Original Article

Development of Tapentadol Hydrochloride Loaded Proniosomal Gel using Main Effects Screening Design and *in silico* Verification using Parameter Sensitivity Analysis

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ABSTRACT

Aim: Tapentadol Hydrochloride is a centrally acting opioid analgesic of biopharmaceutical classification system class I drug. Oral administration of tapentadol hydrochloride undergoes extensive first-pass metabolism leads to poor bioavailability. The present study was aimed to screen the critical material attributes to deliver the tapentadol hydrochloride through the transdermal route using a carrier proniosomal gel. **Methodology:** Main effect screening design has been constructed to screen the choice of surfactant used for the formulation of tapentadol hydrochloride proniosomal gel. The critical material attributes selected were surfactant, cholesterol, and soya lecithin, with responses entrapment efficiency (%), vesicle size (nm), and zeta potential (mV). All 24 runs of experiments were performed and evaluated to check the model fit. The *in silico* verification was analyzed using parameter sensitivity analysis. **Results:** The prediction profiler showed maximum desirability for Kolliphore RH 40 against the set goals. The design diagnostic efficiency was measured better for the constructed MESD. Also, parameter sensitivity analysis confirms that the vesicle size would play a principal role in the permeation of tapentadol hydrochloride proniosomal gel. **Conclusion:** Hence, Kolliphore RH 40 was considered for the further optimization process. The tapentadol hydrochloride proniosomal gel would be a better alternative to oral therapy.

Key words: Tapentadol, Main effect screening design, Parameter sensitivity analysis, Proniosomal gel, Kolliphore RH 40.

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INTRODUCTION

Every individual encounters pain as the most common associated manifestation, after an injury. According to the International Association for Study of Pain (IASP), pain is defined as "An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage".[1] To manage the pain with analgesics, a three-step analgesic ladder strategy[2] was designed by World Health Organization found to be inappropriate to practice to the current pain management scenario in the specific condition of Chronic Non-Cancer Pain (CNCP). So, a four-step integrative therapy was proposed with interventional therapy at the third step followed by strong opioids at the fourth step of the analgesic ladder for CNCP.[3] In both, three steps and four-step analgesic ladder strategy, the opioids were retained on the ladder and are regarded as the gold standard to treat pains of moderate to severe conditions.[4] Tapentadol Hydrochloride (TPL) is a novel central analgesic that acts

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with a dual mechanism; as an agonist to μ-opioid receptor and as a Nor adrenaline Re-uptake Inhibitor.[5] TPL remained as the first new opioid drug nearly for a quarter of the century and was approved by (November 2008) the United States Food and Drug Administration.[6] The Biopharmaceutical Classification System (BCS) categorizes TPL, as a class I drug, though its lipid solubility (2.8) has been comparatively lower.[7] TPL can be administered to manage the nociceptive and neuropathic pain,[8] Cancer pain,[9] osteoarthritis, and low back pain,[10] etc, TPL is commercially available as tablets; as an immediate release (IM) tablets for acute pain of moderate to severe conditions at different dose strength of 50 mg, 75 mg, 100 mg and as an extended-release (ER) tablets for chronic pain of moderate to severe conditions at different dose strength as 50 mg, 100 mg, 150 mg, 200 mg, 250 mg.[11] After a single oral dose, the TPL extensively undergoes first-pass hepatic metabolism that accounts for its 32% oral bioavailability. The biological half-life is very short 4 h, it requires a frequency dose for every 4 to 6 h.[4] About 97% of the administered dose of TPL, gets metabolized to an inactive form and none of those resultant metabolites exhibited analgesic activity.[12] The pharmacokinetics study of TPL through oral route is not well augmented due to the limitations such as frequent dosing, poor bio-availability that hampers the desired therapeutic efficacy of the drug. Nausea, vomiting, and constipation are the main adverse effects ensued for the opioid analgesic therapy intended to alleviate the pain, therefore anti-emetics and laxatives should be co-administered with the opioid analgesics.[13] Drug abuse, Drug tolerance, withdrawal symptoms, and liability are a few of the other adverse effects cited after administering the oral opioids.[4] To overcome these challenges encountered by the oral administration, an attempt was made to deliver the TPL via the topical route using a vesicular drug delivery system. The Drugs delivered through the transdermal add many benefits such as bypass the first-pass hepatic metabolism, thereby augments the bio-availability, ease of self-administration, improved patient compliance, and evades from the bitter taste of the drug.[14] The vesicular drug delivery system alters the skin properties that enhance the permeation of the drug through the stratum corneum.[15] Numerous studies on the vesicles as a drug carrier for Transdermal Drug Delivery System (TDDS) developed were found to be flexible, carrier beneficial,[16] better in terms of absorption and bioavailability,[17] increased effectiveness, and reduced toxic effects of TDDS.[18]

The present research study emphasizes, Main Effects Screening Design (MESD) to screen the selected surfactants in the development of proniosomal gel. The screening study of pharmaceutical formulations helps to choose the ideal formula and Parameter Sensitivity Analysis (PSA) was simulated to estimate the factor that would affect the formulator decisions simulating *in silico* performance of the formulation by Gastroplus® software.

MATERIALS AND METHODS

Materials

The gift sample of TPL was received from Symed labs, Hyderabad, India, Kolliphore RH 40 (Polyoxyl 40 hydrogenated castor oil) was obtained from BASF, India, Labrafil M 1944 CS (Oleoyl macrogol-6glycerides), and Labrafil M 2125 CS (Linoleoyl macrogol-6 glycerides) were received from Gattefosse, France. Soya lecithin Pharma grade was obtained from VitaeGen life sciences, India. Span 60 (Sorbitan Monostearate) and cholesterol were procured from Himedia. All the other chemicals, reagents, solvents used were of analytical grade.

Methods

Main effect screening design

The standard screening design like the Fractional factorial design was found to be impractical to carry out the experiment due to a large number of runs (64-128). To reduce the cost-effectiveness, time utilization, and number of runs as a constraint, the MESD was intended to screen the Critical Material Attributes (CMA). MESD is a design of experiment used for the screening studies when there is no existence of a standard design. It is a near orthogonal design that consents for categorical, discrete numeric factors with any number of levels besides, two-level continuous factors.[19,20] This design is cost-effective and conceptually reveals the important effects of CMAs that greatly influence the responses. Hence, these most important effects are called main effects.[21] MESD was constructed using the JMP® Pro 15.0.0 software (Trial version). It is an efficient method for assessing main effects when the interactions are insignificant.[22] In the interest of selecting the best suitable surfactant in the formulation of TPL loaded proniosomal gel, four surfactants were selected and screened. The factors / independent variables are selected in two-level factors, the categorical factors were the surfactants choices, namely Span 60 (HLB= 4.7), Kolliphore RH 40 (HLB= 14-16), Labrafil M 1944 CS (HLB= 9), Labrafil M 2125 CS (HLB= 9) and the continuous factors are Cholesterol and soya lecithin. The categorical and continuous factors varied in two levels and it was fixed based on the preliminary

trials of TPL proniosomal gel (Table 1). Critical Quality Attributes (CQA) identified for the screening designs are Entrapment efficiency (EE) (%), Vesicle size (nm), Zeta potential (mV). This is also called responses or dependent variable (Table 2). The responses, upper and lower limits were fixed based on the literature to form a stable formulation. The formulations structured by the MESD with 24 trials were evaluated to screen the best surfactant and summarized (Table 3).

Preparation of TPL proniosomal gel

The proniosomal gel was prepared by the coacervation phase separation method with a slight modification of the earlier reported methods.[23] Accurately weighed quantities (as detailed in Table 3) of TPL, surfactants, cholesterol, soya lecithin and ethanol were taken in a beaker. The components were mixed together and covered with an aluminum foil sheet to avoid the evaporation of the solvent. The beaker was kept in a water bath maintaining the temperature between 65 ± 5°C for 5 m till the homogeneous solution is formed. Followed by aqueous phase was added to the above mixture and again kept in the water bath for 2 m till the clear solution was formed.[24] The solution was mixed and stored at room temperature to form a gel. The formed proniosomal gel was stored in a dark place and used for further characterization.

Characterization of proniosomal gel formulations

Percentage entrapment efficiency

100 mg of TPL proniosomal gel was weighed accurately and diluted with 5 ml Phosphate buffer saline pH 7.4.[25]

Table 1: Screened CMAs and their ranges of MESD.

Factors	Applied value	
	Lower limit	Higher limit
Kolliphore RH 40 (mg)	700	800
Span 60 (mg)	700	800
Labrafil M 1944 CS (mg)	800	900
Labrafil M 2125 CS (mg)	800	900
Cholesterol (mg)	100	150
Soya lecithin (mg)	50	100

Table 2: Responses in the Main Effect Screening Design.

Responses	Goal	Applied value	
		Lower limit	Higher limit
Entrapment efficiency (%)	Maximize	85	100
Vesicle size (nm)	Match Target	100	400
Zeta potential (mV)	Match Target	-50	-40

The hydrated gel was sonicated for 15 m to make a uniform dispersion and centrifuged (Remi Motors, India) at 25,000 revolutions per minute for 30 m.[26] The supernatant liquid was drained, the absorbance was measured using a UV spectrophotometer (Shimadzu, UV-1800 spectrophotometer, Japan) at λ= 272 nm. The Entrapment efficiency (Remi Motors, India) was measured using the following formula,

$$\text{Entrapment efficiency (\%)} = \frac{(\text{Total amount of drug} - \text{Amount of the free drug}) * 100}{\text{The total amount of drug}}$$

The experiment was performed in triplicate.

Determination of vesicle size

100 mg of TPL proniosomal gel was measured and diluted with double distilled water. The aqueous suspension was sonicated for 15 m to form a uniform dispersion.[27] The vesicle size was determined using Horiba SZ-100 Nanoparticle analyzer dynamic light scattering system. All the samples were measured in triplicate.

Determination of Zeta potential

100 mg TPL proniosomal gel was diluted with double distilled water. The surface charge of the niosomes derived from the TPL proniosomal gel was measured using Laser Doppler electrophoretic mobility measurements using Horiba SZ-100. The measurements were done in triplicate.

Evaluation of MESD

The experimental results obtained for all 24 formulations responses were applied in the design to assess the properties of the generated design. The effect summary gives the factor that influences the response of the formulations. The scaled estimates give the individual factors, significance on the individual response. The predicted plots were studied in correlation to individual response. There are three factors (choice of surfactant, cholesterol, soya lecithin) and responses (EE %, Vesicle size nm, Zeta potential mV). The prediction profiler visualizes the predicted response in contrast to the one factor while the others are held constant. The desirability platform measures the desirability of each factor and overall against the response. The overall desirability measures on a scale of 0 to 1.[28] The goal was assigned to maximize entrapment efficiency and match the target for vesicle size and zeta potential.

Parameter Sensitivity Analysis using Gastroplus®

Parameter Sensitivity Analysis (PSA) mode in Gastroplus®, Simulations Plus GastroPlus 8.7 (academic

Table 3: Formulations of TPL Proniosomal and its responses.

Formulation Code	Cholesterol (mg)	Soya lecithin (mg)	Surfactant (categorical factors)	Entrapment efficiency (%)*	Vesicle size (nm)*	Zeta potential (mV)*
1	150	100	Span 60 700 mg	77.85 ± 0.49	311.67 ± 2.36	-68.6 ± 1.11
2	100	100	Span 60 800 mg	86.98± 1.16	570 ± 1.36	-59.3 ±1.47
3	150	50	Kolliphore RH 40 700 mg	97.87± 0.097	142.63 ± 1.69	-53.1 ±1.38
4	100	50	Kolliphore RH 40 800 mg	98.55 ± 0.637	141.51 ± 1.68	-50.1± 1.53
5	100	100	Labrafil M 2125cs 800 mg	55.45 ± 1.53	69.83 ± 2.69	-74.6 ±2.18
6	100	50	Labrafil M 2125cs 900 mg	63.88 ± 3.04	98.64 ± 1.98	-84.4 ±2.36
7	150	100	Labrafil M1944cs 800 mg	72.15 ± 1.56	98.97 ± 2.68	-69.5 ±1.69
8	150	100	Labrafil M1944cs 900 mg	72.15 ± 1.98	94.65 ± 2.45	-61.3 ±1.45
9	100	50	Span 60 700 mg	87.98 ± 0.986	321.51 ± 3.69	-79.5 ± 1.31
10	150	50	Span 60 800 mg	88.98 ± 1.115	483.68 ± 1.65	-59.3 ± 0.36
11	150	100	Kolliphore RH 40 700 mg	92.03 ± 0.635	142.45 ± 2.56	-44.6 ±1.94
12	150	100	Kolliphore RH 40 800 mg	98.87 ± 1.36	161.56 ± 1.89	-49.4 ±1.96
13	150	50	Labrafil M 2125cs 800 mg	65.77 ± 1.458	85.50 ± 1.65	-63.7 ±1.36
14	150	100	Labrafil M 2125cs 900 mg	72.15 ± 0.786	98.95 ± 2.56	-56.8 ±1.18
15	100	100	Labrafil M1944cs 800 mg	55.45 ± 0.658	69.81 ± 1.69	-74.6 ± 1.78
16	100	50	Labrafil M1944cs 900 mg	68.45 ± 1.73	97.73 ± 2.32	-62.1 ± 1.69
17	100	100	Span 60 700 mg	87.98 ± 1.49	321.14 ± 3.53	-79.5 ± 2.36
18	150	50	Span 60 800 mg	87.67 ± 1.96	684 ± 2.36	-59.6 ± 2. 23
19	100	50	Kolliphore RH 40 700 mg	95.69 ± 2.49	141 ± 1.96	-58.5 ± 1.68
20	100	50	Kolliphore RH 40 800 mg	98.55 ± 1.86	140.5 ± 1.6	-46.8 ± 1.56
21	150	50	Labrafil M 2125cs 800 mg	61.34 ± 2.69	91.8 ± 2.1	-56.4 ± 2.35
22	100	100	Labrafil M 2125cs 900 mg	71.56 ± 1.697	86.1 ± 1.36	-61.7 ± 1.96
23	150	50	Labrafil M1944cs 800 mg	65.77 ± 1.32	78.5 ± 1.65	-56.7 ± 1.36
24	100	100	Labrafil M1944cs 900 mg	68.45 ± 2.13	97.7 ± 1.49	-62.1 ± 1.13

*expressed as Mean ±SD, *n*=3

evaluation version) allows to leverage and model the importance of a variety of molecular parameters in predicting absorption, pharmacokinetics, and pharmacodynamics. A PSA consists of multiple simulations wherein one parameter is varied at a time. PSA is an effective tool to predict the performance of a formulation development candidate at an early stage instead of relying on *in vitro* parameters alone. PSA has been increasingly used as an application to augment the QbD based development which provides an extra dimensionality in predicting the *in vivo* performance, which helps the formulator to choose the best runs of the experiment.

RESULTS AND DISCUSSION

MESD is an efficient screening design, which is applied when there is no proper design in existence for the selected variables and responses. The present design inputs, one categorical factor, and two continuous factors. The main effects of the CMAs on the dependent variables viz. Vesicle size, % EE and zeta potential were studied to develop a stable TPL proniosomal gel and were found that the main effects of the CMAs profoundly influenced its performance. MESD is orthogonal or near orthogonal, as this design fits aptly to the mathematical model of the Design of Experiments (DOE) when there is no proper design exists. The effect summary shows that the surfactant has statistically significant (P = 0.000), cholesterol refers statistically significant (P =0.01804) and soya lecithin did not have any significance (P =0.442) on the responses (Table 4).

Proniosomal gel was prepared using the coacervation phase separation method. Proniosomal gel is also called liquid crystalline granules. The coacervation phase separation method was adopted to produce a lyotropic liquid crystalline state. This state was achieved during the

Table 4: Effect summary of Independent variables.

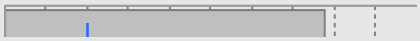
Source	Log Worth		*P* Value
Surfactant	7.786		0.00000
Cholesterol (mg) (100,150)	1.744		0.01804
Soya lecithin (mg) (50,100)	0.354		0.44270

Table 5: Scaled estimate value of entrapment efficiency.

Term	Scaled Estimate		Std Error	t Ratio	Prob>|t|
Intercept	78.815417		0.92475	85.23	<.0001*
Cholesterol (mg)(100,150)	1.3322745		0.982767	1.36	0.1967
Soya lecithin (mg)(50,100)	-0.776392		0.982767	-0.79	0.4427
Surfactant [Span 60 700 mg]	6.4908056		2.492826	2.60	0.0208*
Surfactant [Span 60 800 mg]	8.3583611		2.492826	3.35	0.0047*
Surfactant [Kolliphore RH 40 700 mg]	15.678361		2.492826	6.29	<.0001*
Surfactant [Kolliphore RH 40 800 mg]	20.026544		2.487439	8.05	<.0001*
Surfactant [Labrafil M 2125cs 800 mg]	-18.66497		2.492826	-7.49	<.0001*
Surfactant [Labrafil M 2125cs 900 mg]	-8.915861		2.492826	-3.58	0.0030*
Surfactant [Labrafil M1944cs 800 mg]	-14.54404		2.487439	-5.85	<.0001*
Surfactant [Labrafil M1944cs 900 mg]	-8.429194		2.492826	-3.38	0.0045*

interaction between surfactant and water by extending the temperature at Kraft point, the addition of solvent, which liquefies lipids, and the use of both temperature and solvent. Liquid crystalline proniosomes, function as a carrier for transdermal drug delivery systems.[29] It provides substantial entrapment efficiency, stability, and functions as a permeation enhancer. Excipients (surfactants, cholesterol, and soya lecithin) concentrations were selected according to the FDA inactive ingredient database and GRAS listed.[30] Ethanol was selected as a solvent to form an appropriate vesicle size.

Percentage Entrapment efficiency

Effect of surfactants on EE

The EE is a paramount characterization for proniosomes, which denotes the amount of drug retains in the vesicles. Higher the EE of the drug could lower the dose of the drug. EE is influenced by the alkyl chain length, HLB value, and phase transition temperature of the surfactant. The surfactant concentration has an impact on the EE. The EE of all 24 formulations were ranged from 55.45± 0.658 to 98.87 ± 1.36 %.

The reports of EE reveal that higher EE was seen in the formulations containing Kolliphore RH 40 800 mg. As concentration Kolliphore RH 40 800 mg increases, the hydrophobicity of bilayer increases; therefore volume casing the drug also increases. The higher the concentration of surfactant, the higher the formation of vesicles, which leads to higher EE as reported earlier.[27] Though Kolliphore RH 40 is a hydrophilic surfactant, the inclusion of an appropriate amount of cholesterol aids in the formation of stable vesicles. The scaled estimates show Kolliphore RH 40 is statistically significant ($P = 0.0001$) Table 5.

Span 60 also showed significant EE. The literature confirms that this might be due to the high length of the alkyl chain (C_{18}) and phase transition temperature (56-58°C). Labrafil (HLB-9) a non-ionic surfactant, also acts as a solubilizing agent and the wetting agent shows very less entrapment owing to a drippy bilayer.

The scaled estimates value confirms that Kolliphore RH 40 in both the concentration 700 and 800 mg were statistically significant ($P = 0.0001$) and followed by span 60 and Labrafil (Table 5).

Effect of cholesterol on EE

Cholesterol did not show any statistically significant result on EE (Table 5), but literature confirms its influence in the formation of stable vesicles. The concentration utilized may vary based on the HLB value. The Hydrophobic surfactant (Span 60) requires a lesser amount of cholesterol. It acts as vesicular cement in the bilayer, results in less leaky vesicles and higher EE. Whereas, hydrophilic surfactant (Kolliphore RH 40) capability to form vesicles with the help of an appropriate amount of cholesterol. If it exceeds beyond the concentration of cholesterol, it competes with the drug for space within the bilayer.[31] Kolliphore RH 40 (HLB=14-16) with the requisite amount of cholesterol forms a stable vesicle. There are in concurrence with earlier studies.[32]

Effect of soya lecithin on EE

Soya lecithin was found to be insignificant (Table 5). But the smaller concentration of soya lecithin helps to rigidify cholesterol and helps to form a stable bilayer membrane.

Determination of vesicle size

The vesicle size helps in the permeation of vesicles through the skin. The average range of vesicles for TDDS is 100-500 nm.[32] The vesicle size was found to be influenced by the surfactant. The vesicle size ranges were found to be 69.81± 8.69 to 684 ± 5.69 nm. All the formulations were found to be in the nano-size range. The span 60 -800 mg of formulation 2, vesicle size was found to be 570 ± 1.36 nm (Figure 1). The lower vesicle size of span 60 may be attributed to the hydrophobic nature. Kolliphore RH 40 and Labrafil were shown further decrement in the vesicle size compared to span 60. The vesicle size of Kolliphore RH 40 -800 mg and Labrafil M 2125 -900 mg coded as were found to be 140.5 ± 1.6 nm and 85.50 ± 1.65 nm, respectively (Figures 2 and 3), which may be attributed to the repulsion force between the vesicles owing to discrete vesicles. The higher the amount of surfactant Kolliphore RH -800 mg decreases the vesicle size as reported in previous studies.[27]

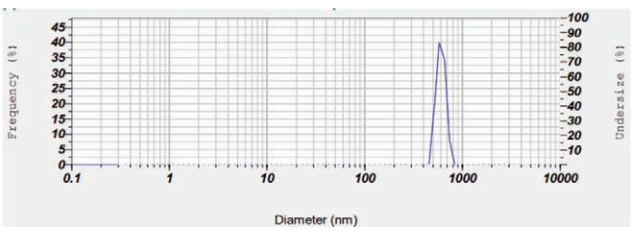


Figure 1: Vesicle size for the coded formulation 2 (span 60- 800mg).

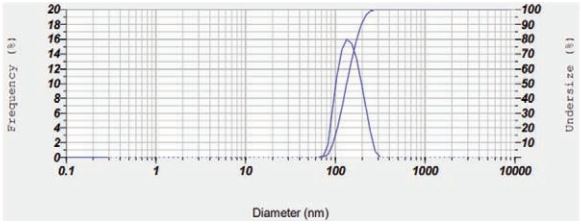


Figure 2: Vesicle size for the coded formulation 19 (Kolliphore RH 40- 800mg).

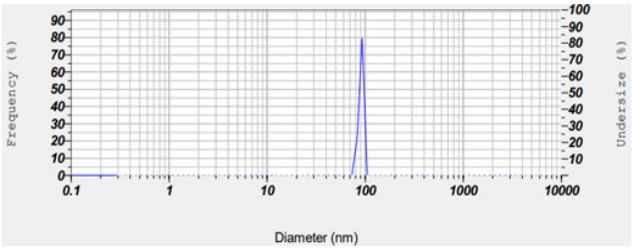


Figure 3: Vesicle size for the coded formulation 19 (Labrafil M 2125 - 800mg).

The scaled estimate value of vesicle size has shown in Table 6. The actual experiments evidenced that Kolliphore RH 40 produced better vesicle sizes than that of span60 and Labrafil. As stated the vesicle size is the rate-limiting factor for the bioavailability of the formulation. Hence, the choice to Kolliphore RH 40, which produced the best vesicle size and was deemed fit for the formulation as it produced much better vesicle size in comparison to span 60 and Labrafil.

Effect of cholesterol on vesicle size

Cholesterol helps to maintain the stability of the bilayer and vesicle shape of proniosomes. It was noticed; a higher concentration of surfactant and cholesterol supports the construction of the rigid bilayer structure. The surfactant functions in the formation of vesicles and cholesterol to maintain the rigidity of the bilayer. In consequence, finding the precise ratio between cholesterol and surfactant is thought-provoking during the formulation development.

Effect of soya lecithin on vesicle size

Lecithin has a negligible effect on vesicle size and did not show any significant effect on vesicle size (Table 6).

Zeta Potential

The surface and interfacial properties of non-ionic surfactant vesicles show distinct parameters. It helps in enhancing the stability of the vesicular system. Higher the zeta potential of the vesicles leads to higher electrical response forces between the particle, which prevents the coalescence. The zeta potential ranges were found to

Table 6: Scaled estimate value of Vesicle size.

Term	Scaled Estimate		Std Error	t Ratio	Prob>|t|
Intercept	188.92083		8.654934	21.83	<.0001*
Cholesterol (mg)(100,150)	9.7584314		9.197926	1.06	0.3067
Soya lecithin (mg)(50,100)	-6.984902		9.197926	-0.76	0.4602
Surfactant [Span 60 700 mg]	134.59361		23.33089	5.77	<.0001*
Surfactant [Span 60 800 mg]	355.49806		23.33089	15.24	<.0001*
Surfactant [Kolliphore RH 40 700 mg]	-52.53528		23.33089	-2.25	0.0409*
Surfactant [Kolliphore RH 40 800 mg]	-40.16299		23.28048	-1.73	0.1065
Surfactant [Labrafil M 2125cs 800 mg]	-114.4686		23.33089	-4.91	0.0002*
Surfactant [Labrafil M 2125cs 900 mg]	-88.80639		23.33089	-3.81	0.0019*
Surfactant [Labrafil M1944cs 800 mg]	-107.4453		23.28048	-4.62	0.0004*
Surfactant [Labrafil M1944cs 900 mg]	-86.67306		23.33089	-3.71	0.0023*

Table 7: Scaled estimate value of zeta potential.

Term	Scaled Estimate		Std Error	t Ratio	Prob>|t|
Intercept	-62.175		1.363467	-45.60	<.0001*
Cholesterol (mg)(100,150)	3.8796078		1.449008	2.68	0.0180*
Soya lecithin (mg)(50,100)	0.4762745		1.449008	0.33	0.7473
Surfactant [Span 60 700 mg]	-12.55722		3.675464	-3.42	0.0042*
Surfactant [Span 60 800 mg]	1.6405556		3.675464	0.45	0.6622
Surfactant [Kolliphore RH 40 700 mg]	8.9738889		3.675464	2.44	0.0285*
Surfactant [Kolliphore RH 40 800 mg]	14.860294		3.667521	4.05	0.0012*
Surfactant [Labrafil M 2125cs 800 mg]	-3.859444		3.675464	-1.05	0.3115
Surfactant [Labrafil M 2125cs 900 mg]	-4.323889		3.675464	-1.18	0.2590
Surfactant [Labrafil M1944cs 800 mg]	-6.210294		3.667521	-1.69	0.1125
Surfactant [Labrafil M1944cs 900 mg]	1.4761111		3.675464	0.40	0.6940

be -44.6 ± 3.69 (Kolliphore RH 40) and -84.4± 1.36 (Labrafil M 2125). The study reveals that surfactants (Kolliphore RH 40 and span 60) and cholesterol have a significant effect on zeta potential. The scaled estimate of zeta potential has been represented in Table 7.

Evaluation of model

Model evaluation was done after substituting the obtained responses in the design and model evaluation parameters were assessed. The actual vs predicted plot was evaluated for the individual responses of the multiple regression models. The R^2 and p values obtained from all the responses like EE (R^2=0.94, p<0.0001), vesicle size (R^2=0.96, p<0.0001), and zeta potential (R^2=0.76, p<0.0045) were statistically significant (Figures 4-6). The prediction profiler reveals the desirability of individual response on the desirability scale. The EE and vesicle

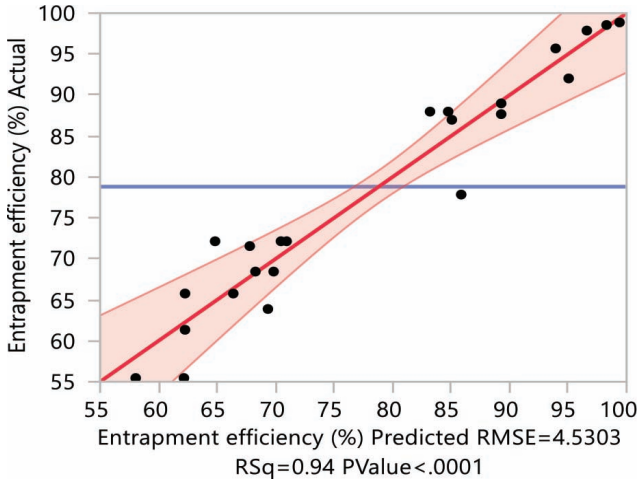


Figure 4: Actual Vs Predicted plot of Entrapment efficiency.

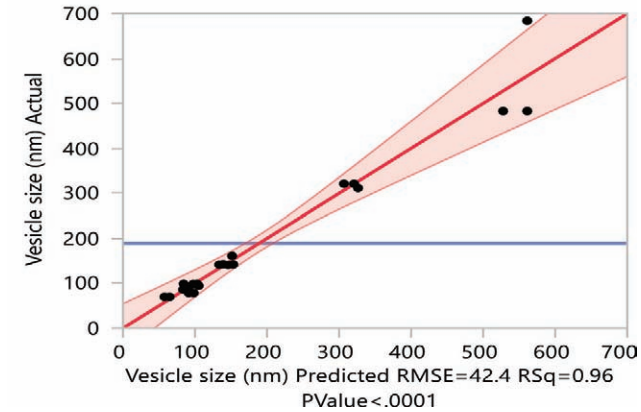


Figure 5: Actual Vs Predicted plot of vesicle size.

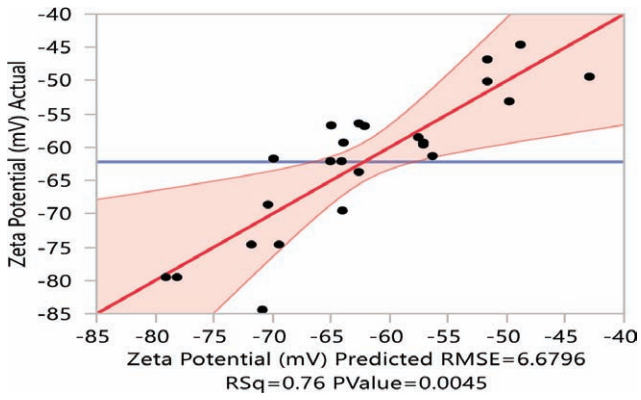


Figure 6: Actual Vs Predicted plot of zeta potential.

size have shown the highest desirability (nearer to 1) while zeta potential was recorded (nearer to 0). But the overall desirability predicted in the design was 0.5468. Hence, all responses were predicted to be within the desired limit (0-1). The prediction profiler predicted the composition of proniosomal gel containing

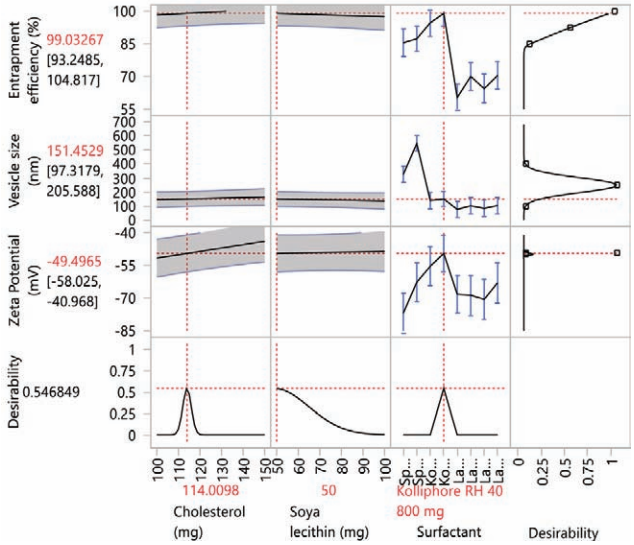


Figure 7: Prediction profiler of desirability.

Table 8: Design diagnostic.

Design diagnostic efficiency	% estimation
D Efficiency	97.63365
G Efficiency	81.52174
A Efficiency	95.0783

Kolliphore Rh 40-800 mg, cholesterol 114.00 mg, and soya lecithin 50 mg was best fit to the model design (Figure 7).

Design diagnostic measures the efficiency of the constructed design. The maximum efficiency is 100 % for any criterion. The constructed MESD shows the highest efficiency measures better. (Table 8).

Parameter Sensitivity Analysis

The PSA a modeling feature in GastoPlus® 9.7 was used to assess the *in vivo* behaviour of the designed formulation. The PSA was modeled on the responses of the design and the likely *in vivo* behaviour they are likely to elicit. The dermal physiology model and population size parameters were depicted in Figures 8 and 9. The model revealed the particle size plays a significant role in the *in vivo* characteristics and performance of the formulation. The response of particle size and its associated factors have a significant effect on dissolution (Figure 10) and strongly suggest that the dissolution of the drug is rate limited by the particle size of the formulation. The percentage of the drug absorbed by the topical route (Figure 11) too suggested the % of drug absorbed by the topical route has an ideal range between 10–70 nm to more likely cause a better absorption profile likewise the percent bioavailable too

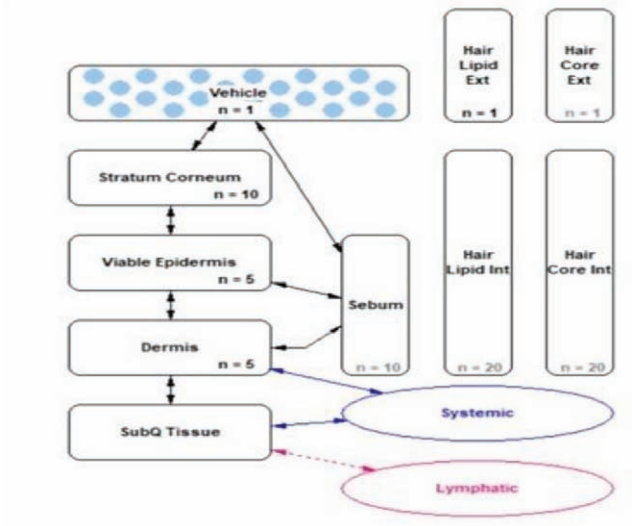


Figure 8: Dermal Physiology Model.

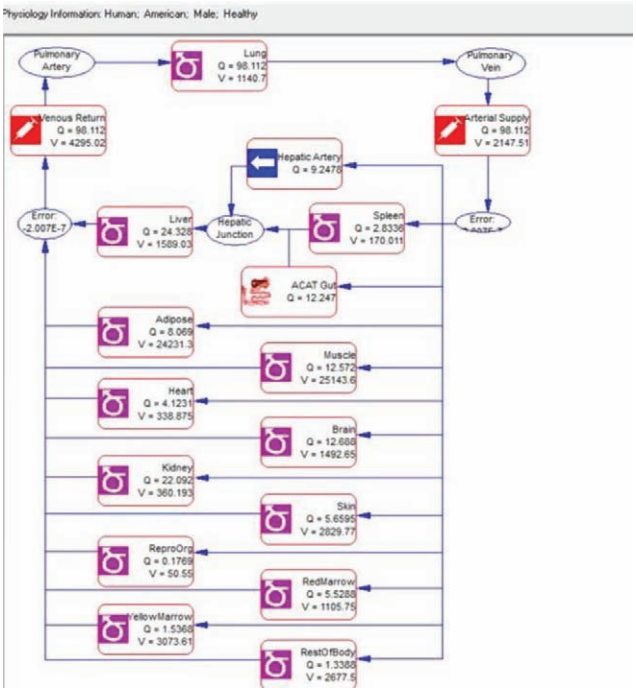


Figure 9: PBPK based dermal absorption model for 30 y male.

is dependent on the same particle size range (Figure 12). The disposition of TPL *in vivo* shows a distinct normal distribution which is particle size-dependent and the most likely accumulation of the vesicles and their disposition is particle size-dependent (Figure 13). The model suggests that the mean particle radii play a pivotal role in the absorption and disposition of TPL as a proniosomal gel.

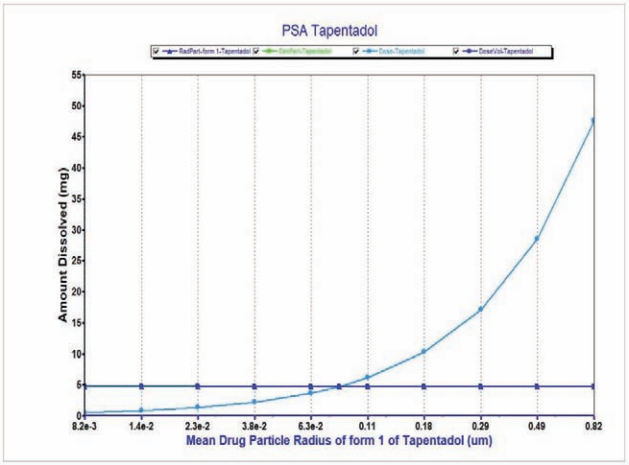


Figure 10: PSA of drug dissolution.

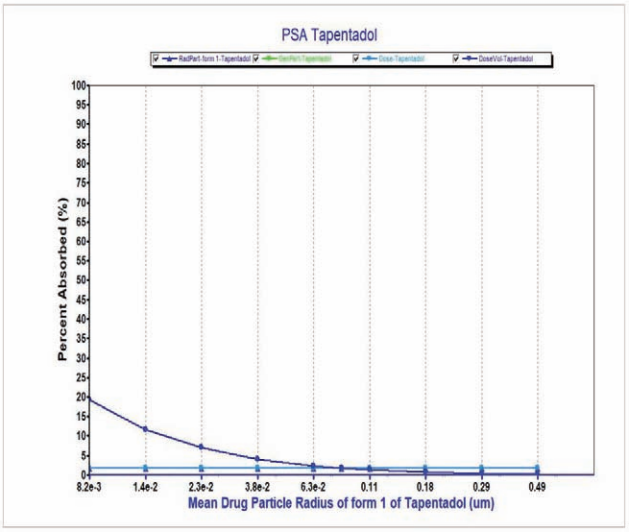


Figure 11: PSA of percentage drug absorption.

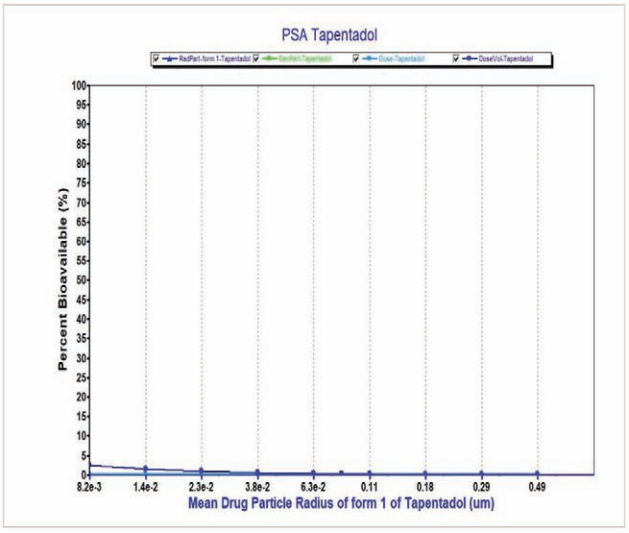


Figure 12: PSA of percentage bioavailability.

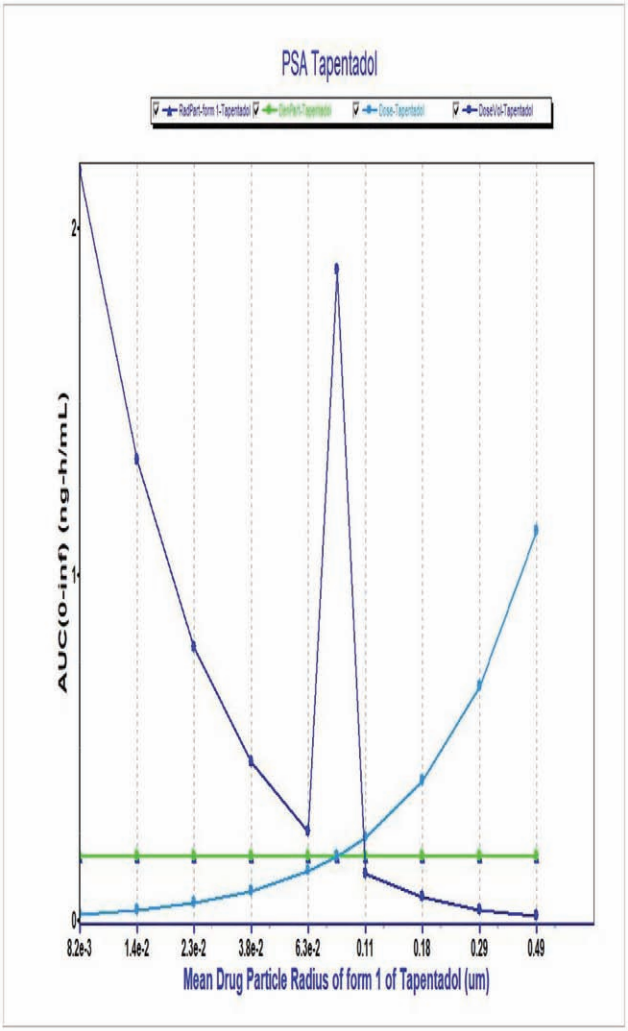


Figure 13: PSA of AUC of TPL.

CONCLUSION

The MESD was devised to study the effect of the ideal surfactant that will affect favorably the formulatory response of the TPL proniosomal gel. Kolliphore RH 40 showed a good response in EE and vesicle size and the further investigation established by a multivariate relationship between factors and responses augmented by the PSA model, confirms that particle size plays a pivotal role in permeation bioavailability and disposition of TPL. The MESD devised establishes the choice of excipient and its likely response and its predicted *in vivo* behaviour based on the particle size of the vesicles. Hence MESD augmented by PSA forms a viable and economic and scientifically sound approach to optimize formualtory response with a constrained set of input factors.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest

ABBREVIATIONS

IASP: International Association for Study of Pain; **CNCP:** Chronic Non-Cancer Pain; **TPL:** Tapentadol Hydrochloride; **BCS:** Biopharmaceutical Classification System; **IM:** Immediate Release; **ER:** Extended Release; **TDDS:** Transdermal Drug Delivery System; **MESD:** Main Effects Screening Design; **PSA:** Parameter Sensitivity Analysis; **CQA:** Critical Quality Attributes; **CMA:** Critical Material Attributes; **EE:** Entrapment Efficiency.

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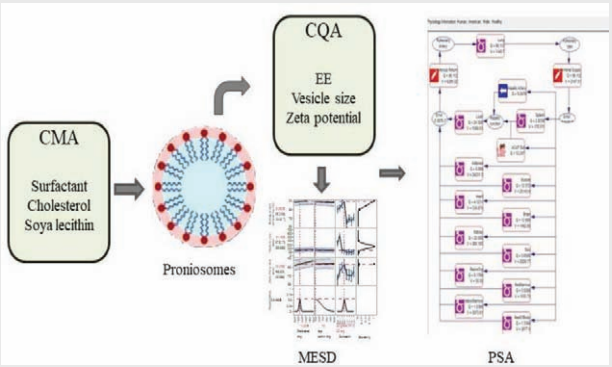
PICTORIAL ABSTRACT



SUMMARY

To formulate a stable tapentadol-loaded proniosomal gel, the MESD design was constructed to screen the choice of surfactants for the selected CMAs and CQAs. The model fit was evaluated. The prediction profiler showed the maximum desirability for Kolliphore RH 40. *In silico* verification was performed using GastoPlus® 9.7 to assess the *in vivo* behaviour of the designed formulation. The chosen surfactant Kolliphore RH 40 would be used for the further optimization of tapentadol-loaded proniosomal gel.

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