

BIOLOGICAL SAFETY EVALUATION OF STEALTH SOLID LIPID NANOPARTICLES OF RISPERIDONE: EFFECT OF SURFACE CHARACTERISTICS ON THEIR HYDROPHOBICITY, HAEMOLYSIS AND MACROPHAGE PHAGOCYTOSIS

JALEH VARSHOSAZ^{1*}, HOJJAT SADEGHI², FAEZEH SHAFIPOUR¹

¹ *Department of Pharmaceutics, Faculty of Pharmacy and Isfahan Pharmaceutical Sciences Research Centre, Isfahan University of Medical Sciences, Isfahan, Iran.*

² *Department of Biotechnology, Faculty of Pharmacy and Isfahan Pharmaceutical Sciences Research Centre, Isfahan University of Medical Sciences, Isfahan, Iran.*

*Corresponding author: varshosaz@pharm.mui.ac.ir

Abstract

The aim of this study was to prepare stealth solid lipid nanoparticles (SLN_s) of risperidone, for controlled delivery through the intravenous (i.v.) route to reduce the frequency of administration, dose and adverse effects during the short-term management of manifestation of psychotic disorders. Stealth SLN_s were prepared by emulsification-solvent diffusion and sonication method by adding acetone/ethanol containing drug, lipid and stabilizer to aqueous phase, containing surfactant, under homogenization. The effect of lipid type, lipid percentage, stabilizer type and stabilizer percentage were evaluated on the particle size, zeta potential, drug loading efficiency and drug release for optimization of SLN_s. Dialysis bag membrane was used to determine drug release, the Rose Bengal binding constant for surface hydrophobicity and serum protein adsorption. The cytotoxicity of SLN_s on macrophages and red blood cells were also assessed in order to evaluate the impact of surface modifications on toxicity of the different formulations. The optimized formulation was composed of 0.05% stearyl alcohol (relative to the total volume of dispersion) and 25% PEG₄₀ stearate (relative to the weight of lipid) using a homogenization speed of 1000 rpm and 4 minutes sonication. The results showed that the *in vitro* specifications of stealth SLN_s of risperidone are suitable for i.v. administration.

Rezumat

Studiul și-a propus prepararea și caracterizarea farmaco-tehnologică a unor noi nanoparticule cu risperidonă, în scopul eficientizării farmacoterapiei acestui medicament antipsihotic. Formularea risperidonei sub forma de nanoparticule cu cedare lentă poate reduce frecvența administrării dozelor de risperidonă necesare obținerii efectului antipsihotic, precum și scăderea incidenței apariției reacțiilor adverse.

S-au evaluat următorii parametri: mărimea nanoparticulelor, potențialul zeta, cedarea substanței active din noua formulă farmaceutică, constanta de legare Rose Bengal, precum și efectul hemolitic al noilor nanoparticule obținute.

Keywords: Risperidone, Stealth solid lipid nanoparticles, Parenteral sustained release, Solvent evaporation method.

Introduction

Risperidone is an atypical antipsychotic agent with low extrapyramidal side effects. It is used in the treatment of psychotic disorders in low-doses of 2–4 mg/day orally for short-term management while long-term treatment is needed to treat schizophrenia. It has significant “first-pass” metabolism with an oral bioavailability of 70% and a half-life of 3 hours. Its metabolite 9-hydroxy risperidone has a half-life of 21 hours. To minimize its adverse effects the lowest possible effective doses are recommended [1-3].

Risperidone depot injectable formulation is the first long-acting formulation of an atypical antipsychotic drug that was introduced on the market and is based on microparticulate formulation of poly(D,L-lactide-co-glycolide) (PLGA) with doses of 25 and 50 mg for intramuscular injection every 2 weeks [4]. The steady-state level of drug is attained within 6–8 weeks. However, less than 1% of the dose is released after the first 3 weeks of injection, hence oral antipsychotic dosage forms are required during the first 3 weeks of injection [5]. Consequently as nanoparticles avoid the first-pass effect and have extended-release pattern, it seems risperidone nanoparticles can dramatically reduce drug dose *via* parenteral route. Considering this thermal-responsive aspect, *in situ* gel containing extended-release PLGA nanoparticles of risperidone were prepared for parenteral (subcutaneous) delivery to reduce the dose-dependent extrapyramidal side effects of risperidone. It prolonged the antipsychotic effect of the drug for up to 72 hours with fewer extrapyramidal side effects [6]. Poly (D, L-Lactide) nanoparticle suspensions of risperidone were also designed by nanoprecipitation method using as Pluronic F-68 or Pluronic F-127 stabilizer. Risperidone release was continued for more than 24 h with 75% drug release [7]. Another polymeric nanoparticle of risperidone was prepared by poly (epsilon-caprolactone) by the nanoprecipitation method for intravenous delivery which reduced the dose-dependent extrapyramidal side effects of risperidone with prolonged antipsychotic effects [8, 9].

Mucoadhesive nasal nanoemulsions were other nanometric dosage forms of risperidone that were designed and showed stability for 3 months with no nasal ciliotoxicity [10]. The mucoadhesive nasal nanoemulsion was more effective with high brain targeting of risperidone. The studies conclusively demonstrated that nasal mucoadhesive nanoemulsion could rapidly transport the drug in larger extent when compared to intranasal and i.v. solution and nanoemulsions [11].

Solid lipid nanoparticles (SLNs) are colloidal nanoparticles made from solid lipids, surfactant(s) and water. SLNs have the advantages of traditional systems while they avoid their major disadvantages [12, 13]. Some advantages of SLNs are: possibility of large scale production, sterilization, drug targeting and controlled drug delivery, enhancing drug stability, high drug payload, possibility of incorporation of drugs with lipophilic and hydrophilic properties, lack of biotoxicity and biocompatibility, avoidance of organic solvents in the case of production by high pressure homogenization, and good tolerability [13-15]. Considering these advantages reported for SLNs, risperidone SLNs were prepared by solvent emulsification-solvent evaporation method from Compritol 888 ATO and sodium lauryl sulphate as lipid and surfactant [16].

The intranasal risperidone loaded SLNs designed for brain targeting, showed higher values for hind limb retraction time or brain targeting in the paw test using Perspex platform, in SLNs compared with solution of risperidone. The neuronal localization of the drug was also confirmed by gamma scintigraphy imaging [17].

In spite of preparation of SLNs with risperidone, depot parenteral formulations of atypical antipsychotics are not yet widely available and there is no report on the production of stealth SLNs of risperidone with long lasting circulation and no study has been done on the biological safety of these depot parenteral formulations. Serum protein adsorption, haemolysis of blood cells and phagocytic uptake of nanoparticles by macrophages are among the most important incompatibilities of nanoparticles affected by their surface properties when entered in the systemic circulation. The aim of the present study was the obtaining of stealth SLNs and assessing the impact of surface modifications of SLNs with risperidone using stabilizers on the surface hydrophobicity, serum protein adsorption and their cytotoxicity with regard to human macrophages and red blood cells.

Materials and Methods

Materials

Cetyl alcohol, stearyl alcohol, acetone, ethanol, polyethylene glycol 40 stearate (PEG40 stearate), Triton X100, dimethylsulfoxide (DMSO) and Tween 20 were all from Merck Chemical Company (Germany), penicillin/streptomycin solution, Pluronic F127, pyrene and Rose Bengal were purchased from Sigma (USA), RPMI 1640 culture medium, trypsin and FBS (fetal bovine serum) were provided by Gibco (Scotland) and macrophage cell line of J774A1 was supplied by Pasteur Institute (Iran).

Experimental design and analysis

Table I displays the variables and their levels, studied according to irregular fractional factorial design by Design expert software. Table II shows the number of the experimental run formulations used to examine four-factors each in two levels. A run involved the corresponding combination of levels to which the factors in the experiment were set. All experiments were performed in triplicate. Three studied responses included surface hydrophobicity, red blood cell haemolysis and macrophage uptake. However, particle size, zeta potential, polydispersity index, loading efficiency and release efficiency were also considered in the optimization process. The experimental results were then analyzed by the Design Expert software to extract independently the main effects of these factors, followed by the analysis of variance (ANOVA) to determine which factors were statistically significant. Identifying controlling factors and qualifying the magnitude of effects, as well as identifying the statistically significant effects, were emphasized.

Table I

Studied variables and their levels in the irregular fractional factorial design

Variable	Level 1	Level 2
Lipid type (A)	Cetyl alcohol	Stearyl alcohol
Lipid % to the total volume of dispersion (B)	0.05	0.075
Stabilizer type (C)	PEG ₄₀ Stearate	Pluronic F127
Stabilizer % to the total weight of lipid (D)	20	25

Table II

Prepared formulations of SLNs according to irregular fractional factorial design

Formulation code	Lipid type	Lipid % to the total volume of dispersion	Stabilizer type	Stabilizer % to the total weight of lipid
C _{0.075} P ₂₀	Cetyl alcohol	0.075	Pluronic F127	20
C _{0.05} P ₂₅	Cetyl alcohol	0.05	Pluronic F127	25
C _{0.05} E ₂₀	Cetyl alcohol	0.05	PEG ₄₀ Stearate	20
S _{0.05} E ₂₅	Cetyl alcohol	0.05	PEG ₄₀ Stearate	25
S _{0.075} E ₂₅	Stearyl alcohol	0.075	PEG ₄₀ Stearate	25
S _{0.075} E ₂₀	Stearyl alcohol	0.075	PEG ₄₀ Stearate	20
S _{0.05} P ₂₀	Stearyl alcohol	0.05	Pluronic F127	20
S _{0.05} E ₂₀	Stearyl alcohol	0.05	PEG ₄₀ Stearate	20
S _{0.075} P ₂₅	Stearyl alcohol	0.075	Pluronic F127	25
S _{0.05} P ₂₅	Stearyl alcohol	0.05	Pluronic F127	25
C _{0.075} P ₂₅	Cetyl alcohol	0.075	Pluronic F127	25
C _{0.075} E ₂₀	Cetyl alcohol	0.075	PEG ₄₀ Stearate	20

Preparation of risperidone loaded stealth SLNs

SLNs of risperidone were prepared by an emulsion-solvent diffusion and evaporation method followed by ultrasonication as reported before [18, 19], according to Table II. Briefly, lipid (cetyl alcohol or stearyl alcohol), stabilizing agent (Pluronic F127 or PEG40 stearate) (with 1:4 or 1:5 ratio regarding to the lipid weight) and drug (lipid to drug ratio was 2:1) were dissolved in 4mL of a mixture of acetone and absolute ethanol with 1:1 ratio. In the second step, the oily phase was dispersed in 40 mL of aqueous phase containing 3v/v% of Tween 20 and premixed by magnetic stirrer for 5 min at 1000 rpm. The resulting pre-emulsion was then ultrasonicated for 4 minutes using a probe sonicator (Bandelin, Germany) by probe TT13 in amplitude 10% to produce an O/W nanoemulsion. In the last step the obtained nanoemulsion (O/W) was stirred at room temperature on a magnetic stirrer at 600 rpm for about 1 hour.

Determination of particle size, and zeta potential of nanoparticles

Size and zeta potential of all drug loaded SLN samples were measured by photon correlation spectroscopy (PCS, Zetasizer 3000, Malvern, UK). All samples were diluted 1:10 ratio with distilled water to get optimum 50-200 kilo counts per second (Kcps) for measurements. Z-Average particle size, polydispersity index and zeta potential were measured in triplicate.

Determination of entrapment efficiency and drug loading in the SLNs

Drug loading percent was determined by measuring the concentration of untrapped free drug in aqueous medium [20]. The aqueous medium was separated through centrifugation (Sigma 3K30, Germany). About 1.5 mL of the SLN dispersion was placed in the Eppendorf tubes and centrifuged at 25000 rpm for 60 min. The amount of risperidone in the aqueous phase was estimated spectrophotometrically at 276.8 nm. The loading percentage was calculated using Eq. (1):

$$\text{Loading \%} = \left(\frac{\text{Analyzed weight of drug in SLNs}}{\text{Analyzed weight of SLNs}} \right) \times 100 \quad \text{Eq. (1)}$$

In- vitro release of risperidone from stealth SLNs

To determine the release rate of risperidone from nanoparticles, 4 mL of aqueous dispersion of formulation was added to the dialysis bags with a molecular weight cutoff of 12400 Da and the sealed bags were placed in the glass test-tube with 30 mL of the phosphate buffer solution (PBS) 0.1 M (pH 7.4) containing 3% polysorbate 80 to provide sinking conditions with agitation of 150 rpm. Samples were withdrawn at predetermined time intervals until 40 hours and then returned to the medium. The content of risperidone in the samples was determined spectrophotometrically at 276.3 nm.

Surface hydrophobicity of nanoparticles

To describe the surface hydrophobicity of SLNs, the binding constant of Rose Bengal to the surface of different nanoparticles was used [21]. Adsorption isotherms were measured in 0.1 M PBS (pH 7.4). 250 µg/mL of SLNs were incubated with concentrations of 1–45 µg/mL of dye solution for 3 h at 25°C. After centrifugation of SLNs for 1 hour at 20,000 g, the supernatants were determined photometrically at 542.1 nm. Control samples were used in each experiment to omit the effect of binding of Rose Bengal to the centrifuge tubes and pipette tips. The binding constant K_b of Rose Bengal was calculated using Scatchard transformation according to Equation 2, where r is the amount of adsorbed dye, a is the Rose Bengal equilibrium concentration or free dye, and N is the maximum amount of dye bound.

$$\frac{r}{a} = K_b N - K_b r \quad \text{Eq. (2)}$$

The surface hydrophobicity or binding constant of Rose Bengal (K_b) was obtained from the slopes of the straight lines from a plot of Rose Bengal ratio of the amount adsorbed and the free amount (r/a) versus adsorbed Rose Bengal concentration (r). The greater the slope, the greater the (K_b) or the relative hydrophobicity. Repeated analysis ($n=3$) produced consistent slopes.

Study of the red blood cells hemolysis

Human blood was collected from healthy volunteers (under written informed consent) into test tubes containing anticoagulant and centrifuged at 1000 g for 5 min. After discarding the plasma, erythrocytes were washed 3 times with PBS to remove the serum proteins. The stock of erythrocyte dispersion was usable within 24 hours at 6°C. Ethical approval for this study was granted by the Human Ethics Committee of the Isfahan University of Medical Sciences.

100 µL of the erythrocyte dispersions were added to 1000 µL of SLNs suspension, shaken and incubated at 37°C for 1 hour. To remove intact erythrocytes from debris the mixture was centrifuged for 3 min at 750 g and 100 µL of the supernatant were added to 2000 µL of a mixture of absolute ethanol and concentrated HCl (40/1 (v/v)) and centrifuged again. The absorption of the supernatant was measured spectrophotometrically at 398 nm [22]. No lysis (0%) and 100% lysis were taken as the control samples in PBS and bidistilled water respectively [23].

Macrophage cell culture

Macrophages from cell line J774A1 were cultured on RPMI-1640 containing 10% FBS (Fetal Bovine Serum) and 1% antibiotics mixture of penicillin (5000 IU/mL) and streptomycin (5000 µg/mL) at 37°C and in 5% CO₂. The pH of culture medium was adjusted to 7.2–7.4 using HCl.

Preparation of stealth fluorescent SLN

Surface-modified SLNs, *i.e.* stealth SLNs, were then prepared as reported above. Then were loaded with 1mg pyrene in the same way as mentioned for risperidone loaded SLNs. For measuring the amount of pyrene loaded in SLNs, after centrifuging 1.5mL of SLN suspensions at 25000 rpm at 8°C for 60 min, the supernatant was diluted with a mixture of absolute ethanol:acetone:water in ratios of 4.55%:4.55%:90.9% and the intensity of fluorescence was determined in this solution at λ_{ex} =338nm and λ_{em} =375nm using a spectrofluorimeter LS5, Perkin-Elmer, USA.

Uptake of fluorescent SLNs by macrophages

Following incubation of a cell suspension containing 4×10^4 cell/mL for 24 h in 5% CO₂, cells were washed three times with pH 7.4 PBS, and 1 mL of 1:200 diluted SLNs loaded with pyrene was added to each well, of a 24-well culture plate (SPL Lifescience, Korea). The plate was incubated at 37 °C and in 5% CO₂. After 5, 10, 40, 60, 90 and 120 min of incubation the plate, the upper culture medium which contained the non-phagocytated SLNs was removed, the cells were washed with PBS (pH 7.4) and 100 μ L of RPMI culture medium was added to wells. To separate the adherent cells which contained the uptaken SLNs, 200 μ L of a 0.25% solution of trypsin was added to the wells and incubated for 5 min. Then 800 μ L of RPMI culture medium was added to neutralize the cytotoxic effect of trypsin. The contents of each well were then transferred to an Eppendorf tube and 0.5 mL of Triton-X100 diluted with water (1:10) was added to lyse the cells. After 10 min of lysis the intensity of cell lysate fluorescence was measured using the spectrofluorimeter. Uptake was expressed as the percentage of fluorescence associated with cells *versus* the amount of fluorescence developed in the feed solution. Blank SLNs were used by the same procedure as the control to develop fluorescence of the cells.

Results and Discussion

In spite of the reports on the preparation of nanoparticles of risperidone for parenteral applications there is no study on their biological safety. Meanwhile none of the prepared nanoparticles of this drug can circulate for a long time in the blood. For this purpose in the present study the stealth SLNs of risperidone were prepared and evaluated for their biological safety.

Table III demonstrates the physical characteristics of stealth SLNs of risperidone. It can be noticed that among different studied formulations the mean particle size varied between 74-424nm with a reasonable polydispersity index (Pdi) of 0.3-0.5. Almost all formulations were stable due to their high zeta potential that varied from -6 to -22 mV. High drug payload between 34.5-93% was possibly due to the high lipophylicity of the drug. After 40 hours of

release test the release efficiency (RE_{40}) varied between about 17-60% that indicated a sustained release pattern for the drug.

Table III
Physical properties of solid lipid nanoparticles of risperidone

Formulation code	Particle size (nm)	PdI*	Zeta potential (mV)	Loading efficiency (%)	RE_{40}^{**} (%)
C _{0.075} P ₂₀	156.33±4.93	0.48±0.08	-18.47±0.40	73.06±0.59	57.98±0.74
C _{0.05} P ₂₅	262.77±1.17	0.32±0.08	-6.39±2.63	88.49±1.12	51.35±0.94
C _{0.05} E ₂₀	123.17±12.72	0.90±0.04	-17.60±3.01	34.52±4.38	16.78±0.54
S _{0.05} E ₂₅	349.10±3.73	0.36±0.09	-6.18±0.09	67.39±0.92	42.61±1.95
S _{0.075} E ₂₅	146.50±1.18	0.35±0.06	-22.30±1.57	74.84±6.65	37.74±3.84
S _{0.075} E ₂₀	194.87±80.80	0.40±0.06	-21.57±2.95	84.71±2.03	57.64±0.37
S _{0.05} P ₂₀	190.03±30.14	0.35±0.05	-15.33±0.25	69.83±5.59	47.89±0.32
S _{0.05} E ₂₀	73.68±2.48	0.45±0.08	-15.90±0.46	44.53±4.55	37.34±1.34
S _{0.075} P ₂₅	168.57±1.38	0.39±0.03	-21.20±3.04	94.74±2.11	59.90±0.70
S _{0.05} P ₂₅	125.57±13.29	0.38±0.02	-12.80±0.26	92.96±0.46	72.02±1.14
C _{0.075} P ₂₅	332.03±17.93	0.44±0.06	-16.37±4.99	91.67±4.33	48.52±1.07
C _{0.075} E ₂₀	423.67±14.57	0.51±0.04	-15.37±3.80	77.44±9.17	35.78±3.14

* Polydispersity index; ** release efficiency after 40 hours

Table IV also compares the biological safety properties of stealth SLNs of risperidone that varied with their surface characteristics and formulation parameters. Figure 1 summarizes the effect of each individual studied variable and their mutual interactions on these properties. As Fig. 1a indicates, the interaction of lipid type and percent and also the stabilizer type is the most effective variable that affects the serum protein uptake or hydrophobicity of the stealth SLNs that in turn causes higher values for Rose Bengal binding constant. After that the interaction of lipid and stabilizer type and also the lipid percent along with the stabilizer type are the most effective parameters on this response.

Table IV
Observed surface properties of solid lipid nanoparticles of risperidone

Formulation code	Rose Bengal binding constant (K_b)	Haemolysis (%)	Phagocytosis (%) after 2 hours
C _{0.075} P ₂₀	0.037±0.010	8.84±0.67	4.03±0.00
C _{0.05} P ₂₅	0.083±0.016	1.10±0.08	3.43±0.03
C _{0.05} E ₂₀	0.042±0.011	7.12±0.54	2.38±0.05
S _{0.05} E ₂₅	0.180±0.021	4.22±0.32	3.86±0.04
S _{0.075} E ₂₅	0.013±0.005	5.29±0.44	1.33±0.21
S _{0.075} E ₂₀	0.016±0.005	0.38±0.03	1.54±0.03
S _{0.05} P ₂₀	0.060±0.005	1.51±0.12	2.49±0.02
S _{0.05} E ₂₀	0.053±0.014	0.54±0.04	1.66±0.02
S _{0.075} P ₂₅	0.020±0.002	0.17±0.01	2.59±0.00
S _{0.05} P ₂₅	0.078±0.003	0.36±0.02	1.79±0.07
C _{0.075} P ₂₅	0.045±0.008	0.70±0.05	2.67±0.02
C _{0.075} E ₂₀	0.058±0.010	0.76±0.06	3.04±0.02

The stabilizer type is the most important individual variable effective on the K_b so that increasing the stabilizer percent, it reduced the hydrophobicity of the surface on stealth SLNs (Fig. 2). Changing the lipid type from stearyl alcohol to cetyl alcohol had a reverse effect (Fig. 2).

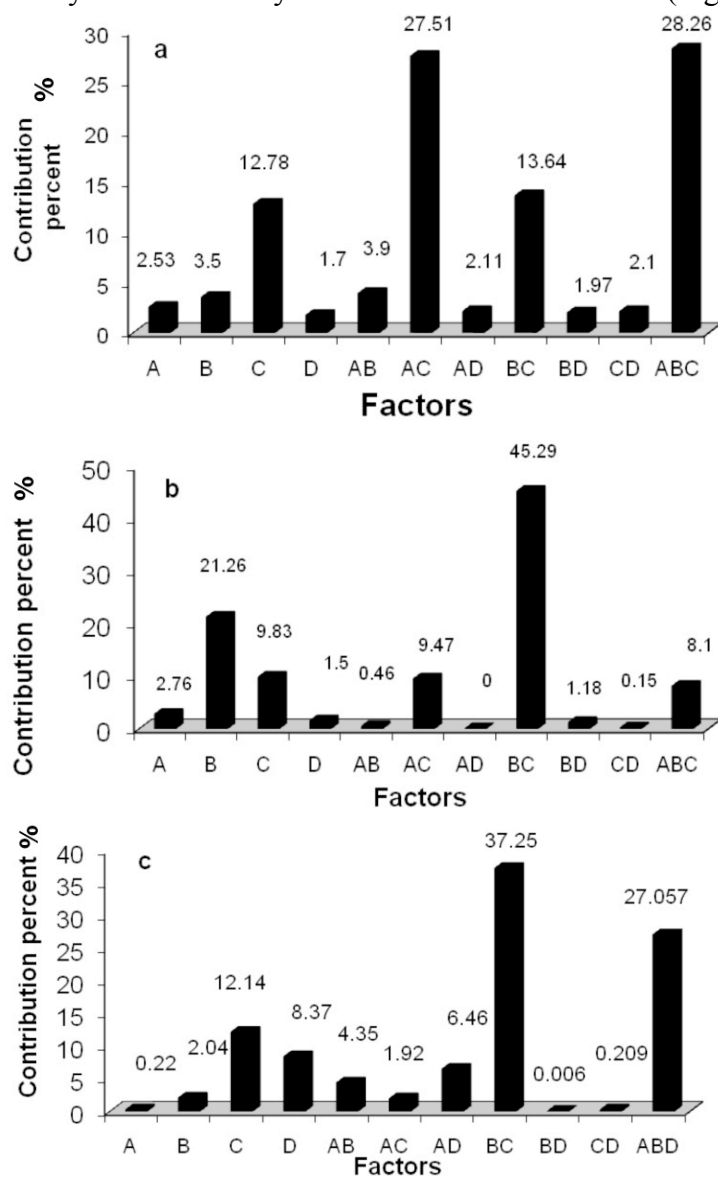


Figure 1

Contribution percent of the effect of each individual studied variable and their interactions on a) the serum protein uptake or hydrophobicity b) haemolysis of human red blood cells and c) macrophage phagocytosis of the stealth SLNs of risperidone

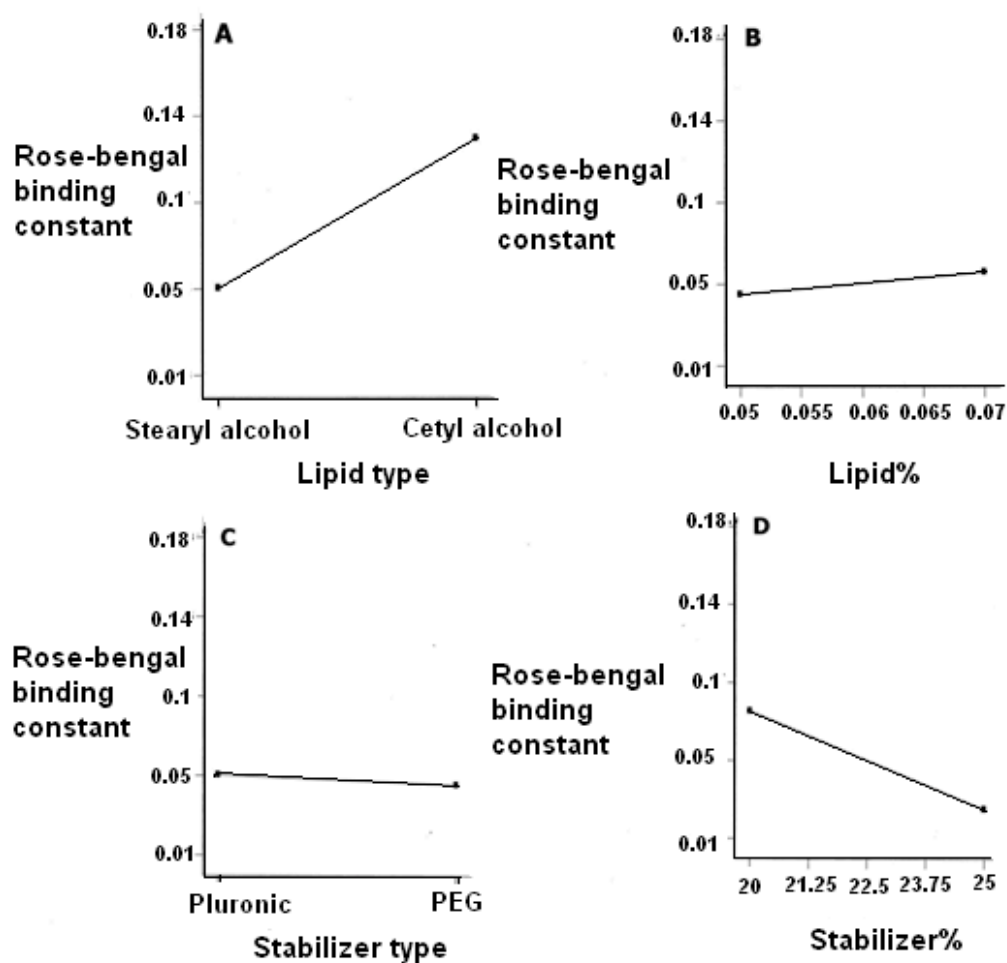


Figure 2

Effect of different levels of A) lipid type, B) lipid %, C) stabilizer type D) stabilizer % on the Rose Bengal binding constant (serum protein uptake or hydrophobicity) of the stealth SLNs of risperidone

Figure 2B shows that the interaction of lipid percent and the stabilizer type is the most effective parameter on the haemolysis of red blood cells by the stealth SLNs. Lipid percent is also effective on this response, so that by increasing the lipid percent, the haemolysis increases (Fig. 3). However, changing the lipid type from stearyl alcohol to cetyl alcohol, decreases the haemolysis (Fig. 3).

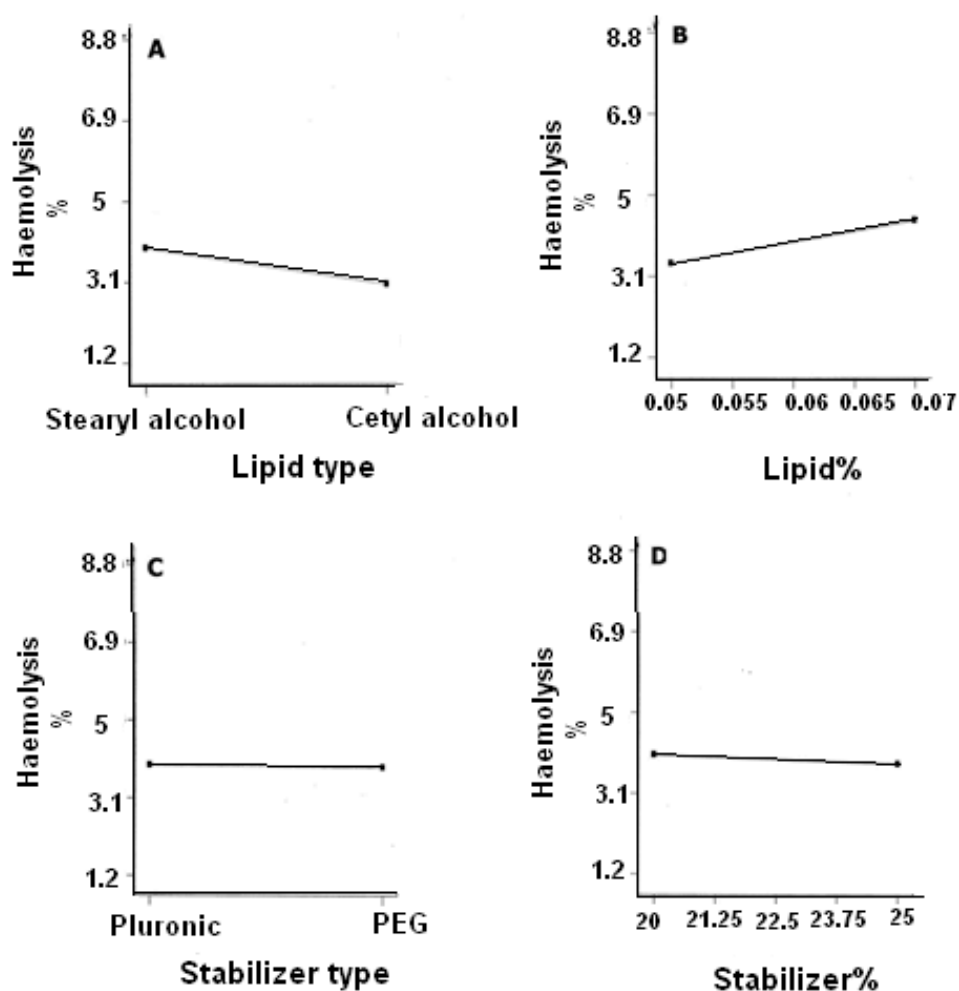


Figure 3

Effect of different levels of A) lipid type, B) lipid %, C) stabilizer type D) stabilizer % on haemolysis of red blood cells of the stealth SLNs of risperidone

Fig. 3C shows that the most effective variables on the phagocytosis of stealth SLNs by macrophages are the interactions of lipid percent and the stabilizer type and also the simultaneous effects of lipid type, lipid and stabilizer percent. Increasing the lipid percent and shifting from stearyl alcohol to cetyl alcohol increased the phagocytosis, but higher amounts of PEG40 stearate can decrease this phenomenon significantly compared to Poloxamer (Fig. 4)

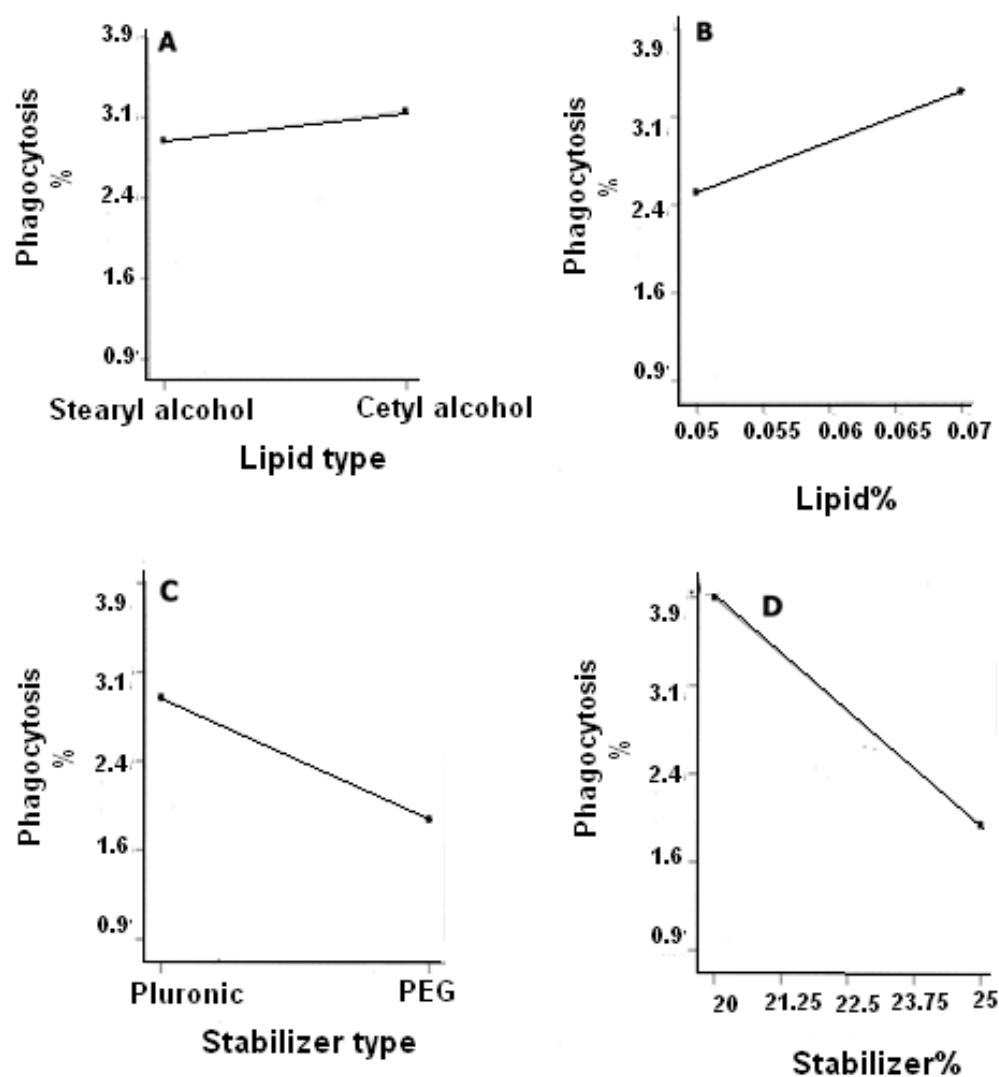


Figure 4

Effect of different levels of A) lipid type, B) lipid %, C) stabilizer type
D) stabilizer % on macrophage phagocytosis of the stealth SLNs of risperidone

After the optimization process of data by Design Expert software the optimum SLNs were prepared and checked for the studied responses. A summary of the results analysed statistically on the observed and expected values for the studied responses on the optimized formulation, are shown in Table V.

Table V
Summary of the statistical results for studied responses

Studied factor	Measured actual value	Estimated value	Error percent
Particle size (nm)	70.0±0.3	50.9	37.52
Zeta potential (mV)	-23.95±1.44	-21.7	10.37
Loading efficiency (%)	82.41±1.65	86.25	4.45
RE ₄₀ (%)	72.57±0.53	74.15	2.13
Rose Bengal K _b	0.036±0.008	0.041	11.55
Hemolysis %	1.865±0.052	1.6	16.56
Phagocytosis %	1.413±0.258	1.15	22.87

The optimized formulation was prepared both in the presence and in absence of the stabilizer, to see its effect on the biological safety parameters. The results are shown in Table VI.

Table VI
The effect of stabilizer on the surface properties of optimized SLNs of risperidone

Studied factor	Optimized SLN with stabilizer	Optimized SLN without stabilizer
Rose Bengal binding constant (K _b)	0.036±0.007	4.25±1.14
Hemolysis %	1.86±0.05	35.00±8.16
Phagocytosis %	1.41±0.26	28.20±4.81

The presence of PEG40 stearate as stabilizer reduced significantly the surface hydrophobicity and hence the Rose Bengal binding constant, hemolysis of red blood cells and the phagocytosis of the optimized stealth SLNs WITH risperidone. Mononuclear phagocyte system can opsonize colloidal drug carriers and so rapid clearance of drug may cause reduction of its effectiveness. To enhance the plasma half-life and circulation of nanoparticles, many researchers have recommended PEG modification of nanoparticles [24, 25]. Colloid surfaces may be modified with hydrophilic molecules that can prolong circulation time *in vivo* [26, 27]. Stealth SLNs have been developed by Bocca et al. [28] who used PEG that reduced SLNs uptake in murine macrophages significantly, and improved their physical stability. Wan et al. [29] also reported the conjugate of PEG₂₀₀₀–stearic acid to prepare PEGylated SLNs of vinorelbine bitartrate by cold homogenization technique. Polyoxyethylene stearate was also used by Wang et al. [30] as stabilizing agent in a stearic acid matrix for the production of stealth SLNs. The longer and more dense the polyethylene glycol chains, the less uptake by macrophages. Poloxamer 188 has also been used for surface modification of SLNs and pharmacokinetic behaviour of the incorporated drug has been modified by changing the characteristics of

SLNs [31]. Schubert and Müller-Goymann [32] modified the surface of SLN by lecithin and nonionic emulsifiers. The results of the present study indicated that PEG₄₀ stearate is more adequate than Pluronic F127 in protecting the nanoparticles from being uptaken by macrophages. It determined the lowest surface hydrophobicity and so the less serum protein absorption. Also their hemolysis on the red blood cells was minimal.

Conclusions

SLNs of risperidone prepared by emulsification-solvent diffusion technique composed of 0.05% stearyl alcohol (relative to the total volume of dispersion) and 25% PEG₄₀ stearate (relative to the weight of lipid) using a homogenization speed of 1000 rpm and 4 minutes sonication are suitable for parenteral applications. These optimized SLNs had particle size of 70 nm, zeta potential of -23.95 mV, drug loading efficiency of 82.41%, release efficiency of 72.57%, Rose Bengal binding constant of 0.036, red blood cell haemolysis of 1.87% and phagocytosis 1.41% which are promising properties for a stable and safe formulation of an antipsychotic depot preparation.

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