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Original Article

FORMULATION AND CHARACTERIZATION OF ROPINIROLE HYDROCHLORIDE LOADED SOLID LIPID NANOPARTICLES

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ABSTRACT

Objective: The aim of the present study was to formulate and evaluate the Solid Lipid Nanoparticles (SLNs) of Ropinirole Hydrochloride (ROP).

Methods: ROP-loaded SLNs were prepared by a double emulsion method using glyceryl monostearate (GMS) as lipid and soya lecithin as a stabilizer. All formulated ROP-loaded SLNs were characterized for its particle size and size distribution, zeta potential, % Entrapment Efficiency (EE) and drug loading. The formulations were optimized in terms of GMS to soya lecithin ratio and sonication time of primary emulsion. Shape and surface morphology of the optimized formulation was studied using optical microscopy and scanning electron microscopy. *In vitro* and *ex vivo* Study of optimized formulation was also performed and compared with a pure drug solution.

Results: The particle size and polydispersity index (PDI), zeta potential and EE of optimized formulation were found to be 320±5.15 nm, 0.260±0.012,-37.9±1.43, 56.13±2.33% respectively. *In vitro* and *ex vivo* permeation study revealed that percentage cumulative drug release of optimized formulation was found to be 58.45±1.75% and 53.75±1.34 % respectively in 24 h and more than 90% drug release from pure drug solution was found to be within 6 h. Drug release from the formulation is sustained as compared to the plain drug solution which release 97.74 % (*in vitro*) and 88.15 % (*ex vivo*) of the drug within 6 h.

Conclusion: From the results, it concludes that drug released from SLNs follows sustained release pattern and it will enhance the overall activity of the drug.

Keywords: Ropinirole hydrochloride, Solid lipid nanoparticles, Double emulsion method.

INTRODUCTION

Parkinson's Disease (PD) is a degenerative disorder of the central nervous system. It occurs due to the death of dopamine-generating cells in the substantia nigra, a region of the midbrain; the cause of this cell death is unknown. PD is characterized typically by motor features of tremor, rigidity, bradykinesia and postural instability and non-motor disorder symptoms such as Dementia, Depression, and falls or emerges with the progression of the disease [1]. Ropinirole Hydrochloride (ROP) has been a recently introduced selective non-ergoline dopamine D2 receptor agonist, which stimulates striatal dopamine receptors to produce dopamine, for the treatment of PD.

ROP is a low molecular weight, highly water soluble drug. It is rapidly absorbed from the gastrointestinal tract and mean peak plasma concentrations have been achieved within 1.5 h after oral doses. The oral bioavailability of ROP is 50% due to extensive first pass metabolism by the liver. Its mean plasma half-life is 5–6 h [2, 3]. The starting dose of ROP is 2 mg taken once daily for 1 to 2 weeks, followed by increases of 2 mg/d for one week and so on. For that, the patient has to take a conventional IR tablet 3-4 times a day [4]. The drawback of conventional dosages can be overcome by formulating sustained release Solid Lipid Nanoparticles (SLNs) of ROP. Sustained drug release reduces the dosing frequency, minimizing the side effect, enhanced therapeutic efficacy and prevention of hepatic first pass metabolism of the drug and improves the bioavailability of drugs. The treatment of all neurodegenerative diseases is a big challenge because of the numerous protective barriers surrounding the central nervous system. Pharmaceutical agents that are used to treat neurodegenerative diseases are usually administered orally, such as donepezil, memantine, rivastigmine etc., However, most of the ingested drugs do not reach the brain entirely because some of them are not able to cross the blood brain barrier (BBB) in adequate quantity and are, instead, metabolized totally or partially by the liver. This inefficient utilization of drugs may require ingestions of higher drug concentrations that can produce toxic effects in the heart, liver, or kidney [5]. The targeting of drug to the Central Nervous System (CNS), for the therapeutic advancement of neurodegenerative disorders such as Alzheimer's, Parkinson's disease, etc. can be done by administering the drug formulation like polymer nanoparticle [6], SLNs [7], liposome [8] etc., which can cross the BBB [9] or by administering the drug formulation through intranasal route which can bypass the BBB [10, 11].

SLNs are the rapidly developing field of nanotechnology with several potential applications in drug delivery and research. Due to their unique size dependent properties, lipid nanoparticles offer the possibility to develop new therapeutics. The ability to incorporate drugs into nanocarriers offers a new prototype in drug delivery that could use for drug targeting. Hence SLNs hold great promise for reaching the goal of controlled and site specific drug delivery and hence attracted wide attention of researchers [12, 13]. Hence the present study was aimed to formulate the SLNs of ROP, to overcome the drawbacks of the conventional tablet dosage form and also ensure the sustained release profile of the drug from dosages from.

MATERIALS AND METHODS

Materials

Ropinirole Hydrochloride (ROP) was received as a generous gift from Orchid Pharmaceuticals Pvt. Ltd., (Chennai, India). Glycerol monostearate (GMS), Soya lecithin and tween 80 (sorbitate monooleate) were procured from Sigma Aldrich Chemicals Pvt. Ltd., USA. Chloroform was obtained from Loba chemie Pvt. Ltd. (Mumbai, India). Dialysis membrane procured from Hi media Laboratories Pvt. Ltd., (Mumbai, India). All other chemicals used were of analytical grade and procured from S. D. Fine Chemicals (Mumbai, India).

Methods

Preparation of ROP loaded SLNs (ROP-SLNs)

ROP-loaded SLNs were prepared using double emulsion methods [14] which were earlier reported for encapsulation of hydrophilic drugs, with slight modifications as per our laboratory setup.

Preparation of primary emulsion (W/O emulsion)

1 ml of aqueous ROP solution containing 2% (w/v) Tween 80 was added drop wise into 10 ml of hot chloroform (50 °C) containing different ratios of GMS: Soya lecithin with continuous stirring at 500 rpm using a magnetic stirrer followed by Probe sonication at an amplitude of 50, pulse 5 sec on and 3 sec off for 5 min. The temperature was maintained at 50 °C throughout this experiment (table 1).

Preparation of double emulsion (W/O/W emulsion)

The primary W/O emulsion from the previous step was added drop wise to 50 ml of hot aqueous phase (50 °C) containing stabilizer (2% (w/v) Tween 80) with continuous stirring at 1,000 rpm. This mixture was then probed sonicated at amplitude of 50, pulse 5 sec on and 3 sec off for 10 min. The resultant W/O/W double emulsion was further added to 50 ml of cold water (2–4 °C) for solidification of lipid nanoparticles. Chloroform was removed from keeping the formulation on a magnetic stirrer for few hrs. The nanoparticles dispersion was then centrifuged at 35,000×g for 30 min and washed twice with purified water.

Optimization of process variables

The W/O/W double emulsion was optimized in terms of GMS to soya lecithin ratio and droplet size reduction method by sonication. A 5 % (w/w) theoretical loading of drug with respect to the GMS was taken as a constant parameter unless specified.

For optimization of GMS to soya lecithin ratio, SLNs with different lipid ratios (90:10, 85:15, 80:20, and 75:25 (w/w)) were prepared (keeping other parameters constant) and their effect of particle size and entrapment efficiency was studied (table 2) [14].

Table 1: Composition of ROP loaded SLNs

Formulation code	Drug loaded (%)	GMS/Soya lecithin ratio (W/W)	Tween 80 (%w/v)
F1	5	90:10	2
F2	5	85:15	2
F3	5	80:20	2
F4	5	75:25	2

For optimization of GMS to soya lecithin ratio, the primary emulsion was prepared by sonication at 50 Hz amplitude for 5 min while double emulsion was by sonication at 50 Hz amplitude for 10 min. However, these standard conditions resulted in relatively large particles, which necessitated further optimization of droplet size reduction by modifying the sonication time (2.5, 5.0, 10, and 12.5 min with other parameters maintained constant) (table 3) for primary emulsion.

Characterization of ROP-SLNs

Particle size and size distribution and Zeta potential

The particle size, PDI and Zeta potential of SLNs was determined by using Zeta Sizer by dynamic light scattering (Nano ZS, Malvern Instruments, UK). Six replicates were measured and values were measured as mean±standard deviation (SD). The zeta potential of a particle is the overall charge that the particle acquires in a particular medium. Knowledge of the zeta potential of SLNs helps to assess the stability of the formulation during storage [14, 15].

Optical photo microscopy

Colloidal dispersion of SLNs was placed on a clean glass slide and viewed by MOTIC digital photographic microscope under 45X magnification. Size (μ) of the MLVs was also measured using the microscopic scale.

Scanning electron microscopy (SEM)

Shape and surface morphology of the SLNs were studied using SEM (JEOL, JSM 50A, Tokyo, Japan). An appropriate amount of colloidal dispersion of SLNs was mounted onto metal (aluminium) using double-sided adhesive tape and fractured with a razor blade. The samples were sputter-coated with gold/palladium for 120 sec at 14 mA under argon atmosphere for secondary electron emissive SEM and observed for morphology, at an acceleration voltage of 15 KV [14].

Entrapment efficiency (EE) and drug loading

For determining the EE, SLNs dispersion was centrifuged at 20000 rpm for 30 min using a cold centrifuge at 4 °C. The clear supernatant was decanted. Pellets of ROP-SLNs were washed with water and recent rifuged to remove an un entrapped drug. The two supernatant were combined and after suitable dilution the absorbance was recorded at 250 nm by keeping blank as drug free SLNs [13, 15].

$$\text{Entrapment Efficiency (\%)} = \frac{\text{Total amount of Drug added- amount of Drug is supernatant}}{\text{Totan amount of Drug added}} \times 100$$

$$\text{Drug Loading (\%)} = \frac{\text{Amount of ROP in the SLN}}{\text{Weight of Lipid}} \times 100$$

***In vitro* drug release**

An *in vitro* drug release from ROP-SLNs formulation was performed using Franz diffusion cell across the dialysis membrane (12 Kda, Hi Mediam). The membrane was soaked in deionized water for 12 h before use. The donor compartment containing 1 ml of ROP-SLNs and the receptor compartment was filled with 80 ml of phosphate buffer solution (pH 6.4), within the pH range in the nasal cavity, as the dissolution medium (n=6).

The dissolution medium was maintained at 37±0.5 ºC using circulating water bath and the medium was stirred at 200 rpm with the help of small teflon coated magnetic bead. The beaker was kept covered throughout the study to preclude evaporation of the medium. Aliquots of the medium were withdrawn at suitable time intervals and were replaced with the same volume of fresh medium to maintain the sink condition. These samples were assayed using UV-Vis spectrophotometer (1700, Shimadzu®, Tokyo, Japan) at a wavelength of 250 nm [13].

***Ex vivo* drug release**

Ex vivo drug permeation studies were performed using Franz diffusion cell across the sheep nasal mucosa as the permeation barrier, obtained from a local slaughterhouse within 1 h of sacrificing the animal. Nasal mucosa was carefully cut with a scalpel and mounted on the diffusion chamber with mucosal and serosal surfaces facing donor and receptor compartments, respectively. Other experimental and sample collection procedures were performed in same fashion as *in vitro* diffusion studies [13].

Drug release kinetics

To study drug release kinetics of SLNs formulation, data obtained from *in vitro* and *ex vivo* drug release studies were plotted in various kinetic models: zero order (see Equation: 1) as cumulative percentage of drug released versus time, first order (see Equation: 2) as log cumulative percentage of drug remaining versus time [16].

Zero order equation $Qt = K_o t$ (1)

First order equation $\text{In } Q_t = \text{In } Q_0 + K_1 t$ (2)

Where Q_t is the percentage of drug release at time t and K_0 and K_t are the coefficients of the equation.

Mechanism of drug release

Mechanism of drug release from drug-loaded SLN was evaluated by subjecting the data obtained from *in vitro* drug diffusion studies in Higuchi's model (see Equation: 3) as cumulative percentage of drug released versus square root of time and Korsmeyer–Peppa's model (see equation: 4) as log cumulative percentage drug released versus log time.

Higuchi equation $Q_t = Kt^{1/2}$(3)

Korsmeyer and peppas equation $Q_t = K_p\ t^n$(4)

K_p is constantly incorporating structural and geometrical characteristics of the release device and n is the release exponent indicate the release mechanism [16].

Drug excipient compatibility studies by using fourier transform infrared spectroscopy

IR spectroscopy was studied using a Shimadzu FTIR 8300 Spectrophotometer and the spectrum was recorded in the region of 2000 to 400 cm^{-1}. The procedure consisted of dispersing a sample (table 1) in KBr (200-400 mg) and compressing into the discs by applying a pressure of 5 tons for 5 min in a hydraulic press. The pellet was placed in the light path and the spectrum was obtained [15].

RESULTS AND DISCUSSION

Preparation and optimization of ROP-SLN

ROP-SLNs were formulated by using different ratios of GMS and Soya lecithin. The particle size and EE of different formulation was shown in table 2. The particle size and PDI of SLNs decreased as the GMS: Soya lecithin ratio was decreased in the final dispersion. Highest drug entrapment was found at 80:20 (w/w; GMS/soya lecithin) ratio and, therefore, used for optimization of sonication time. In table 3 showed the effect of droplet size reduction method on the particle characteristics of SLNs. As sonication time increased (from 2.5 to 10.0 min) led to a significant decreased in the particle size (534±6.75 to 320±5.15) and PDI (0.553±0.016 to 0.260±0.012) of the resultant emulsion droplets. Further increase in sonication time from 10 to 12.5 min was accompanied by increased droplet size. Hence, a sonication time of 10 min was taken as optimum for the preparation of primary W/O emulsions.

Characterization of SLNs

Particle size, PDI, zeta potential and EE

The mean±SD particle size, PDI and zeta potential and EE of the all formulations are given in table 2. Optimization of sonication time for primary W/O emulsion for F3 formulation based on particle size, PDI and Zeta potential are given in table 3. Not much change was found in % EE (56 to 57 %) after altering the sonication time for primary W/O emulsion. Based on particle size and % EE, 80:20 (W/W) ratio of GMS/Soya lecithin was selected as optimized ratio for SLNs formulation (F3 formulation) and primary emulsion sonication time was selected as 10 min based on particle size ($F3_b$ Formulation).

Shape and morphology

From optical photomicrography, it was clear that ROP-SLNs formed were spherical in shape and uniform in size. The SEM study reveals that SLNs were spherical in shape with an average particle size around 320±5.15 nm (n=200). Optical and SEM image of SLNs is shown in fig. 3_a & 3_b. Occasional clumps observed in the images might be due association occur with the shrinkage of SLNs during drying or concentration of dispersion medium.

Table 2: Effect of GMS: soya lecithin ratio on particle size, PDI, zeta potential and EE

Formulation code	GMS/soya lecithin ratio (W/W)	Particle size (nm)	PDI	Zeta potential (mV)	EE (%)	Drug loading (%)
F1	90:10	867±6.75	0.753±0.016	-36.4±2.32	29.33±2.32	1.45
F2	85:15	571.6±6.27	0.685±0.005	-33.0±1.24	43.29±1.67	2.17
F3	80:20	462.2±4.55	0.547±0.013	-31.6±1.13	56.13±2.13	2.81
F4	75:25	433.1±5.95	0.288±0.006	-36.7±1.42	36.12±1.98	1.82

Table 3: Optimization of sonication time for primary W/O emulsion for F3 based on particle size, PDI and zeta Potential

Formulation no	Sonication time (min)	Particle Size (nm)	PDI	Zeta potential (mV)
$F3_a$	2.5	534±6.75	0.553±0.016	-35.4±1.32
F3	5	462.2±4.55	0.495±0.005	-31.6±1.113
$F3_b$	10	320±5.15	0.260±0.012	-37.9±1.43
$F3_c$	12.5	357.1±6.95	0.388±0.006	-36.76±2.11

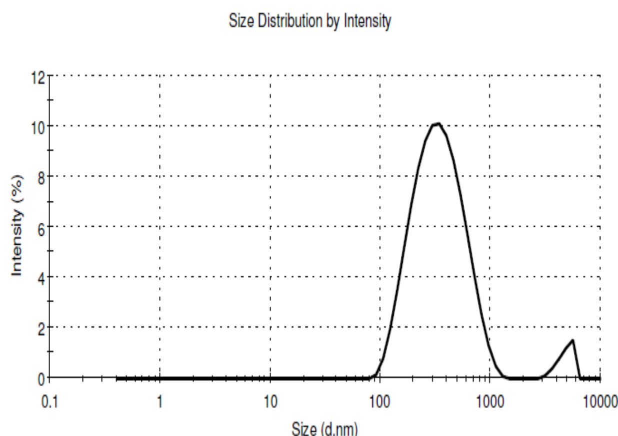


Fig. 1: Graph showing the mean particle size of optimized formulation ($F3_b$)

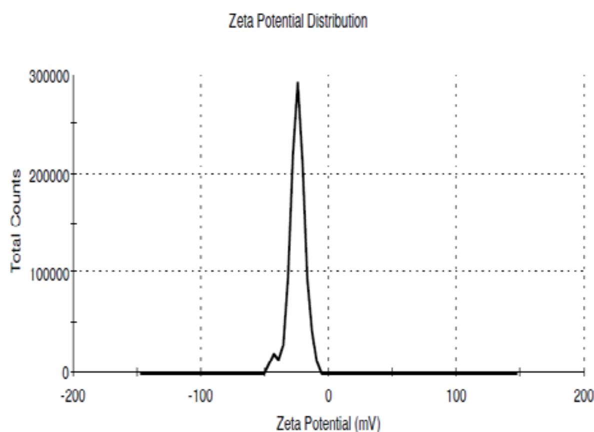
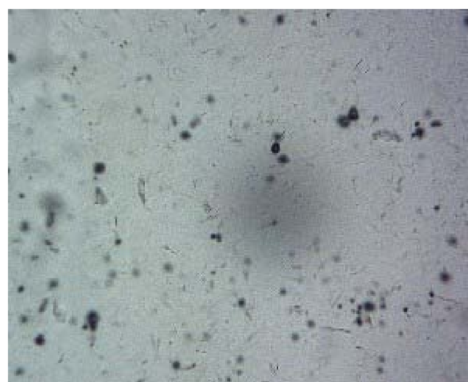
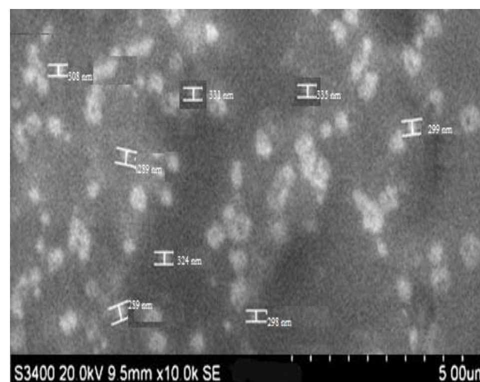


Fig. 2: Graph showing the zeta potential of optimized formulation ($F3_b$)





3A **3B**

Fig. 3A: Optical micrographs 3B: SEM image of optimized formulation of ROP-SLNs

In vitro and ex *vivo* drug release studies

In vitro and *ex vivo* release of ROP from SLNs formulation and pure drug solution are illustrated in fig. 4a & 4b. The release curve of ROP-SLNs exhibited a biphasic pattern in both *in vitro* and *ex vivo* release. There was an initial burst drug release through *in vitro* was 39.13% and ex *vivo* was 30.45 % within the initial 6 h, followed by a slow and sustained release.

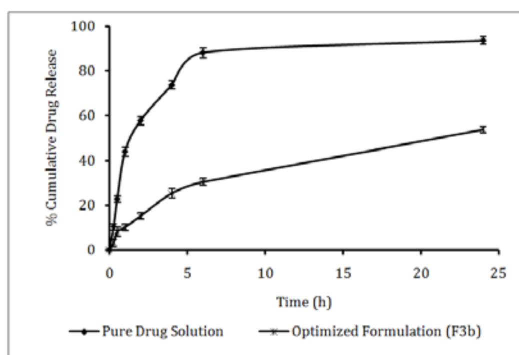


Fig. 4a: *In vitro* release profile of optimized formulation and pure drug solution

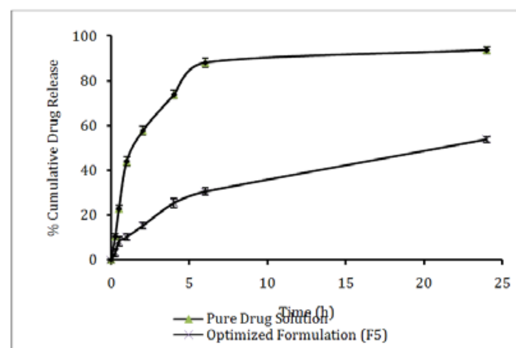


Fig. 4b: *Ex vivo* release profile of optimized formulation and pure drug solution

The amount of cumulated drug release over 24 h through *in vitro* was 58.51% and *ex vivo* was 53.75 %. The *in vitro* and *ex vivo* drug release from the pure drug solution were found to be 97.74 % and 88.15 %, respectively within 6 h and reached 99.38 % and 93.66 % by 24 h.

Kinetic parameter for *in vitro* and ex vivo drug release from pure drug solution and optimized formulation ($F3_b$) were given in table 4

Table 4: Kinetic parameter for *in vitro* and *ex vivo* drug release from pure drug solution and optimized formulation ($F3_b$)

Kinetic m. odel	*In vitro* release		*Ex vivo* release	
	Pure drug solution	**Optimized formulation (F5)**	**Pure drug solution**	**Optimized formulation (F5)**
Zero Order	y = 3.164x+39.47 R^2 = 0.462	y = 2.117x+12.63 R^2 = 0.790	y = 2.651x+41.49 R^2 = 0.494	y = 1.794x+11.06 R^2 = 0.887
First Order	y =-0.075x+1.655 R^2 = 0.728	y =-0.014x+1.943 R^2 = 0.875	y =-0.043x+1.737 R^2 = 0.747	y =-0.011x+1.952 R^2 = 0.940
Higuchi	y = 18.19x+28.46 R^2 = 0.701	y = 12.88x+0.177 R^2 = 0.934	y = 17.92x+22.59 R^2 = 0.721	y = 10.66x+1.264 R^2 = 0.975
Korsmeyer Peppas model	y = 0.410x+1.593 R^2 = 0.854	y = 0.602x+1.040 R^2 = 0.962 n= 0.602	y = 0.475x+1.505 R^2 = 0.836	y = 0.494x+1.053 R^2 = 0.987 n=0.494

Table 5: Major IR peak of pure ROP and Optimized Formulation of ROP-SLNs

Samples	Composition	Major peak (wave numbers. cm^{-1})
A	Pure ROP	3415.93, 2968.45, 2881.65, 1729.36, 1703.14, 1614.42, 1382.96, 1242.94, 1051.20, 557.42
B	Optimized formulation of ROP-SLNs	3415.93, 291813, 2850.76, 1712.79, 1641.41, 1359.82, 1222.87, 1093.64, 536.21

Drug excipients compatibility studies (FTIR)

No considerable changes in the IR peaks of the drug were observed in the optimized formulation when compared with the pure drug, which indicate the absence of any chemical incompatibility between drug and excipients.

DISCUSSION

Formulation optimization

For the preparation of ROP loaded SLNs, in the present study, double emulsion method has been chosen since it is a very well known

technique for the encapsulation of hydrophilic molecules in SLNs [14]. GMS was used as the solid lipid and soya lecithin as a stabilizer. Amphiphilic soya lecithin has the ability to modify the lipid matrix and results in enhanced drug loading capacity of SLNs [17]. As shown in table 2, a decrease in GMS/soya lecithin ratio from 95:5 to 80:20 (w/w) led to a significant reduction in particle size. As the concentration of soya lecithin increased, particle size and PDI of SLN decreased considerably. This might be recognized to be the amphiphilic nature of the soya lecithin, which facilitated its adsorption onto the surface of emulsion droplets and aided in the reduction of droplet size [18]

In present study sonication was employed as a tool for reducing droplet size of primary W/O emulsion preparation because the volume of primary emotion is less (1 ml). From table 3, it is observed that an increase in sonication time led to a significant decrease in droplet size. However, this phenomenon was observed up to 10 min because higher sonication led to excessive input of energy and shear during the emulsion formation which might have caused the formation of non-uniform layer of stabilizer, and hence led to increase particle size of SLNs [14]. Zeta potential of all formulations was more than±30 mV, therefore particles are normally considered as stable [19]. PDI is an indicator of homogeneity of the size distribution. Lower the PDI higher will be homogeneity of the size distribution. PDI of optimized formulation was found to be 0.260±0.012, conclude that particle was uniform in size and it was uniformly distributed [20]. As the ratio of GMS/Soya lecithin decreases entrapment efficiency increases, because as the concentration of soya lecithin was increases the film forming capacity of it was increased and the result of that % EE was increased (see in table 2).

In vitro and *ex vivo* release studies and kinetics model

The release pattern of drug revealed a biphasic pattern in both *in vitro* and *ex vivo* curve. They initially showed bust release, which may be due to retention of small unentrapped free drug on the surface of SLNs [15], followed by sustained release, which may be due to loading and deposition of the drug inside the cavities of GSM and results in low diffusion of drug molecules through the lipid matrix of the nano particles [13]. Sustain release of drug contributes to maintaining the effective therapeutic drug concentrations. *Ex vivo* % drug release is less when compared to *in vitro*, because some amount of drug is retained in mucosal layer.

Released data were fitted to kinetic models in order to investigate the drug release kinetics. It was found that, *in vitro* and *ex vivo* drug release was best fitted to First order as the plot showed highest linearity regression coefficient (R^2) of 0.875 and 0.940 respectively compared to zero order kinetic model. Release data were also fitted to Korsmeyer Peppa's exponential model to investigate the mechanism of drug release from SLNs. The corresponding *in vitro* and *ex vivo* drug release plot of Korsmeyer–Peppa's equation indicated a good linearity of regression coefficients (R^2) 0.962 and 0.987 respectively. Release exponent (n) was found to be 0.602 (*in vitro*) and 0.492 (*ex vivo*) respectively which is more above 0.45, so that the release can be characterized by Non–Fickian (anomalous) diffusion, which may indicate that the drug release rate is controlled by more than one mechanism i.e. Diffusion coupled with erosion mechanism for and drug release [16]

CONCLUSION

SLNs of ROP (a hydrophilic drug) were prepared by an optimized double emulsion process using GMS as solid lipid and soya lecithin as the stabilizer. Desired particle size of SLNs was obtained by increasing soya lecithin concentration as well as increasing sonication time of primary emulsion. The ROP-SLN obtained *in vitro* and *ex vivo* release experiments, exhibited a biphasic release pattern with burst release at the initial phase followed by sustained release compared to pure drug solution. However, *in vivo* studies for ROP-SLN should be performed to determine its brain delivery efficacy.

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CONFLICT OF INTERESTS

Declared None

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