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Review

Solid lipid nanoparticles as new drug delivery system

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The main challenge in cancer chemotherapy is toxic side-effects induced by chemotherapeutic drugs. Thus, alternative methods of drug administration like appropriate drug carrier system is needed to overcome this problem. The main objective of new drug delivery systems is to improve the anti-tumor efficacy of drug and reduce their toxic effects on normal tissues. Recently Solid Lipid Nanoparticles (SLN) as colloidal particulate drug delivery system have received much attention from drug development researchers. The use of solid lipid nanoparticle opens up new perspectives for the formulation of poorly soluble drugs. The SLN is a very complex system with some advantages and disadvantages over other colloidal carrier systems. Tamoxifen, an antiestrogen molecule and strong hydrophobic drug is used as a chemotherapy drug against breast cancers. When tamoxifen encapsulated within solid lipid nanoparticles, it is like free TAM display antitumoral activity against human breast cancer cells. The biological availability of drug is not affected when incorporated into SLN. Therefore SLN could be applied as a drug delivery system for cancer treatments.

Key words: Drug delivery systems, solid lipid nanoparticles.

INTRODUCTION

Breast cancer is one of the most important health concerns of the modern society (Ferlay et al., 2007). Worldwide, it is estimated that over one million new cases of breast cancer were diagnosed every year and more than 400,000 will die from the breast cancer (Coughlin and Ekwueme, 2009). The life-time risk in women contracting breast cancers was estimated to be 1 in 8, which is the highest among all forms of cancers (DevCan5.2, 2004). Although, the mortality rates from breast cancers have decreased in most developed countries because more frequent mammographic screening and extensive use of tamoxifen, it still remains the second highest in women (Clark, 2008). The main options for breast cancer treatment include surgery, radiation therapy and chemotherapy (Mirshahidi and Abraham, 2004). Surgical procedures usually lead to significant morbidity such as lymph edema, muscle wasting, neuropathy and chronic pain (Paci et al., 1996). Radiation therapy is useful for cancer which is more localized, but it also carry a number of acute and chronic side- effects such as nausea, diarrhea, pain and fatigue (Ewesuedo and Ratain, 2003). Endocrine therapy may be used as a supplementary treatment; this method of therapy is applied to specific group of patients, for example women after menopause with hormone-responsive disease (Gradishar, 2005). In hormone-sensitive cancer patients receive chemotherapy with cytotoxic drugs. The cytotoxic drugs treat cancers by causing cell death or growth arrest. Effective cancer chemotherapy is able either to shrink a tumor or to help destroy cancer cells (Ewesuedo and Ratain, 2003).

A number of obstacles such as drug toxicity, possible undesirable drug interactions and various forms of drug resistance have to be overcome to achieve effective chemotherapy (Cardosa et al., 2009). Drug resistance is a general problem in the chemotherapy of several cancers including breast cancers (Wong et al., 2006). Failures in treatment of cancers are common. Development of new drugs is also slow to progress. Among the reasons contributing to this are weak absorption, high rate of metabolism and elimination of

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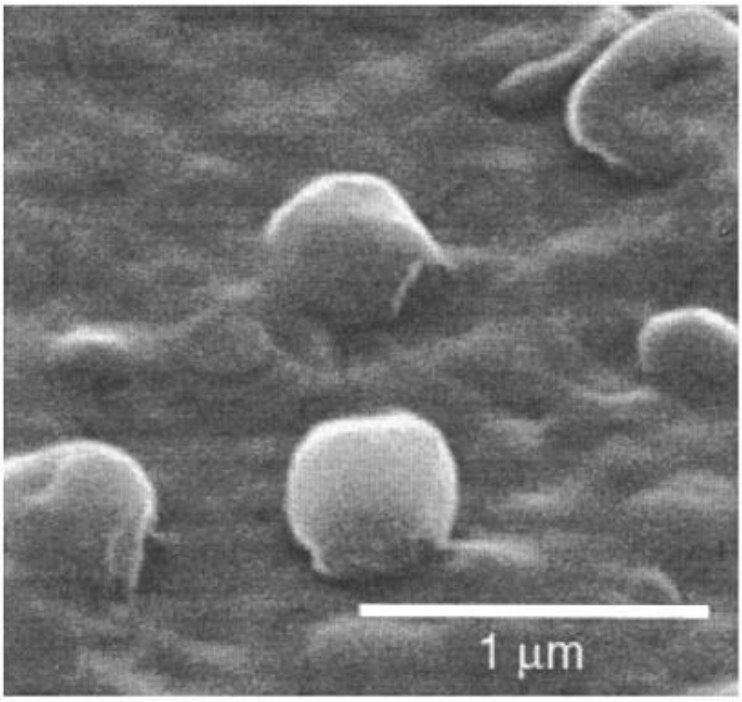

Figure 1. Electron microscopy picture of solid lipid nanoparticles made from Compritol® stabilized with poloxamer 188, diameter 400 nm (Müller et al., 2000).

drugs per oral administration resulting in less or variable concentrations in blood, poor drug solubility, unpredictable bioavailability of oral drugs due to food and tissue toxicity (Sipos et al., 1997). Thus, alternative methods of drug administration like appropriate drug carrier system is needed to overcome this problem. Targeting of unhealthy tissues and organs of the body is one of the important challenges of the drug delivery systems (Kayser et al., 2005). Depending on the route of administration, the size of drug carriers may range from a few nanometers (colloidal carriers), to micrometers (microparticles) and to several millimeters (implants). Among these carriers, nanoparticles had shown great promise for parenteral application of chemotherapeutic drugs (Mehnert et al., 2001). Nanoparticles seem to show promise as a drug targeting systems supplying drug to target tissues at the right time (Kayser et al., 2005). Nanotechnology has found application in drug delivery because with drug-loading of nanoparticles, delivery becomes effective and more specific (Brannon-Peppas and Blanchette, 2004).

Particulate colloidal delivery systems such as liposomes, nanospheres and nanocapsules have been developed and are now being studied as new carriers for drugs and vaccines (Brigger et al., 2002; Koping-Hoggard et al., 2005). These new delivery systems can be powerful, particularly in intravenous administration because they can target the macrophages of the reticuloendothelial system. The targeting of macrophages using these systems is due to the opsonization of the particles and recognition by the macrophages. Among the applications of these systems are in treatment of malignancies and infectious diseases (Youssef et al., 1988). The main objective of new drug delivery systems is to improve the anti-tumor efficacy of drug and reduce their toxic effects on normal tissues. Nanoparticle is expected to be able to diminish toxicity of chemotherapy drug. Nanoparticles based on lipids that are solid at room temperature, namely solid lipid nanoparticle (SLN) using physiological well-tolerable lipids have potentially wide application (Figure 1) (Müller et al., 2000).

Solid lipid nanoparticles (SLNs)

Beginning from early 1990s, pharmaceutical researches began to shift towards production of nanoparticles from solid lipids which were named the solid lipid nanoparticles (SLN) or lipospheres or nanospheres (Siekmann and Westesen, 1994). The SLN is a drug delivery system that loads lipophilic or chemically unstable drugs. This system for drug delivery has all the advantages of other developed delivery systems that are physical stability, protection of encapsulated labile drugs from degradation, controlled-release and high tolerability. Among the advantages of SLN are high potential for management of

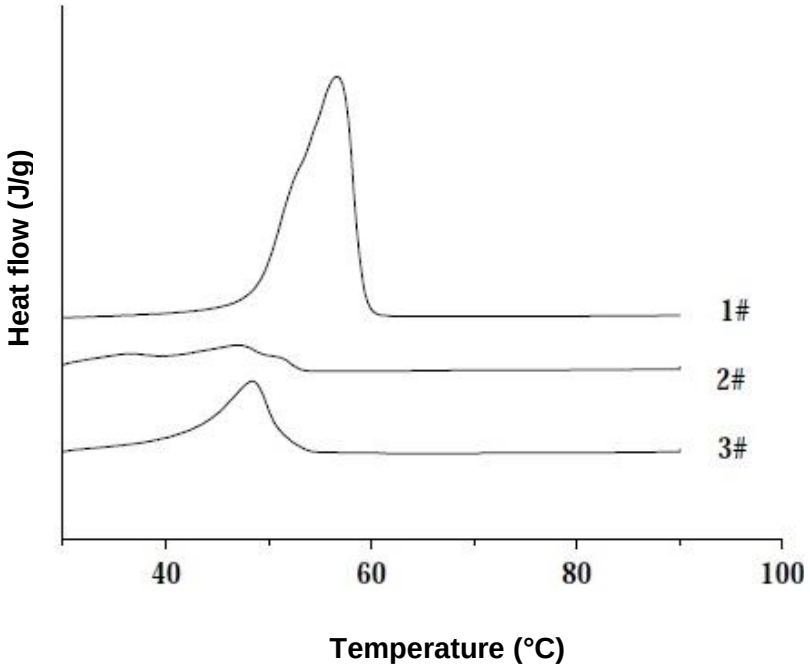

Figure 2. DSC scan of lyophilization SLNs powder heating from 301 to 901°C at a rate of 5 k/min. 1#-Bulk matrix material, 2#-Placebo SLNs, 3#-drug loaded SLNs powder containing 2 mg/ml model drug (Hou et al., 2003).

drug release and drug targeting, high stability for drug loading and high capacity for drug payload. This delivery system makes possible the encapsulation of lipophilic and hydrophilic drugs without the toxic effect of the carriers. SLN also avoids the use organic solvents and has potential for large scale production. One of the major advantages of SLNs as a drug carrier is the high rate of drug entrapment efficiency (EE) that can result in long-term physical stability of the drug. The crystalline structure of the lipid phase of SLN that represents its chemical nature plays an important role in determining whether or not a guest molecule can be strongly encapsulated within delivery system. If the lipid structure of the nanoparticle delivery system is composed of a high crystalline structure with perfect lattice, the incorporated drug would be expelled. In other words, the existence of imperfect crystalline structure or lattice in the lipid phase of SLN makes it possible for stronger drug incorporation. Therefore, the lipids with lesser ordered arrangements in the chemical structure of SLN would be useful for high-rate drug loading.

This observation is supported by another study using differential scanning calorimetric (DSC) and x-ray diffraction (XRD) measurements which show that most of the SLNs have less ordered arrangement crystalline structure favoring increasing capacity drug-loading. Hou et al. (2003) showed when the bulk matrix material is turned into SLNs, the melting point was depressed no matter whether it was drug-free or not (Figure 2). The decrease of the onset and maximum temperature could be attributed to the small size effect and explained by the Thomson equation. Also the presence of the reflections in X-ray diffraction pattern of the SLN compared to the bulk materials (Figure 3) confirm that lipid within nanoparticles are arranged in low ordered crystalline structure. It was clear that in the drug-free SLNs, the amorphous state would contribute to the higher drug loading capacity. However, several disadvantages are associated with SLNs to include particle growth, particle aggregation, unpredictable gelation tendency, polymorphic transitions, burst drug release and inherently low incorporation capacities due to the crystalline structure of the solid lipid (Mehnert and Mäder, 2001).

PRODUCTION OF SOLID LIPID NANOPARTICLES

Ingredients

The ingredients used for production of SLN include solid lipids, emulsifiers and water. The lipids used cover a wide range from triglycerides (for example tristearin), partial glycerides (for example imwitor) and fatty acids (for example stearic acid), to steroids (for example cholesterol) and waxes (for example cetyl palmitate). Many emulsifiers of varying charges and molecular weights can potentially be used to stabilize the lipid dispersion. If a combination of certain emulsifiers were

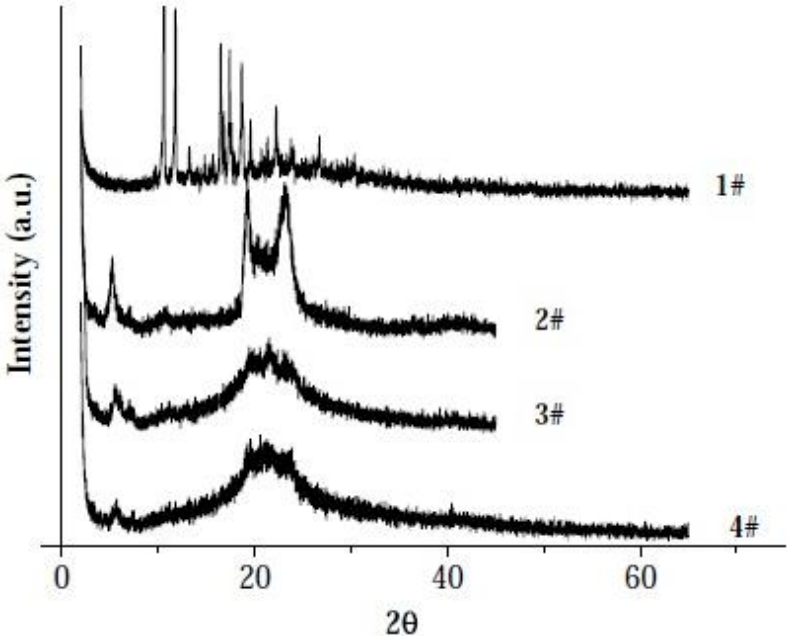

Figure 3. X-ray diffraction pattern. 1#-Pure mifepristone (model drug), 2#-bulk material, 3#-Placebo SLNs, 4#-lyophilization SLNs powder containing 2 mg/ml model drug (Hou et al., 2003).

used together, particles aggregation may be prevented or minimized. Physiological lipids which are used for production of SLN will reduce acute and chronic toxicity of chemical components (Abbasalipourkabir et al., 2011a). Emulsifiers are generally toxic and rarely used for parenteral administrations. Therefore the use of this ingredient in production of SLN should take into consideration their toxic effects and route of administration. It has been shown that formulations with a mixture of surfactants would produce a more stable SLN with lower particle size than formulations with only one surfactant. Table 1 shows ingredients that are generally used in the production of SLN.

Lyophilization is the best way to increase chemical and physical stability for long-term storage of SLN and for improvement of SLN-incorporation into pellets, tablets and capsules (Mehnert and Mäder, 2001).

Technique

The most common production technique of SLNs are high-pressure homogenization (HPH) of lipids in the fluid phase, precipitation of microemulsions, high-shear homogenization combined with ultrasound, solvent emulsification/evaporation and microemulsion techniques. The HPH is the predominant production method because it is easy to handle and scale-up. Scale up methods has been developed to produce SLN in commercial quantities (Copland et al., 2005; He et al., 2007). High pressure homogenization (HPH) is a relevant method for the preparation of SLN and can be conducted above or below room temperature (hot or cold HPH technique) (Schwarz et al., 1994). Homogenization provides cavitations forces which break down the particles into smaller sizes (Schwarz and Mehnert, 1997). Harivardhan et al. (2006) found homogenization at 15,000 psi for 3 cycles resulted in smaller sized nanoparticles. High pressure leads to increase particle size. It is postulated that at high homogenization pressure, the particles may have coalesced as a result of the high kinetic energy of the particles which impaired the homogenization process.

Increasing the kinetic energy increases particle collision and consequently resulting in coagulation. High particle collisions also distort the surfactant film coating the particle surface and hence, enhance particle aggregation (Siekmann and Westesen, 1994).

CHARACTERIZATION OF SLNS

Characterization of SLN is necessary for control of the quality of the product. The parameters in the characterization of SLN are directly involved in stability and release kinetics are particle size, particle size distribution (PI) and zeta potential, degree of crystallinity and lipid modification, co-existence of additional colloidal structures like micelles, liposomes, supercooled melts, drug nanoparticles and dynamic phenomena (Laggner, 1999). Particle size and particle size distribution are

Table 1. Lipids and emulsifiers for preparation of SLN.

Lipids	Emulsifiers/coemulsifiers
Triglycerides	
Tricaprin	Soybean lecithin
Trilaurin	Lipoid® S75, Lipoid® S100)
Trimyristin	Egg lecithin (Lipoid® E80)
Tripalmitin	Phosphatidylcholine
Tristearin	Epikuron® 170, Epikuron® 200)
Hydrogenated coco-glycerides	
(Softisan®142)	Poloxamer 188
	Poloxamer 182
	Poloxamer 407
	Poloxamine 908
Hard fat types	
Witepsol W 35	
Witepsol H 35	Tyloxapol
Witepsol 42	Polysorbate 20
Witepsol E 85	Polysorbate 60
Glyceryl monostearate (Imwitor®900)	Polysorbate 80
Glyceryl behenate (Comporitol 888®ATO)	Sodium cholate
Glyceryl palmitostearate (Precirol®ATO5)	Sodium glycocholate
Cetyl palmitate	Taurocholic acid sodium salt
Stearic acid Palmitic acid	Taurodeoxycholic acid sodium salt
Decanoic acid	Butanol
Behenic acid	Butyric acid
Acidan N12	Dioctyle sodium sulfosuccinate
	Monooctylphosphoric acid sodium

Mehnert and Mäder (2001).

important factors in the physical stability of nanodispersions (Attama and Müller-Goymann, 2007). Schubert and Müller-Goymann (2005) showed in the preparation of SLN, at least 10% emulsifier such as lecithin should be incorporated in the lipid matrix. As lecithin concentration increased, particles size decreased to 95.5 ± 13.1 nm. The presence of lecithin within the multilayer structure of the particle surfaces provides interface between oil and water (Westesen and Siekmann, 1997). Since lecithin has limited mobility, aggregation of particles that could lead to particle growth does not occur (Heiati et al., 1996). Schubert and Müller-Goymann (2005) also found high concentrations of lecithin content lead to significant increases in particle sizes. It is possible that beyond this critical concentration of lecithin, presence of other colloidal structure within the aqueous phase and/or around the particles will cause increased mobility of lecithin resulting in particle size increase (Westesen and Wehler, 1993).

A similar result obtained high concentrations of poloxamer 188 was incorporated into the SLN (Sanjula et al., 2009). This again may be attributed to the increase in particle size as well as reduction in hydrophobicity. Small particle sizes and presence of emulsifiers may retard lipid crystallization and modification changes (Mehnert and Mäder, 2001).

Incorporation of drugs in SLNs

Incorporation of the drug into a particulate carrier can protect it from degradation *in vitro* and *in vivo* (Figure 4). The release of the incorporated drug can be controlled and greater specific targeting is possible (Müller and Keck, 2004). A potent delivery system should be able to load drug with high capacity. The incorporated drugs can be accommodated between the fatty acid chains, lipid bilayers or in imperfect spaces. The drugs are usually located in the core of the particles or shell or molecularly dispersed throughout the matrix. The location of drugs in SLN is generally depending on ratio of drug to lipid and solubility of drug in lipid. The solid lipid core of SLN has the capability to increase the chemical stability of incorporated drugs (Müller and Bohm, 1998). The

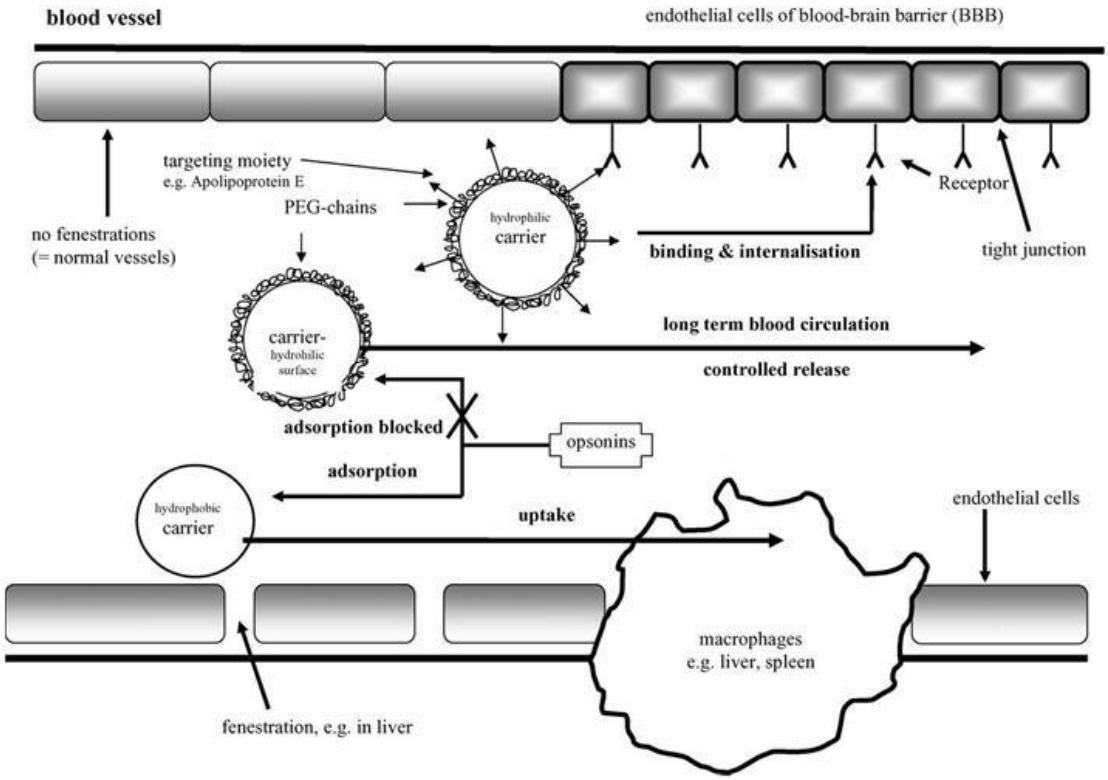

Figure 4. Approaches to achieve passive targeting, long-circulating carriers for prolonged drug release and target-specific carriers (Müller and Keck, 2004).

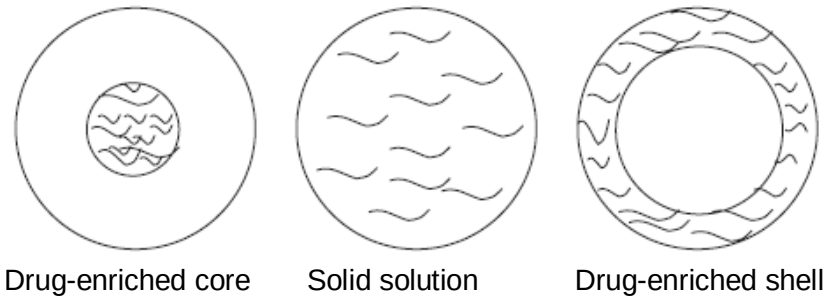

Figure 5. Proposed structural models for drug loading in SLN (Mühlen et al., 1998).

proposed structural models are shown in Figure 5. Abbasalipourkabir et al. (2011b) studied the incorporation of an antiestrogen molecule and strong hydrophobic drug, tamoxifen (TAM) in palm oil SLN. They found the palm oil which consist of triglyceride mixture of natural fatty acids produce the highest entrapment efficiency (89.98 to 90.25%). Increasing drug concentration led to significant increase ($P<0.05$) in drug loading (DL) (Figure 6) and significant decrease ($P<0.05$) in entrapment efficiency (EE) (Figure 7). These effects are due to the reduced SLN dispersions and higher solubility of the drug in higher lipid concentrations.

Factors affecting loading capacity of drug in nanoparticles include solubility of drug in melted lipid, miscibility of drug and lipid melt, chemical and physical structure of solid lipid matrix and polymorphic state of lipid material. Therefore, high solubility of the drug in the lipid matrix leads to adequate loading capacity (Westesen et al., 1997). Increasing lipid matrices lead to increased EE and DL suggesting that greater solubility of the drug in the higher lipid concentrations (Harivardhan et al., 2006). Mühlen et al. (1998) reported that drug incorporation can accelerate the transformation to the stable modification in comparison to drug free particles. However, when

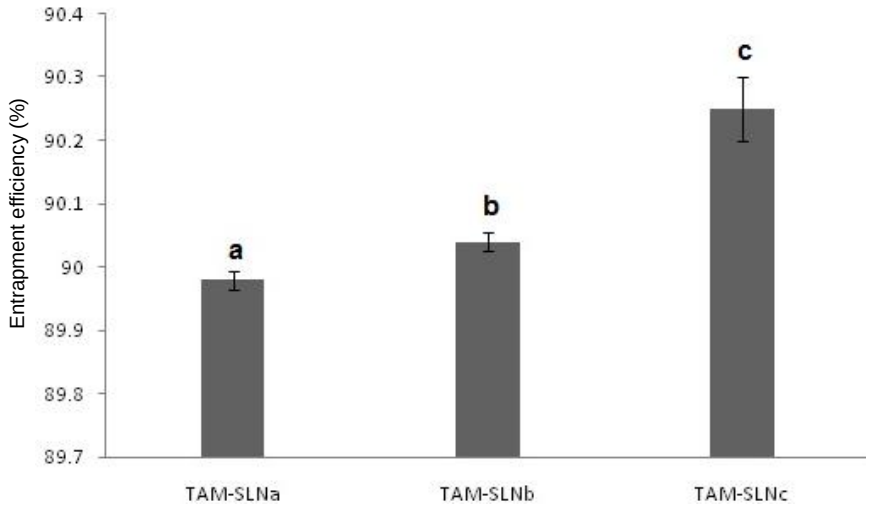

Figure 6. Entrapment efficiency of tamoxifen-loaded solid lipid nanoparticles. TAM-SLNa, TAM-SLNb and TAM-SLNc represent 1 mg TAM incorporated in 5, 10 and 20 mg SLN respectively. Means with superscripts a to c are significantly different ($p<0.05$), ($n = 5$) (Abbasalipourkabir et al., 2011b).

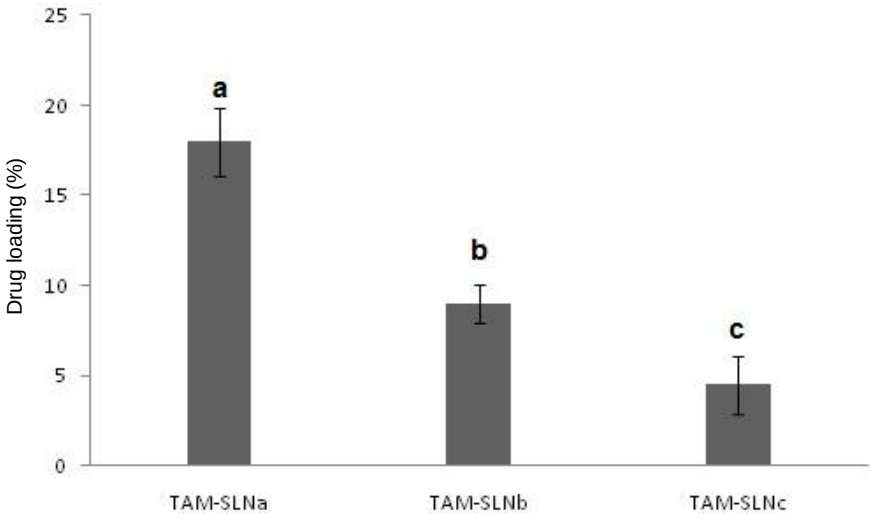

Figure 7. Drug loading of tamoxifen-loaded solid lipid nanoparticles. TAM-SLNa, TAM-SLNb and TAM-SLNc represent 1 mg TAM incorporated in 5, 10 and 20 mg SLN respectively. Means with superscripts a to c are significantly different ($p<0.05$), ($n = 5$) (Abbasalipourkabir et al., 2011b).

thermodynamic stability and lipid packing density increase, drug incorporation rates decrease in the order: supercooled melt, α-modification, ß–modification, β'-modification (Müller et al., 2000).

Release of drugs from SLNs

The profile of drug release depends on some parameters such as modification in lipid matrix, surfactant concentration and production conditions. The probable mechanism for drug release from SLN is that drug loading in SLN is associated with the partitioning of the drug in the water phase. During the cooling step that phase separation occurs, the lipid precipitates and partitions into liquid-lipid phase. The drug which is present at high concentrations in the outer shell of the nanoparticles crystallizes. If the drug is primarily loaded in the outer shell of the particles, a burst release mechanism may occur. Huang et al. (2008) reported that

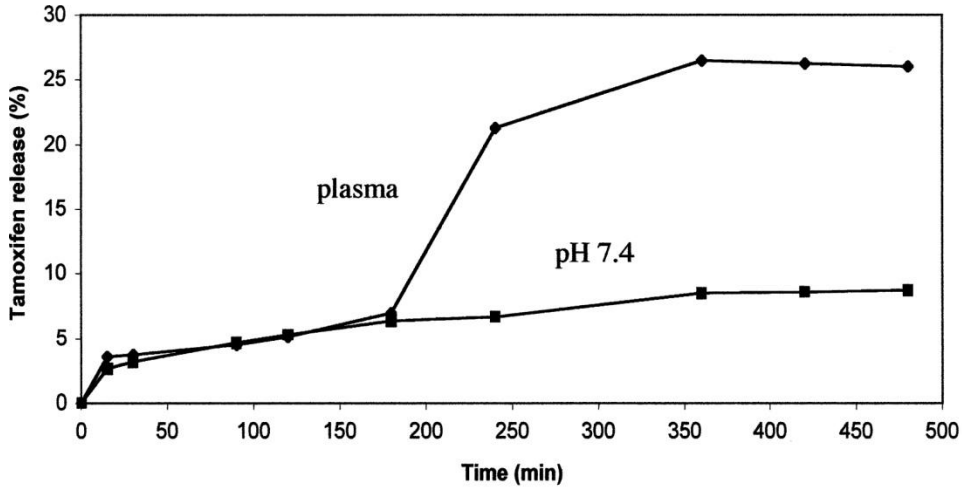

Figure 8. Release profiles of tamoxifen at pH 7.4 and in plasma from palmitic acid nanoparticles. Each value is the mean of three experiments (Fontana et al., 2005).

SLN made of 'precirol' may have the potential to serve as a delivery system for parenteral camptothecin administration because of the sustained drug release, strong cytotoxicity, limited hemolysis and good storage stability.

Memisoglu-Bilensoy et al. (2005) obtained a delay for tamoxifen citrate pre-loaded nanospheres based on 'amphiphilic h-cyclodextrin' that liberate the drug within 6 h while Fontana et al. (2005) showed the release rate of tamoxifen from SLN based on 'palmitic acid' is quite low until 3 h after the beginning of the experiment and then the successively release rates increase quickly and the amount of drug released reaches 21% after 4 h and 26% after 6 h (Figure 8) suggesting other factors such as large surface area, high diffusion coefficient (small molecular size), low matrix viscosity and short-diffusion distance of the drug are also contributory to the fast release of the drug (Mühlen et al., 1998).

Antitumor efficacy of drug-loaded SLNs

The basis of drug-loaded SLN is to obtain the necessary dose of drug at tumor location for a known period of time and reducing adverse effects on normal organs in the body (Chawla and Amiji, 2003). According to Abbasalipourkabir et al. (2010), tamoxifen-loaded SLN has similar effect on the tumor as free TAM which promotes apoptosis in the rat mammary gland tumor. The efficacy of free-TAM and TAM-loaded SLN is similar. However, the TAM-loaded SLN show a more prolonged effect suggesting that incorporation of TAM in SLN is suitable for delayed drug release in the chemotherapy of breast cancers while decreasing the hepatotoxic effects. According to Fontana et al. (2005), *in vitro* anti-proliferative activity of SLNs containing tamoxifen on MCF-7 cell line (human breast cancer cells), maintain an antitumoral activity comparable to free drug (Figure 9). These results, demonstrate that drug activity is not reduced in the presence of nanoparticles carrier.

SUMMARY AND OUTLOOK

The use of solid lipid nanoparticle (SLN) opens up new perspectives for the formulation of poorly soluble drugs. The SLN is a very complex system with some advantages and disadvantages over other colloidal carrier systems. Our study showed that lipophilic drugs can be encapsulated into the SLN formulations. However, further research should be conducted to determine the structure and dynamics of drug-loaded SLN at molecular level, so as to further understand the mechanism of drug delivery using this system. This study may be conducted using NMR, ESR and synchrotron irradiation techniques. This will throw some light on the question whether the drug is really incorporated in the solid lipid or the lipid and drug nanosuspensions coexist in the formulation. The lipid matrix of SLN is more mobile than polylactide-co-glycolide based nanoparticles and diffusion of the drug within the lipid matrix is limited, therefore the mechanism of controlled-release of drug and factors influencing release need to be further investigated. Further study is needed to determine the interaction (adsorption, desorption processes, enzymatic degradation, agglomeration) of SLN with biological environment. A better understanding of the plasma protein adsorption patterns and the *in vivo* fate of this drug delivery system need to be further studied to ascertain effectiveness of tissue-targeting by the drug.

The main challenge in cancer chemotherapy is toxic side-effects induced by chemotherapeutic drugs. Single

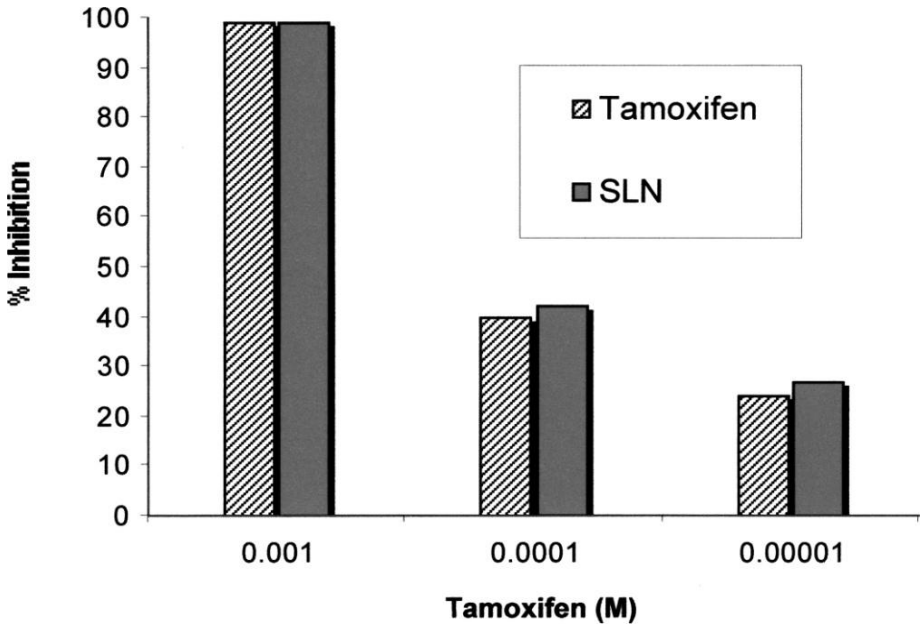

Figure 9. Antiproliferative activity of free tamoxifen and tamoxifen-loaded SLN on human breast cancer MCF-7 cells. Each value is the mean of three experiments (Fontana et al., 2005).

dose or short-time application (1 to 2 weeks) will probably cause serious health problems, but the use of biodegradable nano-sized particles for long-term or life-time therapy may produce other serious side-effects. Increasing the encapsulation efficiency of poorly water-soluble molecules will lead to the development of improved SLN formulations. In the near future, it is expected that more studies will focus on improving SLN and drug-loaded SLN formulations to increase the efficacy and reduce the side-effects of chemotherapeutic drugs for anticancer treatment. These studies should include preparation of formulations with different particle size and distributions, different matrix lipids and additional ingredients. Thus, if nanoparticulate drug delivery systems to be used effectively and routinely, the matter of toxicity of the components of nanoparticles must be addressed. Thus, SLN warrants further development before it can be used as a new drug delivery system for chemotherapy drugs in treatment of human cancers.

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