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DESIGN AND EVALUATION OF GLYBURIDE LOADED SOLID LIPID NANOPARTICULATE SYSTEM

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ABSTRACT

The objective of the study was to design and evaluate glyburide loaded solid lipid nanoparticles (SLNs) drug delivery system, where glyburide nanoparticles with suitable size ranges are expected to improve the therapeutic efficacy. Components of the SLNs were lipid (palmitic acid) and surfactants (Pluronic F68 and Tween 40). The glyburide loaded nanoparticles were prepared by microemulsion dispersion method. Experiments were carried out with optimized ratio of excipients, where drug-lipid ratio and surfactant-cosurfactant ratio (Km) were varied to optimize the formulation characteristics. The effects of dispersion media, its pH, ionic content, etc. were investigated to optimize the SLNs production. Particles size analysis and zeta potential measurements were done using Malvern Mastersizer Hydro 2000G. The particles were also subjected to DSC, IR and XRD analyses. The *in vitro* drug release profile from nanoparticles was found to prolong up to 12h. Kinetic analysis of release indicated that nanoparticles formed were matrix in nature, in which glyburide dispersed uniformly. Optimized formulations were found to have a lipid-drug ratio of 1.5:1 and prepared at a Km ratio of 1:2 to maximize drug loading, modulate release and minimized particle size. The microemulsion mediated nanoparticle preparation methodology ensured high drug loading (ca. 80%), low and narrow size distribution and provided a reproducible and fast production method. The study elaborates on the feasibility and suitability of lipid based colloidal drug delivery system, employing optimize design to develop a clinically useful nanoparticle system with targeting potential.

KEYWORDS: SLN, glyburide, Pluronic, micrormulsion, optimization, XRD, DSC.

INTRODUCTION

Solid lipid nanoparticles (SLN) are colloidal carriers, which were developed at the beginning of the 1990s as an alternative system to the existing traditional carriers (emulsions, liposomes and polymeric nanoparticles), especially for the delivery of lipophilic compounds.[1,2] These types of colloidal drug delivery systems are useful for different routes of administration. They show some potential advantages like drug leakage during storage and insufficient drug load. To overcome the limitation of SLN, nanostructured lipid carriers (NLC) have been developed.[3] Both carrier types are submicron size particles (50-1000 nm) and are based on solid lipids but they can be distinguishing by their inner structure. SLNs consist of solid lipids while NLC are made of solid matrix entrapping variable liquid lipid noncompartments.[4] NLC is also called as an upgrade of the solid lipid nanoparticles even though SLNs is still intended to indicate the nanostructered lipid carriers, creating no clear differentiation. The NLCs have mainly been investigated in the topical and dermatological preparations[5] in the delivery of clotrimazole,[6-8] other antifungal imidazoles and ascorbyl palmitate. The successful implementation of lipid nanoparticles for drug delivery depends on their ability to penetrate through several anatomical barriers, sustained release of their contents and their stability in the nanometer size. SLN offer unique properties like small size, large surface area, interaction of phases at the interfaces, and are attractive for their potential to improve performance of pharmaceuticals, neutraceuticals and other materials.[9] SLNs are drawing major attention as novel colloidal drug carrier for intravenous applications.[10,11]

The SLNs are submicron colloidal carrier, which is composed of physiological lipid, dispersed in water or in an aqueous surfactant solution. It has advantages of good tolerability, scalability to large-scale preparation, excellent biocompatibility and protection of incorporated drugs against chemical/ enzymatic degradation. If systematically investigated, SLNs may open new avenues in research and therapy.[12] Selection of suitable lipids and surfactants for formulating Solid Lipid Nanoparticles (SLNs), have made it possible to cross lipoidal Blood Brain Barrier (BBB) and deliver the desired drugs to highly restricted cerebral site. Further, numerous applications have been reported in literature and excellent reviews covered the clinical applications of lipid nanoparticles. As such they hold a great promise in materializing legendary Ehrlich′s dream of a Magic Bullet. In the present investigation, the focus was on the development of suitable colloidal dispersed system (solid lipid nanoparticles) of glyburide, along with physiologically compatible lipid, palmitic acid and FDA approved surfactant, Pluronic F68. Glyburide is a weakly basic (pKa = 3.7) and highly

hydrophobic (octanol/water partition coefficient at pH = 8.1, logP = 5.66). Its aqueous insolubility resulted large interindividual and intraindividual variation of its oral bioavailability.

Therefore, in this investigation, an attempt has been made to prepare homophasic or heterophasic colloidal dispersed system from suitable rate modulating macromolecules and surfactants, to entrap glyburide, and to study different formulations and processing characteristics like particle size determination, measurement of zeta potential, x-ray diffractometry (XRD), diffrential scanning colorimetry (DSC), atomic force microscopy (AFM) etc of the system. The solid lipid matrix based systems such as nanoparticles/lipospheres or their suspension form is envisaged as the target formulation. Such a dispersed system would be expected to improve the bioavailability, stability and make it more useful against diabetes. The toxicity profile of the potent drug is also expected to be reduced significantly when delivered in colloidal system, presumably due to reduction in effective dose and better distribution profile.[2,6]

Although various methods have been reported in literature, the followed method of solid lipid nanoparticle (SLN) preparation is a thermodynamically spontaneous technique of SLNs preparations. It is also less equipment intensive and has good commercial scale-up feasibility of the master formula. The formulations have been developed keeping in view their reproducibility to characterize the effect of various formulations and processing parameters on the quality and performance of lipid nanoparticles.[13] It is expected that this type of glyburide loaded lipid nanoparticulate system could be clinically effective in better management of diabetes with greater degree of safety and efficacy.

MATERIALS AND METHODS

Materials

Glyburide was obtained as gift sample from Hetero Drugs Pvt .Ltd, Hyderabad and surfactant (Pluronic-F 68) and Lipid (Palmitic acid) were received as gift samples from Sun Pharmaceuticals Ltd., Baroda. Acetonitrile, dichloromethane and all other reagents were procured commercially and used as obtained. Double distilled water was used in the experiment.

Method

Method of Preparation

We optimized the maximum amount of drug loaded in the molten lipid (1:1.5 drug to lipid ratio). Then the molten lipid was used to develop a clear microemulsion above the melting temperature of the lipid using the water titration method. Briefly, molten lipid and surfactants and/or cosurfactants were mixed in different proportions ranging from 0% to 100% lipid and each such mixture was titrated with water till the clear mixture turned turbid. The appearance of the turbidity was taken as the limit of the microemulsion zone. The surfactant to cosurfactant ratio (Km) was optimized to obtain the microemulsion with the broadest clear zone in the pseudo-ternary phase diagram (Figure 1). The optimized Km ratio was found to be 1:2.

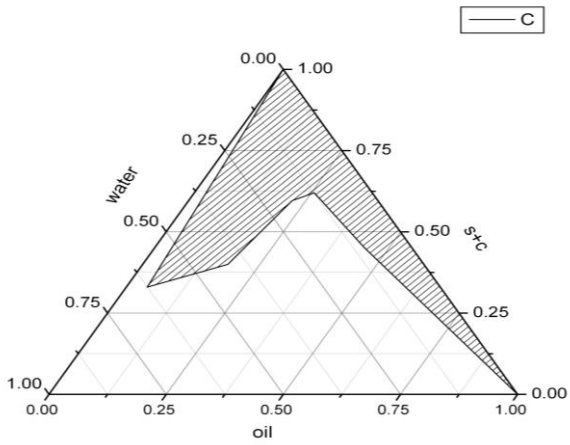

Fig. 1: Pseudoternary diagrams at 2:1 ratio.

The brief procedure adopted was as follows

In the method of preparation palmitic acid and glyburide in a ratio of 1.5:1 was taken in an Eppendrof tube. Next pluronic F-68 and tween 40 (w/w) in the ratio of 1:1 were weighed and added in that Eppendrof tube. Then co-surfactant (butanol+ethanol = 1:1 w/w) was added in that tube. At last water was added (w/w) with micropipette in that eppendrof tube. The tube was then placed in a water bath which was maintained at a temperature of 80^0C for 5 minutes for melting the solid substance. The clear warm microemulsion was then mixed properly with vortex mixture and again placed in the water bath.

Solid Lipid Nanoparticle (SLN) preparation via microemulsion method

The warm microemulsion was dispersed in the different types of aqueous media in different ratio ranging from 1:25, 1:50, 1:75 and 1:100. The best dispersion ratio was optimized from

these ratios. The 1:50 ratio was found to be optimum based on the visual appearance and particle size distribution study.

The dispersion medium used was both chilled and hot. Through variation in the temperature of the dispersion medium, the effect of this process variable was ascertained. It was found that the chilled medium usually resulted in lipid aggregates. Hence, the dispersion medium used was kept hot during dispersion and then slowly cooled to room temperature with stirring at 2500rpm for 30 minutes. It yielded a nanosuspension which was homogenized using high speed homogenizer (Polytron 1200E) at 30000 rpm for 5 minutes.

The various formulations prepared by varying drug, lipid, surfactants, cosurfactants and dispersion medium are given in Tables 1.

Table 1: Master batch formulae.

Code	Composition (%)					Dispersion Medium	Vol. of Dispersion medium (ml)
	Palmitic acid (Lipid)	Glyburide (Drug)	Pluronic F68 + Tween 40 (Surfactant)	Butanol + Ethanol (co-surfactant)	Water		
F1	17.6	10.1	17.2	35.1	17.37	d. Water	50
F2	17.12	11.2	16.25	29.2	17.2	d.water +0.1N NaOH	50
F3	17.3	11.1	17.59	33.57	18.57	d.water +0.1N Kcl	50
F4	17.2	10.07	16.99	33.25	18.51	d.water +0.1N $CaCl_2$	50
F5	17.25	10.72	17.28	34.23	18.23	d.water+0.1 NNaOH+0. 1N$CaCl_2$	50
F6	17.25	11.21	17.2	34.48	18.25	0.1M $CaCl_2$ + PBS pH 5.4	50
F7	17.67	10.7	16.25	33.25	17.31	0.1 N HCI	50
F8	18.15	10.84	17.35	34.21	17.24	0.1 N NaOH	50
F9	17.63	12.25	17.31	34.24	17.56	0.2M $CaCl_2$+0.1N NaOH	50
F10	17.23	11.37	18.1	33.25	18	0.05M $CaCl_2$+0.1N NaOH	50

d.water – double distilled water, PBS – Phosphate buffer soln.

Particle size analysis

Particle size was determined by using Malvern Mastersizer Hydro 2000G at 25°C using disposable sizing cuvette. The count rate was kept at around 200 kcps with varying duration greater than 50s. The dispersant used was water and its RI (1.33), viscosity (0.8872 cP) and Dielectric constant (78.5) were kept constant for all determinations. In this method, 1 ml sample was taken from formulated nanosuspension. Then it was dispersed in double distilled water. The samples were ultrasonicated for 5 min prior to size determination to measure the primary particle size. Then it was taken in the disposable sizing cuvette and placed in the instrument for size and zeta potential measurements.

Drug Release Study

Release studies were carried out for all the formulations by using the HIMEDIA DM70 dialysis membrane having a pore diameter of 2.4 nm and cut off of 12-14 kD. Medium used was 200ml of phosphate buffer pH 7.4 containing 2% SLS. The membrane was then activated by incubating in 5% EDTA solution for 30 minutes and then in boiling water for 1 hr prior to use. Then one side of the membrane was tied off with thread and 10ml of nanosuspension was then placed inside the membrane. The other side was tied off properly and placed in 200ml release medium in a beaker with magnetic stirring. The tests were carried out for 12hrs. 2ml of the aliquot was withdrawn at different predetermined intervals. The required dilutions were made with dissolution medium and the solution was analyzed for the drug content spectrophotometrically at 299.5 nm against appropriate blank. Equal volume of the medium was replaced after each withdrawal to maintain sink condition. The release studies were carried out in triplicate for all the formulations and the average values were calculated. From this percentage drug release was calculated and this was plotted against function of time to study the pattern of drug release.

Measurement of Zeta Potential

Zeta Potential was determined at room temperature (25^0C) by using Malvern Mastersizer Hydro 2000 G. 1 ml nanosuspension sample was taken and dispersed in double distilled water. To prevent the agglomeration the dispersed solution was placed for 5 minutes in ultra sonicator bath. Then the sample was taken in the glass cuvette and placed in instrument to measure the zeta potential.

Crystal properties

Polymorphic composition and degree of crystallinity were evaluated by X-ray powder diffraction (XRPD). X-ray powder diffraction experiments were carried out using a siemens D-5000 X-ray diffractometer with Co Kα radiation (λ = 1.7890 Å) at scanning speed of S^{-1} 2θ over a range of 3 – 50^0. Silicon served as the internal standard. Diffrac AT software was used to display the X-ray diffraction patterns.

Differential scanning calorimetry

Samples were analysed for crystallinity of lipid by differential scanning calorimetry (DSC), with a Mettler-Toledo DSC 822^0 instrument (Mettler-Toledo GmbH Analytical, Schwerzenbach, Switzerland) using nitrogen gas. The sample (4 mg) was poured in a 40 ụl Al crucible, which was then sealed. The sample was then kept at 25^0C for 10 min, and heated from 25 to 250^0C at a scan rate of 5^0C/min.

RESULTS AND DISCUSSION

Particle size determination

Particle size measurement with Malvern mastersizer was performed at room temperature (25^0C) after dilution of the samples. It was found that the systems preserved their colloidal particle size between 305-817nm. The particle size was less (305 nm) when the distilled water used as a dispersion medium. The particle size was bigger in case of the other electrolyte medium. But the observed particle size of all of the investigated system was in the colloidal ranges (Table 2).

Table 2: Particle size determination.

Record	Sample	Z-Ave	PDI	Zeta potential	Pk 1 Mean Int
		d. nm		mV	d. nm
1	F1	860	0.705	-33.5	305
2	F2	912	0.615	-28.3	502
3	F3	973	0.611	-27.7	528
4	F4	1060	0.611	-29.3	746
5	F5	775	0.917	-21.3	505
6	F6	612	0.942	-26.5	406
7	F7	1120	0.605	-26.8	817
8	F8	602	0.816	-27.4	352
9	F9	811	0.657	-25.9	523
10	F10	846	0.601	-19.8	523

Drug Release Study

It was found that the optimum drug release was observed from the formulation (F1), was up to 12hr. About 88% drug released from nanoparticles while dispersed in distilled water. The release studies of different formulations are given in Table 3.

Table 3: *In vitro* cumulative drug release (%) of different formulations.

Time (Hour)	% CDR									
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
0.5	9.3	8.73	6.77	8.04	6.08	8.04	6.31	7.77	6.43	10.01
1	16.2	14.78	13.04	11.73	10.43	10.86	17.51	14.54	16.35	12.78
2	26.21	23.58	22.28	22.93	19.13	17.39	27.35	18.97	20.36	17.26
3	42.28	37.93	35.97	35.32	35.97	29.89	34.45	23.69	24.80	20.03
4	54.78	44.78	41.63	42.28	41.63	40.97	40.14	27.99	29.37	22.80
5	65.54	59.13	56.73	54.13	53.47	52.82	46.18	32.84	34.09	25.22
6	72.06	62.82	59.78	59.13	59.78	59.78	52.93	37.83	39.08	28.33
7	75.32	69.89	62.17	62.82	62.82	63.47	57.56	42.27	43.80	31.09
8	78.58	71.63	68.8	67.93	69.89	69.89	61.47	47.13	48.79	33.51
9	80.97	74.23	70.97	70.97	71.63	70.97	64.67	51.43	53.37	36.28
10	83.36	75.32	73.58	73.58	74.23	73.58	67.51	55.04	57.53	38.70
11	85.32	78.32	75.32	75.32	74.67	75.32	69.65	57.95	61.42	40.43
12	87.82	80.32	81.63	78.58	79.67	80.32	71.78	60.32	63.78	41.81

Measurement of Zeta Potential

The surface charge of the particle was changed by altering the dispersion medium (Table 2). In case of the distilled water the zeta potential was found to -33.5mV (Fig 12) where the zeta deviation was 8.5mV. As we know the standard zeta potential was +40 to -40mV. So the zeta potential -33.5mV which was near the range contains the very small particle size in the formulation (F1). The variation of zeta potential was related with nature of dispersion medium.

Crystal properties

The crystalline properties of drug was reduced by forming in the SLNs, however the drug retains its crystallinity and has not completely changed into amorphous form (Fig.2).

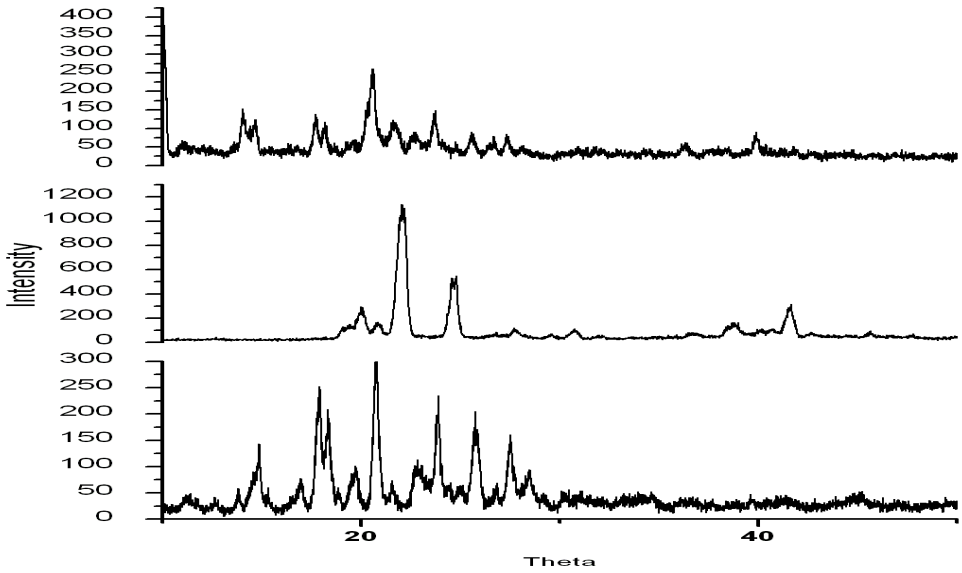

Fig. 2: X-ray diffractometry of the drug (below), lipid (middle) and F1 (top).

The decreasing intensity of the XRD peaks might be attributed to lower drug content in the nanoparticles. There was no appreciable change in the position of the peak with respect to 2θ values. Due to comparatively high drug content, it was expected that the drug did not get enough space for dilution amorphization within the nanomatrix of SLNs. Coupled with this, relatively fast cooling rate (from 85^0C TO RT) might not allow conversion into amorphous form.

As regards the lipids, there has been shift/disappearance of few of the peaks in the X-ray diffractogram which might be an indication of formation of different polymorph of the lipid, presumably due to the heating and cooling process.

Overall, the drug loaded SLNs retain the crystallinity of drug, which was indicative of a heterogeneous drug distribution in the lipid matrix. This might be the cause for a steady release since as per classical matrix dissolution theory, the drug must dissociated from their crystal structure prior to diffusion through matrix. The initial burst release (up to 17%' F1) at 1^{st} hr. might be attributed to the drug crystal embedded to SLNs surface and their dissolution enhancement due to co-existence of hydrophilic surfactant (pluronic F-68).

Differential scanning calorimetry

The DSC thermogram of pure drug, lipid and drug loaded SLNs showed marked variations in their thermal profiles (Fig.3).

The melting endotherm of lipid as well as the degradation shoulders disappeared in the formulation and they are appearing to a shift of Tg towards lower temperature in the SLNs. This might be due to the presence of drug and Pluronic F68 acting as plasticizer, also the appearance of different polymorph of lipid, as a consequence of thermal cycling during SLNs preparation.

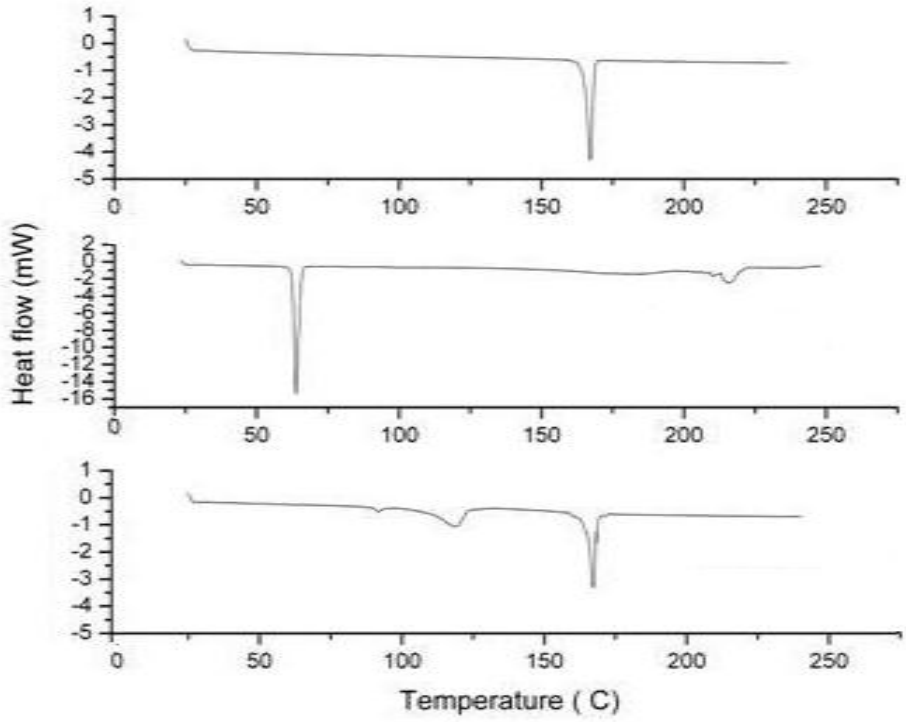

Fig. 3: Differential Scanning Calorimetry of the drug, lipid and formulation (F1).

However the drug melting peak was retained almost same position like pure drug indicated further crystallinity of glyburide which was complimentary to the same finding of XRD. The presumed polymorphic modification of lipid might be instrumental to provide a steady but almost complete release up to 12hrs study.

CONCLUSIONS

The formulations can be economically manufactured from relatively cheap raw material, like palmitic acid, Pluronic F68, Tween 40 etc. Lipid nanoparticles with suitable and desired characteristics may be prepared by microemulsion dispersion technique. The release profiles of glyburide from the SLNs are amenable to slow delivery of the drug to afford at least twice daily administration. The formulation profile of nanoparticles of glyburide was mediated through pseudo-ternary phase diagrams for developing the microemulsion concentrate before dispersing it in suitable quantity of the aqueous congealing phase. The optimized Km ratio

was 1:2 and the optimized drug-lipid ratio was 1:1.5. The microemulsions have inherent droplet size of the internal phase (molten lipid in this case) in the range <200nm, which makes the ideal process for preparing nanoparticles of low and narrow size range. The developed SLNs offers the advantages of high drug-lipid ratio, drug loading, minimal particle size and size-distribution and a moderate zeta potential of the particles.

REFERENCES

1. Han SF, Yao TT, Zhang XX, Gan L, Zhu C, Yu HZ. et al. Lipid-based formulations to enhance oral bioavailability of the poorly water-soluble drug anethol trithione: effects of lipid composition and formulation. Int J Pharm, 2009; 379(1): 18-24.
2. Nepal PR, Han HK, Choi HK. Enhancement of solubility and dissolution of Coenzyme Q10 using solid dispersion formulation. Int J Pharm, 2010; 383(1-2): 147-53.
3. Singh AK, Chaurasiya A, Awasthi A, Mishra G, Asati D, Khar RK. et al. Oral bioavailability enhancement of exemestane from self-microemulsifying drug delivery system (SMEDDS). AAPS Pharm Sci Tech, 2009; 10(3): 906-16.
4. Chen H, Xiao L, Du D, Mou D, Xu H, Yang X. A facile construction strategy of stable lipid nanoparticles for drug delivery using a hydrogel-thickened microemulsion system. Nanotechnology, 2010; 21(1): 015101.
5. Mrestani Y, Behbood L, Härtl A, Neubert RH. Microemulsion and mixed micelle for oral administration as new drug formulations for highly hydrophilic drugs. Eur J Pharm Biopharm, 2010; 74(2): 219-22.
6. Kalam MA, Sultana Y, Ali A, Aqil M, Mishra AK, Chuttani K. Preparation, characterization, and evaluation of gatifloxacin loaded solid lipid nanoparticles as colloidal ocular drug delivery system. J Drug Target, 2010; 18(3): 191-204.
7. Madan J, Dhiman N, Parmar VK, Sardana S, Bharatam PV, Aneja R. Inclusion complexes of noscapine in beta-cyclodextrin offer better solubility and improved pharmacokinetics. Cancer Chemother Pharmacol, 2010; 65(3): 537-48.
8. Varshosaz J, Tabbakhian M, Mohammadi MY. Formulation and optimization of solid lipid nanoparticles of buspirone HCl for enhancement of its oral bioavailability. J Liposome Res, 2010; 20(4): 286-96.
9. Buyukozturk F, Benneyan JC, Carrier RL. Impact of emulsion-based drug delivery systems on intestinal permeability and drug release kinetics. J Control Release, 2010; 142(1): 22-30.

10. Tanaka Y, Inkyo M, Yumoto R, Nagai J, Takano M, Nagata S. Nanoparticulation of poorly water soluble drugs using a wet-mill process and physicochemical properties of the nanopowders. Chem Pharm Bull, 2009; 57(10): 1050-7.
11. Muchow M, Schmitz EI, Despatova N, Maincent P, Müller RH. Omega-3 fatty acids-loaded lipid nanoparticles for patient-convenient oral bioavailability enhancement. Pharmazie, 2009; 64(8): 499-504.
12. Yin YM, Cui FD, Mu CF, Choi MK, Kim JS, Chung SJ, et al. Docetaxel microemulsion for enhanced oral bioavailability: preparation and in vitro and in vivo evaluation. J Control Release, 2009; 140(2): 86-94.
13. Bu P, Ji Y, Narayanan S, Dalrymple D, Cheng X, Serajuddin AT. Assessment of cell viability and permeation enhancement in presence of lipid-based self-emulsifying drug delivery systems using Caco-2 cell model: Polysorbate 80 as the surfactant. Eur J Pharm Sci, 2017; 1(99): 350-360.