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FORMULATION AND CHARACTERIZATION OF BIOCOMPATIBLE MICROEMULSIONS FOR TOPICAL ADMINISTRATION OF SODIUM DICLOFENAC

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ABSTRACT. The purpose of this study was to develop microemulsion-based drug vehicles for topical administration of sodium diclofenac (SD), using Tween 80 and 1-octanol as surfactant and cosurfactant, respectively. Three commonly pharmaceutical oils (isopropyl myristate, arachis oil and canola oil) were investigated as oil phases. Microemulsion existence region was identified through the construction of the pseudoternary phase diagrams, using water titration method. The prepared microemulsions were evaluated regarding their transparency/translucency, optical birefringence, electrical conductivity, viscosity, pH and stability. The isopropyl myristate-based systems showed a slightly higher area of microemulsion existence and permitted the formation of microemulsions at lower concentrations of surfactants. The effect of added active substance on the stability of microemulsions was also evaluated for 1% SD (w/w).

Keywords: topical microemulsion, sodium diclofenac, viscosity, electrical conductivity, optical birefringence

INTRODUCTION

Microemulsions are optically transparent, isotropic and thermodynamically stable mixtures of oil, water and amphiphile molecule(s), with a droplet size smaller than 200 nm (Danielsson *et al.*, 1981). They are characterized by ultralow interfacial tension between water and oil, usually achieved when using a surfactant in combination with a cosurfactant. Thus, microemulsions form spontaneously and present a long shelf life, attributes that make them ideal drug delivery vehicles (Talegaonkar *et al.*, 2008). In correlation with the low interfacial tension and different packing parameters characterizing the surfactant molecules, microemulsions are, in general, divided into 2 types, based upon their internal structure: droplet-like microemulsions (oil-in-water or water-in-oil, respectively) and bicontinuous microemulsions, which present different behavior regarding the release of solubilized drug (Paul *et al.*, 1997). Although stable preparations, microemulsions are not inert vehicles, being influenced to a greater or smaller extent by variations in composition, including the addition of a drug to pharmaceutical microemulsions. Therefore, the microstructure and stability of the system can be affected (Bagwe *et al.*, 2001).

Understanding the relationship between composition of a microemulsion and its microstructure is a fundamental aspect when formulating microemulsions with pharmaceutical applications, especially when considering the dilution of a microemulsion introduced in a physiological fluid. Characterization of the internal structure of microemulsions is far from being a simple process, usually the best conclusions being drawn after using several techniques. The experimental techniques reported in literature used for characterizing microemulsion's microstructure include electrical conductivity and viscosity measurements, differential scanning calorimetry (DSC), small-angle X-ray scattering (SAXS), small-angle neutron scattering (SANS), cryo-transmission electron microscopy (Cryo-TEM), fluorescence correlation spectroscopy (FCS) and nuclear magnetic resonance (NMR) (Podlogar *et al.*, 2004; Boonme *et al.*, 2006; Alany *et al.*, 2001; Bumajdad *et al.*, 2004; Mitra *et al.*, 2005).

In this study three biocompatible microemulsions comprising in isopropyl myristate/arachis oil/canola oil, Tween 80, 1-octanol and distilled water were investigated as potential vehicles for topical administration of sodium diclofenac (SD). Microemulsion existence regions for each system were identified through construction of pseudoternary phase diagrams. Changes in the internal structure of microemulsions placed on a dilution line have been evaluated regarding their visual appearance, electrical conductivity, viscosity and pH. Additionally, the influence of sodium diclofenac on the stability of drug-loaded microemulsions was investigated.

MATERIALS AND METHODS

Materials

Isopropyl myristate (IPM) was purchased from Titolchimica. Arachis vegetable oil was purchased from Solaris. Canola (rapeseed) oil was purchased from Biologicoils. 1-octanol was purchased from Merck-Schuchardt. Tween 80 was kindly gifted by Actavis. Distilled water was used as hydrophilic phase.

Methods

Construction of pseudoternary phase diagrams

Biocompatible components were used for the formulation of microemulsion systems. Pseudoternary phase diagram were constructed at room temperature (25±3°C) by admixing appropriate amounts of the various components. Tween 80 (surfactant) and n-

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octanol (cosurfactant) were used at a weight ratio of 1:1. At the beginning, the Tween 80/n-octanol mixture was mixed with the oil phase at weight ratios of 95:5, 90:10, 85:15, 80:20, 75:25, 70:30, 60:40, 50:50, 30:70 and 10:90. The obtained mixtures were diluted with water using a microsyringe and stirred vigorously for a sufficient length of time for homogenization. After mixing the components, each sample was sonicated for approximately 20 minutes to remove air bubbles, then let to settle for 24 h before further analysis. The obtained samples were classified as either microemulsions or multiphase systems/liquid crystals based on their visual appearance and their microscopic appearance under cross-polarized light. The triangular phase diagrams were constructed using Triplot 4.1.2 software.

Transparency/Translucency

Having a droplet size smaller than the wavelength of visible light, microemulsions appear transparent when white light pass through. The obtained microemulsion systems were inspected for transparency and homogeneity by visual observation against strong light. Only the samples that presented an optically clear single phase were classified as microemulsions. Further, the samples were examined for the absence of birefringence using a Motic B1 optical microscope with polarized light module (Friberg, 1990).

Preparation of microemulsion samples

Based on the obtained pseudoternary phase diagrams, five microemulsion formulations with different water content (corresponding to the 85:15 dilution line) were selected for each of the three oils and were used for further investigations. The composition of the samples is given in Table 1.

Table 1
Composition of the microemulsion samples along the dilution line

Sample	Water [%]	Oil [%]	Surfactant mixture [%]
1	0.00	15.00	85.00
2	1.64	14.76	83.60
3	3.61	14.45	81.94
4	13.04	13.04	73.92
5	18.37	12.24	69.39

Conductivity measurements

Electrical conductivity of microemulsion samples was measured using a Cole Parmer Conductivity meter CON 500. The readings were taken at room temperature, after the value had remained constant for at least 2 minutes.

Viscosity measurements

Viscosity measurements were performed using an Ubbelohde viscometer. The instrument was placed in a water thermostat with the temperature controlled at 25 ± 0.1°C. The calibration of the viscometer was made using 1-hexanol and 1-heptanol as references.

pH measurements

The pH of microemulsion samples was measured at room temperature, by direct immersion of the electrode of Hanna Instruments 991001 pH meter.

Stability studies

The physical stability of the samples was assessed during one month storing at room temperature. Additionally, a centrifuge stress test was carried out at 9000 rpm for 20 minutes in order to evaluate the thermodynamic stability of the microemulsion samples. The influence of added sodium diclofenac on the stability of the samples was evaluated over a 10 days period by adding the active substance in a concentration of 1% (w/w).

RESULTS AND DISCUSSION

Phase diagrams

The high amount of surfactants necessary for the formation of microemulsions represents an important disadvantage, as it can lead to tissue irritation when topical administered or even systemic toxic effects for oral preparations. However, in order to prepare a stable microemulsion it is necessary to achieve a low interfacial tension that cannot be otherwise achieved. For this reason, when considering formulating pharmaceutical microemulsions, it is recommended to use biocompatible components.

Phase diagrams were constructed to determine the microemulsion regions. The pseudoternary phase diagrams corresponding to isopropyl myristate (IPM), arachis oil (ARH) and canola oil (CAN), respectively, are presented in Figure 1. The IPM-based system shows a higher microemulsion area when compared to the vegetable oils systems. The arachis oil and canola oil-based systems present a similar behavior, leading to identical phase diagrams. The IPM-water-Tween80/1-octanol system permits the formation of microemulsions at a concentration of surfactants as low as 47%, whereas for vegetable oils systems the same is observed at concentrations higher than 54%. Thus, IPM-based microemulsions could be more promising drug delivery systems.

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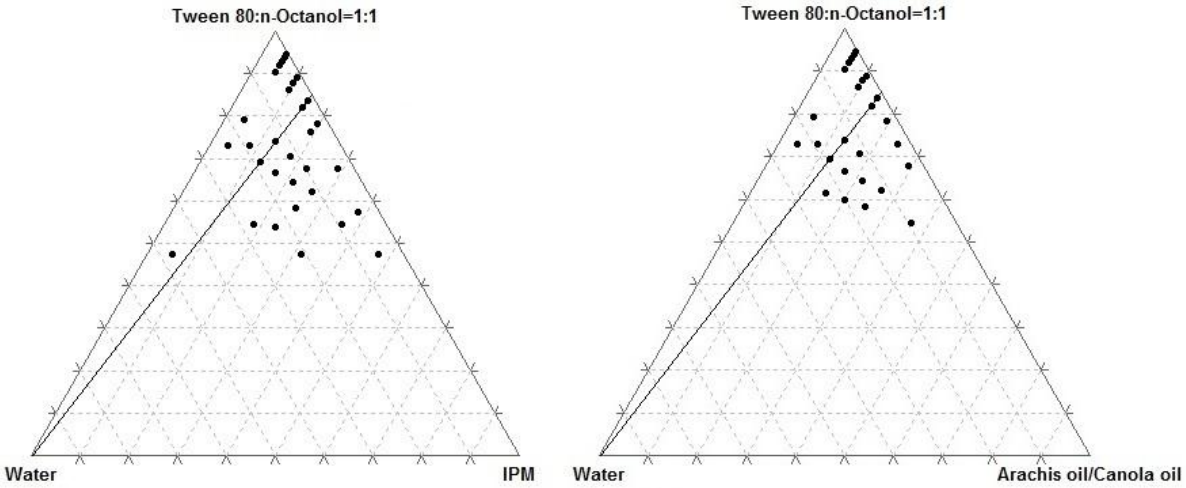

Fig. 1 The pseudoternary phase diagrams (microemulsion ●) and the investigated dilution line

Transparency/Translucency

All selected samples corresponding to the 85:15 dilution line were transparent solution-like liquids, when observed for visual clarity against strong light. A simple method generally used to differentiate a microemulsion system from a liquid crystal is to observe the sample under a cross-polarized light microscope. Thus, when analyzed under polarized light, the selected microemulsion samples appeared completely dark, suggesting the lack of birefringence and also sample homogeneity and optical isotropy (Friberg, 1990; Junyapraser *et al.*, 2007; Shah *et al.*, 1971).

Conductivity and viscosity

Water present in a microemulsion can be either free or bound water, depending on the microemulsion internal microstructure. Free water is assumed to have properties similar to those of pure water. On the other hand, bound water (i.e. water-in-oil microemulsions) is strongly influenced by the amphiphilic molecules (Podlogar *et al.*, 2004; Garti *et al.*, 2000).

Although electric conductivity measurements should be studied in the presence of a dissolved electrolyte (Weigert *et al.*, 1997), there is evidence that its addition may influence and change the type of the system (see later). Despite the fact that the samples were prepared using non-ionic surfactants, the electrical conductivity values were high enough for providing accurate measurements.

The conductivity of microemulsions showed an increase tendency along the dilution line, starting with values around 4 µS/cm for the samples with 0% water content (possibly due to the impurities present in Tween 80). As the water fraction increases so does the electrical conductivity. However, the values for conductivity are lower than those observed for pure water, indicating the presence of a water-in-oil droplet structure for samples 2, 3 and 4, for each of the three investigated systems (as shown in Figure 2). It can be pointed out that all three systems showed a similar conductivity profile, suggesting the formation of the same type of internal structures. However, sample IPM 5 (~18% water content) presented a lower conductivity compared to samples ARH 5 and CAN 5 as a result of the much higher viscosity exhibited (Figure 3). Nevertheless, the sharp increase in electrical conductivity and the higher then previous viscosity values for samples IPM 5, ARH 5 and CAN 5 are arguments for the bicontinuous nature of these formulations, as shown by others (Gradzielski *et al.*, 1999; Zvonar *et al.*, 2009). All viscosity results indicate low-viscosity formulations, characteristic to microemulsions.

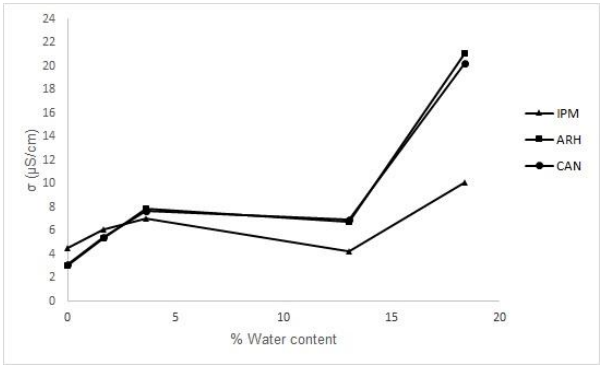

Fig. 2 Electrical conductivity (σ) of the selected microemulsions as a function of the water content (%)

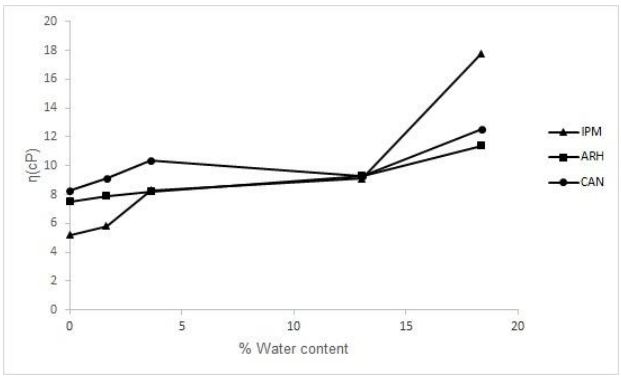

Fig. 3 Viscosity (η) of the selected microemulsions as a function of the water content (%)

pH

When considering using a microemulsion for pharmaceutical use it is necessary to design the delivery vehicle starting from the properties of the active substance intended to be embedded, thus assuring both drug's stability and the stability of the pharmaceutical formulation. Also, physiological requirements have to be met.

The results of pH measurements are shown in Table 2. As it can be observed, all the investigated systems are within the required physiologic pH interval for dermal preparations (4.5-8.5 pH units). Lower pH values are characterizing the vegetable oils-based samples. Also, the pH decreases with the increase of water content in microemulsion ($pH_{used\ water}$=5.63).

Tabel 2

pH measurements of microemulsion samples

Sample	pH	Sample	pH	Sample	pH
IPM1	6.38	ARH1	6.23	CAN1	6.18
IPM2	6.33	ARH2	6.21	CAN2	6.11
IPM3	6.22	ARH3	6.08	CAN3	6.03
IPM4	5.82	ARH4	5.74	CAN4	5.62
IPM5	5.36	ARH5	5.27	CAN5	5.22

Stability

After 30 days storage at room temperature (25±3°C), all samples remained transparent and no turbidity or phase separation was observed. Microemulsion stability was assessed by visual inspection and optical microscopy using polarized light. No appreciable structure changes were detected. Also, the centrifuge stress indicates thermodynamic stability of prepared microemulsions, as no phase separation occurred.

Effect of added sodium diclofenac

The effect of adding 1% (w/w) sodium diclofenac to the prepared samples was evaluated. The active substance was added under continuously stirring until it dissolved. No change in transparency and cross-polarized light microscopy aspect was detected over a 10 days period, at room temperature (25±3°C), indicating that sodium diclofenac-loaded samples remained microemulsions. No attempt was made to distinguish between oil-in-water, water-in-oil or bicontinuous type microemulsions.

The effect of sodium diclofenac (SD) on the stability of three more samples (one for each investigated system) was evaluated, in order to determine the influence of the drug on microemulsion-liquid crystal delimitation line from the obtained phase diagrams. The composition of the sample (Table 3) corresponds to the selected dilution line.

Table 3

Composition of the sample across the microemulsion-liquid crystal delimitation line

Water [%]	Oil [%]	Surfactant mixture [%]
25.93	11.11	62.96

Initially, all three samples were considered liquid crystals, being turbid birefringent systems. After the addition of sodium diclofenac and vigorously stirring, all three samples became clear and showed a reduction in viscosity. No other measurements were conducted. Addition of sodium diclofenac appears to modify the nature of the investigated water-oil-surfactants systems, leading to an increase in microemulsion area. A possible explanation is the solubility of SD in both aqueous and oil phases. Thus, SD may concentrate at the oil-water interface, leading to further stabilization and tension reduction of the interfacial film.

CONCLUSIONS

Three pseudoternary systems based on biocompatible components were compared. From the corresponding phase diagrams, it can be concluded that IPM-based system has a higher area of microemulsion existence and requires a lesser concentration of surfactants for microemulsion formation compared to vegetable oils formulations. The internal structure of selected microemulsions from a dilution line was investigated using conductometric and viscosity measurements. The pharmaceutical acceptance of prepared microemulsions was evaluated based on pH determination, stability studies and evaluation of the effect of adding sodium diclofenac over the stability of the samples. Future studies will be carried out in order to successfully select a microemulsion formulation for optimal embedding of sodium diclofenac.

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