

Scientific paper

Formulation and Evaluation of Multidose Propofol Nanoemulsion Using Statistically Designed Experiments

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Abstract

Despite of the clinical, scientific, and commercial development, many patients complain about pain on the intravenous injection of propofol. Present work was undertaken to develop a stable multi-dose propofol nano-emulsion using 3² full factorial design which is supposed to be associated with less anticipated pain during intravenous administration. Propofol was incorporated in the mixture of disodium edetate, sodium oleate, thioglycerol, glycerol, egg lecithin, soyabean oil and medium chain triglyceride oil, and homogenization was continued at controlled temperature of 20 °C. The product did not show any significant change in visible extraneous particulate matter, pH, osmolality, bacterial endo-toxin, sterility, high performance liquid chromatography (HPLC) their stability and impurities after exposing at 40 °C for 3 and 6 months. Homogenization at 850 bar pressure of 30 min duration produced 174 nm particles with –53.6 mV zeta potential indicating its stability.

Keywords: Homogenization; nano-emulsion; propofol; statistical design of experiment.

1. Introduction

From the time of World War–II, anesthesiology got its importance for induction in combating casualties. The core objectives in developing injectable formulation are solubility, stability, and systemic tolerability. Osmolality is a potential factor for local tissue tolerability of an injectable drug product which would ideally be similar to that of the body fluid. Propofol is widely used for anesthesia.^{1,2} However, propofol is associated with pain at the administration site of the intravenous (IV) injection which is the substantial setback in clinical anesthesiology for smooth induction of general anesthesia.^{3,4} Various strategies have been reported to diminish the prevalence of pain by the addition of lidocaine, pethidine, fentanyl, alfentanil, remifentanil, butorphanol, thiopental etc. to propofol but no significant success has yet been achieved.^{5–7} The induction of anesthesia needs a single dose of 1–5 mg/kg and maintenance dose of 50–250 mcg/Kg/min. Frequent dosing may be required for the entire operation period of in-

fusion.⁸ Attempts were also taken to minimize the pain by increasing the rate of infusion at 2.5 to 5 ml every 5 seconds compared to the normal rate of infusion (20 drops/minute) but managing pain was still not possible.^{9–11}

Commercially available propofol emulsions vary in particle size and lies somewhere around 300 nm.^{5,12,13} Reduction in particle size of propofol may reduce pain upon its intravenous administration. Particle size reduction presented lower free fraction of propofol and a tendency to a lower incidence of pain.¹⁴ A lot of momentum in the recent past has been observed in fabricating nano-sized drug particles by top-down method using high pressure homogenization. Li et al., formulated microemulsion using pluronic F68, propylene glycol and saline, and produced a droplet size of about 300 nm.¹³ Ethyl butyrate being a short chain fatty (C-4, fewer than six carbons) acid showed higher drug release but deprived stability. Whereas, soybean oil, a larger molecule exhibited lower partitioning with excellent emulsion stability. Propofol emulsions formulated with mixtures of ethyl butyrate and soybean oil

were kinetically stable with reduced aqueous phase drug concentration.^{15–17} No significant differences in pharmacokinetics and sedation properties were observed on comparison to a commercial soy-based propofol emulsion.¹⁸ To decrease the amount of free propofol in the aqueous phase propofol emulsions were fabricated with medium and long-chain triglycerides (MCT/LCT).^{19–22} Heat generated during processing of a propofol product can swiftly induce microbial growth.²⁰ It was evident from a randomized double-blind clinical trial that pain on injection was reduced with small particle size lipid emulsion of propofol.²³ Soybean oil, glycerol, egg lecithin, and disodium edetate are present in the propofol innovator product (Diprivan, Astra Zeneca), and the combination of triglycerides and phospholipids in the formulation reduces the chance of free propofol concentration.^{24–26}

Multi-dose propofol oil in water stable nano-emulsion has been developed complying USP specifications with an intention to decrease the particle size, which is supposed to cause less pain. Propofol nano-emulsions were fabricated with and without sodium oleate using controlled homogenization technique. Process duration and homogenization pressure have been optimized for obtaining stable nano-emulsion of reduced particle size.

2. Experimental

2.1. Materials

Propofol was received as a kind gift sample from Themis Medicare, Gujarat, India. Soybean oil (Lipoid purified soybean oil 700), MCT (medium chain triglycerides) oil, Egg lecithin (Lipoid E80), and sodium Oleate (Lipoid Sodium Oleate B) were collected from Lipoid GMBH, Germany. Glycerols, thioglycerol, benzyl alcohol, disodium edetate, sodium hydroxide were sourced from Sigma Aldrich / Merck India. Capsule and membrane filters were from Millipore, India. Clear tubular glass vials were procured from Schott Kaisha, India and Bromobutyl stoppers were from West Pharma, Singapore. Freshly prepared double distilled water was used whenever required. All other reagents used were of analytic grade.

2.2. Formulation Development and Optimization

Preliminary studies were performed to characterize the active and inactive substances for their various physicochemical properties. Solubility and compatibility of the drug with other excipients were determined. The drug substance was thoroughly mixed with each of the excipients (except sodium hydroxide) in 1:1 ratio and were exposed at 50 °C for 2 weeks and observed for any change due to the impact of temperature and humidity.

The entire work was carried out in a Class-100 area, under the LAF (Lamellar Air Flow, Accumax, India) cabinet,

pre fumigated with H₂O₂. All the containers and accessories were autoclaved at 121 °C, 15 psi of pressure for 15 min prior to use. The dissolution of each and every formulation ingredient was checked. The speed of stirring, temperature and time were recorded. The manufacturing temperature was fixed by observing the solubility of propofol. The formulation pH was observed by checking the pH at each step with reference to pH drift studies performed by adjusting the pH of the bulk to different pH values within the range.

After execution of several trials of process temperature, homogenization time, formulation stability and microbial attributes the optimized formulation was finalized with the inactive ingredients as: soybean oil (50 mg/mL), MCT oil (50 mg/mL), egg lecithin (13 mg/mL), sodium oleate (0.52 mg/mL), glycerol (22.5 mg/mL), benzyl alcohol (1 mg/mL), disodium edetate (0.05 mg/mL), and thioglycerol (1 mg/mL). Disodium edetate, sodium oleate, thioglycerol, glycerol and Egg lecithin were added to water at about 20 °C and stirred for 10 min with an overhead stirrer and filtered using a 0.45 µm PVDF filter (Durapore, Millipore). Soybean oil and MCT oil were mixed with an overhead stirrer for 5 min in a separate beaker and propofol (10 mg/mL) was added to it and mixed further for 5 min. Both these phases were mixed for 5 min and sodium hydroxide was added for pH adjustment to 6.5. Then homogenized using the Lab scale homogenizer (Lark Ultrasonic Cell Crusher, India) until it was filterable through the 0.22 µm PVDF filter (Durapore, Millipore). Throughout the process, the temperature was maintained at about 25 °C. The laboratory scale optimized process was executed in triplicate for reproducibility and to monitor the robustness of the formulae and process with confirmation of various analytical parameters.

Scale up of the manufacturing process of the optimized batch was carried out in a class-100 area (room temperature 20 ± 2 °C) using large scale equipments, e.g., mixing/pressure vessels, homogenizer (Goma Engineering, Mumbai, India), cooling jackets, vertical stirrers, filling and sealing machines. Homogenization^{27,28} was continued for 30, 60 or 90 min at 650, 750 or 850 bar as per the levels of the factorial design (Table 1).

Benzyl alcohol, thioglycerol and disodium edetate were dissolved in water at about 20 °C and egg lecithin was added with continued stirring for 30 min. Other ingredients of the oil phase were properly mixed and filtered through 0.45 µm filter and was poured in to the aqueous phase for homogenisation from 650 to 850 bar pressure for 30–90 min.

Droplet size distributions and zeta potential of emulsion at each interval of time and pressure were obtained using dynamic light scattering (Malvern Nano-ZS, USA).²⁹

2.3. Statistical Design of Experiment

Varieties of factorial designs of experiments can be used in drug development. These different factors and

their interactions should be evaluated to predict their effects on the final formulation and its robustness.³⁰ An appropriate mathematical model (experimental design) can give necessary information for the fabrication process and product development.³¹

A three-level factorial design was used for optimization of the homogenization process. In the present study, pressure during homogenization (X_1) and duration (time) of homogenization (X_2) were chosen as independent variables. The particle size (Z) following homogenization was estimated as dependent response (Table 1). Homogenization pressure was evaluated at 650 bar (-1), 750 bar (0), and 850 bar (+1), while duration of homogenization was evaluated at 30 min (-1), 60 min (0), and 90 min (+1). From the preliminary trials, the levels for these two parameters were finalized. To evaluate the responses incorporating interactive and polynomial terms in a statistical model was explained.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2 + \dots \quad (1)$$

where, Y is the dependent variable, b_0 is the arithmetic mean response of the nine runs, and b_i (b_1, b_2, b_{12}, b_{11} and b_{22}) are the estimated coefficient for the corresponding factor X_i (X_1, X_2, X_1X_2, X_1^2 and X_2^2), in which the average result of changing one factor at a time from its low to high value is represented. The change in response is shown by the interaction term (X_1X_2) when two factors are changed simultaneously. To investigate nonlinearity the polynomial terms (X_1^2, X_2^2) are included. For the generation and evaluation of the statistical experimental design Design-Expert software (Version. 10.0.1, Stat-Ease Inc., Minneapolis, USA) was used.³²

2. 4. Polyvinylidene Fluoride (PVDF) Filter Integrity Test

Sterilizing-grade hydrophilic PVDF disposable filter (Durapore membrane disc, Millipore Corp.) disc (0.45 and 0.22 μm) was used for product integrity test. The filter was incubated with the product in a closed vessel at 40 °C for a contact time of 4 hours. The filter was then investigated for change in the physical characteristics such as bubble point, membrane thickness and burst pressure. The integrity testing of the filter elements were wetted with the optimized formulation.

2. 5. Leachable Extracts

Presence of any leachable extracts and the loss of components due to adsorption were estimated by soaking the filter membrane with the optimized formulation in relation to the fresh formulation.

2. 6. Filling and Sealing

The product was then passed through a membrane filter holder with 2.0 & 0.45 μm filters at 0.3–0.4 bar pressure, filled in 50 mL sterilized vials under nitrogen and sealed.

2. 7. Color and Clarity

The color and clarity parameters were assessed by comparing the test solution with a standard reference suspension. Briefly, standard stock opalescence was prepared by adding 1% (w/v) hydrazine sulphate in water (25ml) to a 10% (w/v) solution of Hexamine in water (25

Table 1. Process variables and the observed response using 3² factorial design

Run	Process factors		Observed response Particle size ^c (Z) in nm
	Pressure (X_1) ^a	Duration (X_2) ^b	
1	1.00	0.00	182 (\pm 8.1)
2	-1.00	-1.00	328(\pm 4.8)
3	-1.00	1.00	302(\pm 6)
4	0.00	0.00	206(\pm 5)
5	-1.00	0.00	302(\pm 5)
6	0.00	0.00	210(\pm 3)
7	1.00	-1.00	176(\pm 3)
8	0.00	0.00	203(\pm 3)
9	0.00	0.00	205(\pm 5)
10	0.00	0.00	209(\pm 3)
11	0.00	1.00	206(\pm 3)
12	1.00	1.00	207(\pm 5)
13	0.00	-1.00	222(\pm 3)

^a Levels of homogenization pressure (X_1) as 650 bars (-1), 750 bars (0) and 850 bars (+1).

^b Levels of homogenization duration (X_2) as 30 min (-1), 60 min (0) and 90 min (+1).

^c Data shown are mean of three determinations and figure in the parentheses indicates standard error of the mean.

ml); mixed well and allowed to stand for 24 hours. Standard opalescence was prepared by diluting 15 ml of the standard stock opalescence solution to 1000 ml with water and further, the reference suspension was prepared by diluting 5ml standard of opalescence with 95 ml of water. Each of 20 ml of test solution and reference suspension was transferred to a colorless, transparent and neutral glass tube, and the absorbance was measured at 430 nm using a UV-visible spectrophotometer (Jasco V-630, USA). The clarity of the solution was compared with standard opalescence produced by hydrazine sulphate in a transparent, neutral glass tube against a black background by viewing in diffused daylight down the vertical axis.³³

2. 8. pH, Osmolality, Particulate Matter Density and Specific Gravity

pH and Osmolality of propofol nano emulsion were measured employing a pH meter (720A; Thermo Orion, USA) and Cryoscopic Osmometer (Osmomat 030; Gonotec GmbH, Germany) respectively. Presence of any particulate matter was observed through the light obscuration counter sensor (APSS-2000 Liquid Particle Counter, Particle Measuring Systems, USA). Density and specific gravity of the emulsion were tested by using a density / specific gravity meter-DA-100 (Kyoto Electronics, Japan).^{33–35}

2. 9. HPLC Assay

For all types of drug estimation the chromatographic procedure was carried out using high performance liquid chromatography (Jasco LC-Net II/ADC, USA). Stainless steel column of 5 mm internal diameter (15 cm × 4.6 mm long), packed with octadecylsilyl silica gel for chromatography R (5 μm) (Nucleosil C18) with a solvent system of orthophosphoric acid, water and acetonitrile at a ratio of 7:200:300 (by volume) was used as mobile phase at a flow rate of 1.2 mL/min. Each solution of 25 μL was injected and the detector of the spectrophotometer was set at 210 nm. Same method was followed taking 10 μL of reference solution and continuing the chromatography for 15 min for the impurities.

2. 10. Multiple Piercing Resistance Study

The final sterile formulation was filled in a sterile 50 mL clear tubular glass vial capped with a sterile bromobutyl stopper. The stopper was punctured with a sterile 22 gauge needle at 0th, 8th, 24th, 32nd, 48th hours with a storage condition of 2–8 °C and sample (2 mL each) was withdrawn for evaluation of sterility and bacterial endotoxin (BET) loads to ensure the products resistance to multiple piercing as BET are lipopolysaccharides associated with pathogenic Gram-negative shock

2. 11. Bacterial Challenge Test

The product was collected from the sealed vials and filtered through a 0.45μ membrane. The membrane was rinsed with 0.1% peptone water and placed over the pre incubated tryptone soya agar plates. The agar plates were incubated at 20–25 °C for 3 days followed by 30–35 °C for further 2 days and the numbers of colonies forming units were counted. Aseptic techniques were followed throughout the process.

Limulus Amoebocyte Lysate (LAL) test was performed to detect the presence of any bacterial endotoxin. All the test tubes, micropipette tips and volumetric pipettes were depyrogenated by autoclaving (121 °C and 15 psi of pressure for 30 min) before testing. Glassware were rinsed with water and are made endotoxin free by dry heat sterilization at 250 °C for one hour. Both NaOH and HCl solution (each 0.1 N) were prepared and autoclaved at 121 °C, 15 psi of pressure for 30 min.⁵¹

2. 12. Stability Studies

Droplet size distribution of the emulsion after 3 freeze–thaw cycles (16 hours at –5 °C, 8 hours at 25 °C) were examined using dynamic light scattering (Malvern Nano-ZS, USA). The hydrodynamic size and zeta potential obtained were compared to that of the freshly prepared samples. Filled and sealed vials (50 mL) were stored at 40 °C ± 2 °C / 75% RH ± 5% RH for 6 months at upright and inverted positions and evaluated for stability. Emulsions were investigated visually at the end of the 3rd and 6th month for any phase separation and creaming and allowed for further testing.¹⁵

3. Results and Discussion

3. 1. Formulation Development

Three reproducible batches of propofol nano-emulsions were developed varying the formulation ingredients and homogenization parameters (Table 1) to optimize the product. Taking the ingredients, pre mixing of oil and water phases with an overhead shaft stirrer resulted in foam formation which hindered a proper mixing of the ingredients. Oil soluble ingredients were mixed and filtered using 0.45 μm filter. Aqueous soluble ingredients were mixed separately and filtered through 2.0 μm filter as egg lecithin micelles cannot be filtered through 0.45 μm filter. Then both phases were mixed separately and then homogenized at up to 850 bar pressure for up to 90 min as per (Table 1). Formulations were fabricated with and without sodium oleate to interpret its interference in BET aggravation. However, sodium oleate was found to be a non-interfering substance. The single dose commercial products may not contain anti-oxidant/preservative; even if the ingredients are susceptible to oxidation and support microbial

Table 2. Stability analysis of the product

Test parameters	Specification	Initial sample	Stability sample	
			3 rd Month	6 th Month
Description	Clear colorless solution free from visible extraneous particulate matter	Complies	Complies	Complies
Absorbance at 430 nm (Color and clarity)	Not more than 0.200	0.108	0.112	0.120
Clarity of the solution	Opalescence not more pronounced than the reference suspension	Complies	Complies	Complies
pH	Between 6.0 and 8.5	6.4	6.5	6.8
Osmolality	Between 260–320 mOsm/Kg	304	306	310
Hydrodynamic size (nm)	–	174	222	227
Zeta potential (mV)	–	– 53.6	– 47.1	– 45.8
Bacterial Endotoxins	Not more than 0.5 EU/mg of Propofol	None detected	None detected	None detected
Particulate matter i) ≥ 10 μ per container ii) ≥ 25 μ	Not more than 6000	120	148	129
	Not more than 600	11	14	11
Sterility	No microbial growth should be observed in the test sample after 14 days of preparation	No growth	No growth	No growth
HPLC assay	Between 90–110	100.02	98.96	96.74
Impurities (%) Total impurities	Not more than 1.0	0.56	0.7	0.71

growth.^{47–48} Formulation trials were also executed incorporating an anti-oxidant and a preservative which are generally recognized as safe (GRAS) for parenteral use. The product characterization data are presented in Table 2.

In the fabrication process addition of egg lecithin to cooled water and stirring for 30 min may promote the growth of microorganisms as egg lecithin is a supportive nutrient.^{36,49} This process was revised by the addition of preservatives first and then egg lecithin. This had a bacteriostatic effect on the liquid and microbial growth was arrested leading to improvement in BET values (Table 3).

With an objective to select a sterilization filter, PVDF capsule and membrane filters were tested to establish a product wetted bubble point values in contrast to the water wetted values. The levels of leachable substances were examined at this part of the study that might be associated with the use of filters in contact with the product.

Initially, the liquid bulk product was tried for sterilization by filtration using capsule filter. However, difficulties were observed in passing this product through the capsule filter. The product was then passed through 2.0 & 0.45 μm membrane filters using a membrane filter holder

at low pressure (0.3–0.4 bar). The bubble point (filter integrity) value was found to be 1.5 bar which was in agreement with its viscosity.^{37,38,46} Based on this confidence levels, the filtered product was filled into vials. Product failed sterility testing as the filtration train with 2.0 & 0.45 μm filter did not meet the usage of a series of filters sufficiently. The microbiological studies for sterility and endotoxin loads confirmed that the product remained intact as of its initial potency.

The sample meets the requirements of the test if the observation of the test is negative when tested at a dilution not exceeding Maximum Valid Dilution (MVD). No microbial growth was observed in the test sample after 14 days of preparation. BET levels were found acceptable in the product i.e., less than the fixed limits of 0.5 EU/mg (Table 2 and Table 3). The addition of the preservative and the method of separate filtration process of the different phases were able to reduce the microbial load of soyabean oil (12 CFU/gm) and egg lecithin (40 CFU/gm). This result enabled the product to resist multiple piercing for up to 48 hours. This confirmed the content integrity of the product for multiple dosing for over a period of 48 hours in a controlled environment.

$$MVD = \frac{\text{Endotoxin limit} * \text{concentration of product} \left(\frac{\text{mg}}{\text{mL}}\right)}{\text{Lysate sensitivity} (\lambda)} \quad (2)$$

Table 3. Bacterial endotoxin testing after multiple piercing of the same vial

Time point of piercing the vial (h)	Endotoxin dilution (EU/mL)				LRW (Negative Control)	End point
	2.0λ (0.06)	1.0λ (0.03)	0.5λ (0.015)	0.25λ (0.007)		
0	+	+	–	–	–	0.12
8	+	+	–	–	–	0.12
24	+	+	+	–	–	0.06
32	+	+	+	–	–	0.06
48	+	+	+	–	–	0.06

Where, λ = labelled sensitivity of LAL Reagent (EU per mL) LRW = LAL (Limulus Amoebocyte Lysate) reagent water

The levels of pH and Osmolality were well in their specific limits and have not changed significantly in accelerated stability condition, and supposed to be in the tolerable level.³⁹ Non appearances of creaming in the product till 1 month were further exposed to freeze thaw cycles and other accelerated conditions. A continuous homogenization with cooling jackets for 30 minutes at 850 bar pressure generated droplets of 174 nm mean diameter and –53.6 mV zeta potential. Under the stressed conditions of freezing and thawing the droplet size distribution of the product remained almost unchanged, and exhibited a size of 175 nm and –47.1 mV zeta potential.

Prior to Bacterial Challenge Test (BCT), a viability test was performed in order to establish the bactericidal effect of the product. The viability test helps determine the most appropriate BCT methodology. After the total exposure time, no more than 1 log reduction of the initial bacterial concentration was observed and hence the product was considered to be non-bactericidal.

3. 2. Homogenization Process Optimization Using DoE

Statistically designed experiments have been exploited widely for pharmaceutical process optimization includ-

ing formulation parameter screening activities.^{40–42} In the present research a 3² full factorial design was used to optimize the homogenization process parameters.⁴³ The homogenization process is very crucial in the development of a stable nano-emulsion, and the key process variables are homogenization duration and pressure. As per DoE software suggestion 13 runs were taken with the selected two numeric continuous process variables. The response variable chosen was the particle size of propofol post homogenization. Since, no aliases were found for quadratic model, response surface quadratic model was used safely for navigation through the model.⁴⁴ The highest order polynomial was selected as the sequential model sum of squares (Type 1) where the additional terms were significant (p<0.05) and the model was not aliased. The cubic model was found to be aliased and hence the model was not selected (Table 4). Quadratic model was fitted to the data (P<0.05) based on the model summary statistics including the adjusted R² and predicted R² for interpretation of the homogenization process response and particle size (Z) (Table 4).

The model and the model terms like X₁, X₁X₂, X₁² and X₂² are significant as understood by the analysis of variance (ANOVA) for the response surface quadratic

Table 4. Statistical model analysis of particle size

Sequential model sum of squares for				
Source	Sum of Squares	Degree of freedom (Df)	F-Value	p-Value
Mean vs Total	6.749E+005	1		
Linear vs Mean	21999.00	2	16.52	0.0007
2FI vs Linear	702.25	1	1.06	0.3298
Quadratic vs 2FI	5813.52	2	144.39	< 0.0001
Cubic ^a vs Quadratic	91.50	2	4.63	0.0728
Residual	49.42	5		
Total	7.035E+005	13		
Design model summary statistics				
Source		Adjusted R ²		Predicted R ²
Linear		0.7212		0.5013
2FI		0.7229		0.0023
Quadratic		0.9916		0.9619
Cubic		0.9959		0.9325

^aCubic model is aliased and hence is not selected.

Table 5. Analysis of variance

Source	Response Surface Quadratic model			Standardized mean effect		
	Sum of Squares	F-Value	p-Value*	Coefficient Estimate	Standard Error	Standardized mean effect
Model	28514.77	283.28	< 0.0001	205.93	1.86	110.71
X1	21961.50	1090.89	< 0.0001	-60.50	1.83	-33.06
X2	37.50	1.86	0.2146	-2.50	1.83	-1.36
X1X2	702.25	34.88	0.0006	13.25	2.24	5.91
X12	3934.09	195.42	< 0.0001	37.74	2.70	13.97
X22	262.09	13.02	0.0086	9.74	2.70	3.60

*p-Values <0.05 indicates significant terms

model (Table 5). The fitted polynomial equations relating to the responses were generated using Design Expert software and are given below.

$$\text{Particle Size} = +205.93 - 60.49 * X_1 - 2.49 * X_2 + 13.24 * X_1X_2 + 37.74 * X_1^2 + 9.74 * X_2^2$$

The coefficient estimate and standardized main effects (SMEs) for the dependent responses are listed in Table 6. SME values were calculated by dividing the main effects by the standard error of the main effects. In addition, three dimensional response surface plots were presented to estimate the effects of the mixture components on each response. Results of multiple regression analysis and SMEs revealed that the process variables had statistically significant influence on the dependent variables ($p < 0.05$, Table 5). The SME values clearly indicated that the homogenization pressure (X_1) is the most influential factor compared to the duration of homogenization (X_2) on the average particle size and can be visualized from the 3D surface plots (Figure 1) and contour plot (Figure 2). The surface response and contour plots are indicative of an op-

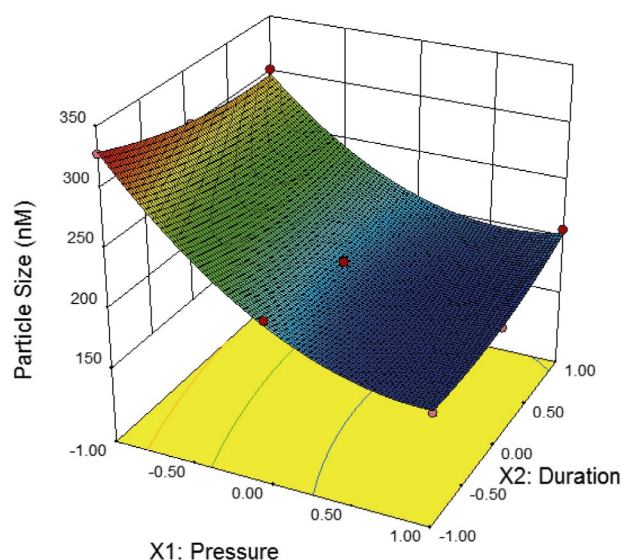


Figure 1. 3D Surface response plot indicates the effect of homogenization pressure (X_1) and the duration of homogenization (X_2) on average particle size.

timal point of resultant particle size beforehand of homogenization. Both the plots of DOE potentially predicted the systematic workflow. Average particle size yield was found to be 174 nm (Figure 3) with 30 min of homogenization at 850 bar pressure. Similar marketed formulations were reported of having much larger particles of around 300 nm⁵ compared to the present formulation and supposed to cause less pain while passing through the arteries.²³

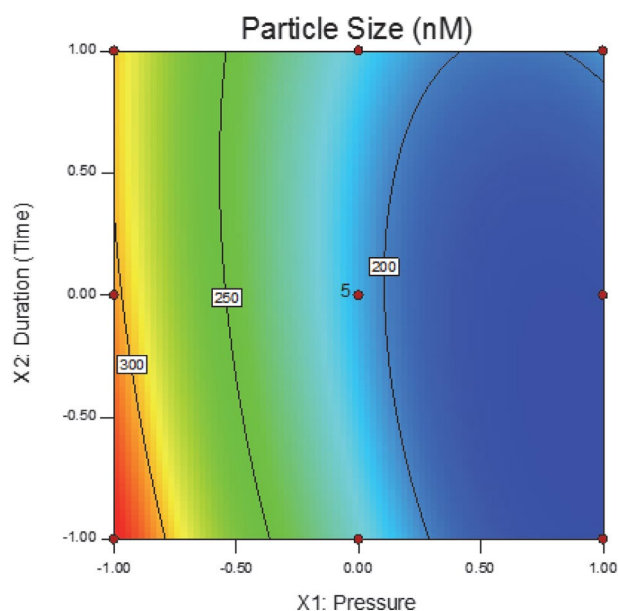


Figure 2. Contour plot indicating that the homogenization pressure (X_1) is the most influential factor compared to the duration of homogenization (X_2) on average particle size.

For parenteral emulsions droplet size and polydispersity index (PI) are vital physicochemical parameters as large particle size is clinically unacceptable. The formulation exhibited PI of 0.096 (Figure 3) which is well below the acceptable limit for parenteral emulsions^{44,45} indicating high stability. The initial zeta potential (-53.6 mV, Figure 4) of the product was found above -40 mV in accelerated stability condition of 6 months, which also implied its better stability. The stability indicating factors of the accel-

erated condition confirmed with those of the initial strategic values and hence can have a better shelf life in controlled environmental conditions.

The HPLC chromatogram of initial formulation are reported in the Figures 5 indicating the retention time of

the active substance and other negligible peaks due to inactive substances and impurities. The assay of the formulation has shown the value in the prescribed range of 90–110 % (Table 3, Figure 5) and the impurity levels (Table 3) remained below the threshold throughout the testing period.

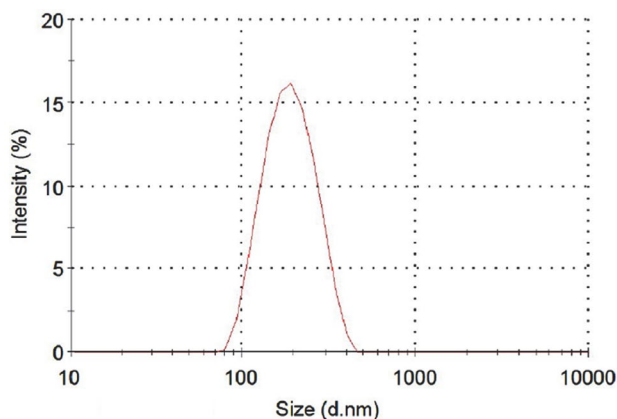


Figure 3. Hydrodynamic size distribution of the initial formulation

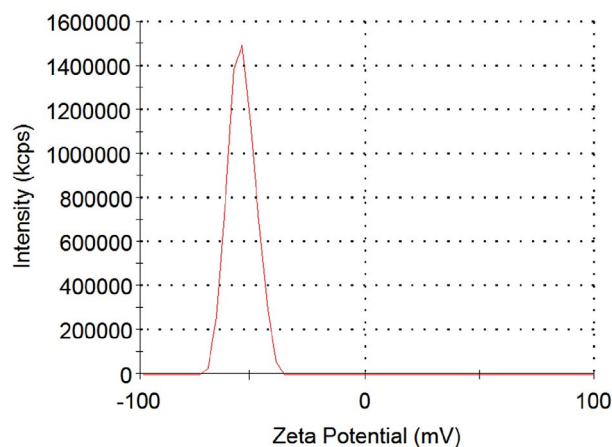


Figure 4. Zeta potential distribution of the initial formulation

4. Conclusions

Multi-dose propofol nano-emulsion was prepared with and without sodium oleate complying USP specifications and evaluated successfully using design of experiments. Controlled homogenization process could unable to influence the particle size significantly. The optimized process of homogenization at 850 bar of 30 min of duration produced 174 nm particles. PI and zeta potential of 0.096 and -53.6 mV respectively indicated highly stable product. The accelerated stress testing including freezing and thawing exhibited no significant change in the product. Further studies could be done to perform preclinical investigations in suitable animal models to assess the product.

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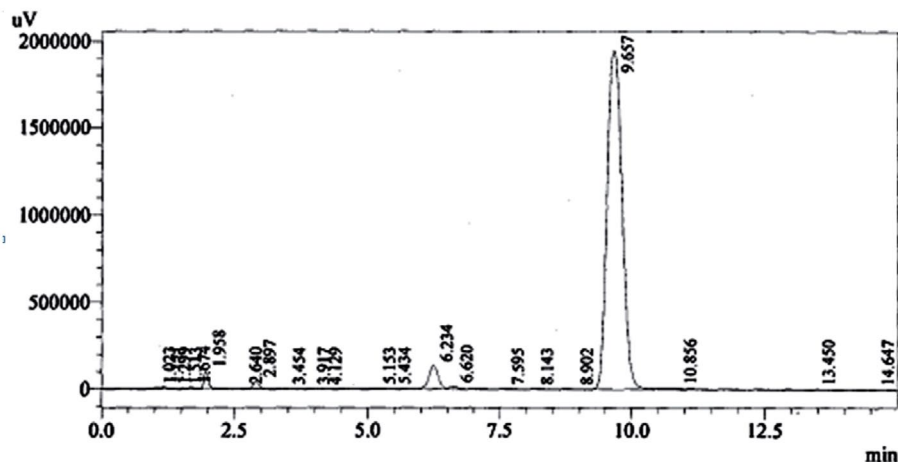


Figure 5. HPLC analysis for assay of the initial formulation

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Povzetek

Kljub kliničnemu, znanstvenemu in komercialnemu razvoju se mnogi bolniki pritožujejo zaradi bolečin pri intravenski injekciji propofola. Pričujoča raziskava je bila izvedena z namenom razviti stabilne večodmerne nano-emulzije propofola z uporabo celovitega 3^2 faktorialnega modela, ki naj bi bil povezane z manj pričakovanimi bolečinami med intravenskim dajanjem propofola. Propofol je bil vgrajen v mešanico dinatrijevega edetata, natrijevega oleata, tioglicerola, glicerola, jajčnega lecitina, sojinega olja in trigliceridnih olj s srednjo dolžino verige. Homogenizacijo smo nadaljevali pri nadzorovani temperaturi 20 °C. Po izpostavitvi temperaturi 40 °C za 3 in 6 mesecev izdelek ni vidno spremenil zunanjega videza, vrednosti pH, osmolalnosti, bakterijskega endotoksina, sterilnosti in določitev nečistoč. Homogenizacija pri tlaku 850 bar je po 30 minutah vodila do delcev velikosti 174 nm in zeta potenciala –63,6 mV, kar kaže na njegovo stabilnost.



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