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Formulation and *ex vivo* Evaluation of Solid Lipid Nanoparticles (SLNS) Based Hydrogel for Intranasal Drug Delivery

Pramod Jagtap, Kisan Jadhav, Neha Dand

Abstract**—**Risperidone (RISP) is an antipsychotic agent and has low water solubility and nontargeted delivery results in numerous side effects. Hence, an attempt was made to develop SLNs hydrogel for intranasal delivery of RISP to achieve maximum bioavailability and reduction of side effects. RISP loaded SLNs composed of 1.65% (w/v) lipid mass were produced by high shear homogenization (HSH) coupled ultrasound (US) method using glycerylmonostearate (GMS) or Imwitor 900K (solid lipid). The particles were loaded with 0.2% (w/v) of the RISP & surface-tailored with a 2.02% (w/v) non-ionic surfactant Tween® 80. Optimization was done using 32 factorial design using Design Expert® software. The prepared SLNs dispersion incorporated into Polycarbophil AA1 hydrogel (0.5% w/v). The final gel formulation was evaluated for entrapment efficiency, particle size, rheological properties, X ray diffraction, in vitro diffusion, *ex vivo* permeation using sheep nasal mucosa and histopathological studies for nasocilliary toxicity. The entrapment efficiency of optimized SLNs was found to be 76 ± 2%, polydispersity index <0.3., particle size 278 ± 5 nm. This optimized batch was incorporated into hydrogel. The pH was found to be 6.4 ± 0.14. The rheological behaviour of hydrogel formulation revealed no thixotropic behaviour. In histopathology study, there was no nasocilliary toxicity observed in nasal mucosa after *ex vivo* permeation. X-ray diffraction data shows drug was in amorphous form. *Ex vivo* permeation study shows controlled release profile of drug.

Keywords**—***Ex vivo*, particle size, risperidone, solid lipid nanoparticles.

I. Introduction

A large variety of antipsychotic drugs acts on the receptors present in the brain. The entry of drug molecules into the brain is limited by one of the strictest barrier: the blood-brain barrier (BBB). The BBB consists of a continuous layer of endothelial cells joined together by tight junctions (zonulaeoccludens) which severely restrict paracellular transport across the barrier [1]. The BBB allows for passive diffusion of small lipid-soluble molecules, whereas hydrophilic substances or molecules with high molecular weight have minimal passive permeation [2], [3]. A number of strategies that have been utilized for targeting of drug to the brain, include invasive approaches (BBB disruption, intracerebral implants), physiological approaches (pseudo-nutrients, ligand-binding proteins, chimeric peptides) and pharmacological approaches (liposome, nanoparticles, nano-conjugates, chemical drug delivery) [4]-[6].

In recent years the nasal route has gained importance as a non-invasive drug application route that offers many advantages for the introduction of drugs into systemic circulation. Its major advantage is the rapid absorption of drugs and therefore quick onset of their effect. The most efficient area for drug administration is the lateral walls of the nasal cavity, which consist of highly vascularized tissue, the mucosa. The surface of the nasal cavity is approximately 150 cm^2. Paracellular and transcellular mechanisms are involved in the absorption from nasal mucosa. Olfactory region of nasal cavity is responsible for direct nose to brain transport of therapeutic agents [7], [8].

Colloidal carrier systems have been receiving growing interest in the field of drug delivery because they can offer several advantages in this area like, increase the solubility and hence the bioavailability of poorly water soluble actives, which belong to the classes II and IV in the biopharmaceutical classification system (BCS), protection of drugs against degradation or alteration of their biodistribution after i.v. administration, have a sufficient drug loading capacity, the possibility of drug targeting and controlled release characteristics [9]-[12]. At the beginning of the 1990s, Solid Lipid Nanoparticles (SLNs) have been introduced as an alternative carrier system to emulsions, liposomes and polymeric nanoparticles. Lipid nanoparticles have been reported as useful tools in development of oral, injectable, topical dosage forms for poorly water soluble drugs [13], [14]. SLNs are produced by replacing the oil of an o/w emulsion by a solid lipid or a blend of solid lipids, i.e. the lipid particle matrix being solid at both room and body temperatures. SLNs are composed of 0.1% w/w to 30% w/w solid lipid dispersed in an aqueous medium and if necessary stabilized with preferably 0.5% w/w to 5% w/w surfactant. The incorporation of cosmetic and pharmaceutical actives is feasible. The mean particle size of SLNs is in the submicron rage, ranging from about 40 nm to 1000 nm [15], [16]. SLNs have the combine advantages of polymeric nanoparticles and o/w emulsions for drug administration such as good tolerability, lower cytotoxicity, higher bioavailability by oral administration, and increase in the drug stability [17]. Other advantage of lipid excipients, such as biodegradability and cost effectiveness, lipid nanoparticles are suitable for the incorporation of

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lipophilic, hydrophilic and poorly water soluble drug within the lipid matrix in considerable amount [18].

Risperidone (RISP) is an approved antipsychotic drug belonging to the chemical class of benzisoxazole derivative and is available as tablet, oral liquid (Risperidal®) and orally disintegrating tablet (Risperidal® M-TAB). These dosage forms exhibit low bioavailability as RISP belongs to BCS class II thereby demonstrating poor water solubility and high intestinal permeability. An improvement in bioavailability of RISP has been directly related to improvement in solubility and dissolution rate [19]-[21]. RISP is one of the representative atypical antipsychotic drug which has a potent antagonist effect on serotonin 5-HT2 and dopamine D2 receptors present in the mesolimbic and mesocortical region of the brain [22]. As the target site of the RISP is brain, thereby a strategy is desirable to improve the bioavailability by targeting to the receptor site and bypassing the BBB so as to achieve desired drug concentration at the site of action. Hence preventing the availability of drug at non-targeting sites and reducing the side effects [23].

Considering all these premises, in the present work, we thought it worthy of interest to investigate the feasibility of developing a new drug delivery system for RISP nasal administration based on combined strategy which exploits both SLNs preparation, to enhance drug solubility and increase permeation and loading of SLNs into hydrogel system and to improve drug delivery through the nasal mucosa. Delivery to the nasal mucosa is limited by mucociliary clearance; thereby, formulation of a mucoadhesive hydrogel would be the most effective solution [24]. A variety of gelling agents have been reported: chitosan, carbopol, polycarbophil AA1, xanthan gum [25], [26], out of which polycarbophil AA1 which is polyacrylic acid derivative, has demonstrated good gelling properties and superlative mucoadhesion [27]. In the framework of this project the SLNs production was studied using a modified high shear homogenization (HSH) coupled ultrasound (US) method. The aim is to systematically asses various parameters, such as lipid concentration, emulsifier concentration, influence the size of lipid nanoparticles and entrapment efficiency. This has been addressed using factorial analysis, which allows not only to extract the maximum amount of information from the collected data, resorting to a limited number of experiments, but also to establish the influence of multiple factors on the formulation properties. The direct effect of each factor is studied and the respective interaction with other factor is also assessed in detail. The SLNs systems were evaluated for entrapment efficiency (EE) and particle size. The best optimized SLNs system were formulated into a polycarbophil hydrogel and assessed for rheological analysis; X-ray diffraction (XRD); histopathological study of nasal mucosa, assessment of *in vitro* RISP diffusion profiles and *ex vivo* permeation study.

II. Materials & Methods

A. Materials

Risperidone (RISP) was kindly provided by Alkem laboratories (Mumbai). Imwitor® 900 K or glyceryl monostearate (GMS), 40–55% and Dynasan® 114 were gift sample from Sasol (Germany). Compritol® 888 ATO and Precirol® ATO 5 samples were gifted by Gattefosse (France). Glyceryltristerate, glyceryltripalmitate and Tween® 80 of highly pure grade were purchased from Sigma-Aldrich (Mumbai). HPLC grade methanol and glacial acetic acid (100%) were obtained from SD Fine chemicals (Mumbai). For the hydrogels preparation, gelling agent Polycarbophil® AA1 (polyacrylic acid derivative, pharmagrade, MW-3.50 billion) was given as gift sample by Lubrizol India Pvt. Ltd. (Mumbai). The water used in all experiments was purified and distilled.

B. Methods

1. Determination of Drug Solubility in Lipids

Various lipids like Compritol® 888 ATO**,** Precirol® ATO 5, Imwitor® 900K, glyceryltristerate, Dynasan® 114 and glyceryl tripalmitate were screened by physical method [28] to access the solubility of RISP in them. Weighed quantity of RISP was taken in series of test tubes and different lipids in increasing amounts were added to it until drug is completely solubilized. The temperature of test tubes was maintained 10°C above the melting point of lipid used. The test tubes were intermittently shaken on cyclone mixer and observed visually for any drug residue. The amount of lipid required for solubilizing fixed amount of drug was determined. Some researchers have reported drug solubility in lipids using differential scanning calorimertry analysis (DSC) [29], [30]. The lipid in which the drug showed maximum solubility was selected for further studies.

2. Preparation of SLNs

The preparation of SLNs was based on the principle of 'HSH coupled US' method [31]. Drug and GMS (in various ratios) were heated to 70-80°C in a water bath. The total drug: lipid ratio was maintained at 1:5, 1:7.5 and 1:10 % w/w. The resulting solution was poured into 10 ml of aqueous Tween® 80 solution which was maintained at same temperature as lipid melt. The mixture was high shear homogenised using Ultra Turrax® T25 digital (IKA, Germany) at 10,000 rpm for 5 min. The pre-emulsion is formed which was then sonicated for 5 min at 20 Watt using Sonapros® PR-250 M (Oscar Ultrasonics, Andheri). The resulting hot dispersion was cooled under magnetic stirring at 4-8°C in order to allow for lipid solidification and formation of SLNs.

3. Optimization of SLNs

A 3^2 randomized full factorial design was used in the present study for optimizing the concentration of lipid and surfactant. In this design 2 factors are evaluated, each at 3 levels and experimental trials are performed in all 9 possible combinations. The amount of Drug: Lipid ratio (X_1), and the amount of surfactant (X_2), were selected as independent

variables. The entrapment efficiency and particle size were selected as dependent variables in a 3^2 randomized full factorial design to evaluate the responses. The design matrix and coded levels are mentioned in actual values as shown in Table I.

TABLE I
FORMULATION OF SLNS USING FACTORIAL DESIGN

Formulations	Drug: lipid ratio	Weight of drug (mg)	Weight of lipid (mg) X1	Aqueous phase volume q.s. 10 (ml)	Tween® 80 (mg) X2
F1	1:5 (1)	20	100	10	200
F2	1:5 (2)	20	100	10	300
F3	1:5 (3)	20	100	10	400
F4	1:7.5 (1)	20	150	10	200
F5	1:7.5 (2)	20	150	10	300
F6	1:7.5 (3)	20	150	10	400
F7	1:10 (1)	20	200	10	200
F8	1:10 (2)	20	200	10	300
F9	1:10 (3)	20	200	10	400

So, nine formulations were formulated and evaluated for particle size and EE. Based on those results overlay plots and surface response plots were obtained. Tables II and III show coded and actual value of design matrix for factorial design experiment.

TABLE II
DESIGN MATRIX OF INDEPENDENT VARIABLES

FORMULATION	CODED LEVELS X_1 (mg)	CODED LEVELS X_2 (mg)
F1	-1	-1
F2	-1	0
F3	-1	+1
F4	0	-1
F5	0	0
F6	0	+1
F7	+1	-1
F8	+1	0
F9	+1	+1

X_1. Drug: Lipid Ratio, X_2–Surfactant Concentration

TABLE III
CODED LEVELS IN ACTUAL VALUES

CODED LEVELS	ACTUAL VALUES X_1	ACTUAL VALUES X_2 (mg)
-1, -1	1:5	200
-1, 0	1:5	300
-1, +1	1:5	400
0, -1	1:7.5	200
0, 0	1:7.5	300
0, +1	1:7.5	400
+1, -1	1:10	200
+1, 0	1:10	300
+1, +1	1:10	400

4. Preparation of SLNs Hydrogel

The gelling agent was pre-soaked for 5-6 h then the nanoparticulate dispersion was poured under stirring into the pre-soaked gelling agent. The pH of dispersion was adjusted in between 5.5 to 6.5 by triethanolamine to form gel with good consistency. For optimization of concentration of gelling agent, various concentrations of gelling agents used are 0.1, 0.5, 1, and 1.5%. Composition of trial batches were shown in Table IV.

TABLE IV
OPTIMIZATION OF GELLING AGENT CONCENTRATION

Batch code	Polycarbophil® AA1 concentration (%)	Drug in mg	Lipid Concentration (mg)	Surfactant Concentration (mg)	Amount of water q.s. (ml)
SG1	0.1	20	165	202	10
SG2	0.5	20	165	202	10
SG3	1	20	165	202	10
SG4	1.5	20	165	202	10

5. Chromatographic Conditions

RISP was quantified by modified HPLC method described by [32]. For required retention time slight modifications are made in mobile phase i.e. the ratio of Methanol: 40 mM Ammonium acetate buffer pH 5 changed from 80:20 v/v to 60:40 v/v and the method was partially validated (System suitability (Table V), linearity: r^2= 0.999, accuracy and precision (Table VI)). The HPLC assay was carried out using on an Agilent Technologies 1200 series system, based on quaternary pump, autosampler, Ultra Violet detector (UV detector) and EZ-Chrome software. The column HiQSil® C18HS (250 X 4.6 mm, 5 µm) was maintained at 20° C. The injection volume was 65 µl. An isocratic mobile phase used Methanol: Ammonium acetate buffer pH 5 (60:40 v/v) with the flow rate of 1ml/min.

TABLE V
SYSTEM SUITABILITY PARAMETERS

Parameter	RISP (10 µg/ml) Retention time (min)	RISP (10 µg/ml) Peak area (µv.sec)
Mean (n=5)	7.329	1103077
S.D	0.05568	301.23
%CV	0.178	0.512

C. Evaluation of SLNs Dispersion and SLNs Based Hydrogel

1. Particle Size Analysis

TABLE VI
INTRA AND INTER DAY ACCURACY AND PRECISION OF HPLC ASSAY FOR RISP

Day	Parameter	Nominal concentration 3 µg/ml	Nominal concentration 7 µg/ml	Nominal concentration 15 µg/ml
	Mean (n=5)	2.811	6.940	15.00
Day-1	**S.D**	0.1069	0.1637	0.1762
	% CV	2.80	2.36	1.17
	Mean (n=5)	2.843	6.917	14.90
Day-2	**S.D**	0.1676	0.2005	0.2646
	% CV	5.89	2.90	1.78
	Mean (n=5)	2.832	6.994	15.07
Day-3	**S.D**	0.07132	0.2045	0.1528
	% CV	2.52	2.92	1.01
	Mean (n=5)	2.828667	6.950333	14.99
Inter day	**S.D**	0.016258	0.039526	0.08544
	% CV	0.57477	0.568697	0.56998

The particle size analysis of the selected formulation was performed using NANOPHOX® (NX0073) particle size analyzer (Sympatec GmbH, Germany). The principle behind the particle size measurement is cross correlation laser diffraction. An aliquot of SLNs was diluted in deionised water prior to measurements. Analysis was performed at a fixed angle of 90° to the incident light and data were collected over a period of 2 min. All measurements were done in triplicate.

The mean diameter of each batch and particle size distribution curve was recorded.

2. Entrapment Efficiency

EE corresponds to the percentage of drug encapsulated within and adsorbed onto the nanoparticles. Nanoparticle dispersion (1 ml) was centrifuged at 14000 RPM (Remi instruments, India) for 20 min to separate the nanoparticles. To facilitate the separation of the nanoparticles, electrolyte such as NaCl was used. The supernatant was analyzed for unencapsulated drug by using UV-spectrophotometric method after suitable dilution with methanol [33].

The EE was calculated

$$\%\,\mathrm{EE} = \frac{M_{\text{initial drug}} - M_{\text{free drug}}}{M_{\text{initial drug}}} * 100 \quad (1)$$

where, '$M_{\text{initial drug}}$' is the weight of the drug used for the fabrication of the nanocarriers and '$M_{\text{free drug}}$' is the weight of free drug detected in the supernatant after centrifugation of the nanoparticulate dispersion. The wavelength of analysis for RISP is 280 nm. All the experiments were performed in triplicate.

3. Rheological Behavior

Viscosity measurements were carried out using a Brookfield® digital viscometer (Model 5.0, Brookfield Engineering Labs. Inc., Middleboro, USA) equipped with S62 spindle. 7.5 g of hydrogel was filled in the cylindrical tube and the viscosity was noted at 0.5, 1, 2.5, 3, 5, 5, 10, and 20 RPM. The speed was then successively lowered and the corresponding dial readings were noted to know the thixotropic behaviour of the system. The experiments were performed in triplicates (n = 3). To reduce the effect of temperature on the rheological behaviour the temperature was maintained with the help of thermostatic water bath (25 ± 0.5°C), during all experiments.

4. X-ray Diffraction Study

X-ray scattering measurements were carried out with a Pan-analytical Xpert PRO® MPD X-ray diffractometer. Anode copper K alpha and radiation wavelength 1.5405 Angstrom was used with power of 45 KV and 40 mA and detected using Xcelerator deffracted beam monochromator. XRD measurements were carried out for RISP, drug loaded SLNs hydrogel.

5. *In vitro* and *ex vivo* Permeation Studies

In vitro permeation experiments were carried out using dialysis membrane (M. Wt. cutoff: 12,000–14,000, Himedia, India) as the permeation barrier using the same experimental set up as mentioned below for the *ex vivo* study using sheep nasal mucosa.

In vitro drug permeation study was carried out using a Franz diffusion cell [34]. The cell consists of two chambers, the donor and the receptor. The Franz diffusion cells are having a surface area 3.14 cm^2 and receptor compartment having a capacity of 20 ml. The receptor chamber was filled with freshly prepared phosphate buffer pH 6.4 to ensure perfect sink conditions and was provided with a sampling port. The 2 × 2 cm^2 piece of activated cellophane membrane was mounted over diffusion cells and then air bubbles were removed. Recirculating water bath was maintained at 37 ± 0.5ºC and the solution in the receptor chambers was stirred using a small magnetic bar for uniform mixing of the contents. Prior to application of formulations the membrane was allowed to equilibrate for 30 min, donor compartments were filled with dose or volume of SLNs hydrogel equivalent to 1.0 mg of RISP. 1 ml of the receptor phase was removed and immediately replaced by the equal volume of buffer at set interval of 1, 2, 3, 4, 6, 8 and 10 h. All the experiments were performed in triplicate. The amount of RISP permeated into the receptor phase from the formulations was determined by a validated HPLC method. By determining the amount of RISP permeated at various time intervals, the % release versus time graphs were plotted for optimized batch of RISP loaded SLNs hydrogel.

Preparation of sheep nasal mucosa for *ex vivo* permeation study: The freshly excised sheep nasal except the septum part was collected from the slaughter house. The membrane was kept in PBS pH 6.4 for 15 min to equilibrate. The superior nasal conche was identified and separated from the nasal membrane. The excised superior nasal membrane was then mounted on Franz diffusion cell. The tissue was stabilized using phosphate buffer pH 6.4 in both the compartments and allowed to stir for 30 min on a magnetic stirrer. After 30 min, solution from both the compartments was removed and fresh phosphate buffer pH. 6.4 were filled in the acceptor compartment. The mounting of the nasal membrane was done on acceptor compartment using clamp [35].

6. Histopathological Examination of Nasal Mucosa for Nasocilio Toxicity

Freshly excised sheep nasal mucosa, except for the septum was collected from the slaughter house in PBS pH 6.4. Two sheep nasal mucosa pieces (P1 and P2) with uniform thickness were selected. P1 was treated with 100 mg of PBS pH 6.4 (negative control), P2 with 100 mg of SLNs hydrogel for 10 h. After 10 h, the mucosa was rinsed with PBS pH 6.4 solutions. The mucosa was fixed in 10% neutral formalin for 24 hours and then cut vertically against the surface at the central region (4 mm width). Each section was dehydrated using graded solutions of ethanol and then embedded in paraffin wax. Tissues were divided into small pieces and stained with haematoxylin and eosin. The sections were observed under 100X magnification and photographed [36], [37].

III. Results & Discussion

Before the experimental design was constructed, various preformulation studies were carried out for the selection of the appropriate lipid phase and emulsifier type and are described in what as follows.

A. Determination of Drug Solubility in Lipids

Lipid solubility study carried out to determine the maximum solubility of drug in particular lipid. The aim was to select a lipid and study the affinity of drug for the lipid matrix type. The solubility of drug in lipid determines the drug loading capacity of lipid as well as entrapment efficiency. Highest drug solubility in lipid also reduces chances of drug expulsion from lipid particles. It was found that the Imwitor® 900K lipid shows highest drug solubilising capacity over other lipids. Fig. 1 shows the comparative solubility of drug in different lipids. Imwitor® 900K is selected as the model lipid for the formulation and development. The concentration of lipid was taken for the pre-optimization study on the basis of drug solubility in lipid. A study performed by Silva et al on the solubility of RISP in various solid lipids by DSC method showed superiority of Imwitor 900K over other solid lipids [29].These results concurred with our findings from the physical method of solubility determination.

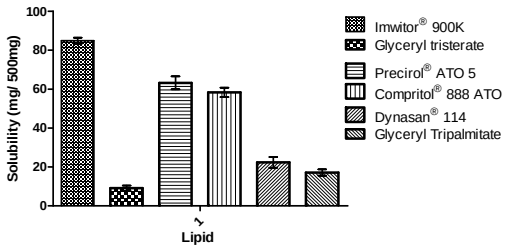

Fig. 1 Comparative drug solubility in different lipids (n=3±SD)

B. Surfactant Selection

The choice of surfactant is of at most important in the development and optimization of nanoparticle formulation. The role of this component is not only to control the particle size and stability of dispersion but also to control the crystallization behaviour of the lipid particles including polymorphs [14]. The most commonly used surfactants were Tween® 80, Solutol® HS 15, Phospholipon® 90G, Poloxamer® 188 were screened. These surfactants were screened in the wide concentration range. The pre-optimization batches were taken and it was observed for various parameters like appearance, stability and particle size was shown in Table VII. These batches indicate that the Tween® 80 leads to smaller particle size with good stability. It was decided to choose Tween® 80 as a model surfactant for optimization study. Reported researchers have developed SLNs using Tween® 80 as model surfactant [38], [39].

C. Optimization

The preliminary factor screening results reflects that this system is highly influenced by the lipid type, surfactant type and the concentration. It should be noted that these are widely recognised as major factors influencing the size of lipid nanoparticles. Statistical software, Statease Design-Expert® 8.0.6 was utilized to evaluate the response. A 3^2 factorial experimental design (Table VIII) was used to reduce the number of experiments to nine. The selection of formulation variables such as drug: lipid ratio, concentration of Tween® 80 and their levels in the design were decided from the results of preliminary investigations. The expected responses were EE = NLT 70 % and particle size = Less than 350 nm.

TABLE VII
PRE-OPTIMIZATION BATCHES OBSERVATIONS

Sr No.	Concentration of surfactant (%)		Appearance	Stability		Particle size (nm)
				Immediately	After 24 h	
A	Tween® 80	1	+	*	#	---
B		1.5	+	*	*	650
C		2	++	*	*	444
D		2.5	+++	*	*	250
E		3	+++	*	*	120
F	Poloxamer® 188	1	-	#	#	---
G		1.5	+	#	#	934
H		2	++	*	#	---
I		2.5	+++	*	#	---
J		3	+++	*	*	400
K	Solutol® HS 15	1	-	#	#	---
L		1.5	+	#	#	---
M		2	+	#	#	---
N		2.5	++	*	#	600
O		3	++	*	#	387
P	Phospholipon® 90G	1	+	#	#	---
Q		1.5	+	#	#	---
R		2	+	#	#	---
S		2.5	++	*	#	---
T		3	++	*	*	465

(+) Turbid, (++) Translucent, (+++) Transparent, (-) Separated, (*) Stable, (#) Unstable

The analysis of the data obtained above using the Stat-ease Design Expert 8.0.6 software showed that the formulation containing Imwitor® 900K (165 mg), Tween® 80 (202 mg) was optimum and was subjected to further dosage form development.

TABLE VIII
RESPONSES OF VARIOUS FORMULATIONS INCLUDING SD (N=3)

Formulation code	Coded levels	% EE	Particle size (nm)
F1	-1, -1	64.675	228
F2	-1, 0	22.975	73
F3	-1, +1	21.775	25
F4	0, -1	71.725	278
F5	0, 0	66.475	263
F6	0, +1	27.325	86
F7	+1, -1	81.225	320
F8	+1, 0	71.30	305
F9	+1, +1	62.725	248

1. Overlay Plot

An overlay plot was obtained using Stat-ease Design-Expert® software, to determine the total amount of Imwitor® 900K and amount of Tween® 80 required to obtain formulations having EE above 70 % and particle size less than 350 nm. Fig. 2 shows the overlay plot of the EE (Y_1) and particle size (Y_2) at different values of total lipid amount (X_1) and amount of surfactant (X_2).

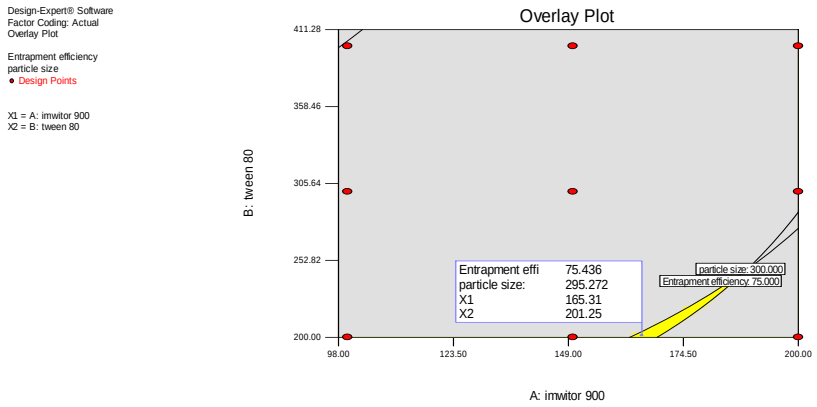

Fig. 2 Overlay plot

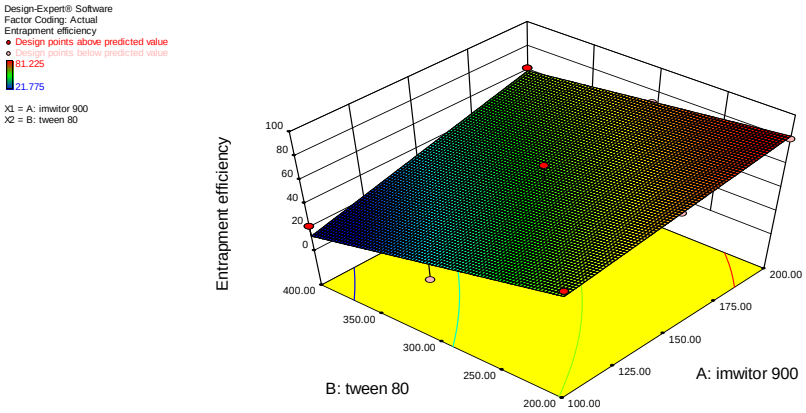

Fig. 3 Response surface plots showing the effect of DRUG:GMS and concentration of Tween® 80 on the % EE

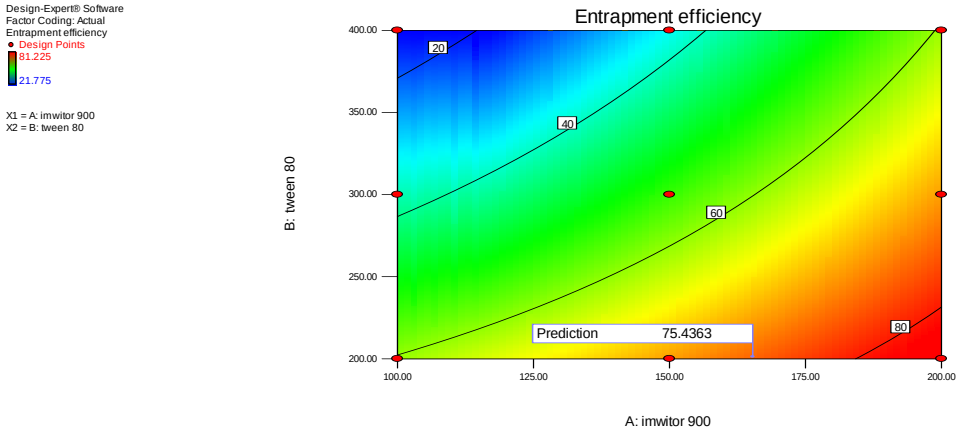

Fig. 4 Contour plots showing the effect of DRUG:GMS and Concentration of Tween® 80 on the % EE

The overlay plot indicated that optimal level of lipid concentration (165 mg) and amount of Tween® 80 (202 mg) favors the desired EE and particle size of the formulation. The plot also gives a theoretical idea about the optimized formulation which coincides with optimum formulation selected after the experimental work. Further, the overlay plot also gives a range of amount of Imwitor® 900K and Tween® 80 concentration that can be used in formulations to obtain desirable EE and particle size.

2. Response Surface Plot: Entrapment Efficiency

Three-dimensional response surface plots are presented in Fig. 3 These types of plots are useful in the study of the effects of two factors on the response at one time. Fig. 4 shows that EE increases with increasing concentrations of DRUG: GMS

ratios and there is a significant difference on EE of increasing concentration of Tween® 80.

3. Response Surface Plots: Particle Size

Three-dimensional response surface plots are presented in Fig. 5. These types of plots are useful in the study of the effects of two factors on the response at one time. Fig. 6 shows that particle size increases with increasing concentrations of DRUG: GMS ratios and there is a significant decrease in particle size by increasing concentration of Tween® 80.

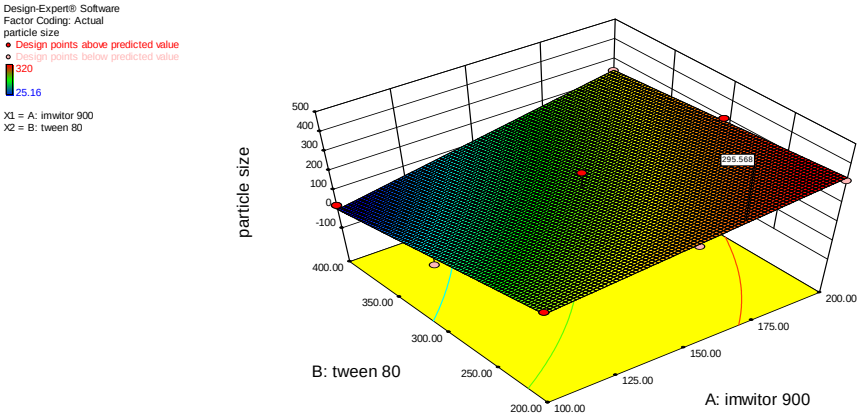

Fig. 5 Response surface plots showing the effect of DRUG:GMS and concentration of Tween® 80 on the particle size

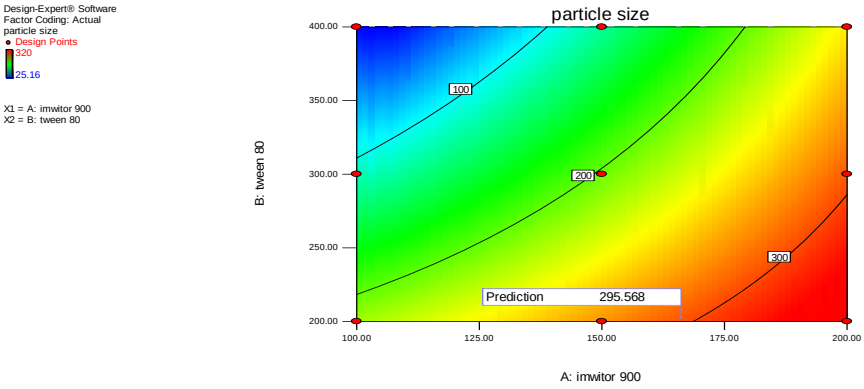

Fig. 6 Contour plots showing the effect of DRUG:GMS and Concentration of Tween® 80 on the particle size

D. Evaluation of SLNs

1. Particle Size

Particle size distribution is one of the most important characteristics for the evaluation of the stability of colloidal systems. Therefore particle size parameters have been evaluated immediately after production of SLNs. The predicted particle size of optimised formulation was 295 nm but in actual the particle size was found to be 278 ± 5 nm. The polydispersity index is a ratio that gives information about the homogeneity of particle size distribution in a system. Ideally it should be <0.3 [13]. A small value of polydispersity index indicates narrow size distribution of the system. The polydispersity index of SLN system was found to be 0.230. The distribution diagram of optimized SLN formulation was shown in Fig. 7.

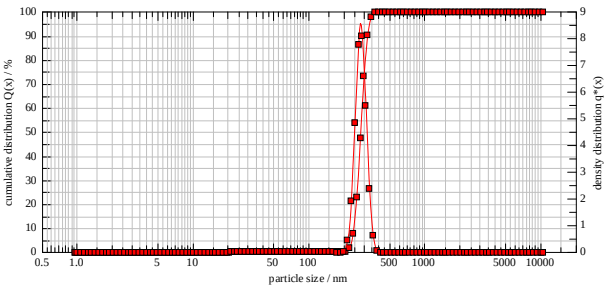

Fig. 7 Distribution diagram of optimized SLNs formulation

2. Entrapment Efficiency

EE is expressed as fraction of drug incorporated into formulations relative to the total amount of drug used. The EE of RISP loaded SLNs varied from 21.27% to 81.22% for various factor level combinations. The independent factors affecting the EE were lipid concentration (X_1) and surfactant concentration (X_2) used in manufacturing of SLNs.

The % EE increased with increase in concentration of lipid. This is probably due to increase in viscosity of medium resulting in faster solidification of nanoparticles. This would further prevent the drug diffusion to external phase of medium [31]. The EE of optimized formulation predicted was 75.43% but in actual it was found to be 76 ± 2%.

The surfactant level (X_2) has significant but negative impact on the EE. This could be explained by partition phenomena. High surfactant concentration in the external phase might increase the partition of drug from internal to external phase of the medium. This increased partition of the drug results in the increased solubilisation of drug in the external phase [34].

E. Evaluation of Effect of Various Parameters on SLNs

1. Effect of Homogenization Time

The effect of homogenization on the particle size, % EE and polydispersity index was checked (Table IX). Homogenization is a state of pre-emulsion formation. By varying the homogenization time 1, 5 and 10 min; the effect on the % EE, Particle size (nm) and polydispersity index was checked. It was found that there was no significant effect on the % EE at various homogenization times. The particle size of 1 min homogenized batch was significantly higher than 5 and 10 min homogenized batches. So, optimum homogenization time of 5 min was selected because there was no significant decrease in particle size when time increased to 10 min. The batch was obtained with least polydispersity index of 0.025 ± 0.007.

TABLE IX
EFFECT OF HOMOGENIZATION TIME ON SLNS

Time (min)	Parameter ± SD		
	% EE	Particle size (nm)	Polydispersity index
1	66.74 ± 5.441	297.3 ± 2.517	0.790 ± 0.055
5	70.26 ± 2.94	209.0 ± 2.660	0.025 ± 0.007
10	65.89 ± 2.174	190.7 ± 12.10	0.170 ± 0.050

2. Effect of Homogenization Speed

TABLE X
EFFECT OF HOMOGENIZATION SPEED ON SLNS

Speed (RPM)	Parameter ± SD		
	% EE	Particle size (nm)	Polydispersity index
5000	63.77 ± 1.613	280.3 ± 9.504	0.525 ± 0.255
10,000	72.07 ± 3.94	280.0 ± 12.60	0.035 ± 0.004
15,000	65.66 ± 0.678	197.3 ± 33.10	0.457 ± 0.225

Homogenization speed was varied 5000 to 15,000 RPM. It was observed that EE of second formulation was highest and there was no difference between particle size of first and second formulation (Table X). The polydispersity index of second formulation was very low i.e. 0.035 ± 0.004. So, the 10,000 RPM selected as homogenization speed. Another advantage of selecting optimum speed it may help in scale up of small scale batch which ensures uniform homogenization.

3. Effect of Sonication Time

Effect of sonication time on % EE, particle size and polydispersity index is shown in Table XI. As the sonication time was increased the particle size was decreased. It was desirable to keep the sonication time minimum to avoid metal shedding of probe tip during sonication. As we were found that the 5 min sonication time is optimal to produce the desirable particle size (283.0 ± 10.61) with less polydispersity index (0.1270 ± 0.0341).

TABLE XI
EFFECT OF SONICATION TIME ON SLNS

Time (min)	Parameter ± SD		
	% EE	Particle size (nm)	Polydispersity index
2.5	70.47 ± 2.538	341.3 ± 11.50	0.6793 ± 0.0561
5	75.69 ± 0.289	283.0 ± 10.61	0.1270 ± 0.0341
7.5	65.19 ± 6.718	175.3 ± 25.51	0.6784 ± 0.2757

4. Effect of Power of Sonication

The effect of power of sonication on % EE, particle size and polydispersity index was checked (Table XII). It was observed that as the sonication power increases from 10, 20 and 30; the particle size decreases significantly. Sonication power of 20 Watt was found to be optimum by looking at EE, particle size and polydispersity index.

TABLE XII
EFFECT OF POWER OF SONICATION ON SLNS

Power (Watt)	Parameter ± SD		
	% EE	Particle size (nm)	Polydispersity index
10	63.49 ± 3.438	1790 ± 59.43	0.6777 ± 0.059
20	76.51 ± 0.600	228.3 ± 10.69	0.1200 ± 0.0200
30	59.74 ± 24.76	143.6 ± 63.73	0.5202 ± 0.007

F. Hydrogel System

SLNs aqueous dispersions can be incorporated into hydrogels consisting of one or more uncharged polymer without significant changes in particle size and zeta potential. Polycarbophil® AA1 selected as model gelling agent for the preparation of SLNs hydrogel. Various concentration of gelling agent were selected and checked for its desirable consistency of gel and gel permeation of SLNs. It was found that the concentration of 0.5

G. Evaluation of Hydrogel

1. Appearance, pH, Chemical Assay

The physical appearance of SLNs based hydrogel is semitransparent to milky white in colour and pH was found to be 6.4 ± 0.14. Chemical assay done as per USP method of optimized SLNs hydrogel and it was found to be 98.92 ± 0.134 (n=3).

2. Viscosity

Rheological behaviour of SLNs is shown in Fig. 8. The viscosity curves were plotted Viscosity (cP) verses shear rate (RPM). It was found that the optimized hydrogel formulation of SLNs shows pseudo plastic non-Newtonian behaviour, whereas the viscosity decreases with increasing shear rate. Ascending and descending curve overlaps and show no thixotropic behaviour. The lipid particles in the semisolid system tend to align with increasing shear stress which is elevating the flow [40].

3. XRD Study

In XRD study has been used for the study of molecular structure and polymorphism of lipid nanoparticles. The characteristic XRD pattern of drug (Fig. 9) was not seen in the diffraction pattern of SLNs (Fig. 10). XRD data allows differentiation between crystalline and amorphous material. It was concluded that the drug is incorporated into SLNs and remains in the amorphous form [29], [40]. Similar findings have been described by Rahman et al who studied the entrapment of RISP in Compritol® 888 ATO by XRD studies [34].

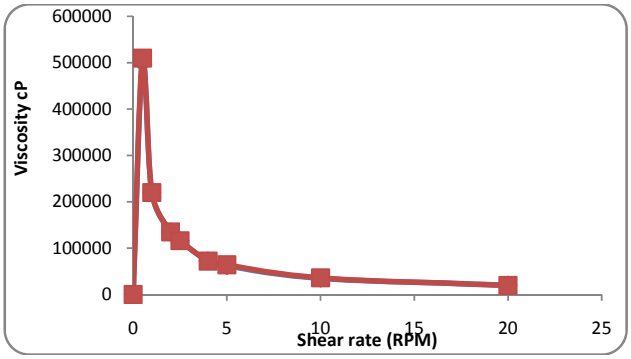

Fig. 8 Rheological behavior of SLNs hydrogel (n=3±SD)

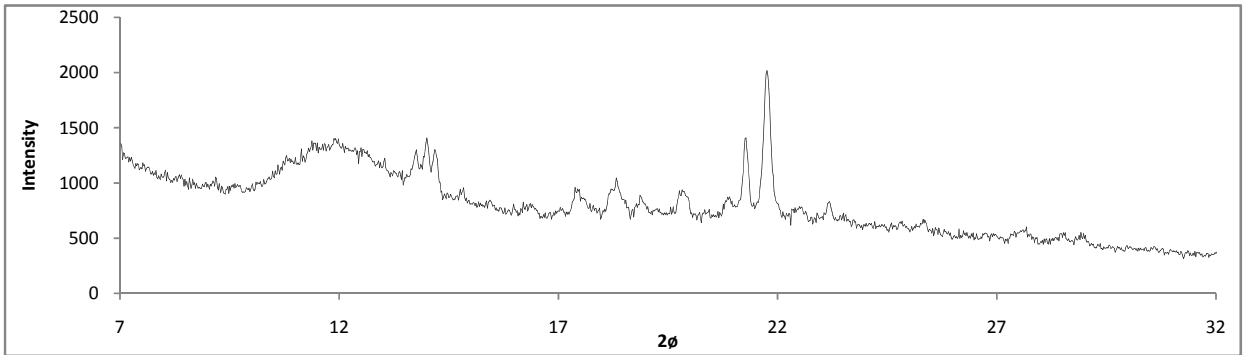

Fig. 9 Diffraction pattern of RISP

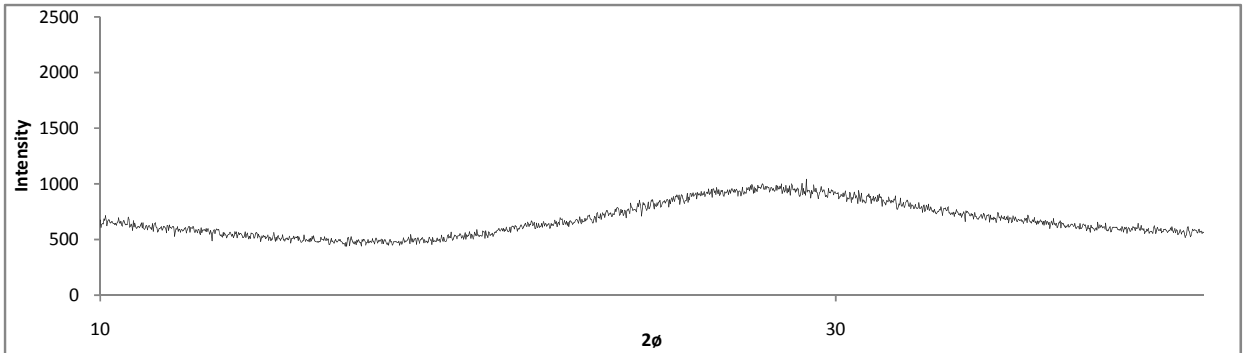

Fig. 10 Diffraction pattern of SLNs hydrogel

4. *In vitro* Gel Permeation

The release profile of SLNs hydrogel is shown in Fig. 11. *In vitro* gel permeation was checked toget idea about release pattern of SLNs polycarbophil hydrogel through nasal mucosa. It was observed that there was no significant retardation of release of SLNs from gel matrix. Release profile was observed over the period of 10 h. 63 % of drug permeated from SLNs hydrogel at the end of 10 h. Sustained release profile suggests the diffusion of RISP from core of lipid matrix to the release medium.

5. *Ex vivo* Permeation Study

The release profile of SLNs hydrogel is shown in Fig. 12. *Ex vivo* study was performed to confirm the permeation across the living tissue i.e. isolated nasal mucosa. It was observed that there was no significant difference between release profile of *in vitro* and *ex vivo* study. Sheep nasal mucosa used with uniform thickness of 0.2 ± 0.1 mm, to avoid release profile variation.

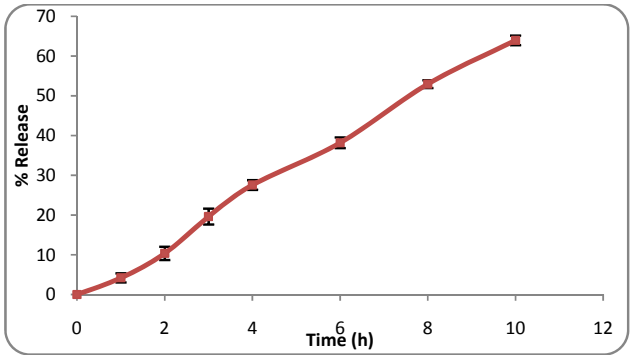

Fig. 11 *In vitro* gel permeation through dialysis membrane (M. Wt. cutoff: 12,000–14,000), effective surface area= 3.14 cm^2 (n=3±SD)

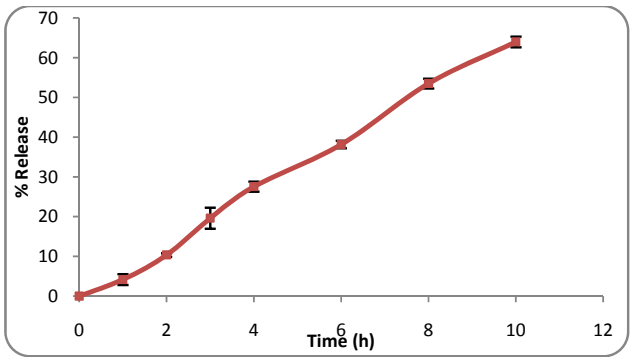

Fig. 12 *Ex vivo* release profile of SLNs hydrogel through sheep nasal mucosa with effective surface area of 3.14 cm^2 (n=3±SD)

6. Flux

The flux calculated directly using the PCP disso software. Fig. 13 shows the graph plotted (average flux versus time) for SLNs hydrogel system. For SLNs hydrogel absorption is passive diffusion process can be described by Fick's law of diffusion (2). The graph shows

$$\frac{dQr}{Adt} = J \quad (2)$$

where, J is the flux across nasal mucosa (µg/cm^2/h), dQr is the change in quantity of material passing through the membrane into the receptor compartment expressed in µg. A is the active diffusion area in cm^2 and dt is change in time.

7. Histopathological Examination of Nasal Mucosa for Nasocilio Toxicity

The histology of excised nasal mucosa treated with optimized SLNs hydrogel (P2) and untreated mucosa (P1) is shown in Fig. 14. The microscopic observations indicate that the optimized SLNs hydrogel has no significant effect on the microscopic structure of the nasal mucosa. The surface epithelium lining and the cellular structure of the nasal mucosa were totally intact. No major changes in the ultra structure of nasal mucosa morphology could be seen and the epithelial cells appeared mostly unchanged. Therefore the hydrogel formulations are safe for application on nasal mucosa; no signs of nasociliary toxicity observed.

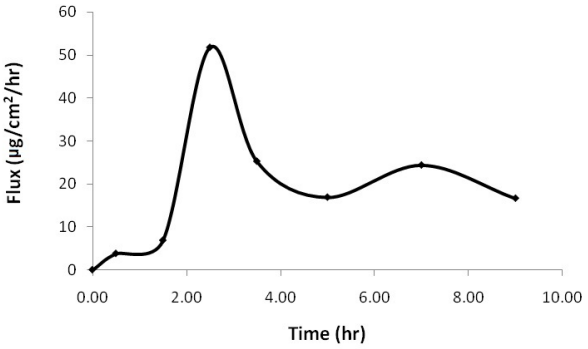

Fig. 13 *Ex vivo* permeation of RISP loaded SLNs hydrogel through sheep nasal mucosa, effective surface area 3.14 cm^2 (n=3±SD).

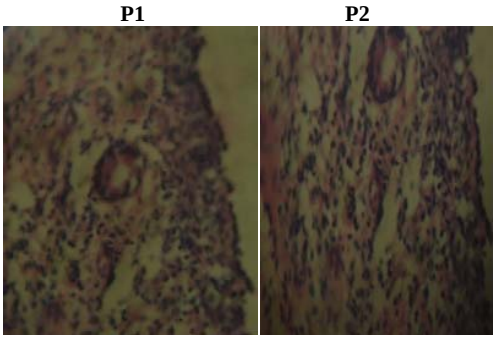

Fig. 14 Histological photomicrograph of eosin-hematoxylin stained nasal mucosa P1=Untreated mucosa, P2= Mucosa treated with 1mg equivalent hydrogel

IV. Conclusion

This study gives a novel approach for treatment of psychotic diseases, decreased side effects, increased bioavailability and targeting of drug to the brain. The following major conclusions are drawn from the above study; Amongst various solid lipids (Compritol® 888 ATO**,** Precirol® ATO 5, Imwitor® 900K, Glyceryltristerate, Dynasan® 114, Glyceryltripalmitate) screened, Imwitor® 900K or GMS showed 3-4 fold higher drug solubility. SLNs dispersions of RISP were developed by HSH coupled US method which is the simplest method of preparation of SLNs. A series of SLNs batches coded (F_1-F_9) were prepared using different combination and ratio of various excipients in order to optimize the % EE and particle size. A randomized 3^2 full factorial design was applied. A RISP loaded SLNs formulation containing 165 mg of GMS and 202 mg Tween® 80 was found to be optimum in terms of EE and particle size. Polycarbophil® AA1 (0.5%) was successfully used to formulate SLNs hydrogel with desired consistency in order to increase residence time and reduce the possibility of mucocilliary clearance. Polycarbophil®AA1 which exhibits excellent mucoadhesive property was chosen to guarantee prolonged residence time. The *in vitro* release profile of SLNs hydrogel and *ex vivo* release showed controlled release of RISP through excised nasal mucosa. XRD study of nasal hydrogel indicates that the crystalline form of the drug have

been changed to amorphous form which indicates increasing the solubility of RISP. Histopathology study revealed that the formulation has no toxic effect on nasal mucosa even after a longer contact and all the surface epithelium lining and the cellular structure remain intact. This ensures the safety of developed SLNs based hydrogel formulation for intranasal delivery of RISP.

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