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Short Communication

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Preparation, Characterization and *In-Vitro* Evaluation of Sunflower oil-Tween 80-Glycerol-based Microemulsion Formulation of a BCS Class-II Drug

Madan M. KAMILA*, Nita MONDAL, Bijan K. GUPTA & Lakshmi K. GHOSH

Division of Pharmaceutics, Department of Pharmaceutical Technology,
Jadavpur University, Kolkata - 700 032, INDIA

***SUMMARY*. The aim of the present study was to prepare, characterize and *in-vitro* evaluation of a Winsor-IV type microemulsion based drug delivery system incorporating celecoxib as BCS class–II model drug. Attempts were made to prepare cost effective O/W microemulsion using Tween 80, Glycerol, sunflower oil and water. The existence of microemulsion zone was investigated using phase diagrams. The systems were characterized by polarized light microscopy, viscosity, refractive index, droplet size of dispersed phase by dynamic light scattering technique, thermal and centrifugal stability and the drug release profile. The obtained microemulsion was found optically isotropic with non-Newtonian behavior. The average droplet size was 100-300 nm. Microemulsions showed reversibility of transparency at ambient temperature after storage at 5 °C. The solubility enhancement of formulated products was apparent from higher release rate from microemulsion as compared to commercial product. The drug release profile was demonstrated to be promising for oral delivery of celecoxib.**

INTRODUCTION

A microemulsion can be defined as a system of water, oil and amphiphile which is an optically isotropic and thermodynamically stable micro-heterogeneous liquid dispersion [1]. Among the four types of microemulsion system Winsor-IV type is of pharmaceutical interest [2]. Their stability and unique solubilization properties have drawn attention for their use as vehicles for drug delivery [3]. They have the ability to protect labile drugs, control their release, increase their solubility and bioavailability [4,5] and reduce patient variability. It has been proven that it is possible to formulate microemulsion preparations suitable for different routes of administration, viz oral, topical, ocular, pulmonary and intravenous. Microemulsion contains nanometer-sized droplets of oil or water. Although various types of microemulsions have been reported as being nontoxic, but microemulsion systems composed of regulatory approved excipients for pharmaceutical uses are limited. Since required concentration to form microemulsion is higher the choice of pharmaceutically acceptable excipients creates challenges to prepare an efficient and cost-effective microemulsion vehicle. Moreover most of the reported excipients used for the preparation of microemulsion for drug delivery system are very costly [6-9].

Celecoxib is a specific cyclooxygenase-2 (COX-2) inhibitor with no inhibition of cyclooxygenase-1 at therapeutic doses. It is a hydrophobic drug (log P = 3.5) and its aqueous solubility is very low. This results in high variability in absorption of the drug after oral administration. According to the Biopharmaceutical Classification System (BCS), the drug belongs to class II category showing low solubility and high permeability [10]. Poor bioavailabilities of such drugs are mainly due to their low solubility. Microemulsion formulation of such drugs increases the solubility of the drugs and thereby improves its oral bioavailability.

In the present work an attempt has been made to explore the potential of microemulsion as therapeutic drug carrier system to improve

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* Author to whom correspondence should be addressed. *E-mail:* mmkamila@research.jdvu.ac.in, or mmkamila@gmail.com

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oral bioavailability of BCS class-II drugs through improvement of solubility by formulating Winsor-IV type microemulsion capable of carrying poorly water soluble celecoxib as model drug employing cheap and biocompatible materials whose safety is widely accepted so that an efficient, safe drug delivery system could be developed.

MATERIALS & METHODS

Celecoxib was obtained as gift sample from Sun Pharma Advanced Research Centre (Vadodara, India). Tween 80 (SRL, India), Glycerol (Rankem, India), Sunflower oil (ITC, India), Milli-Q water.

Screening of oils and surfactants

The solubility study was used to identify the suitable oil and surfactant that possess good solubilizing capacity for celecoxib. Solubility of celecoxib in vegetable oils (soyabean oil, olive oil, and castor oil), and surfactant (Tween 80) was determined by adding excess amount of drug and continuously stirring for at least 72 h at 30 °C. The mixtures were centrifuged (2500 xg, 30 min) and supernatant was filtered through 0.45 µm membrane filter. Amount of excess drug remained undissolved was determined spectrophotometrically.

Preparation of microemulsion

The microemulsion system prepared comprises of Sunflower oil-Tween 80-Glycerol-water. First a surfactant and cosurfactant blend (Tween 80 and Glycerol) with fixed weight ratio (known as K_m ratio) was prepared with the help of magnetic stirrer. This blend of surfactant and cosurfactant is further mixed with definite amount of Sunflower oil by cyclomixer. The final mixture is titrated by adding water drop by drop from microburette to obtain optically transparent microemulsion. During the titration the mixture of four components was stirred to allow equilibrium. Following the addition of aqueous phase drop by drop the mixture was visually examined for transparency. The changes in the sample visual aspect from turbid to transparent and inversely were observed. Transparent and single-phase mixture was designated as microemulsion.

Solubility determination of Celecoxib

The solubility of the drug was determined spectrophotometrically at 253 nm using double beam UV-VIS spectrophotometer (UV-2450, Shimadzu, Japan) connected to computer loaded with spectra manager software UV Probe with spectral bandwidth of 1nm, wavelength accuracy of ± 0.3 nm with a pair of 10 mm matched quartz cells.

Physical Characterization

Phase behavior: Construction of Phase diagram

The pseudo-ternary phase diagram was constructed by titration of homogenous liquid mixtures of oil, surfactant, and cosurfactant, with water at room temperature. Required amount of Tween 80, the surfactant (S), and Glycerol, the cosurfactant (C) were weighed in a small glass beaker, mixed with the help of a magnetic stirrer for 1 h, and then stored overnight at room temperature. Eight surfactant-cosurfactant blends were prepared with different K_m ratio (w/w) such as 4:1, 3:2, 2.5:2.5, 2:3, 1:4, 1:2, 1:3 and 2:1. Then each surfactant-cosurfactant blend (say K_m= 4:1) was mixed with Sunflower oil in the ratio 9:1, 8:2, 7:3, 6:4, 5:6, 4:6, 3:7, 2:8 and 1:9 separately and titrated by adding water drop by drop from microburette. During the titration, samples were stirred to allow equilibration. Following the addition of aliquot of water the mixture was visually examined for transparency. The changes in the sample visual aspect from turbid to transparent and inversely were observed. Transparent, single-phase mixtures were designated as microemulsions. After the water titration, in order to establish the microemulsion region borders, titration of water and surfactant/cosurfactant mixtures with oil were performed, in the same manner. From the results of the titration, which produced visually transparent microemulsion, phase diagram was constructed.

Polarized light microscopy

Drug free microemulsion vehicles were examined by polarized light microscopy (Leitz, Germany) in order to determine optical isotropy of the samples. The observation whether the sample rotates the plane of polarization of polarized light is very useful tool to distinguish isotropic microemulsions from anisotropic lamellar and hexagonal mesophases. When the isotropic microemulsions were observed under polarized light they look black whereas anisotropic lamellar and hexagonal mesophases are found radiant.

Type of microemulsion

The emulsion type was defined by dilution

with water and was confirmed by dye solubility test. Amaranth was used for dyeing the aqueous phase and Sudan-III for dyeing the oil phase.

Optical clarity: Refractive index

Constant transparency is one of the stability indicator of the microemulsion system. An Abbe refractometer was used to determine the refractive index of the microemulsion samples measured at ambient temperature. Measurements were taken in presence of visible light as the light source.

Droplet size determination

Microemulsion vehicle for the determination of droplet size, dynamic light scattering (DLS) method was adopted. The droplet size of the microemulsion vehicle was calculated using Stokes-Einstein equation measuring diffusion coefficients at room temperature at 90 ° in a DLS spectrophotometer (Model DLS700, Otsuka Electronics Company Ltd., Japan) using a neon laser of wavelength 632 nm. The data processing was carried out in a computer interfaced to the instrument. The measurements were duplicated for checking reproducibility. The solutions were repeatedly filtered of 0.45 micrometer pore size prior to taking measurements.

Rheological behavior

Rheological behavior of the drug free microemulsions was evaluated using a rotational viscometer (Viscometer TV-10MW, Toki Sangyo Co Ltd., Tokyo, Japan). Apparent viscosity data were obtained using spindle number M1 and cord number 20.

Drug loading

Celecoxib was chosen as the model drug for the formulation development. The drug is practically insoluble in water. Before loading the drug into the microemulsion vehicle, the drug solubility in oil phase (Sunflower oil) was determined by slowly increasing the amount of drug with continuous stirring and the transparency of the oil phase was checked visually.

The drug loading capacity of the microemulsion vehicle was determined by gradual increase in the amount of celecoxib added to mixture of oil phase and Tween 80-Glycerol blend and subsequently titration by water to form microemulsion.

Drug release study [11] ***and dissolution efficiency%***

Dissolution Studies were performed according to the USP XXII paddle method. Microemulsion containing 20 mg of celecoxib was filled in hard gelatin capsules and introduced into 500 ml of a dissolution medium consisting of 0.25% sodium dodecyl sulphate in double distilled water and maintained at 37 °C. The Revolution speed of the paddle was kept constant at 100 rpm. The aliquot of 5 ml was withdrawn periodically and filtered through 0.45 micrometer membrane filters. The concentration of celecoxib was determined spectrophotometrically at 253 nm.

Dissolution efficiency (ED) can be used as important parameter for comparison of drug dissolution from immediate release formulations [12]. The ED % allows more reliable comparison between two products. It can be calculated from the values of area under the curve (AUC) of the profile of dissolution of celecoxib in the interval of time (t), through the method of trapezoid rule [12]. ED% is determined by the ratio of the area under the curve of dissolution of celecoxib in the interval of time between zero and 90 min ($AUC_{0\text{-}90}$ min) and the total area of the rectangle (AUC_{TR}) defined by ordinate (100% of dissolution) and the abscissa (time equal to 90 min). The ED has been expressed as a percentage and can be defined by the following equation [Eq. **1**]:

$$ED = [AUC_{0\text{-}90} \text{ min} / AUC_{TR}] \times 100\ \% \quad [\mathbf{1}]$$

Stability studies

Thermal Stability

Thermal stability was also carried for prepared different batches at 5 °C and ambient temperature (25 °C). Samples (10 ml) were kept in an ambered colored container for three months. At the end of three months they were checked visually for their transparency and optical clarity measuring refractive index.

Centrifugal Stability

Centrifugal stress was adopted as a means to study the stability of the prepared microemulsions. Since it is a quick and reproducible method where the test parameters can be stringently controlled. The centrifuge method was used to determine whether the system was one phase or multiphase. Microemulsions of different batches were subjected to centrifugal stress using ultra-centrifuge (Sigma, Germany) at 10,000 rpm for 15 minutes.

RESULTS AND DISCUSSION

Screening of Oils and Surfactants

Development of microemulsion systems for

poorly water-soluble drugs is critical. Drug loading per formulation is a very critical design factor, which is dependent on the drug solubility in various formulation components. The volume of the formulation should be as minimized as possible to deliver the therapeutic dose of the drug in an encapsulated form. Components selected for the formulation should have the ability to solubilize the drug in high level to obtain a concentrate form of microemulsions. Non-ionic surfactant is used in this study since they are known to be less affected by pH and changes in ionic strength [13]. As Celecoxib showed better solubility in Tween 80 and Sunflower oil among the surfactants (Tween 80, Tween 20 and Tween 60) and the oils (olive oil, soybean oil and Sunflower oil) Sunflower oil and Tween 80 were used for microemulsification. The solubility of the drug was found to be 317.07 ± 5.02 mg/ mL in surfactant.

Phase Diagram

Microemulsion system has well defined domain (Fig. 1) of feasibility within the investigated range of concentration of the components, as a logical step our foremost objective was to identify the actual zone where the envisaged system would be feasible. Therefore, a pseudoternary phase diagram having the weight percentage of oil phase, water, surfactant-cosurfactant phase as three vertices of the triangle was developed. Having characterized the zone of microemulsion, the feasibility of the system was ascertained and reproducibility of the formulation was tested by developing different formulations within the zone of interest along with their replicates. A successful pharmaceutical microemulsion system should not only be stable and transparent but also should be of relatively low viscosity, low surfactant content and capable of carrying a large amount of poorly water-soluble drugs. Based on these criteria three formulations ME1, ME2 and ME3 were selected for further investigation (Table 1).

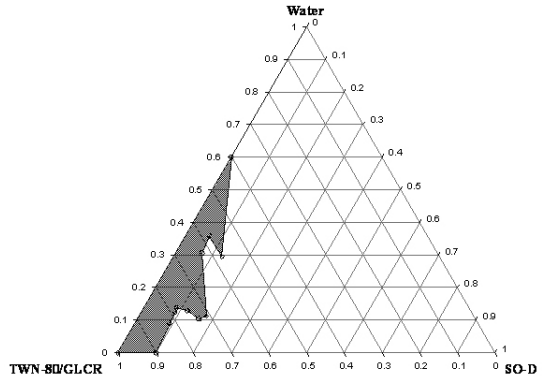

Figure 1. Phase Diagram of Tween 80 (TWN-80)-Glycerol (GLCR)/ Sunflower oil (SO)-Drug (D)/Water. The scale magnitudes have been reduced to 1/100th in the plot.

Batch No.	Composition (w/w) SO-Tween 80-Glycerol-Water	Refractive Index
ME1	8.71/ 19.53/58.58 /13.18	1.449
ME2	8.71/19.61/ 58.84/ 12.85	1.446
ME3	8.80/ 39.65 /39.65/ 11.90	1.445

Table 1. Refractive index of selected microemulsion formulations. SO represents composition of sunflower oil.

Type of Microemulsion

Dilution and dye solubility test confirmed that the prepared microemulsion was oil-in-water type. Since the dilution of the microemulsion with water showed that microemulsion was miscible with water aqueous phase must be the external phase. The continuous colored background of microemulsion mixed with dye confirmed its o/w nature when observed under microscope. However, it should be emphasized that the developed microemulsion system did not form any distinct layer indicating the presence of Winsor-IV type of microemulsion in which theoretically the oil and water domains are randomly oriented throughout the system.

Optical Clarity

Since it was apparent from the preformulation and formulation endeavor that the stability of the system has a direct relationship with optical clarity it was thought logical to monitor these parameters through refractive index measurements as a part of the investigation. It was found that the formulations had a refractive index of around 1.44 which was quite close to that of glycerin (Table 1).

Droplet Size Determination

The DLS method was employed to quantify the droplet size of the microemulsion assuming a monodispersed phase based on spherical globules and the size range was found to vary from as low as 100 nm to 300 nm implying component's concentration did have a considerable effect in determining the droplet size of the system. Although not investigated it is also expected that the energy input during the preparation in the form of mechanical agitation has an effect on particle size. It could be expected from the results that if the system droplet size could be controlled at 200 nm then the vehicle may be

safely used as a vehicle for poorly water-soluble drugs through parenteral routes without the danger of embolism.

Rheological Behavior

Rheograms of the three selected vehicles were studied to know the effect of shear stress in terms of rpm of spindle number M1 and chord number 20. It was clear that there was reduction in the magnitude of viscosity upon increase of shear stress thus showing non-Newtonian fluid behavior (Fig. 2).

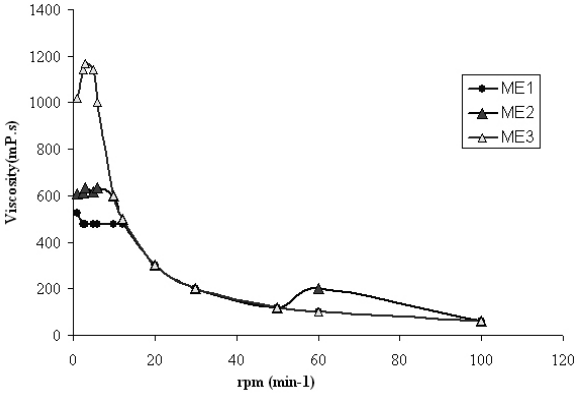

Figure 2. Rheological study of three selected microemulsion formulations.

Drug Release Studies and Dissolution Efficiency

Drug release from the selected microemulsion formulations was compared with that of the market formulation (Capsules). Its was found that drug release from the selected formulations were better than that of market formulation. (Fig. 3). Drug release of the three microemulsion formulations were compared with market formulation calculating ED% of each formulation. All of microemulsion formulations showed higher ED% (89.2%, 77.58% and 77.04% for ME1, ME2 and ME3 respectively) than that of market formulation (69.02%).

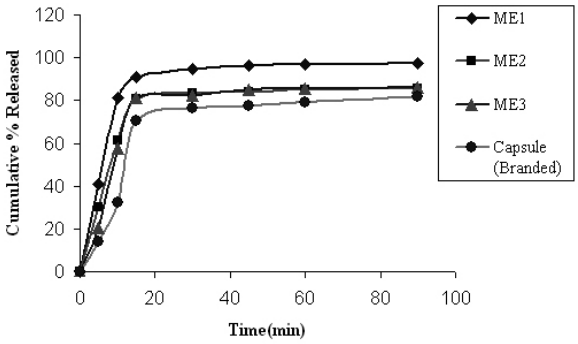

Figure 3. Release of Celecoxib from selected microemulsion formulations and conventional capsule.

From the ED% data it is clear that developed microemulsion formulation would help to improve the solubility of the drug and hence the oral bioavailability since the oral bioavailability of BCS class-II drugs is limited by their solubility in aqueous media.

Thermal Stability Studies

The formulations developed were tested for thermal stability at room temperature and at 5 °C to see whether they could withstand different climatic conditions. It was found that although some of the formulation lost their optical clarity (Table 2) near 5 °C, but effect was reversible where temperature was elevated to normal. It was further observed that the formulation has a most favorable range of transparency beyond which the optical quality of system changed reversibly. It may be due to the rearrangements of the oil/water/surfactants domaining within the Winsor-IV phase due to which the light scattering behavior of the system it undergoes a transition from transparency to translucent to opacity as temperature is varied to most favorable zone. However, being a thermodynamically stable system, the formulation could be buffered in temperature effect by reversing to the transparent state undergoing favorable microscopic rearrangements under ambient conditions. It may be emphasized that these conjecture need to be proved through enthalpy and entropy measurements as a part of the future endeavor.

Sl No.	Transparency at 5 °C	Reversibility of transparency at ambient temperature	Transparency at ambient temperature
ME1	+	+	+
ME2	-	+	+
ME3	-	+	+

Table 2. Thermal Stability study of selected microemulsion formulations at 5 °C and ambient temperature (25 °C). "+" indicates presence of transparency and "–" indicates absence of transparency.

Centrifugal Stability

The formulation showed excellent centrifugal stability where stress tested at 10000 rpm for 15 minutes. They retained their transparency and showed no phase separation for all the systems. The excellent centrifugal stability may be attributed to the attainment of Winsor-IV mi-

croemulsion system where there is an isotropic distribution of contrasting ingredients could be achieved on which the gravity and kinetic energy input has least effect.

CONCLUSION

Developed Winsor-IV type microemulsion formulations have the potential to improve the solubility of poorly water-soluble drug celecoxib and thereby can improve its oral bioavailability. Among the three formulations reported, ME1 showed highest dissolution efficiency and thermal stability with low content of surfactant. The excipients used for the preparation of microemulsion are easily available and low in price as compared to excipients used in reported formulations in literature for therapeutic use.

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