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FORMULATION AND EVALUATION OF LORAZEPAM MICROEMULSION FOR PARENTERAL DELIVERY SYSTEM

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ABSTRACT: The objective of this investigation was to develop Lorazepam microemulsion without a cosolvent. Solubility of Lorazepam in various oils and Solutol HS 15 was determined. The ternary diagram was plotted to identify area of microemulsion existence and a suitable composition with Miglyol 810 and Solutol HS 15 was identified. Process variables like mixing speed and time were optimized.The lorazepam microemulsion was evaluated for globule size, zeta potential, polydispersity index, pH, *in vitro* haemolysis and stability study. Lorazepam microemulsion with Miglyol 810 and Solutol HS 15 showed optimum particle size negligible haemolysis and optimum stability.

Keywords: lorazepam, miglyol 810, solutol HS 15, microemulsion, stability.

INTRODUCTION

Lorazepam is a high-potency, intermediate-duration, 3-hydroxy benzodiazepine drug, often used as a sedative (Tripathi, 2007). It is used for the short-term treatment of anxiety, insomnia, acute seizures including status epilepticus, and sedation of hospitalized patients, as well as sedation of aggressive patients (Riss Jet al., 2008). Since conditions like epilepsy require immediate treatment, most of the important antiepileptic agents including Lorazepam are formulated in a suitable parenteral dosage form. Due to poor aqueous solubility of Lorazepam, co solvents such as polyethylene glycol 400, propylene glycol and glycerin are employed for the development of parenteral formulation. Cosolvent based parenteral formulations suffer from several disadvantages such as pain and tissue damage at the site of injection and precipitation of drug on dilution in several cases. Furthermore, parenteral administration of the organic co solvents can also cause haemolysis (Yalin et al., 1997). Recently, micro emulsions have gained considerable interest in parenteral delivery of hydrophobic drugs and are being preferred over emulsions in several cases. Micro emulsions (Abhijitet al., 2008)are clear, transparent, thermodynamically stable dispersion of oil and water, stabilized by interfacial film of surfactant frequently in combination with a co-surfactant. Recently there has been a considerable interest for microemulsion formulation, for delivery of hydrophilic as well as lipophilic drug as drug carriers because of its improved drug solubilisation capacity, long shelf life, and ease of preparation and improvement of bioavailability (Arcangeliet al., 2005).

MATERIALS AND METHODS

Lorazepam was gifted by CIPLA, Mumbai. Solutol HS 15 was received from Sigma, Aldrich.Miglyol810 was gifted by Sasol Germany GmbH, Mumbai. All other chemicals used were of AR grade and used without further purification.

Selection of oil and surfactant was done based on solubility of drug. The formulation was prepared using titration method by phase diagram and optimized based on percentage transmittance, particle size and zeta potential.

Solubility determination

Solubility of drug lorazepam was determined in different oils and surfactants by using shake flask method (Amit et al., 2008). An excess amount of lorazepam was added to each vial containing 1ml of either oil or surfactant. After sealing, the mixture was vortexed using a cyclomixer for 10 mins to facilitate proper mixing of lorazepam with the vehicles. Mixtures were shaken for 24hrs in an isothermal shaker (Remi, Mumbai, India) and maintained at 37±1°C. Mixtures were centrifuged at 5000 rpm for 15 mins, followed by filtration through a membrane filter (0.22µ, 13 mm Millipore, Bangalore, India). Then the concentration of the mixture was determined by UV spectroscopy.

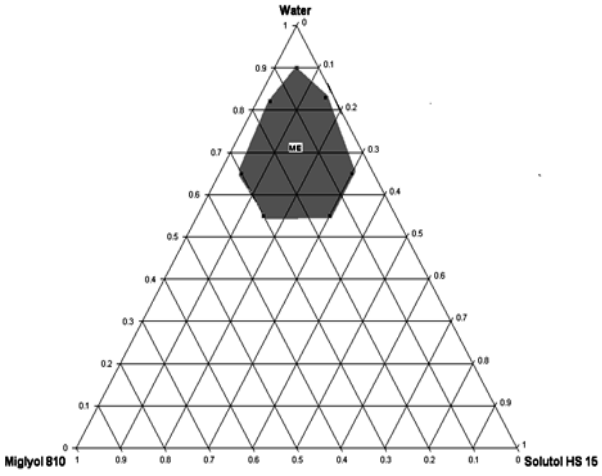

Fig. 1 Ternary Phase diagram for Microemulsion

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FORMULATION METHODOLOGY

The pseudo-ternary phase diagram was constructed (as shown in Fig. 1) using Miglyol 810, Solutol HS 15 and aqueous phase at 25 ± 0.01°C.

The highest solubility of lorazepam was found Miglyol 810 and Solutol HS 15 at a ratio of 1:2 and hence this combination was selected as suitable combination of oil and surfactant. Selection of oil and surfactant, and the mixing ratio of oil to Surfactant can be ascertained by pseudoternary phase diagram as it differentiates the microemulsion region from that of macroemulsion region. Optimized Lorazepam micro emulsion composed of Lorazepam (0.2%), Miglyol 810 (2.5%), Solutol HS 15 (5%), Benzyl Alcohol (1%), Ethanol (0.5%) was selected.

Lorazepam was solubilized in Solutol HS 15 by vortexing. Thereafter, Miglyol 810 was mixed, followed by ethanol and Benzyl Alcohol. The resultant solution was mixed uniformly by vortexing. Then, Water for Injection (WFI) was added and gently mixed to obtain a microemulsion. The resultant microemulsion was sterilized by autoclaving at 121°C and 15 psi for 15 min.

Optimization of process parameters

The stirring speed and the time of rotation were optimized at high speed (2500 RPM) and 5 minutes respectively (Polydispersity index: 0.133).

CHARACTERIZATION OF ICROEMULSION

Measurement of droplet size and zeta potential

The average droplet size and zeta potential of the microemulsion were measured using a Malvern zeta sizer instrument. The measurement was performed at 25°C. Measurementswere made at an angle of 90° for all the microemulsion.

Polydispersity index

2ml of sample was withdrawn from the vial and transferred to 10ml volumetric flask and volume was made with WFI. 1ml of the above solution was taken in the disposable cuvette and size was measured in the zeta sizer instrument. Samples were measured at 25^0C and the light scattering was detected at 173 ^{0}C and collected in automatic mode, typically requiring measurement duration of 150 seconds. It was measured by using the instrument Malvern Zeta sizer.

Assay of lorazepam by HPLC

The amount of lorazepam in receptor compartment was determined by HPLC. The column used was Inertsil ODS, C_{18} column having dimensions of 4.6 mmφ×250 mm i.d. and a particle size of 5 µm (GL. Sciences INC, JAPAN).The samples were chromatographed using an isocratic mobile phase consisting of a 50:50 v/v mixture of a acetonitrile and glacial acetic acid. The flow rate was 2 mL/min and the detection wavelength was 230 nm. All operations were carried out at ambient temperature.

Determination of pH

The pH values of the samples were measured by a pH meter at 20±1°C.All measurements were carried out in triplicate.

In vitro haemolysis study

An *in vitro* hemolytic study was carried out using fresh rat blood (2 mL) by puncturing the orbital sinus (Soniet al., 2014). Clearance for the handling of experimental animals was obtained from the Institutional Animal Ethical Committee (IAEC) constituted for the purpose. Erythrocytes were separated by centrifugation at 3000 rpm for 15 min and washed three times with 10 mMphosphate buffer saline(PBS)pH 7.4 to remove any protein remaining and other debris. Erythrocyte stock dispersion was prepared by making up the volume to 5 mL with PBS and allowed to equilibrate at 2-8°C for 24 h. Test sample (1mL) was added to 100 µL of stock erythrocyte dispersion and was incubated at 37°C for 1 h. The sample was centrifuged at 3000 rpm and 100 µL of supernatant was mixed with 2 mL of ethanol-HCl mixture (39:1 ethanol 99% v/v and HC1 37%, w/w). The absorbance of the mixture was recorded at 230 nm on a UV visible spectrophotometer against a blank sample. The blank sample was prepared using same procedure excluding the erythrocyte to eliminate the drug or excipients related interference. Triton X 5% v/v was used as a positive control and 0.9% w/v NaCl was used as a negative control.

Stability study

Stability of the microemulsion was assessed at various storage conditions viz. 25±2 °C at 60±5% RH(Relative Humidity) and 2-8°C for three months. All Lorazepam (LZM) formulations were stored in glass vials with rubber stoppers and aluminium-crimped tops. Three such vials were stored at various aforementioned storage conditions. Samples were removed periodically and were assessed for content of Lorazepam (LZM), mean globule size, Polydispersityindex andpH.

RESULTS AND DISCUSSION

Solubility studies

Among the various oils that were screened Miglyol 810 exhibited highest solubility withlorazepam.Miglyol 810 is a mixture of Caproic Acid, Caprylic Acid, Capric Acid, Lauric Acid and Myristic Acid. Miglyol 810 is a medium chain tri-glyceride and has been used in parenteral formulations, in the production of emulsions, solutions, or suspensions intended for intravenous administration. Hence Miglyol 810 was selected as the oil phase.Solutol HS 15 is a non ionicsolubilizer and emulsifying agent with Hydrophilic Lipophilic Balance (HLB)between 14-16 and showed good solubility with Lorazepam as mentioned in Table 1.

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Table 1
Solubility study

Phase type	Excipients	Solubility (mg/mL)
Oil	oleic acid	48.16
	Soybean oil	46.32
Surfactant	Miglyol 810	95.29
	Solutol HS 15	198.53

Ternary phase diagram

Solutol HS 15 has good emulsifying potential and Miglyol 810 also has some self- emulsifying properties. The area of microemulsion existence for Solutol HS 15-Miglyol 810-Water system is shown inFigure1. Selection of oil and surfactant was ascertained by pseudoternary phase diagram as it differentiates the microemulsion region from that of macroemulsion region. Microemulsion region from pseudoternary phase diagram was selected for Miglyol 810: Solutol HS 15 system highest region was found in 1:2 ratios and hence this ratio was selected for further preparation.

Characterization of microemulsion

The formulated microemulsion exhibited mean droplet size of 35.74nm. Particle size determination was done from Malvern zeta sizer instrument (Fig. 2) which gave particle size range of nm with maximum intensity and volume. The Polydispersity index was found to be 0.125, showed a narrow size distribution and contributed to the stability of microemulsion. The pH was found to be 7.4. The Zeta potential was found to be 31.08 nm as shown in Table 2. All these studies were carried three times and the results were expressed as the mean ± standard deviation.

Table 2
Characterization of microemulsion

Characterization of microemulsion	Results
Droplet size(nm)	35.74 ±0.05
Zeta Potential (nm)	31.08±0.001
Polydispersity index	0.125±0.001
% Assay	100.805±0.02
Ph	7.4±0.01
Viscosity (cP)	0.8872

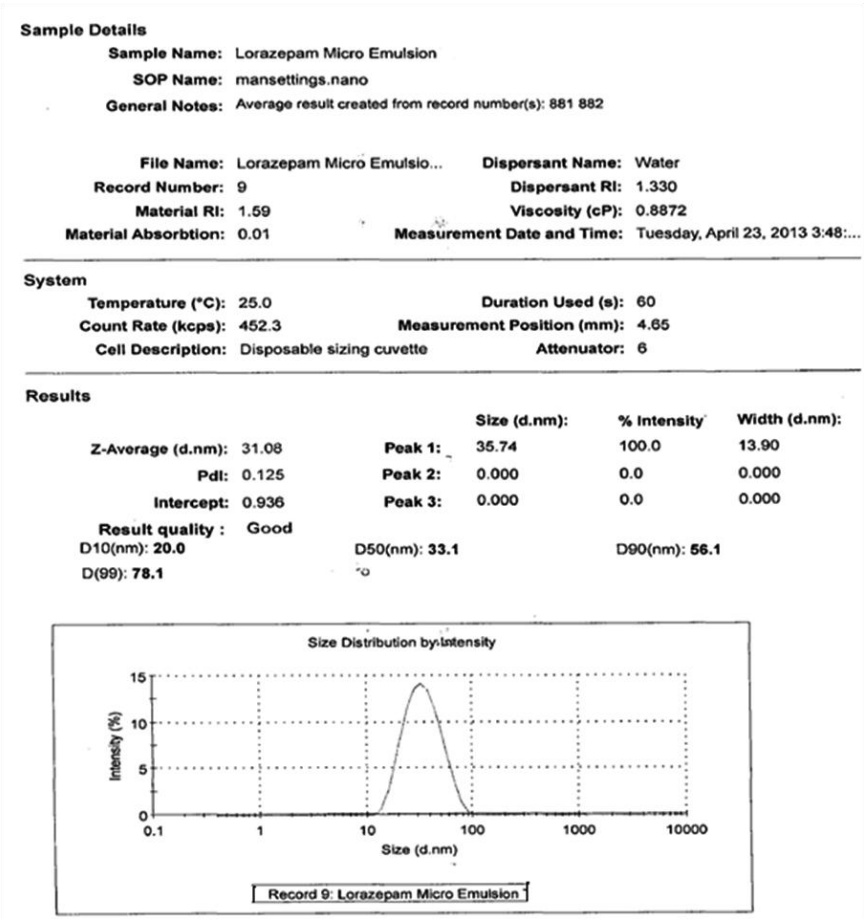

Fig. 2 Particle size distribution

In vitro haemolysis study

The microemulsion showed negligible haemolysis of the erythrocytes (0.16%) indicating its suitability in the parenteral formulations without addition of co solvents. This showed the safety of the micro emulsion for parenteral administration.

Stability study

Prepared microemulsion was found to be stable for three months at ambient temperature and refrigerated condition, as no phase separation or flocculation was observed during storage period. The globule size and zeta potential measurements after three months of storage were found to be satisfactory and hence the microemulsion was found to be stable during this period.

CONCLUSION

Lorazepam microemulsion was prepared successfully with Miglyol 810 and Solutol HS 15. The prepared formulation exhibited all the desirable properties of an ideal microemulsion and was also found to be stable for 3 months.

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