Review Article
ISSN: 0974-6943

Biswajit Basu et al. / Journal of Pharmacy Research 2010, 3(1),84-92

Available online through
www.jpronline.info

Solid lipid nanoparticles :A promising tool for drug delivery system

Biswajit Basu*, Kevin Garala, Ravi Bhalodia, Bhavik Joshi, Kuldeep Mehta

**Department of Pharmaceutics, Atmiya Institute of Pharmacy, Kalawad Road, Rajkot – 360005, Gujarat, India*

Received on: 20-09-2009; Revised on: 16-10-2009; Accepted on:15-12-2009

ABSTRACT

Solid lipid nanoparticles (SLNs) technology represents a promising new approach to lipophilic drug delivery. Solid lipid nanoparticles are important advancement in this area. The biodegradable and bioacceptable nature of SLNs makes them less toxic as compared to polymeric nanoparticles. SLNs can also be used to improve the bioavalability of drugs. In this present review this new approach is discussed in terms of their advantages, characterization, *in-vivo* studies, *in-vitro* studies, pharmacokinetic studies and special features.

Keywords: Solid lipid nanoparticles, *in-vivo* studies, *in-vitro* studies.

INTRODUCTION

For many decades, treatment of an acute disease or a chronic illness has been mostly accomplished by delivering drugs using various pharmaceutical dosage forms, including tablets, capsules, pills, suppositories, creams, ointments, liquids, aerosols and injectables as vehicles. Amongst various routes of drug delivery, oral route is perhaps the most common, and most preferred to the patient and the clinician alike. However, this route possesses some problems for a few drugs such as poor solubility, chemical instability in the gastrointestinal tract, poor permeability through the biological membranes or sensitivity to metabolism. The factors that can limit the oral bioavailability of drugs include first-pass metabolism, lack of the drug solubility and dissolution. Poorly soluble compounds tend to be eliminated from the GIT before they had opportunity to fully dissolve and absorb into the circulation[1]. The inherent problems associated with the drug, in some cases, can be solved by modifying the formulation or changing the routes of administration.

Targeted delivery of a drug molecule to organ or special sites is one of the most challenging research areas in pharmaceutical sciences. By developing colloidal delivery systems such as liposomes, micelles and nanoparticles a few frontier was opened for improving drug delivery. Nanoparticles with their special characteristics small particle size, large surface area and the capability of changing their surface properties have numerous advantages compared with other delivery systems. Nanoemulsions are nanometric-sized emulsion, typically exhibiting diameters of upto 500 nm.

Nanoparticles are solid colloidal particlesranging from 10 to 1000 nm (1.0 µm), in which the active principles (drug or biologically active material) are dissolved, entrapped, and/or to which the active principle is adsorbed or attached[2]. In recent years, significant effort has been devoted to develop nanotechnology for drug delivery, since it offers a suitable means of delivering small molecular weight drugs, as well as macromolecules such as proteins, peptides or genes to cells and tissue[3]. Nanoparticles hold promise for peroral drug delivery, which represents so far the most common and convenient route of administration. The nanoparticles are much more stable and therefore are not rapidly digested in the GIT. The advantages of nanoparticles as drug delivery systems are that they are biodegradable, non-toxic, and capable of being stored for longer periods[2].

It was realized that the nanoparticles loaded bioactives could not only deliver drugs to specific organs within the body, but delivery rate could be controlled as being bystanders, burst, controlled, pulsatile or modulated[4]. Nanoparticles, in general, can be used to provide targeted delivery of drugs, to sustain drug effect in target tissue, to improve oral bioavailability to solubilize drugs for intravascular delivery and to improve the stability of therapeutic agents against enzymatic degradation[3]. Important factor that governs the intestinal uptake of particulates is their particle size. Smaller particles are taken up to a significantly higher degree than larger particles[5].

In addition, their potential uptake as well as their stability in the gastrointestinal tract indicates that nanoparticles are expected to be promising carriers for the transport of drugs. These attributes make nanoparticles more suitable for the purpose of sustained release and improvement of bioavailability.

***Corresponding author.**
Mr. Biswajit Basu,
Department of Pharmaceutics,
Atmiya Institute of Pharmacy,
Kalawad Road,
Rajkot – 360005, Gujarat, India.
Tel.: + 91-9724142699
E-mail: basu.biswajit@yahoo.com

Since a decade, trials are being made to utilize solid lipid nanoparticles (SLN) as alternative drug delivery system to colloidal drug delivery systems such as lipid emulsions, liposomes and polymeric nanoparticles. SLN combines the advantages of different colloidal carriers and also avoids some of their disadvantages. SLN can be used to improve the bioavailability of drugs, e.g. cyclosporine A [6], and to obtain sustained release of lipophilic drugs like camptothecin [7]. It was reported that Idarubicin-loaded SLN acted as a prolonged release system after duodenal administration to rats [8]. Clozapine, a lipophilic drug, is rapidly absorbed orally with a bioavailability of 0.27 [9]. In vitro drug release studies are important to understand the in vivo performance of the dosage form. Drug release studies help in evaluation of sustained and prolonged release dispersion systems [10].

2.1. Role of Nanomedicines in Cancer

Currently in cancer therapy there is the lack of selectivity of anticancer drugs towards neoplastic cells. Generally rapidly proliferating cells such as those of bone marrow or the gastrointestinal tract are affected by the cytotoxic action of these drugs. This results in the narrow therapeutic index of most anticancer compounds. Also the emergence of resistant cell sublines during the chemotherapeutic treatment may require the use of higher doses of these drugs. This may enhance the toxicity of the treatment. In order to decrease the toxicity of the drugs and enhance the selectivity many drug delivery systems have been developed[11,12]. This includes nanoparticles [13] that have received a greater interest for drug targeting, as they can be easily prepared with well – defined biodegradable polymers. The reason of targeting tumors with nanoparticles is because certain neoplastic cells show enhanced endocytotic activity[14]. In addition since some particular tumors are blood supplied by capillaries having an increased vascular permeability, it can be anticipated that nanoparticles will gain access to extravascular tumor cells [15,16]. The ultimate goal of nanomedicines is to create medically useful nanodevices that can function inside the body. Among the newly developed nanomedicine and nanodevices quantum dots, nanowires, nanotubes, nanocantilevers, nanoshells are the most promising applications for various cancer treatments. They would serve the two important purposes of localized drug delivery and specific targeting[17]. Nanoscale devices have the potential to radically change cancer therapy by increasing the number of highly effective therapeutic agents. Nanoparticles can serve as customizable, targeted drug delivery vehicles capable of ferrying chemotherapeutic agents or therapeutic genes into malignant cells while sparing healthy cells. This may allow for small doses of toxic substances as the drugs are delivered directly into the target tissue.

2.2. Targeting

Currently, cancer - fighting drugs are toxic to both tumor and normal cells, thus the efficacy of chemotherapy is always limited by the side effects of the drug[18]. Some nanoscale devices can be targets to the cancer cells. This increases the selectivity of the drugs toward the cancer cells and will reduce the toxicity for normal tissue. Attaching monoclonal antibodies that bind specifically to the cancer cells does this. Surface modification of nanoparticles can also enhance the permeability of the drugs to create high permeability nanoparticle based cancer therapeutics. Barriers to the cancer drugs can be in the form of the cell's plasma membrane or endothelial layers of the cell. Research on the covalent attachment of peptide- membrane translocation sequences, peptides with the ability to pass through the membrane, to nanoparticles has shown increased permeability through membranes [19]. With improved cell permeability, nanoparticles can become more therapeutically effective drug transport vehicles. The activity of nanomedicines at various target sites is discussed in the further points. Targeting macrophage with nanoparticles: macrophage is a specialized host defense cell wherein any alterations in its clearance may contribute to disorders like atherosclerosis, autoimmunity, and major infections. Passive targeting of nanoparticulate vehicles with encapsulated antimicrobial agents to infected macrophages is hence a logical strategy for effective microbial killing[20-24]. Endothelium as the target: endothelium plays an important role in various pathological processes including cancer, inflammation, oxidative stress and thrombosis. Recent studies have shown that cationic liposomes are eternalized into endosomes and lysosomes of endothelial cells in a characteristic organ and vessel specific manner[25]. Extravasation: targeting of solid tumors: there are regulatory approved formulations of long circulating liposomes with entrapped doxorubicin for management of AIDS related Kaposis sarcoma, refractory ovarian cancer and metastatic breast cancer[20,26].

2.3. SLNs: a novel drug delivery system of choice

Chen et al. have aptly discussed polymeric nanoparticles as suitable delivery systems for brain [27]. They have outlined various mechanisms for nanoparticle mediated drug uptake by the brain. These include:

1. Enhanced retention in the brain–blood capillaries, with an adsorption on to the capillary walls, resulting in a high concentration gradient across the BBB.
2. Opening of tight junctions due to the presence of nanoparticles.
3. Transcytosis of nanoparticles through the endothelium.

Furthermore, coating of these polymeric nanoparticles with polysorbate has been reported to improve the brain bioavailability[27]. Some of the proposed mechanisms by which the polysorbate coating is effective, include:

1. Solubilization of endothelial cell membrane lipids and membrane fluidization, due to surfactant effects of polysorbates.
2. Endocytosis of polymeric nanoparticles due to facilitated interaction with BBB endothelial cells.
3. Inhibition of efflux system, especially P-gp. But, there are various problems associated with the use of these polymeric nanoparticles like residual contamination from the production process, for example by organic solvents, polymerization initiation, large polymer aggregates, toxic monomers and toxic degradation products [28]. Other problems include the expensive production methods[29], a lack of large scale production method[30] and a suitable sterilization method e.g. auto-

claving. Considering the success of nanoparticles to pass through the BBB and their limitation(s) especially toxicity and stability, another suitable option for drug delivery into the brain would be SLNs. SLNs constitute an attractive colloidal drug carrier system. SLNs consist of spherical solid lipid particles in the nanometer range, which are dispersed in water or in aqueous surfactant solution. They are generally made up of solid hydrophobic core having a monolayer of phospholipid coating. The solid core may contain the drug dissolved or dispersed in the solid high melting fat matrix with the hydrophobic end of the phospholipids chains embedded in the fat matrix. They have thus the potential to carry lipophilic or hydrophilic drug(s) or diagnostics[32-34]. Wang et al. have reported the synthesis of 3', 5,-dioctanoyl-5 flouro-2,-deoxyuridine to overcome the limited access of the drug 5-flouro-2,-deoxyuridine (FUdR) and its incorporation into solid lipid nanoparticles (DO–FUdR)[35]. The brain area under the concentration/time curve of DO–FUdR–SLN and DO–FUdR were 10.97-fold and 5.32-fold higher than that of FUdR, respectively. These results indicated that DO–FUdR–SLN had a good (2 times the free drug) brain targeting efficiency in vivo. These authors report that SLN can improve the ability of the drug to penetrate through the blood–brain-barrier and is a promising drug targeting system for the treatment of central nervous system disorders.

2.4. Advantages of SLNs over polymeric nanoparticles (and other delivery systems like liposomes).

SLNs combine the advantages of polymeric nanoparticles, fat emulsions and liposomes while simultaneously avoiding their disadvantages[31]. The advantages of SLNs include the following:

1. The nanoparticles and the SLNs particularly those in the range of 120–200 nm are not taken up readily by the cells of the RES (Reticulo Endothelial System) and thus bypass liver and spleen filtratio[36].
2. Controlled release of the incorporated drug can be achieved for upto several weeks.[36-38] Further, by coating with or attaching ligands to SLNs, there is an increased scope of drug targeting[39,40,].
3. SLN formulations stable for even three years have been developed. This is of paramount importance with respect to the other colloidal carrier systems[41,42].
4. High drug payload.
5. Excellent reproducibility with a cost effective high pressure homogenization method as the preparation procedure[31].
6. The feasibility of incorporating both hydrophilic and hydrophobic drugs[32-34].
7. The carrier lipids are biodegradable and hence safe[43-45].
8. Avoidance of organic solvents[40].
9. Feasible large scale production and sterilization[36,46].

2.5. Methods to prolong brain retention of SLNs

The body distribution of SLNs is strongly dependent on their surface characteristics vis a vis size, surface hydrophobicity, surface mobility etc. The SLNs have been proposed as suitable system to deliver hydrophilic drugs like diminazine and also for other BCS class IV drugs like paclitaxel, vinblastine, camptothecin, etoposide, cyclosporine etc[44,47,48]. These carriers can gain access to the blood compartment easily (because of their small size and lipophilic nature) but the detection of these particles by the cells of the reticuloendothelial system (RES) i.e. the mononuclear phagocytic system; MPS cells of the liver (Kupffer) and that of spleen macrophages is a major limitation for their use. Uptake of nanoparticles by RES could result in therapeutic failure due to insufficient pharmacological drug concentration build up in the plasma and hence at the BBB. To overcome these limitations various researchers have tried to increase the plasma half-life of SLNs by the following methods.

2.5.1. Particle size.

The size and the deformability of particles play a critical role in their clearance by the sinusoidal spleens of humans and rats. Particles must be either small or deformable enough to avoid the splenic filtration process at the interendothelial cell slits (IES) in the walls of venous sinuses[49,50]. The IES in sinusoidal spleens provides resistance to flow through the reticular meshwork. The endothelial cells of the sinus wall have two sets of cytoplasmic filaments: a set of loosely associated tonofilaments and a set of filaments tightly organized into dense bands in the basal cytoplasm containing actin and myosin, which can probably vary the tension in the endothelial cells and, hence, the size of IES[51]. However, the slit size rarely exceeds 200 to 500 nm in width, even with an erythrocyte in transit[49]. Hence, retention of blood cells and blood-borne particles at the IES depends on their bulk properties, such as size, sphericity, and deformability. These cell slits are the sites where erythrocytes containing rigid inclusions (e.g., Heinz bodies, malarial plasmodia) are believed to be “pitted” of their inclusions, which are eventually cleared by the red pulp macrophages[52]. Therefore, the size of an engineered longcirculatory particle should not exceed 200 nm ideally. If larger, then the particle must be deformable enough to bypass IES filtration. Alternatively, long-circulating rigid particles of greater than 200 nm may act as splenotropic agents and removed later on, if they are not rigid[54]. Hence SLNs of size below 200 nm have an increased blood circulation and thus an increase in the time for which the drug remains in contact with BBB and for the drug to be taken up by the brain[54]. The table 1 gives the details of particle size achieved with different combinations of lipids, surfactants and method of preparation.

2.5.2. Surface coating with hydrophilic polymers/surfactants.

The high rates of RES mediated detection and clearance of colloidal carriers by liver, significantly reduce the half-life of the drug. The interaction of the colloidal carriers with blood plasma proteins (opsonins) and thus with the membranes of macrophages (opsonization) is believed to be the major criteria for clearance of these systems from the blood stream. Hence to prevent this clearance and to increase their availability at the target site the RES removal of these particulate systems should be prevented[55]. This RES recognition can be prevented by coating the particles with a hydrophilic or a flexible polymer and/or a surfactant. The RES mediated detection and

clearance by the liver is believed to be facilitated by the MPS cells. Opsonins, including complement proteins, apolipoproteins, fibronectin and Igs interact with specific membrane receptors of monocytes and tissue macrophages, resulting in their recognition and thus phagocytosis. It is generally admitted that hydrophobic surfaces promote protein adsorption and that negative surfaces activate the complement system and coagulation factors[53], any shielding of hydrophobic character of the nanoparticles is thus going to stearically stabilize them by providing a dense conformational cloud and thus reducing opsonization and phagocytosis as well as uptake by neutrophilic granulocytes, thus increasing the blood circulation time and hence the bioavailability[56]. Coating with polyethylene glycol (PEG), a polymer of hydrophilic nature showed promising results. PEG has high hydrophilicity, chain flexibility, electrical neutrality and lack of functional groups, preventing it from interacting unnecessarily with the biological components. It has been suggested that the PEG's with a molecular weight between 2000 to 5000 are necessary to suppress plasma protein adsorption; further it has been observed that the thicker the coat, the slower the clearance, and hence a better protection against liver uptake[54]. Enlarging the molecule/particle slows down kidney ultrafiltration and, thereby allowing better accumulation into the brain and other permeable tissues by the passive enhanced permeation and retention mechanism. It also provides protein shielding which reduces proteolysis within the serum and tissues, and hinders immune surveillance of surface epitopes. Pegylation improves the pharmacokinetic profile of molecules by reducing opsonization, phagocytosis and clearance by the liver and reticulaoendothelial system. Other hydrophilic molecules which have been tried are Brij 78, Poloxamer F68 and Brij 68.

Cavalli et al. found that parenteral administration of nanoparticles of paclitaxel was more bioavailable than an i.v. injection of the plain drug[48]. The chemical nature of the overcoating surfactant is of importance, as only polysorbate-coated particles were found to show results in CNS pharmacological effect while a coating with poloxamers (184, 188, 388, or 407), poloxamine 908, Cremophors (EZ or RH40) or polyoxyethylene(23)-laurylether was not effective. The reported mechanism of action was the transport of polysorbate-coated nanoparticles across the BBB via endocytosis by the brain capillary endothelial cells. This endocytosis would be triggered by a serum protein, apolipoprotein E, reported to adsorb on polysorbate 20, 40, 60, or 80-coated nanoparticles after a 5-min incubation in citrate-stabilized plasma at 37°C, but nanoparticles coated with poloxamers 338, 407, Cremophor EL, or RH 40 could not cross the BBB. The delivery of the drugs to the brain by nanoparticles made of polybutylcyanoacrylate (PBCA) and coated with the nonionic surfactant polysorbate 80 has been intensely investigated. Similarly, polysorbates are investigated to have the highest potential to deliver the solid lipid nanoparticles to the brain[58].

2.6. Preclinical data regarding use of SLNs in various neurological disease states

Zara et al. made nonstealth and stealth SLN of doxorubicin. They used PEG 2000 at various concentrations as the stealthing agent. The i.v. administered SLNs and stealth SLN containing increasing amounts of stealthing agent, allowed doxorubicin loaded nanoparticles to be transported through the BBB. They observed an increase in the brain concentration of doxorubicin on increasing the stealthing agent. The amount of drug present in the rabbit brain ranged from 27.5 ng/g for nonstealth SLN to 242.0 ng/g for stealth SLN preparation loaded with 0.45% PEG after 30 min. After 2 h the pattern was the same, but the amount of doxorubicin in the brain was lower than after 30 min, i.e. 8 ng/g to 58.5 ng/g for SLN and stealth SLN loaded with 0.45% PEG. After 6 h, doxorubicin was only detected after i.v. administration of stealth SLNs loaded with 0.45 PEG[58].

Reddy and Venkateshwarlu studied brain levels after i.v. administration of etoposide loaded tripalmitin nanoparticles, and etoposide solution. The authors found a relationship between the charge on the SLN and the brain drug levels. The etoposide loaded positively charged tripalmitin nanoparticles achieved highest brain concentration (0.07% of injected dose per gram of organ/tissue) when compared to negatively charged etoposide incorporated tripalmitin nanoparticles (0.02% of injected dose per gram of organ/tissue) and etoposide solution (0.01% of injected dose per gram of organ/tissue)[59].

Goppert and Muller made polysorbate stabilized SLNs for delivering drugs to the brain. These workers while proving their hypothesis of brain targeting using polysorbate 80 coated SLNs showed that apo C^2 and apo CII inhibit the receptor mediated binding of apoE containing lipoproteins such as â-VLDl to the LDL receptor. It would thus be advantageous to have a high apoE/apoCII ratio absorbed on the particles to achieve brain targeting. Further the authors found that the SLNs stabilized by polysorbate 80 absorb lowest of apo CII[60].

Manjunath and Venkateshwarlu made SLNs of a lipophilic drug nitrendipine for improving its bioavailability upon i.v. administration. Nitrendipine loaded SLNs were made using different triglycerides (tripalmitin, trimyristin and tristearin), emulsifiers—soy lecithin, poloxamer 188 and charge modifiers (dicetyl phosphate; DCP and stearylamine, SA). Upon i.v. administration of nitrendipine suspension and nitrendipine SLNs, nitrendipine SLNs were found to be taken up to a greater extent by the brain and maintained high drug levels for 6 h as compared to only 3 h with nitrendipine suspension. The Cmax of 3.2, 7.3 and 9.1 times was achieved with nitrendipine tripalmitin, nitrendipine tripalmitin dicetyl phosphate and nitrendipine tripalmitin stearylamine SLNs when compared with nitrendipine suspension[61].

Wang et al. incorporated 3', 5'-dioctanoyl-5-fluoro-2'-deoxyuridine into solid lipid nanoparticles. The loaded SLN and drug solution were then administered intravenously and it was determined that the AUC values achieved with SLN were two folds of that obtained by injecting plain drug solution[62].

Intravenous injection of 1.3 mg/kg of an anticancer drug camptothecin into mice resulted in significantly prolonged drug resistance time in the body when loaded stearic acid solid lipid

Table 1: Literature survey of methods of preparing SLNs of different drugs and the resulting particle size distribution and surfactant used for preparation of the SLNs

Lipid matrix	Drug	Preparation method	Particle size (nm) [polydispersity]	Surfactant
Acidan N 12	Diazepam	Microemulsification	70	Epikuron 200
Dynasan 114	Apolipoprotein E	Hot homogenization	186 Poloxamer=245	Polysorbate
Dynasan 114	Clozapine	Hot homogenization	Poloxamer=150	Poloxamer188 Epikuron 200
Dynasan 116	Clozapine	Hot homogenization	Poloxamer=163.3	Poloxamer188
Glyceryl behanate	Diazepam	Microemulsification	86.0	Epikuron 200
Glyceryl monostearate	Mifepristone	Modified high shearhomogenization	106	Tween 80, glycerol
Glyceryl behenate	Vitamin A	High pressure homogenization	300-500	Hudroxypropyl distarch
Monostearin	Clobetasol propionate	Solvent diffusion	143	PVA
Stearic acid	Diminazine	Hot homogenization	78.5	Polysorbate 80
Stearic acid	Doxorubicin	Microemulsification	Nonstealth=80	Epikuron 200
Tristearin	Clozapine	Hot homozenization	Poloxamer=96.7	Poloxamer
Tricaprin	All trans retinoic acid	Melt homozenization	233-487	Tween 80
Witepsol E 85	Ascorbyl palmitate	High pressure homogenization	228	Tegocare

nanoparticle was administered compared with plain drug solution. An increase of 5 folds in plasma AUC and of 10 folds in brain AUC which further increased on increasing the dose of camptothecin from 1.3 mg/kg to 3.3 mg/kg by another 2.7 folds for plasma AUC and 2.6 folds for brain AUC was observed[44].

3. Preparation of the solid lipid nanoparticles:

The solid lipid nanoparticles can be prepared by various method like Microemulsification, Solidification, Hot homogenization, Modified high shear homogenization, Ultrasound Solvent diffusion, Solvent injection, Melt homogenization, and High pressure homogenization techniques.

4. Various techniques to characterize the solid lipid nanoparticles

4.1. Drug incorporation and loading capacity

The essential ingredients for SLNs include lipids, and a single or a combination of emulsifiers[64]. Depending on the lipid (triglycerides, fatty acids, steroids and waxes etc), emulsifier (anionic, cationic or nonionic) and the method of preparation (hot/cold homogenization, microemulsification etc) the particle size, and the surfactant used for the preparation of SLNs is found to vary (Table 1). Factors determining the loading capacity of a drug in the lipid are:

1. Solubility of drug in the melted lipid.
2. Miscibility of drug melt and the lipid melt.
3. Chemical and physical structure of solid lipid matrix.
4. Polymorphic state of lipid material.

The prerequisite to obtain a sufficient loading capacity is a sufficiently high solubility of the drug in the lipid melt. Typically, the solubility should be higher in the melted state than that required in the solid state because the solubility decreases when the melt cools and might even be lower in the solid lipid. To enhance the solubility in the lipid melt one can add solubilizers. In addition, the presence of mono- and di-glycerides in the lipid used as the matrix material also promotes drug solubilization. The chemical nature of the lipid is also important because lipids which form highly crystalline particles with a perfect lattice (e.g. monoacid triglycerides) lead to drug expulsion[64]. More complex lipids being mixtures of mono-, di- and triglycerides and also containing fatty acids of different chain length form less perfect crystals with many imperfections offering space to accommodate the drugs. Polymorphic form is an important parameter determining drug incorporation. Crystallization of the lipid in nanoparticles is different from the bulk material; lipid nanoparticles recrystallize at least partially in the a-form, whereas bulk lipids tend to recrystallize preferentially in the ß'-modification and transform rapidly to the ß-form[64]. With increasing formation of the more stable modifications the lattice is getting more perfect and the number of imperfections decreases, that means formation of ß'/ß-modification promotes drug expulsion. In general the transformation is slower for long chain than for short chain triglycerides[64]. An optimal SLN carrier can be produced in a controlled way when a certain fraction of ß'-form can be created and preserved during the storage time. By doing this normal SLN carrier transforms to an intelligent drug delivery system by having a built-in triggering mechanism to initiate transformation from ß'- to ß-forms and consequently controlled drug release[65].

4.2. Determination of incorporated drug

It is of prime importance to measure the amount of drug incorporated in SLN, since it influences the release characteristics. The amount of drug encapsulated per unit weight of nanoparticles is determined after separation of the free drug and solid lipids from the aqueous medium. This separation can be carried out using ultracentrifugation, centrifugation filtration or gel permeation chromatography. In centrifugation filtration the filters such as Ultra free-MC (Millipore) or Ultrasort-10 (Sartorious) are used along with classical centrifugation techniques. The degree of encapsulation can be assessed indirectly by determining the amount of drug remaining in supernatant after centrifugation filtration/ultracentrifugation of SLN suspension or alternatively by dissolution of the sediment in an appropriate solvent and subsequent analysis. Standard analytical techniques such as spectrophotometry, spectrofluorophotometry, high performance liquid chromatography, or liquid scintillation counting can be used to assay the drug[66]. In gel permeation chromatography Sephadex® and Sepharose® gels are used for removal of free drug from SLN preparations.

4.3. Storage stability of SLNs

The physical stability of the SLNs during prolonged storage can be determined by monitoring changes in Zeta potential, particle size, drug content, appearance and viscosity as a function of time. External parameters such as temperature and light appear to be of primary importance for long-term stability. The zeta potential should in general remain higher than -60 mV for a dispersion to remain physically stable. Values of approx.-15 mV were reported to lead to a distinct coalescence of emulsion droplets in parenteral nutrition. A rapid growth of particle size was observed when the SLNs were stored at 50 °C, with a decrease in their zeta potential. 4 °C was generally the most favorable storage temperature. However, in some cases, long term storage at 20 °C did not result in drug-loaded SLN aggregation or loss of drug, compared to 4°C storage conditions. Various methods such as lyophilization and spray drying have been proposed to optimize the stability[62].

4.4. Analytical methods for characterization

In order to develop a drug product of high quality, a precise physicochemical characterization of the SLNs is necessary. The size of the nanoparticles is the most important feature; however other parameters such as density, molecular weight, and crystallinity influence the nanoparticle release and degradation properties. The in vivo fate vis-à-vis body distribution and interaction with the body environment are however influenced by surface charge, hydrophilicity, and hydrophobicity etc. The most prevailing method for particle size determination until now is the photon correlation spectroscopy because of its fastness[67]. The mean and the polydispersity Index (P.I.) could be calculated with the help of modern computer programmes but the presence of different particle sizes which may be dust, accidental microbial contamination, crystallization of ingredients, or secondary particle agglomerates can lead to erroneous results. To avoid these erroneous results, it is always advisable to verify the results with other suitable methods like electron microscopy vis a vis TEM/SEM. These later methods are however time consuming such that Photon correlation spectroscopy is the most useful and most prevalent method of particle size analysis [67]. Crystallinity could be determined with the help of DSC, X-ray diffraction, thermal gravimetric analysis and thermal optical analysis. The crystallinity is important to be determined as the incorporated drug may undergo a polymeric transition[68]. Co-existence of additional colloidal structures (micelles, liposomes, supercooled melts, drug-nanoparticles) could be ascertained with the use of techniques like magnetic resonance techniques, NMR and ESR. NMR active nuclei of interest are 1H, 13C, 19F and 35P. Due to the different chemical shifts it is possible to attribute the NMR signals to particular molecules or their segments. Simple 1H-spectroscopy permits an easy and rapid detection of supercooled melts[69]. It permits also the characterization of liquid nanocompartments in recently developed lipid particles, which are made from solid and liquid blends[70]. The method is based on the different proton relaxation times in the liquid and semisolid/solid state. Protons in the liquid state give sharp signals with high signal amplitudes, while semisolid/solid protons give weak and broad NMR signals under these circumstances[70]. ESR requires the addition of paramagnetic spin probes to investigate SLN dispersions. The corresponding ESR spectra give information about the microviscosity and micropolarity. ESR permits the direct, repeatable and noninvasive characterization of the distribution of the spin probe between the aqueous and the lipid phase. Experimental results demonstrate that storage induced crystallization of SLN leads to an expulsion of the probe out of the lipid into the aqueous phase[71]. ESR spectroscopy and imaging is expected to give new insights about the fate of SLN *in vivo*.

4.5. Measurement of size and zeta potential of SLN

Size and zeta potential of SLN is measured by photon correlation spectroscopy (PCS) using Malvern Zetasizer. Samples are diluted appropriately with the aqueous phase of the formulation for the measurements[10]. Zeta potential measurements are done at 25 ^{0}C and the electric field strength was around 23.2 V/cm. The zetasizer measures the zeta potential based on the Smoluchowski equation.

4.6. Assay and entrapment efficiency

The amount of drug entrapped within SLN is measured by UV spectrophotometry or by HPLC[10].

4.6.1. Entrapment efficiency

The entrapment efficiency of the drug is determined by measuring the concentration of free drug in the dispersion medium. Ultracentrifugation was carried out using Centrisart, which consist of filter membrane (molecular weight cutoff 20,000 Da) at the base of the sample recovery chamber. The solid lipid nanoparticles along with encapsulated drug remained in the outer chamber and aqueous phase moved into the sample recovery chamber through filter membrane. The amount of the drug in the aqueous phase is determined by HPLC[10].

4.6.2. Statistical analysis

Size and entrapment efficiency of SLNs are compared using the Student's t-test. Statistical analyses are also performed[10].

4.7. Stability studies

Drug loaded SLNs are stored at 25 °C for 6 months and average size and entrapment efficiency are determined[10].

4.8. Effect of sterilization

To see the effect of sterilization on particle size, zeta potential and entrapment efficiency, blank and drug dispersions are autoclaved at 121 °C for 20 min[10].

4.9. Characterization by differential scanning calorimetry

Differential scanning calorimetry (DSC) (yields information on melting behavior and crystallization behavior of solid and liquid

constituents of the particles) are performed.

DSC analysis is performed using Mettler DSC 822e/200 (Mettler Toledo). The instrument is calibrated with indium (calibration standard, purity >99.999%) for melting point and heat of fusion. A heating rate of 10°C/min is employed in the range of 20–220°C. Analysis is performed under a nitrogen purge (50 ml/min). A standard aluminum sample pans (40 Al) are used. About 10 mg sample are taken for analysis [10].

4.10. Powder X-ray diffractometry (PXRD)

PXRD studies are performed on the samples by exposing them to CuKa radiation (40 kV, 30 mA) and scanned from 2° to 70°, 2h at a step size of 0.045° and step time of 0.5 s. Samples used for PXRD analysis are same as those of DSC analysis [10].

4.11. Transmission electron microscopy (TEM)

The morphology of SLNs are examined using an electronic transmission microscope. After diluting 50-fold with the original dispersion medium of the preparation, the samples are negatively stained with 1.5% (w/v) phosphotungstic acid for observation[72].

5. In-vitro and ex-vivo methods for the assessment of drug release from SLNs

5.1. *In-vitro* drug release

5.1.1. Dialysis tubing

In-vitro drug release could be achieved using dialysis tubing. The solid lipid nanoparticle dispersion is placed in a prewashed dialysis tubing which can be hermetically sealed. The dialysis sac is then dialyzed against a suitable dissolution medium at room temperature, the samples are withdrawn from the dissolution medium at suitable intervals, centrifuged and analyzed for drug content using a suitable analytical method (U.V. spectroscopy, HPLC etc)[55]. The maintenance of sink conditions is essential. This method however suffers from the limitation of a lack of direct dilution of the SLNs by the dissolution medium. The drug release of camptothecin SLN using a dynamic dialysis method in phosphate buffered saline has been reported[47].

5.1.2. Reverse dialysis

In this technique a number of small dialysis sacs containing 1 ml of dissolution medium are placed in SLN dispersion. The SLNs are then displaced into the dissolution medium. The direct dilution of the SLNs is possible with this method; however the rapid release cannot be quantified using this method[55].

5.1.3. Franz diffusion cell

The solid lipid nanoparticle dispersion is placed in the donor chamber of a Franz diffusion cell fitted with a cellophane membrane. The dispersion is then dialyzed against a suitable dissolution medium (simulated gastric medium/simulated intestinal medium/simulated plasma) at room temperature, the samples are withdrawn from the dissolution medium at suitable intervals and analyzed for drug content using a suitable instrumental method (U.V. spectroscopy, HPLC)[55]. The maintenance of sink condition is essential and the method suffers from the limitation of lack of direct dilution of the SLNs by the dissolution medium.

6. Ex-vivo model for determining permeability across the gut

Ahlin et al. demonstrated passage of Enalaprit SLNs across rat jejunum. In short, the rat jejunum was excised from the rats after sacrificing the animal. The jejunum 20–30 cm distal from the pyloric sphincter was used. The jejunum was rinsed to remove the luminal contents after washing with ice cold standard Ringer solution. The tissue was then cut into segments, opened up along the mesenteric border and placed between two Easy Mount side-by-side diffusion chambers with an exposed tissue area of 1 cm^2. The mucosal side was bathed with ringer buffer containing 10mM mannitol and the serosal side with ringer buffer containing 10mM glucose. The enalaprilat loaded nanoparticles were placed on the mucosal side, dispersed in ringer containing the paracellular transporter sodium fluorescein confirming for tissue integrity[73]. Similar type of study will be carried out here also.

6.1. Animals and administration of drug formulations

Male Wistar rats and Swiss albino mice are used for pharmacokinetic and tissue distribution studies, respectively.

6.2. Intravenous administration

Rats are anaesthetized and the selected samples are given. Time taken for administration is 30 sec. Blood samples are drawn by retro-orbital venous plexus puncture at 15, 30, 45, 60, 90, 120, 240 and 480 min post i.v. dose. The samples are centrifuged (5000×g, 15 min) and serum are collected and stored at -20 ^{0}C until analysis [74].

6.3. Intraduodenal administration

Rats are anaesthetized by an intraperitoneal injection of 60 mg/kg of thiopentone sodium (short acting anaesthetic agent). Small incision are made at abdomen, duodenum is located and similar formulations are administered directly into the duodenum with syringe. Blood samples are collected and processed as described in intravenous route[74].

6.4. Biodistribution studies

Tissue distribution studies are carried out in Swiss albino mice after a 7-day acclimatization period. At predetermined time points (like 15, 30, 45, 60, 90, 120, 180, 360 and 720 min) three animals at each time point from each group is given anaesthesia and blood is col-

lected via cardiac puncture. Tissues of interest (brain, liver, spleen, kidney, and heart) are collected immediately after cervical dislocation at different time points and they were blotted dry with tissue paper. Serum and tissue samples are frozen at -20 ^{0}C until analysis [74].

6.5. Serum and tissue sample analysis

Serum and tissue samples are evaluated. The method involves extraction of drug. The data is recorded and calculated using Winchrome software [74].

7. Pharmacokinetic analysis

Serum concentration versus time data for drug in individual rats are analyzed by non-compartmental estimations using WinNonlin software (version 1.1). Relative bioavailability (F) of SLNs are obtained. Maximum serum concentration (Cmax) and the time to reach Cmax (Tmax) are taken directly from the observed concentration versus time profiles. The area under the concentration– time curve (AUC) and the area under the first moment curve (AUMC) is calculated using the linear trapezoidal rule. Mean residence time (MRT) is determined by dividing AUMC by AUC. The relative bioavailability (Fr) is defined as ratio of AUC of drug loaded SLN to the AUC of other drug formulation when same doses are administered and calculated [74].

REFERENCES

1. Liversidge GG, Cundy KC. Particle size reduction for improvement of oral bioavailability of hydrophobic drugs: Absolute oral bioavailability of nanocrystallined danazol in beagle dogs. Int J Pharm 1995; 125:91-7.
2. Chowdary KPR, Rao AS. Nanoparticles as drug carriers. Indian Drugs 1997; 34(10):549-56.
3. Rao GCS, Kumar SM, Mathivanan N, Rao MEB. Advances in nanoparticulate drug delivery systems. Indian Drugs 2004; 41(7):389-95.
4. Vyas SP, Khar RK. Targeted and controlled drug delivery-Novel carrier systems. 1st ed. New Delhi: CBS publishers and distributors; 2002.
5. Kreuter J. Peroral administration of nanoparticles. Adv Drug Del. Rev. 1991; 7:71-86.
6. Olbrich C, Kayser O, Muller RH, Lipase degradation of Dynasan 114 and 116 solid lipid nanoparticles (SLN)—effect of surfactants, storage time and crystallinity, Int. J. Pharm. 237 (2002) 119– 128.
7. Yang S, Zhu J, Lu Y, Liang B, Yang C, Body distribution of camptothecin solid lipid nanoparticles after oral administration, Pharm. Res. 16 (5) (1999) 751–757.
8. Zara GP, Bargoni A, Cavelli R, Fundaro A, Vighetto D, Gasco MR, Pharmacokinetics and tissue distribution of idarubicin-loaded solid lipid nanoparticles after duodenal administration to rats, J. Pharm. Sci. 91 (5) (2002) 1324– 1333.
9. Jann MW, Clozapine, Pharmacotherapy 11 (3) (1991) 179– 195.
10. Venkateswarlu V, Manjunath K. Preparation, characterization and in vitro release kinetics of clozapine solid lipid nanoparticles. Journal of Controlled Release 95 (2004) 627– 638.
11. Gupta PK, Drug targeting in cancer therapy : A Clinical Prespective, J. Pharm. Sci., 79:949 (1990)
12. Zee cheng RKY, & Cheng CC, Delivery of anticancer drugs, Methods Find. Exp. Cin. Pharmacol., 11:439 (1989)
13. Flandroy PMJ, Grandfils C, & Jerome RJ, Clinical applications of microspheres in embolisation and chemoembolisation : A comprehensive review and perspectives, in Pharmaceutical Particulate carriers. Therapeutic applications (A. Rolland, ed) Marcell dekker, New York, 1993., 321
14. Kreuter J, Evaluation of nanoparticles as drug delivery systems: Materials, stability, toxicity, possibilities of targeting, & use, Pharm. Acta Helv., 58 : 242 (1983)
15. Jain RK, Transport of molecules across tumor vasculature, Cancer metastasis Rev ., 6 : 559 (1987)
16. Poste G, Drug targeting in cancer therapy, Receptor mediated Targeting of drugs Plenum press New York, 1984, 427
17. Arayne MS, Sultana N. Nanoparticles in drug delivery for the treatment of cancer; Pak J Pharm Sci, 2006 Jul; 19 (3) : 258-68
18. Brigger I, Dubernet C and Couvreur P, Nanoparticles in cancer therapy and diagnosis, Advanced Drug Delivery Reviews, 2002; 54 : 631-651
19. Koch AM, Reynolds F, Merkle HP, Weissleder R and Josephson L, Transport of surface modified nanoparticles through cell monolayers, ChemBioChem, 6 : 337-345, 2005
20. Allen. TM. Cullis PR. Drug Delivery Systems entering the mainstream. Science 303, 2004, 1818-18220
21. Moghimi SM., Hunter AC., Murray JC. Long Circulating and target specific nanoparticles: theory to practice. Pharmacol. Rev. 2001; 53, 283-318
22. Veerareddy, PR., Vobalabonia, V. Lipid based formulas of amphotericin B. Drugs today 40, 2004; 133-145
23. Lavasanifar, A, Samuel J, Sattari S. Kwon GS. Block copolymer micelles for the encapsulation and delivery of amphotericin B. 2002; Pharm. Res. 19, 418-422
24. Agarwaql AK, Gupta CM. Tufstin bearing liposomes in treatment of macrophage based infections. Adv. Drug delivery. Rev. 41, 2000; 135-146
25. McLean JW, Fox EA, Baluk P, Bolton PB, Haskell A, Pearlman R, Thurston G, Umemoto EY, McDonald DM. Organ-specific endothelial uptake of cationic liposome – DNA complexes in mice. Am. J. Physiol. 1997; 1273
26. Gabzon A, Shmeeda H, Barenholz Y. Pharmacokinetics of pegylated liposomal doxorubicin — review of animal and human studies. Clin. Pharmacokinetics 42, 2003; 419-436.
27. Chen Y, Dalwadi G, Benson HAE, Drug delivery across the blood–brain barrier, Cur. Drug Deliv. 1, 2004; 361–376.
28. Kante BCP, Dubois-Krack G, De Meester C, Toxicity of polyalkylcyanoacrylate nanoparticles, J. Pharm. Sci. 71, 1982; 786–789.
29. Limayem ICC, Fessi H, Purification of nanoparticle suspension by a concentration/diafiltration process, Pur. Techol. 38, 2004; 1–9.
30. Muller RH, Mäder K, Gohla S. Solid lipid nanoparticles (SLN) for controlled drug delivery—a review of the state of the art, Eur. J. Pharm. Biopharm. 50, 2000; 161–177.
31. Gohla SH, Dingler A. Scaling up feasibility of the production of solid lipid nanoparticles (SLN™), Pharmazie 56, 2001; 61–63.
32. Fundaro A, Cavalli R, Bagoni A, Vighetto D, Zara GP, Gasco MR, Non-stealth and stealth solid lipid nanoparticles(sln) carrying doxorubicin: pharmacokinetic and tissue distribution after i.v. administration to rats, Pharm. Res. 42 (4) (2000) 337–343.
33. Chen D, Yang T, Liang W, Zhang Q. In vitro and in vivo study of two types of long circulating solid lipid nanoparticles containing Paclitaxel, Chem. Pharm. Bull. 49, 2001; 1444–1447.
34. Reddy JS, Venkateshwarlu V. Novel delivery systems for drug targeting to the brain, Drugs of Future 29 (1), 2004; 63–83.
35. Wang JX, Sun X, Zhang ZR. Enhanced brain targeting by synthesis of 3',5'-dioctanoyl-5-fluoro-2'-deoxyuridine and incorporation into solid lipid nanoparticles, Eur. J. Pharm. Biopharm. 54 (3), 2002; 285–290.
36. Muller RH, Mäder K, Gohla S. Solid lipid nanoparticles (SLN) for controlled drug delivery—a review of the state of the art, Eur. J. Pharm. Biopharm. 50, 2000; 161–177.
37. Muller RH, Mehnert W, Lucks JS, Schwarz C, Muhlen AZ, Weyhers H, Freitas C, Ruhl D. Solid lipid nanoparticles (SLN) — an alternative colloidal carrier systems for controlled drug delivery, Eur. J. Pharm. Biopharm. 41, 1995; 62–69.
38. Muhlen AZ, Schwarz C, Mehnert W. Solid lipid nanoparticles for controlled drug delivery — drug release and release mechanism, Eur. J.

Pharm. Biopharm. 45, 1998; 149 -155.
39. Allen DD, Lockman PR, Oyewumi MO, Koziara JM, Roder KE, Mumper RJ. Brain uptake of thiamine-coated nanoparticles, J.Control. Release 93, 2003; 271–282.
40. Dingler A, Feste Lipid-Nanopatikel als kolloidale wirkstoff-tragersysteme zur dermalen Applikation, in PhD thesis. 1998: Berlin.
41. Diederichs JE, Muller RH. Liposomes in cosmetics and pharmaceutical products, Pharm. Ind. 56 (3), 1994; 267–275.
42. C. Freitas C, R.H. Müller RH. Effect of light and temperature on zeta potential and physical stability in solid lipid nanoparticles (SLN) dispersions, Int. J. Pharm. 168, 1998; 221–229.
43. Siekmann B, Westesan K. Submicron-sized parenteral carrier systems based on solid lipids, Pharm. Pharmacol. Lett. 1, 1992; 123–126.
44. Yang S, Zhu J, Lu Y, Liang B, Yang C. Body distribution of camptothecin solid lipid nanoparticles after oral administration, Pharm. Res. 16, 1999; 751–757.
45. Tabatt KSM, Olbrich C, Muller RH. Effect of cationic lipid and matrix lipid composition on solid lipid nanoparticles by solvent emulsification–diffusion technique, Eur. J. Pharm. Biopharm. 57, 2004; 155–162.
46. Gasco MR. Method for producing solid lipid microspheres having a narrow size distribution, Editor, U.S., 1993.
47. Chen Y, Lu LF, Cai Y, Zhu JB, Liang BW, Yang CZ. Body distribution in mice of intravenously injected camptothecin solid lipid nanoparticles and targeting effect on brain, J. Control. Release 59, 1999; 299–307.
48. Cavalli R, Caputo O, Gasco MR. Preparation and characterization of solid lipid nanospheres containing paclitaxel, Eur. J. Pharm. Biopharm.10, 2000; 305–330.
49. Chen LT, Weiss L. The role of the sinus wall in the passage of erythrocytes through the spleen, Blood 41, 1973; 529–537.
50. Moghimi SM, Porter CJH, Muir IS, Illum L, Davis SS. Nonphagocytic uptake of intravenously injected microspheres in rat spleen: influence of particle size and hydrophilic coating, Biochem. Biophys. Res. Commun. 177, 1991; 861–866.
51. Drenckhahn D, Wagner J. Stress fibers in the splenic sinus endothelium in situ: molecular structure, relationship to the extracellular matrix and contradictibility, J. Cell Biol. 102, 1986; 1738–1747.
52. Groom AC. Microcirculation of the spleen: new concepts, Microvasc. Res. 34, 1987; 269–289.
53. Moghimi SM, Hunter C, Murray JC. Long-circulating and target-specific nanoparticles: theory to Practice, Pharmacol Rev. 53, 2001; 283–318.
54. Oyewumi MO, Yokel RA, Jay M, Coakley T, Mumper RJ. Comparison of cell uptake, biodistribution and tumor retention of folate coated and PEG coated gadolinium nanoparticles in tumor bearing mice, J. Control. Release 95, 2004; 613–626.
55. Utreja S, Jain NK. Solid Lipid Nanoparticles. Advances in Controlled and Novel Drug Delivery, CBS Publihers & Distributors, New Delhi, 2001; 408–425.
56. Olivier JC. Drug transport to brain with targeted nanoparticles, NeuroRx 2 (1), 2005; 108–119.
57. Vasir JK, Reddy MK, Labhasetwar VD. Nanosystems in drug targeting: opportunities and challenges, Curr. Nanosci. 1 (1), 2005; 47–64.
58. Zara GP, Cavalli R, Bargoni A, Fundaro A, Vighetto D, Gasco PR. Intravenous administration to rabbits of non-stealth and stealth doxorubicinloaded solid lipid nanoparticles at increasing concentrations of stealth agent: pharmacokinetics and distribution of doxorubicin in brain and other tissues, J. Drug Target. 10 (4), 2002; 327–335.
59. Reddy JS, Venkateshwarlu V. Novel delivery systems for drug targeting to the brain, Drugs of Future 29 (1), 2004; 63–83.
60. Goppert TM, Muller RH. Polysorbate-stabilized solid lipid nanoparticles as colloidal carriers for intravenous targeting of drugs to the brain: comparison of plasma protein adsorption patterns, J. Drug Target. 13 (3), 2005; 179–187.
61. Manjunath K, Venkateshwarlu V. Pharmacokinetics, tissue distribution and bioavailability of nitrendipine solid lipid nanoparticles after intravenous and intraduodenal administration, J. Drug Target. 14 (9), 2006; 632–645.
62. Indu PK, Rohit B, Swati B, Vandita K. Potential of solid lipid nanoparticles in brain targeting. Journal of Controlled Release 127, 2008; 97–109
63. Westesen K, Bunjes H, Koch MHJ. Physicochemical characterization of lipid nanoparticles and evaluation of their drug loading capacity and sustained release potential, J. Control. Release 48, 1997; 223–236.
64. Westesen K, Siekmann B, Koch MHJ. Investigations on the physical state of lipid nanoparticles by synchrotron X-ray diffraction, Int. J. Pharm. 93, 1993; 189–199.
65. Jenning V, Gohla S. Encapsulation of retinoids in solid lipid nanoparticles, J. Microencapsul 18, 2001; 149–158.
66. Magenheim B, Levy MY, Benita S. A new in vitro technique for evaluation of drug release profile from colloidal carriers—ultrafiltration technique at low pressure, Int. J. Pharm. 94, 1993; 115–123.
67. Kreuter J. Physicochemical characterization of polyacrylic nanoparticles, Int. J. Pharm. 14, 1983; 43–58.
68. Harmia T, Speiser PP, Kreuter J. Optimization of pilocarpine loading on to nanoparticles by sorption procedures, Int. J. Pharm. 33, 1986; 45–54.
69. Zimmermann E, Liedtke S, Muller RH, Mader K. H-NMR as a method to characterize colloidal carrier systems, Proc. Int. Symp. Control. Release Bioact. Mater. 26, 1999; 591–592.
70. Jenning V, Gysler A, Schäfer-Korting M, Gohla SH. Vitamin A loaded solid lipid nanoparticles for topical use: occlusive properties and drug targeting to the upper skin, Eur. J. Pharm. Biopharm. 49, 2000; 211–218.
71. Liedtke S, Zimmermann E, Muller RH, Mader K. Physical characterization of solid lipid nanoparticles (SLNTM), Proc. Int. Symp. Control. Release Bioact. Mater. 26, 1999; 595–596.
72. Luo Y, Chen D, Ren L, Zhao X, Qin J. Solid lipid nanoparticles for enhancing vinpocetine's oral bioavailability. Journal of Controlled Release 114, 2006; 53–59
73. Ahlin P, Kristl J, Kristl A, Vrecer F. Investigation of polymeric nanoparticles as carriers of enalaprilat for oral administration, Int. J. Pharm. 239, 2002; 113–120.
74. Manjunath K, Venkateswarlu V. Pharmacokinetics, tissue distribution and bioavailability of clozapine solid lipid nanoparticles after intravenous and intraduodenal administration. Journal of Controlled Release 107, 2005; 215–228.

Source of support: Nil, Conflict of interest: None Declared