was found to be inversely proportional to the viscosity of the vehicle used but independent of the

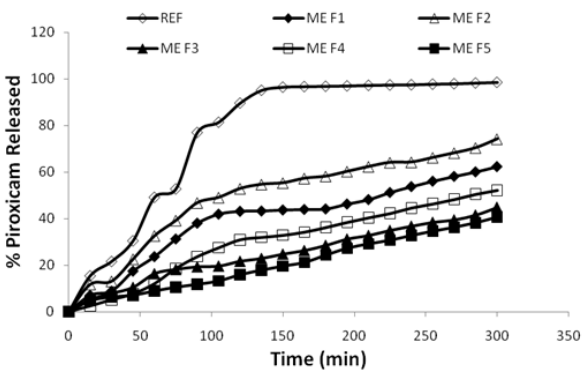

Figure 9. Percent release of piroxicam from microemulsion formulations as compared to a piroxicam solution in phosphate buffer, pH 7.4.

nature of viscolizer used. This conclusion agrees with the data from Mokhtar *et al.* (*16*), who studied the *in vitro* release of atenolol from different microemulsion bases. Mokhtar *et al*. found that the release rates of atenolol depended substantially on the vehicle used, the viscosity of the system, and the existence of surfactant micelles. The present results also agree with data from Dalmora *et al.* (*12*), who studied the effect of incorporation of piroxicam in positively charged microemulsions on the release profile. Dalmora *et al*. found that this incorporation resulted in a maximum release level about 3.6-fold lower than that of the control solution (the same concentration of piroxicam in a phosphate buffer, pH 5.5). Therefore, the previous data imply that the incorporation of piroxicam in the microemulsion formulations may result in less release than from the reference solution (*i.e.,* an aqueous buffer solution of piroxicam) over the same period of time. Another more important characteristic observed in the systems containing a microemulsion is the capacity for the internal phase (the oil) of the microemulsion to retain piroxicam. This allowed a constant and regular release over time in comparison to the reference solution.

4. Conclusion

Solubility studies showed that oleic acid, Tween-80, and propylene glycol resulted in the highest solubilization of piroxicam, and thus a triangular phase diagram was prepared using these components. In accordance with the prepared triangular phase diagrams, the most suitable microemulsion formulations were selected for further investigation. The selected microemulsion formulations were then characterized. Particle size measurement proved that all the investigated formulations, regardless of whether they had only microemulsion bases (*i.e.,* piroxicam-free formulations) or contained piroxicam, had a particle size of 500 nm, which falls within the range for the particle size of a microemulsion. Both physical and thermodynamic

stability tests proved that all the selected microemulsion formulations were physically and thermodynamically stable. Finally, an *in vitro* study of piroxicam release from these formulations allowed the selection of the most suitable microemulsion formulations with the greatest *in vitro* release of piroxicam. The most suitable microemulsion formulation in this study was microemulsion formulation 2, which consisted of 10% oleic acid, 60% Tween-80, 30% propylene glycol, 50% water of the total pre-microemulsion weight, and 0.5% piroxicam. This formulation had the best particle size as well as good physical and thermodynamic stability in addition to the best and the greatest *in vitro* release among all of the investigated formulations. The incorporation of piroxicam in microemulsion formulations led to enhancement of the piroxicam release profile by allowing constant and regular *in vitro* release as well as reducing piroxicam's particle size to that suited to a microemulsion. Thus, the usage of a microemulsion technique led to improvement in piroxicam availability, suggesting the potential for the technique's use as topical vehicle for piroxicam delivery.

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