Design of a Microemulsion-Based Drug Delivery System for Diclofenac Sodium

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Summary: A microemulsion-based drug delivery system has been designed for Diclofenac Sodium (DS) comprising Span 60, 1-Propanol, Water, and Lemon Oil. The microemulsion system has been characterized by a pseudo-ternary phase diagram using the water titration method. The properties and structure of this system have been studied by the use of refractive index, electrical conductivity, viscosity and UV-Visible spectroscopy. The conductivity (σ) and viscosity ($k\eta$) measurements have provided evidence for percolation behavior with variation in Φ (weight fraction of aqueous phase). This phase transition corresponds to the structural change from water-in-oil to a bicontinuous microemulsion system. The percolation threshold (Φ_C) obtained from conductivity measurements was in accordance with that obtained by viscosity measurements. Five microemulsion samples were selected and the changes in microstructure after incorporation of the drug, Diclofenac Sodium (DS) were examined by centrifugation, conductivity measurements, viscosity measurements and spectroscopic studies. The conductivity measurements showed that DS-loaded samples have higher conductivity values when compared to non-loaded samples. It was also found that DS is interfacially active. In addition, loading of DS had no negative effect on the stability of the system.

Introduction

The objectives of this study were: (a) derivation of a pseudoternary phase diagram of the microemulsion system, (b) evaluation of the water and oil distribution in the microemulsion system using electrical conductivity and viscosity measurements, and (c) use of the prepared microemulsion system as a vehicle for the transdermal application of Diclofenac Sodium, a non-steriodal anti-inflammatory drug (NSAID).

Microemulsions, as drug delivery systems, are becoming more and more important due to controlled release of drug and improved bioavailability. Microemulsions are optically trans-parent. These are low viscosity, thermodynamically stable dispersions of oil and water stabilized by surfactant, usually in combination with a co-surfactant [1-3]. Microemulsions can be different-tiated into three types' oil-in-water, water-in-oil and bicontinuous. Microemulsion types depend on the dispersed and continuous phases [4]. The structure of microemulsion is important in drug release. Hydrophilic drugs solubilize mainly in water phase in the water-in-oil microemulsion droplets and hydrophobic drugs in oil droplets of oil-in-water microemulsions [4].

Microemulsions, as drug delivery systems, offer advantages for topical and transdermal drug delivery due to their unique properties. Some of the microemulsions advantages are their spontaneous formation, thermodynamic stability and high solubilizing capacity for various drugs. The high solubilizing capacity of microemulsion enables them to increase the solubility of many compounds particularly the solubility of hydrophobic drugs. Microemulsion systems are a useful vehicle to increase transdermal drug permeability [5]. To investigate the possibility of transdermal delivery of a drug incorporated in microemulsion system, Diclofenac Sodium was used as a model drug. The chemical structure of Diclofenac Sodium is shown in Fig. 1.

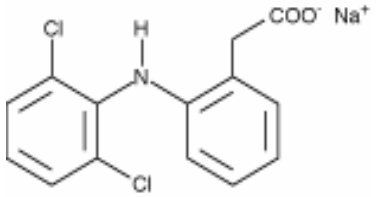

Fig. 1: Chemical structure of Diclofenac Sodium.

Diclofenac Sodium (DS) is a commonly used highly effective non-steriodal anti-inflammatory drug (NSAID). It is used for acute conditions of inflammation and pain, musculoskeletal disorders and arthritis [5]. Diclofenac Sodium is a relatively safe and tolerable NSAID. Serious gastrointestinal adverse effects occasionally appear after oral administration. Due to its adverse effects as well as its short biological half life, topical application of Diclofenac Sodium provides a preferred alternative to

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oral ingestion [5]. In contrast to their ease of preparation, characterization of microemulsion is complicated and requires the use of several techniques. The experimental techniques that are routinely used for characterizing microemulsion microstructure include electrical conductivity, viscosity, differential scanning calorimetery (DSC), nuclear magnetic resonance (NMR), freeze-fracture transmission electron microscopy (FF-TEM) and cryo-field scanning electron microscopy (Cryo-FESEM) [6]. To investigate the drug delivery potential of microemulsion vehicle, it is necessary to characterize the micro-structure of pure and drug-loaded microemulsion system. The aim of the characterization performed was to determine the type and structure of colloidal samples formed in Span 60/1-Propanol/Water/Lemon Oil system (Table-1). Span 60 is a non-ionic surfactant. Non-ionic surfactants are frequently used in microemulsion preparation because of their minimal toxicity [6].

Table-1: The contents of selected Microemulsion formulations in mole fraction ratios.

System	S/CoS	Water	Oil
ME-A	0.8	0.02	0.18
ME-B	0.74	0.04	0.22
ME-C	0.68	0.08	0.24
ME-D	0.62	0.12	0.26
ME-E	0.56	0.14	0.30

Results and Discussion

Construction of a Phase Diagram

The behavior of the four component system was characterized by drawing a pseudo-ternary phase diagram (Fig. 2). A mixture of surfactant, alcohol (co-surfactant) and oil was prepared in test tubes at pre-determined volume ratios. These samples were then titrated against the aqueous phase to get a clear solution with constant stirring. The tubes were then visually inspected. An appearance of turbidity was considered an indication of phase separation. All samples which remained transparent and homogenous after vigorous stirring were considered to belong to the mono-phasic area in the phase diagram. Also, the dilution line was studied by adding water until solution became turbid. Similarly five samples were selected from the microemulsion region for further study (Fig. 3). A pseudo-ternary phase diagram was constructed using units of mole fraction.

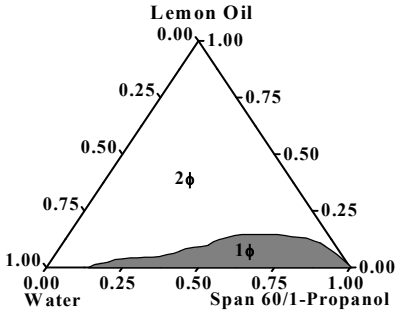

Fig. 2: Complete pseudoternary phase diagram for the system Span 60/1-propanol/lemon oil/water. 1ϕ is the area of w/o microemulsion (one phase region) and 2ϕ is the area of biphase region.

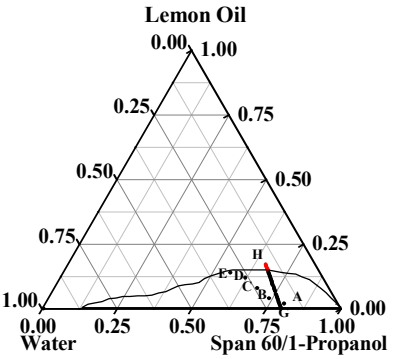

Fig. 3: GH represents the dilution line and compositions A-E were selected for further study by conductivity measurements.

Refractive Index Measurements

Refractive index values of pure oil, water and selected microemulsion were studied. The comparison of these values shows that water-in-oil microemulsion has been formed. Also, drug loading has no significant effect on the refractive index of the microemulsion system.

Conductivity Measurements

One of the key methods used for studying microemulsion is measurement of electrical conductivity. Conductivity measurement is a way to determine whether a microemulsion system is oil-continuous or water-continuous. Also it is a tool to monitor percolation or phase inversion phenomena [4]. Percolation is often referred to transition of isolated droplets to an interconnected bicontinuous structure. Structure of microemulsion depends on the water-oil ratio.

The system investigated is (Span 60/1-Propanol)/Lemon Oil/Water along dilution line GH (Fig. 2, Table-2). Electrical conductivity "σ" was measured as a function of water content Φ (wt %) for oil, S/CoS mixture along dilution line GH as shown in Fig. 3. The results of variation of σ as a function of water content Φ (wt %) are shown in (Fig. 4).

Table-2: Different selected microemulsion systems and their characteristic conductivity values are shown.

System	Φ	σ (μS/cm) Non-loaded system	σ (μS/cm) Drug- Loaded system
ME-A	2	0.79	14.14
ME-B	4	0.84	19.52
ME-C	8	0.87	30.60
ME-D	12	0.83	41.30
ME-E	14	0.77	44.00

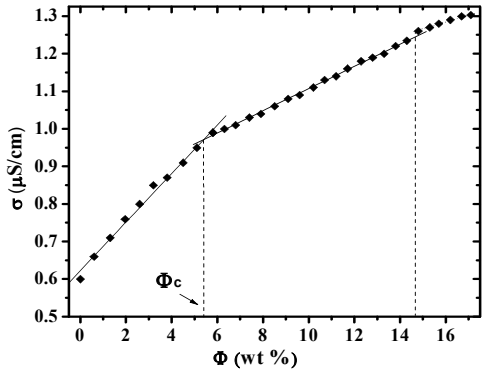

Fig. 4: Variation of electrical conductivity "σ" of the four component microemulsion system as a function of water phase Φ (w/v %) along the dilution line.

For the system under investigation, low conductivity values were obtained without addition of water volume fraction. As the volume fraction of water increased the electrical conductivity increased. Fig. 3 shows that there is an increase in electrical conductivity till approximately 5 wt % of water. This can be attributed to the occurrence of a percolation transition [7]. The conductivity of water-in-oil microemulsion system showed quite a remarkable change. Below percolation transition, the conductivity of water-in-oil microemulsion tends to decrease with decreasing volume fraction of water. As has been reported previously that in the case of non-ionic surfactants, a small amount of aqueous electrolyte has to be added to transport the charges. However, addition of salts can significantly change the phase behavior and structural properties and even results in phase separation [7]. For this reason, conductivity measurements were done without the incorporation of an electrolyte. In this study, short chain alcohols were used as co-surfactant. It was found that for these solutions, the electrical conductivity increased continuously with the increase in water content [8].

It must be emphasized that these w/o droplets remain isolated from each other below Φ_C and are embedded in a non-conducting oil phase and therefore contribute very little to electrical conductivity. However, as the weight percent of water reaches approximately 5% (percolation threshold), some of these droplets began to form clusters [8].

In the present study, with the increase in water content of the microemulsion system, the interactions between aqueous domains became increasingly important. Water forms a network of conducting channels (bicontinuous microemulsion). These channels increases rapidly above the percolation threshold Φ_C = 5% and give rise to observed changes in properties, in particular conductivity. This percolation behavior can be explained by charge fluctuation model. The σ above Φ_C has been attributed to either hopping of surfactant ion from drop to droplet within droplet cluster or transfer of counterions from one droplet to another through water channels opening between droplets [8]. Among the selected microemulsion samples: A to E (Table-1), ME-A and ME-E were found to be unfavorable because of high surfactant content and non-dilutability respectively whereas ME-B, C, D showed electroconductive behavior inspite of non-ionic nature of amphiphile. Among ME-B to D, ME-C is the best composition selected for further physicochemical studies and drug delivery. The variation of electrical conductivity as a function of Φ is shown in Figs. 4-6, respectively. A comparison of these systems shows that incorporation of drug does not affect the microstructure of microemulsion.

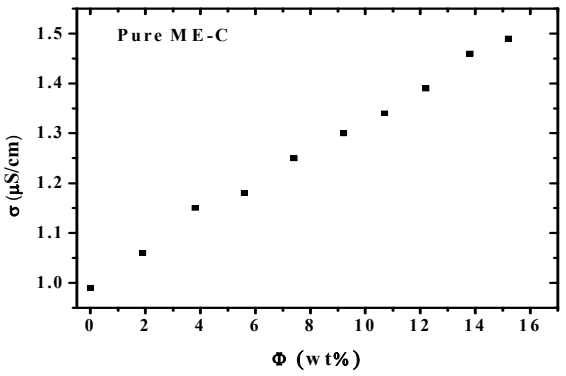

Fig. 5: Variation of electrical conductivity "σ" of pure ME-C as a function of Φ added weight fraction of water.

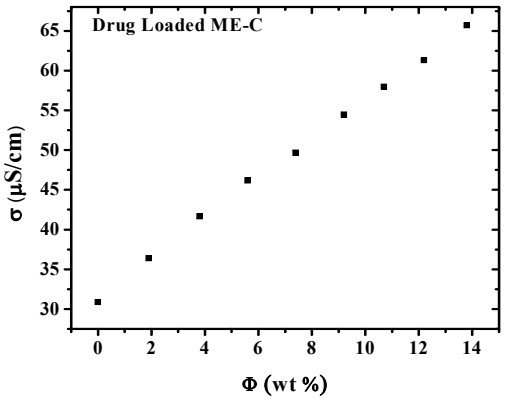

Fig. 6: Variation of electrical conductivity "σ" of drug loaded ME-C as a function of Φ added weight fraction of water.

Viscosity Measurements

Viscosity is a characteristic property of any fluid. It largely depends on aggregates present, on their interaction and on the concentration. Thus it is mainly used to get insight of microstructure of the system. Fig. 7 represents the flow time of the oil, S/CoS mixture as a function of the weight fraction of the aqueous phase (Φ % wt) along the dilution line as represented in Fig. 3.

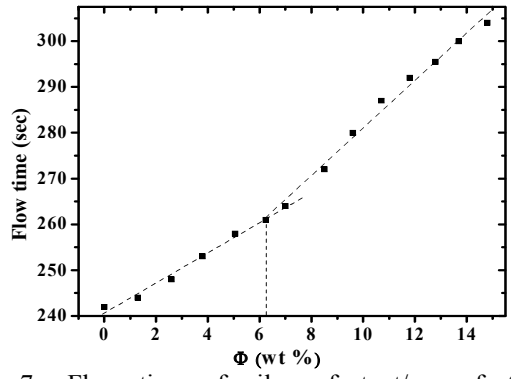

Fig. 7: Flow time of oil, surfactant/co-surfactant mixture as a function of weight fraction of aqueous phase (Φ % wt).

Fig. 8 shows that there is increase in kinematic viscosity approximately at 6 % of water weight fraction. The viscosity profiles reflect structural transitions and structural boundaries. When the weight fraction of water is low, the microemulsion consists of water droplets dispersed in oil and the interaction between the droplets is weak as reflected by the low viscosity values. Once the droplets swell, the droplet-droplet interaction increases and viscosity value climbs. This increase in viscosity indicates change in the shape of microemulsion droplets since the growth of spherical droplets into larger non-spherical droplets is followed by a significant increase in the solution viscosity [9]. This clustering of droplets into water channels at percolation threshold leads to an increase in viscosity [6]. The microemulsion thus shows a structural change from oil-continuous system to water-continuous which has higher viscosity than the former. Similar trends have been observed for the viscosity of oil, S/CoS mixture as a function of Φ as shown in Fig. 8. The break in viscosity curve is due to change in microstructure of microemulsion.

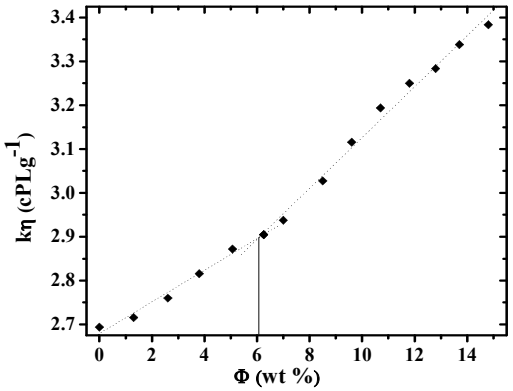

Fig. 8: Variation of kinematic viscosity as a function of weight fraction of water (Φ wt%).

Spectroscopic Studies

The turbidity of the microemulsion system 'C' was investigated using UV-Vis spectro-photometer. UV-Visible spectrum of pure ME-C was recorded in range of 350-800 nm. This technique has been used to study the turbidity and the maximum uptake of water. The pure microemulsion system showed an absorbance of less than 0.1. A plot of turbidity values versus weight fraction of added water is shown in Fig. 9. This plot can be clearly divided into three regions. In first region, which ends at 12 % of Φ (water weight fraction), the system remains clear and stable. The state of the region comprising a single phase was transparent. After that the system became turbid.

The initial low viscosity system changed to a dense solution with constant addition of water. This region can be classified as a transient region that resisted the phase separation and became turbid. This behavior continued till the system became approximately 25 wt % in water. But as the amount of water was increased, this turbid system separated into two phase regions as shown in Fig. 9. This result

is in accordance with the results obtained from conductivity and spectroscopic data. The System 'C' was able to retain water till 12 wt % and it remained stable and in one phase.

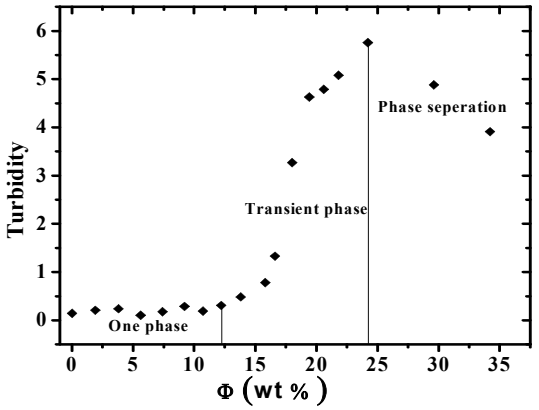

Fig. 9: Plot of turbidity versus weight fraction for the dilution studies of chosen composition 'C' in microemulsion region. The plot shows the three regions.

Experimental

Materials

Span 60 was obtained from MP Bio-medicals, LLC (Germany). Extra pure 1-propanol was obtained from Scharlau (Spain). Lemon oil was obtained from the local market. The water used was deionized and water distillation apparatus was IM 100-IRMECO GmbH purchased from Geesthacht/ Germany.

Methods

Preparation of Water-in-Oil Microemulsion

All the experiments described in this study were performed at room temperature. Span 60 was used as a surfactant and 1-propanol as a co-surfactant (CoS). Lemon oil was used as the oil phase and distilled water was used as the aqueous phase. The hydrophilic-lipophilic balance value was 4.7. A phase diagram was constructed to determine the microemulsion region.

For the microemulsion formulation, surfactant (S) was dissolved in 1-propanol which acts as the co-surfactant (CoS). S/CoS mixture was then added to the appropriate amount of the lemon oil. The mixture was then titrated slowly with distilled water with constant stirring. Ideal S/CoS ratios and microemulsion areas were detected with the aid of the following phase diagram (Fig. 1). Diclofenac sodium was added to microemulsion at the last stage. All samples of microemulsion contained 1% (w/v) Diclofenac Sodium.

Incorporation of Drug in Microemulsion System

Five microemulsion systems ME-A to ME-E has been selected from single phase region of phase diagram (Fig. 2) with the compositions mentioned in table 1 to study their potential use as drug delivery system. Diclofenac Sodium was dissolved in samples at a concentration of 1 % (w/v) under stirring.

Microemulsion Characterization

Centrifugation

The stability of the microemulsion system was checked using centrifugation. The effective gravitational force on a test tube can be increased so as to more rapidly and completely cause the precipitate to gather on the bottom of the tube if the system is not stable. Centrifugation was carried out in this study at 5500 rpm for 10 minutes using a Hermle Z 200 centrifuge.

Refractive Index Measurements

Refractometry is the method of measuring substances' refractive index in order to assess their composition or purity. A refractometer measures the extent to which light is bent (*i.e.* refracted) when it moves from air into a sample and is typically used to determine the index of refraction of a liquid sample. Refractive index values of pure and drug loaded microemulsion droplets were determined using the ATAGO RX-5000 Refractometer.

Optical Transparency Measurements

The isotropic nature of microemulsion and their optical clarity is studied by the use of spectroscopic techniques. UV-Vis. Spectroscopic techniques were used to study the microemulsion system.

Conductivity Measurements

Conductivity measurements provide a means of determining whether a microemulsion is oil-continuous or water-continuous as well as providing a means of monitoring percolation or phase inversion phenomena. The electrical conductivity "σ" was

measured by the use of InoLab Digital Conductivity Meter. Conductivity of selected unloaded and drug loaded microemulsion samples was measured. Also the conductivity of the dilution line was studied (Table-2). For the study of dilution line, initially conductivity of S/CoS and oil mixture was noted. And then, with each addition of water in the mixture conductivity was measured until the solution became turbid.

Viscosity Measurements

Viscosity measurements can provide first hand information on the internal consistency of the colloidal dispersions, as well as furnish knowledge about the overall geometry of the particles of the dispersed phase. Viscosities were measured using a calibrated Ostwald viscometer at 27 °C. For each measurement, the viscometer was washed, rinsed and dried. Flow time was measured for the samples along the dilution line. The error limit for viscosities measurements was +/- 3%.

Turbidity Measurements

The stability of microemulsion was investigated by turbidity measurements using a UV-Vis spectrophotometer. Samples were scanned in quartz cuvettes with thickness of 1 cm. The turbidity of each sample was calculated as: turbidity × path length = 2.303 × absorbance [9].

Conclusions

A low viscosity, single phase water-in-oil microemulsion has been formed using Span 60/1-Propanol/Lemon Oil/Water. A complete pseudo-ternary phase diagram has been constructed and a clear, single-phase microemulsion region has been delineated by constructing a boundary line. The remaining part of phase diagram has been studied and found that all area comprises a biphasic system. The stability of the water-in-oil microemulsion system was checked by performing accelerated stability test and also by allowing the samples to stand at room temperature for extended period of time. The microemulsion system was found to be stable after seven months of storage.

Physico-chemical characterization showed that the system undergoes a structural transition from water-in-oil to bicontinuous microemulsion system upon addition of water. The conductivity and viscosity studies along dilution line (in phase diagram) provided evidence for the structural transition from water-in-oil to bicontinuous phase at ~ 6 % w/v fraction of aqueous phase (Φ) as previously shown.

The sample, ME-C was found to be the best for use as a drug delivery system on the basis of its optimal solubility for Diclofenac Sodium. After the incorporation of the drug, the microemulsion system remained stable and optically clear showing no phase separation. The solubility of the drug was confirmed using conductivity measurements which indicated that the drug may be present at the interface of the oil and aqueous phase. UV-Visible spectroscopic studies indicated that the system was optically clear and the uptake of water was possible till ~ 12 % w/v mole fraction (Φ) of water. We can conclude that our microemulsion system helps increase the solubility of the hydrophobic drug with the help of hydrophobic component of microemulsion and lipophilic part of the surfactant. On the basis of physico-chemical characterization and spectroscopic studies, it may be concluded that microemulsion formulation for the drug, Diclofenac Sodium can be used as a drug delivery system. Further work in this direction is needed.

Acknowledgments

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