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Research Article

Design and Evaluation of Microemulsions for Improved Parenteral Delivery of Propofol

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Abstract. The objective of this investigation was to evaluate the potential of the microemulsions to improve the parenteral delivery of propofol. Pseudo-ternary phase diagrams were plotted to identify microemulsification region of propofol. The propofol microemulsions were evaluated for globule size, physical and chemical stability, osmolarity, *in vitro* hemolysis, pain caused by injection using rat paw-lick test and *in vivo* anesthetic activity. The microemulsions exhibited globule size less than 25 nm and demonstrated good physical and chemical stability. Propofol microemulsions were slightly hypertonic and resulted in less than 1% hemolysis after 2 h of storage with human blood at 37 °C. Rat paw-lick test indicated that propofol microemulsions were significantly less painful as compared to the marketed propofol formulation. The anesthetic activity of the microemulsions was similar to the marketed propofol formulation indicating that they do not compromise the pharmacological action of propofol. The stability studies indicated that the microemulsions were stable for 3 months when stored at 5±3 °C. Thus, microemulsions appeared to be an interesting alternative to the current propofol formulations.

KEY WORDS: biocompatibility; hemolysis; microemulsions; pain on injection; propofol.

INTRODUCTION

Propofol, (2,6-diisopropylphenol) is a short-acting hypnotic agent which is administered by intravenous route during short surgical procedures and mechanical ventilation in the intensive care unit (1,2). It offers various advantages such as favorable pharmacokinetic profile (rapid distribution and high metabolic clearance), rapid onset and recovery even after long periods of anesthesia and low incidence of post-operative nausea and vomiting (3,4). In spite of such attractive clinical advantages, the successful development of injectable propofol formulation is very difficult due to its poor aqueous solubility (154 μg/ml) and high lipophilicity (log P=3.8) (5).

Currently, propofol is formulated as an oil-in-water emulsion (1%, *w*/*v*) that contains soybean oil, glycerol, and purified egg phosphatide (Diprivan®, Astra-Zeneca, USA and the generic Propofol emulsion from Baxter International, USA). Diprivan® contains disodium edetate and has a pH of 7–8.5, while the Baxter propofol emulsion contains sodium metabisulphite and is formulated to a pH of 4.5–6.4 (6). However, both the formulations, due to presence of soybean oil, result in hyperlipidemia after long-term infusion and cause pain at the site of injection, which strongly limits its clinical utility (7). Furthermore, being a lipid-based emulsion, it also suffers from a number of limitations, such as poor physical stability, potential for embolism, need for strictly aseptic handling, and rapid growth of microorganisms (7,8).

These drawbacks have stimulated an active search for better alternatives, the main objective being an improvement in aqueous solubility, stability and patient acceptance of propofol. With this aim, various approaches like complexation with cyclodextrins (5,9,10) and design of prodrugs or water-soluble derivatives of propofol (11–13) have been exploited by several researchers. However, propofol prodrugs would be treated as new chemical entities and would have to undergo exhaustive clinical trials starting from Phase 1. Hence, the utility of this approach is limited. Solubilization of propofol by complexation with various cyclodextrins like hydroxypropyl-β-cyclodextrin, hydroxypropyl-γ-cyclodextrin, sulfobutylether-β-cyclodextrin has also been reported in the literature. However, relatively high concentration of cyclodextrins is required (ranging from 17 to 21% *w*/*w*) to yield propofol solution equivalent to marketed propofol formulations (14). Hence, this formulation approach may not be cost effective.

Microemulsions have gained great attention as a drug delivery vehicle in pharmaceutical research due to their well known advantages such as thermodynamic stability, ability to improve efficacy of hydrophobic drugs and ease of manufacture and scale up (15–17). Microemulsions are thermodynamically stable, transparent, isotropic, low-viscosity colloidal dispersions consisting of microdomains of oil and/or water stabilized by an interfacial film of alternating surfactant and cosurfactant molecules. Microemulsions include swollen micellar (oil-in-water, O/W), reverse micellar (water-in-oil, W/O) and bicontinuous structures and have globule size below

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100 nm. Their applications in improved topical, peroral, ocular, nasal and parenteral delivery of hydrophobic drugs are continuously being unraveled (15–17).

Recently, Morey *et al.* (18) have explored the utility of microemulsions for improving the delivery of propofol. These microemulsion formulations utilized sodium salts of C_8, C_{10} and C_{12} fatty acids. Sodium caprylate (C_8 fatty acid) has been shown to cause significant hemolysis at a concentration of 213 mM (19). Although the microemulsions reported by Morey *et al.* (18) do not contain such a high concentration of sodium caprylate, blood compatibility and parenteral acceptability of sodium salts of C_8, C_{10} and C_{12} fatty acids on long term administration have not been established. Ryoo *et al.* (20) in another investigation have reported formulation of microemulsion based on the Solutol® HS 15 and ethanol. However, the microemulsions were not evaluated for *in vivo* advantages such as 'pain on injection' and anesthetic activity.

As propofol formulations have to be administered as a continuous intravenous (I.V.) infusion without any dilution, biocompatibility of all the components and their safety on long-term administration are prerequisites to the formulation. In the present investigation, formulation of oil-in-water microemulsions of propofol was attempted by employing excipients with established long term parenteral acceptability. Propofol, which exists in liquid state at room and physiologic temperature, served as an oily phase of the microemulsions in the present investigation. This would render lipid-free propofol formulations, which would be devoid of various disadvantages associated with current formulations such as hyperlipidemia and growth of microorganisms due the presence of soybean oil. The propofol microemulsions were evaluated for osmolarity, chemical stability of propofol, *in vitro* hemolytic potential, pain on injection and anesthetic effect. The stability of propofol in microemulsions, stored as preconcentrates was determined.

MATERIALS AND METHODS

Materials

Propofol was kindly provided by Bharat Serums and Vaccines Ltd, (Mumbai, India). Solutol® HS 15 (PEG-660–12-hydroxystearate), Cremophore® RH 40 (PEG-40–hydrogenated castor oil), Lutrol® F-127(Poloxamer 407) from BASF (Mumbai, India) and MYS-40 (Polyoxyl-40-stearate) from Nikkol Chemicals, Japan were received as gift samples. Glycofurol (GF) and Tween 80 (Polysorbate 80) were purchased from Merck (Mumbai, India). Propylene glycol (PG), glycerol, polyethylene glycol 400, sodium chloride, ethanol (all AR grade), were purchased from Qualigens (Mumbai, India). Brij® 96 V (Polyoxyethylene-10-oleyl ether) was purchased from Sigma (USA). All the excipients and reagents were used as received. Double distilled water was prepared freshly whenever required.

Screening of Surfactants for Emulsifying Ability

Surfactants, which have parenteral acceptability, were screened for ability to emulsify propofol by using method proposed in the literature (21). Propofol, 100 mg was added to 1 g of the selected surfactant. The mixture was gently heated at 45 °C for homogenizing the components. This homogenous isotropic mixture was diluted with the 9 ml of double distilled water to yield a fine dispersion. The resulting dispersion was allowed to stand for 2 h and its turbidity was assessed spectrophotometrically (Shimadzu UV-160A double beam spectrophotometer) by measuring the percent transmittance value of the dispersion at 638.2 nm, using double distilled water as blank (n=2). The transmittance values of the various dispersions are indicative of the ability of the surfactant to emulsify propofol.

Screening of Cosurfactants (21)

Briefly, Solutol® HS 15 (SHS 15), 0.5 g was mixed with 0.5 g of selected co-surfactant. Propofol, 100 mg was added to this mixture and the mixture was gently heated at 45 °C for homogenizing the components. The homogenous isotropic mixture was diluted with the 9 ml of double distilled water to yield fine emulsion/microemulsion. The percent transmittance value of the resulting dispersion was measured (n=2) in a similar way described in earlier section. Since the ratio of SHS 15 to all the investigated co-surfactants is same, the percent transmittance value of resulting emulsions/microemulsions was considered indicative of the relative efficacy of the co-surfactants to improve the microemulsification ability of SHS 15.

Phase Diagrams

An oil (propofol) titration method was employed in present investigation to construct phase diagrams (22). Briefly, mixtures of the double distilled water with surfactant + cosurfactant (at 4:1 *w/w* ratio) were prepared at ratios (%*w/w*) of 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9 into different vials. A small amount of propofol in 0.5% (*w/w*) increment was added into the vials. Following each propofol addition, the mixtures in vials were vortexed 2–3 min and were allowed to equilibrate at 25 °C for 30 min. After equilibration, the mixtures were examined visually for phase separation, transparency and flow properties. In addition, the mixtures were observed through crossed polarizers (fabricated in house by using polarizing lenses, Nikkon, Japan) for determining the optical isotropy of the systems. The point at which the mixture became turbid or showed signs of phase separation was considered as the end point of the titration. The area of microemulsion existence was determined and denoted as ME. Based on the phase diagrams, the quantity of the surfactant + cosurfactant blend required to solubilize 1% *w/w* propofol (concentration equivalent to marketed propofol formulations) was extrapolated for all the microemulsion formulations.

Evaluation of Propofol Microemulsions

Effect of Various Vehicles on Globule Size and pH of the Microemulsions

The effect of various vehicles viz. water, 2.25% *w/v* glycerol solution, 5% *w/v* dextrose solution and 0.9% *w/v* saline solution on the globule size and pH of the microemulsions was assessed. All the aforementioned vehicles (except water) are isoosmotic to blood and are employed

for the development of parenteral formulations. Based on their effect on globule size and pH, the suitable vehicle was selected and used as an aqueous phase in further investigation.

Globule Size Analysis

The average globule size and polydispersity index (P.I.) of microemulsions were determined by the photon correlation spectroscopy (PCS; Beckman Coulter N5, Wipro, India). Microemulsions were diluted with double distilled water to ensure that the light scattering intensity (between 6e + 004 to 1e + 006), was within the instrument's sensitivity range. Measurements were made at an angle of 90° for all the microemulsions.

Determination of Osmotic Pressure (14)

Osmolarity of various propofol microemulsions and marketed formulation was determined by the freezing point depression method using Osmomat 030 (Gonastec, Berlin, Germany). The instrument was calibrated using an isotonic solution of NaCl and pure water, to give values of 300 and 0 mOsm, respectively. The osmometer vials were filled by micropipette taking up 50 μl of each solution of various propofol formulations. All the measurements were carried out in triplicate.

***In vitro* Hemolysis (23,24)**

Various propofol microemulsions were assessed for their potential to cause *in vitro* hemolysis of heparinized fresh human whole blood. For hemolytic studies, the propofol microemulsions were diluted with 0.9% saline to obtain a concentration of 50 μg/ml. Blood was obtained from two human volunteers. Both volunteers signed written consent forms. Blood samples were pooled and subdivided into three portions (1.6 ml each) and each of the portions was spiked with various propofol microemulsions diluted with saline (0.4 ml) such that the final concentration of propofol in all the blood samples was 10 μg/ml. The propofol concentration (10 μg/ml) employed in the hemolytic studies is representative of *in vivo* concentration of propofol achieved after intravenous infusion (considering the standard propofol dose).

Immediately after addition, the blood-microemulsion mixture was gently agitated for 5–10 s and incubated at 37 °C for 2 h. After incubation, the mixtures were placed in the ice-cold water for 2 min to quench the hemolysis. The intact red blood cells were separated from the supernatant by centrifugation at 3,000 rpm for 5 min at 5 °C. The negative control was prepared by incubating 1.6 ml of blood with 0.4 ml of 0.9% *w*/*v* saline solution at 37 °C for 2 h. In order to effect 100% hemolysis of the blood, 1.6 ml of blood was diluted with 14.4 ml of double distilled water and the mixture was incubated at 37 °C for 30 min. This sample served as positive control. The controls received same treatment as that of test samples so as to separate intact erythrocytes from the mixture. However, in case of negative control all the erythrocytes remained intact and in case of positive control none of the erythrocytes remained intact.

The supernatant from all the test samples (including positive and negative control) was separated and centrifuged again at 3,000 rpm for 5 min at 5 °C in order to get rid of any intact erythrocytes that may have been inadvertently withdrawn with the supernatant. Supernatant, 0.3 ml was suitably diluted with 4.15 ml of 0.9% *w*/*v* saline solution in case of the test samples and negative control and with double distilled water in case of the positive control and analyzed for the content of hemoglobin by measuring the absorbance of supernatant at 540 nm.

The extent of hemolysis as percentage (% H) was determined by using following equation

$$\%\mathrm{H} = \left[\frac{(\mathrm{Abs}_{\mathrm{test}} - \mathrm{Abs}_{\mathrm{control}})}{(\mathrm{Abs}_{100} - \mathrm{Abs}_{\mathrm{control}})}\right] \times 100 \qquad (1)$$

where, $\mathrm{Abs}_{\mathrm{test}}$ is absorbance of test sample, $\mathrm{Abs}_{\mathrm{control}}$ is absorbance of control sample (negative control) and Abs_{100} is absorbance of sample in which 100% hemolysis occurred (positive control).

Rat Paw-Lick Test (25–27)

The rat paw-lick test has been used to evaluate pain experienced by rats after injecting formulations under investigation. 15 weanling rats (Sprague–Dawley males, 70–120 g each) were obtained from the Experimental Animal Breeding Center of Bombay College of Pharmacy. All the experiments were performed according to the CPCSEA (Committee for the purpose of the control and supervision on experiments on animals) guidelines. Rats were divided in the three groups as follows

1. 0.9% *w*/*v* saline solution
2. Marketed propofol emulsion with composition equivalent to the propofol emulsion being marketed by Baxter International, USA (Propovan®, Bharat Serums Pvt. Ltd., Mumbai, India)
3. SHS-T80 a representative of microemulsion formulation

The propofol microemulsion was filtered through 0.22 μ membrane filter (Pall Life sciences, Mumbai, India) at the beginning of the experiment. Each rat was given a single injection of 100 μl of either, 0.9% *w*/*v* saline solution, Propovan® or SHS-T80 into the footpad of the right hind paw. The stopwatch was started immediately whenever rat started licking and stopped immediately when rat stopped licking. Total licking time was recorded by monitoring each rat for 10 min as the reaction to the 'pain on injection' is usually instantaneous and does not last for very long time. The duration of paw-licks, expressed as mean±SD was subjected to statistical analysis. The statistical significance of differences in the duration of paw-lick data were analyzed utilizing two tailed paired '*t*' test (GraphPad InStat Demo Version). Differences were considered statistically significant at $P<0.05$.

***In vivo* Anesthetic Efficacy (14)**

In vivo anesthetic efficacy of the propofol microemulsions was evaluated in comparison to the marketed propofol emulsion (Propovan®, Bharat Serums Pvt. Ltd., Mumbai).

Table I. The Composition (% *w*/*w*) of the Developed Propofol Microemulsions

Ingredients	SHS-PG	SHS-GF	SHS-T80
Propofol	1	1	1
Solutol HS 15	8	8	8
Propylene glycol	2	–	–
Glycofurol	–	2	–
Tween 80	–	–	2
Double distilled Water[a]	89	89	89

[a] For *in vitro* studies (osmolarity and hemolysis) and *in vivo* studies (rat-paw lick test and anesthetic efficacy) the 0.9% saline solution was used instead of double distilled water

Adult female Wistar rats with body mass of 200–250 g, were obtained from Haffkins Institute, Mumbai at the beginning of the experiments. Rats were maintained under an artificial 12 hours light–dark cycle (lights on from 0800 to 2000 hours) and at a constant temperature of 23±2 °C and 65% humidity. Food and water were freely available, and the animals were acclimatized for >7 days before use. Experiments were performed between 0800 and 1400 hours. Animal care and handling throughout the experimental procedure were performed in accordance to the CPCSEA guidelines. The experimental protocol was approved by the Animal Ethical Committee of the Bombay College of Pharmacy. The propofol microemulsions were filtered through 0.22 μ membrane filter at the beginning of the experiment.

Rats were injected intravenously (single bolus in the lateral tail vein) with the different formulations of propofol, each containing equimolar concentration of propofol [10 mg/(ml kg)]. For intravenous administration, animals were restrained in an appropriate plexiglass cage and a tail vein was used. Following the drug administration, rats (n=6) were observed for the 60 min, and the onset and duration of loss of the righting reflex (LORR) were recorded. The duration of LORR, expressed as mean±S.D. was subjected to statistical analysis. The statistical significance of differences in the duration of LORR data were analyzed utilizing analysis of variance (ANOVA) followed by Dunnet's test (GraphPad InStat Demo Version). Differences were considered statistically significant at $P<0.05$.

Stability Studies

For stability studies, propofol microemulsion preconcentrates were employed. Propofol microemulsion preconcentrates had same composition as described in the Table I, except water. Chemical and physical stability of the propofol microemulsion preconcentrates was assessed at various storage conditions viz. 5±3, 30±2/65±5% RH and 40±2 °C/75±5% RH as per ICH Guidelines. Propofol microemulsion preconcentrates were stored in glass vials with rubber stoppers and aluminum-crimped tops. For each propofol microemulsion preconcentrate, five such vials were stored at various aforementioned storage conditions up to 3 months. Samples were removed at 0, 60 and 90 days of interval and were assessed for content of propofol, mean globule size and polydispersity index. The propofol content of the microemulsion preconcentrates was determined by the HPLC method described by Trapani *et al.* (14). The data obtained at various time points about the propofol content and mean globule size of the microemulsions was subjected to statistical analysis. The statistical significance of differences in the data was analyzed utilizing analysis of variance (ANOVA) followed by Bonferroni's test (GraphPad InStat Demo Version). Differences were considered statistically significant at $P<0.05$.

RESULTS AND DISCUSSION

Screening of Surfactants for Emulsifying Ability

The method used for assessing the ability of the surfactants to emulsify oily phase (propofol in this case) is well explained by Date and Nagarsenker (21). Lutrol® F 127 based emulsions separated within 2 h and Brij 96V formed very viscous gel like structure, hence turbidity of these formulae was not measured. The percent transmittance values observed with the other surfactants were in the following order Tween 80 (99.1) > Cremophore RH 40 (97.8) > Solutol HS 15 (94.6) > MYS-40 (22.7). The percent transmittance values clearly differentiated various surfactants for their ability to emulsify propofol. Tween 80 (T80) appeared to be the best emulsifier for propofol. Nevertheless, Cremophore® RH 40 and Solutol HS 15 (SHS 15) also had good ability to emulsify propofol. 'LD$_{50}$ value of the surfactants on parenteral administration' was also considered

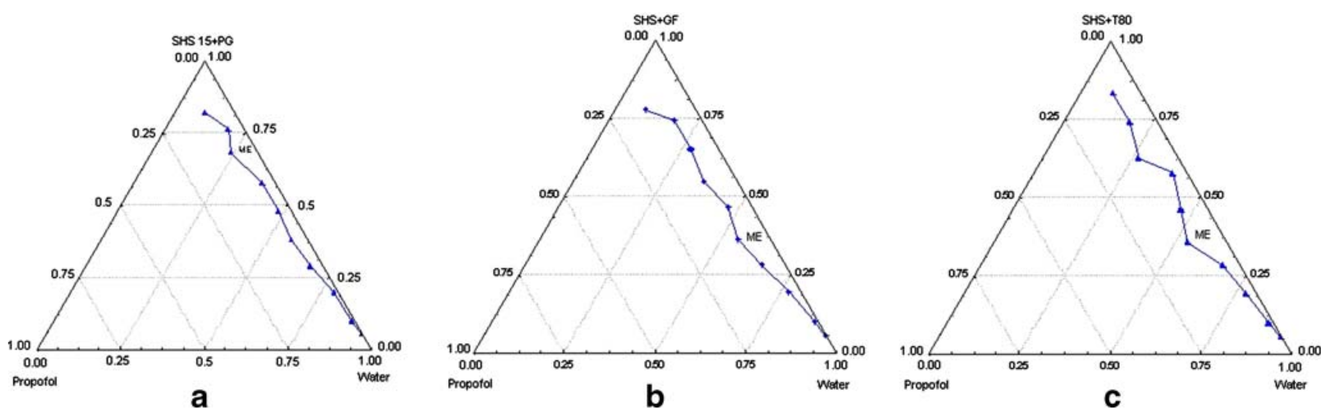

Fig. 1. Pseudo-ternary phase diagrams of propofol microemulsions **a** SHS15-PG-water-propofol system, **b** SHS15-GF-water-propofol system and **c** SHS15-T80-water-propofol system

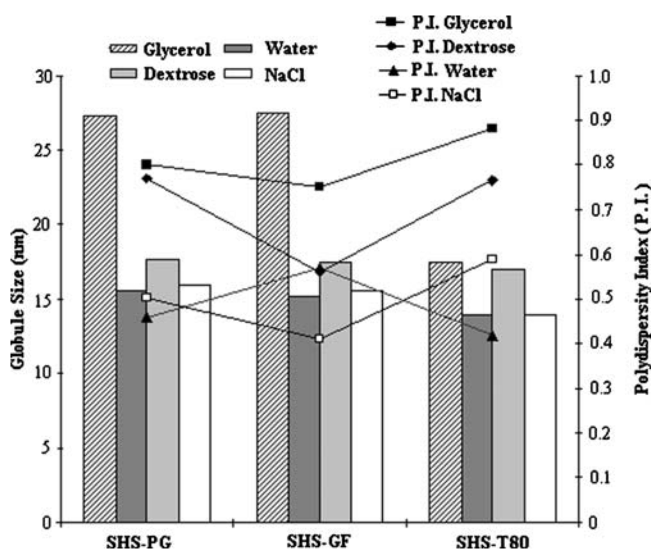

Fig. 2. Effect of vehicles on the mean globule size and polydispersity index of propofol microemulsions; data were expressed as mean (n=2) where relative standard deviation was <10%

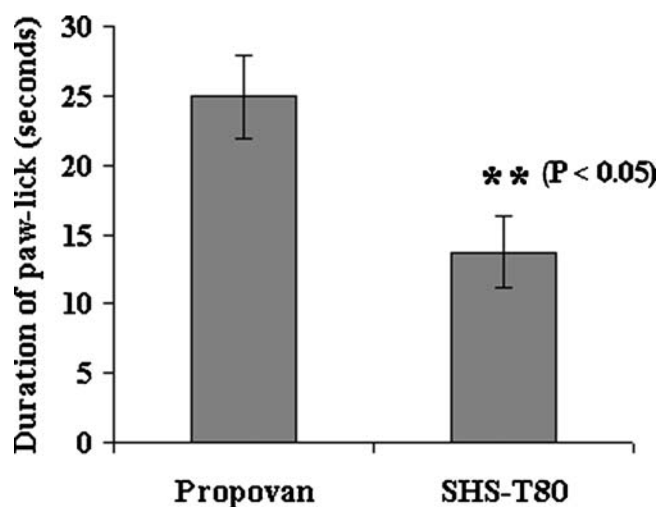

Fig. 3. Results of rat paw-lick test (n=5); the 0.9% saline group did not display any paw-licks

for selecting the surfactant for further studies. Cremophore® RH 40, despite of its good parenteral acceptability, was not selected for further studies as it is known to cause allergic reactions after parenteral administration (28). Hence, SHS 15 and T80 were considered for further studies. Moreover, SHS 15 due to its much better parenteral tolerability than T80 (29,30) was used as a primary surfactant in the further investigation.

Screening of Cosurfactants

The method used for assessing the relative ability of cosurfactants to improve microemulsification of oil by a particular surfactant is well established in the literature (21). The ability of the cosurfactants to improve the microemulsification of propofol by SHS 15 was reflected in the percent transmittance value of the final dispersion and was in the following order Tween 80 (97.8) > Glycofurol (96.2) > Propylene glycol (95.3) > Ethanol (94.9) > Polyethylene glycol (0.6) > Glycerol (0.5). Glycerol and polyethylene glycol resulted in very cloudy emulsions. This could be due to the poor penetration of glycerol and polyethylene glycol in the surfactant layers at the interface (31,32). Propylene glycol (PG), ethanol, glycofurol and Tween 80, all were efficient in improving microemulsification of propofol by SHS 15. Propylene glycol (PG) and ethanol, due to their short chain length, probably penetrated in the surfactant layer at the interface (32) and the dispersions were stable even after 24 h. Glycofurol (GF), due to its amphiphilic nature, would partition in aqueous as well as oily phase and would also penetrate in the surfactant layer leading to stabilization of formed microemulsion. Considering the acceptability on long-term administration, PG and GF were selected for further investigation. Tween 80 (T80) was found to improve micro-emulsification of SHS 15 to greatest extent. Literature indicates that mixture of surfactants can yield stable micro-emulsions (33) and observations in the current investigation are in line with it and the resultant propofol dispersion was absolutely transparent. This could be due to additional stabilization of propofol at interface due to surfactant nature of T80.

Table II. Osmolarity and Hemolytic Potential of the Various Propofol Microemulsions

Formulation	Osmolarity (mOsm/kg)[a]	% Hemolysis[a]
Propovan®	310±1.4	Not determined
SHS-PG	698±2.1[b]	0.6±0.2
SHS-GF	514±2.8[b]	0.2±0.1
SHS-T 80	396±4.2[b]	0.4±0.2

[a] Data expressed as mean±SD (n=3)
[b] Osmolarity values are significantly different from Propovan® (P< 0.05) when evaluated by ANOVA

Phase Diagrams

The phase behavior of all the systems was studied at a fixed ratio of surfactant to cosurfactant 4:1, as it was found to be optimum for all the systems during preliminary investigations (data not shown). The phase diagrams (Fig. 1) have two distinct regions viz. ME region and non-ME region. The ME region represents clear optically isotropic microemulsion area and non-ME region represents the turbid dispersed

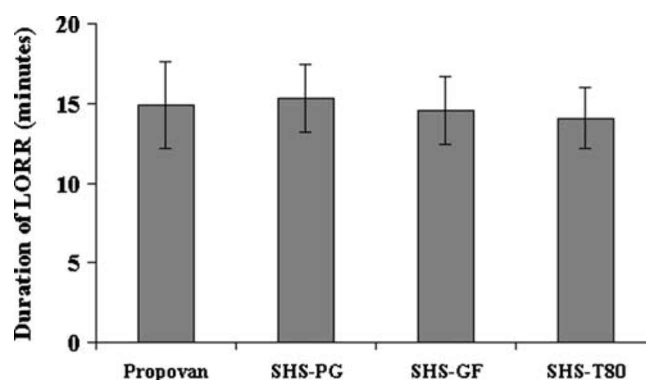

Fig. 4. *In vivo* anesthetic activity of propofol microemulsions in comparison to marketed propofol emulsion, Propovan® (n=6); Microemulsions do not compromise anesthetic effect of propofol (P>0.05 when evaluated with Propovan® by ANOVA)

Table III. Propofol Content[a] in Microemulsions at Various Storage Conditions

Formulation	5±3 °C			30±2 °C/65±5% RH			40±2 °C/75±5% RH		
	0 day	60 days	90 days	0 day	60 days	90 days	0 day	60 days	90 days
SHS-PG	100.4±0.8[a]	100.08±0.6	99.2±0.4	100.4±0.8	100.8±0.5	101±1.2	100.4±0.8	100.6±0.8	99.8±0.4
SHS-GF	101.4±0.6	100.72±0.5	99.24±0.6	101.4±0.6	101±0.6	98.7±1.1	101.4±0.6	100.1±0.7	99±0.9
SHS-T80	100.4±0.9	100.2±0.6	97±2.2	100.4±0.9	99.8±0.7	93±1.4	100.4±0.9	96.6±0.9	92.4±1.6

[a] Data were expressed as mean±SD (n=3).

systems identified based on visual observation. Their properties were further not characterized in detail for any of the investigated systems. Maximum propofol incorporation that was achieved was not more than 20% (at 9:1 ratio of surfactant + co-surfactant to water) for all the investigated systems. Interestingly, these results are similar to those reported by Morey *et al.* (18) although the systems explored by them are different than systems in the present investigation. The area of microemulsion formation was highest in the case of SHS-T80 system and was least for the SHS-PG system but the differences were not very significant. Surprisingly, for all the investigated systems, deflection in the microemulsion boundary line was observed beyond 4:6 ratio of surfactant + cosurfactant mixture to water. This atypical behavior can be attributed to increased interaction/solubilization of propofol with surfactant and cosurfactant at this particular ratio. Phase diagrams clearly indicated that that it was possible to microemulsify 1% *w/w* of propofol at only 10% *w/w* of surfactant + co-surfactant. The composition of microemulsions, which have been used in the further investigation, is given in Table I.

Evaluation of Propofol Microemulsions

Effect of Various Vehicles on Globule Size and pH of Propofol Microemulsions

Identifying suitable parenteral vehicle is very important for the development of parenteral propofol microemulsions. Studies indicated that some of the vehicles did influence the globule size of microemulsions (Fig. 2). Globule size of propofol microemulsions was found to increase appreciably when diluted with 2.25% *w/v* glycerol solution as compared to water. However, the globule size of microemulsions diluted with 0.9% *w/v* saline solution and 5% *w/v* dextrose solution was almost same as the microemulsions containing water. The pH values of the microemulsions produced with various vehicles were in the range of 4.6 to 4.8 (n=3; where relative standard deviation was less than 5%) and are analogous to the pH of the currently available propofol emulsion being marketed by Baxter Inc., USA. For further studies, 0.9% *w/v* saline solution was selected as an aqueous phase.

Determination of Osmotic Pressure

The osmotic pressure of the parenteral formulations should be determined to identify the need for adjustment of the osmolarity. Hypo- or hyperosmolarity of parenteral formulations can cause pain on injection, morphological change of erythrocytes and tissue damage at injection site (34,35). Though the propofol microemulsions were diluted with 0.9% *w/v* saline solution, the presence of surfactants and cosurfactants may alter the osmolarity value of the microemulsions. Hence, it was necessary to determine the osmolarity value of propofol microemulsions. Osmolarity studies indicated that all the propofol microemulsions were slightly hyperosmotic than 0.9% *w/v* saline solution and marketed propofol emulsion (Table II). The rank order of osmolarity was SHS-PG > SHS-GF > SHS-T80. The SHS-PG and SHS-GF exhibited considerably high osmolarity than SHS-T80 as PG and GF themselves exhibit considerable osmotic pressure that is further increased by the addition of 0.9% *w/v* saline solution to the microemulsions.

In Vitro Hemolysis

The hemolytic potential of the parenteral microemulsions should be determined to prove their safety to blood components. It has been demonstrated that commonly employed parenteral cosurfactants such as glycerol or propylene glycol can cause considerable hemolysis on long term contact with the blood (36,37). Identification of hemolytic potential of a parenteral microemulsion is necessary especial-

Table IV. Globule Size of Propofol Microemulsions at Various Storage Conditions

Formulation	5±3 °C			30±2 °C/65±5% RH			40±2 °C/75±5% RH		
	0 day	60 days	90 days	0 day	60 days	90 days	0 day	60 days	90 days
SHS-PG	15.3[a] (0.33)[b]	16.1 (0.64)	16.9 (0.74)	15.3 (0.33)	16.8 (0.59)	16.4 (0.69)	15.3 (0.33)	14.9 (0.83)	16.3 (0.61)
SHS-GF	16.1 (0.59)	14.4 (0.934)	17.1 (0.63)	16.1 (0.59)	17.7 (0.46)	16.7 (0.71)	16.1 (0.59)	15.4 (0.6)	17.5 (0.84)
SHS-T80	12.8 (0.64)	14.9 (0.79)	15.8 (0.72)	12.8 (0.64)	14.2 (0.56)	15.9 (0.86)	12.8 (0.64)	13.7 (0.78)	16.2 (0.77)

[a] Particle size expressed as mean (n=2) where relative standard deviation was < 10%
[b] Polydispersity Index; Data were expressed as mean (n=2)

ly when the formulation is to be administered as a continuous infusion over a long period of time. Hence, though, all the propofol microemulsions were based on the components acceptable for parenteral delivery, their hemolytic potential was determined. The propofol concentration (10 μg/ml) employed in the hemolytic studies is representative of *in vivo* concentration of propofol achieved after intravenous infusion. All the propofol microemulsions caused negligible (<1%) hemolysis (Table II) on contact with human blood for 2 h.

Rat-Paw Lick Test

Interestingly, none of the rats in the group injected with the 0.9% *w*/*v* saline solution displayed any paw-lick. Rat-paw lick test clearly indicated that the rats treated with SHS-T80 microemulsion showed significantly less duration of paw-lick as compared to the marketed formulation (Propovan®; $P < 0.05$ when evaluated by two tailed paired '*t*' test) and the results are shown in Fig. 3. The pain on injection resulting after administration of propofol emulsion is a combined effect of lipid composition and irritating nature of propofol that stems from its phenolic backbone. (7,38) It is noteworthy that rats treated with SHS-T80 microemulsion did exhibit considerable paw licks as compared to 0.9% *w*/*v* saline solution confirming the fact that propofol itself has considerable irritation potential.

It is hypothesized that our propofol microemulsion is less irritating than marketed propofol emulsion due to its lipid-free nature and also due to its much lesser viscosity as compared to marketed formulation (data not shown). However, this observation differs from a recent clinical trial ($n = 130$) conducted by Dubey and Kumar (39) which concluded that lipid free propofol formulation resulted in pain on injection in significantly greater population (89%) as compared to propofol formulation based on medium chain triglycerides (40%). As the nature and composition of the lipid-free formulation of propofol used by the investigators is not disclosed, it is difficult to conclude about the impact of lipid-free propofol formulations (either positive or negative) on the 'pain on injection'. Extending the studies on microemulsions in wider population and to clinical situation will help in providing more conclusive inferences about the reduction in the pain on injection.

In Vivo Anesthetic Efficacy

The results of the *in vivo* anesthetic efficacy test are shown in Fig. 4. For all the formulations, the onset of anesthetic effect was immediate. The duration of loss of righting reflexes (LORR) shown by propofol microemulsions (SHS-PG, SHS-GF and SHS-T80) was statistically not different from the Propovan® ($P > 0.05$) confirming that the microemulsions do not compromise the pharmacodynamic activity of propofol.

Stability Studies

The statistical evaluation of the stability data indicated that propofol content did not undergo any significant change in case of SHS-PG and SHS-GF during the period of 3 months at all the conditions of storage (Table III). In case of SHS-T80 microemulsion, at 5±3 °C, no significant change in the propofol content was observed at the end of 3 months. The same microemulsion, at 30±2 °C/65±5% RH, showed no significant change in the propofol content for first 2 months; but the propofol content considerably decreased (~7%) at the end of 3 month. The similar trend was observed when the SHS-T80 microemulsion was stored at 40±2 °C/75±5% RH (Table III). No significant change was observed in the mean globule size of all the propofol microemulsions even at the end of 3 months at all aforementioned storage conditions (Table IV).

CONCLUSIONS

Microemulsion is a novel and commercially feasible approach to improve the parenteral delivery of propofol and has potential to reduce pain on injection and provide uniformity of content without compromising its pharmacodynamic activity and physicochemical stability.

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