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Tamoxifen Drug Loading Solid Lipid Nanoparticles Prepared by Hot High Pressure Homogenization Techniques

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Abstract: As drug delivery systems Nanoparticulate widely investigated because of many advantages such as smaller size, controlled drug release potential, targeting ability, enhancement of therapeutic efficacy and reduction of toxicity. So, Solid Lipid Nanoparticles have recently received considerable attention as alternative drug delivery carrier. In this study Solid Lipid Nanoparticles (SLNs) containing tamoxifen, nonsteroidal antiestrogens have been loaded and to be used as breast cancer therapy, were prepared by hot High Pressure Homogenization techniques. Tamoxifen loaded SLNs seem to have dimensional properties useful for parenteral administration. Preliminary study of antiproliferative activity *in vitro*, carried out on MCF-7 cell line (human breast cancer cells), demonstrated that SLNs, containing tamoxifen showed an antitumoral activity comparable to free drug. Tamoxifen loaded SLNs seem to have dimensional properties useful for parenteral administration. SLN was characterized by Differential Scanning Calorimetery (DSC), Transmission Electron Microscopy (TEM), Zeta Potential and Particle Size. The results of characterization studies strongly support the potential application of Tamoxifen-loaded SLNs as a carrier system. The SLN presented here are well suited for certain drug delivery applications, particularly breast cancer therapy.

Key words: Solid lipid nanoparticles, high pressure homogenization, drug loading, tamoxifen, cytotoxicity, breast cancer

INTRODUCTION

Recently, it has become more and more evident that the development of new drugs alone was not sufficient to ensure progress in drug therapy. Exciting experimental data obtained *in vitro* were very often followed by disappointing results *in vivo* because of the insufficient drug concentration due to poor absorption, rapid metabolism and elimination, poor water solubility and high fluctuation of plasma levels due to unpredictable bioavailability after peroral administration. A promising strategy to overcome these problems involves the development of suitable drug carrier systems. Solid lipid nanoparticles were developed at the beginning of the 1990s as an alternative carrier system to the existing traditional carriers, such as emulsions, liposomes and polymeric nanoparticles. The SLNs combined the advantages of other innovative carrier systems (e.g., physical stability, protection of incorporated labile drugs from degradation, controlled release and excellent tolerability) while at the same time minimizing the associated problems. Lipophilic drugs, with good compatibility with the lipids, have often been selected to incorporate into the SLNs for high drug loading and entrapment efficiency[1,15,17]. Many methods have been developed to prepare SLNs, such as high pressure homogenization[8-10,14], solvent emulsification or evaporation[4,14], high speed stirring ultrasonication and solvent diffusion method[6,7]. The high pressure homogenization technique has emerged as a reliable and powerful technique for preparation of SLNs. In contrast to other techniques, scaling up represented no problem in most cases. Two general approaches of the homogenization step, the hot and the cold homogenization technique, can be used for the

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production of SLNs. Cold homogenization technique has been developed to overcome the following three problems of the hot homogenization technique[12]: (1) temperature-induced drug degradation; (2) drug distribution into the aqueous phase during homogenization; (3) complexity of the crystallization step of the nanoemulsion leading to several modifications and/or supercooled melts. Oral administration of the nonsteroidal antiestrogen Tamoxifen (partial agonist/antagonist) is today the endocrine treatment of choice for selected patients regardless of the stage of their breast cancer[11]. Despite being quite effective, tamoxifen can have harmful long term side effects such as the development of endometrial cancer, or an acquired Tamoxifen resistance leading to further tumor progression[11]. Thus the aim of this study was to prepare SLNs loading tamoxifen by hot homogenization technique and to determine the physicochemical properties of obtained tamoxifen-loaded SLNs, such as particle size, zeta potential, surface morphology, drug loading capacity and drug entrapment efficiency.

MATERIALS AND METHODS

Lipid Matrices (LM) preparation: Present study was conducted in the Universiti Putra Malaysia April, 2004. Hydrogenated palm oil softisan 154, solid lipid triglyceride mixture, was donated from Condea (Witten, Germany). Phospholipon 90 H is a completely hydrogenated lecithin from Phospholipid (Cologne, Germany). The lipid matrix composition was varied by incorporation of 0-50% phospholipon 90 H (w/w) within Softisan 154. Oleyl alcohol long-chain fatty alcohols and thimerosal used as a preservative were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). The mixtures were stirred with a teflon coated magnet at 80°C until a transparent yellow solution was obtained. The solution was stirred at room temperature until solidification. Bidistilled water was used for all preparations and all chemicals were of pharmacopeial or reagent grade.

Solid Lipid Nanoparticles (SLN) preparation: Basic formulation of SLN contained 10% of hydrogenated palm oil Softisan 154 and hydrogenated lecithin (lipid matrix), 1% oleyl alcohol, 0.005% thimerosal and 89% bidistilled water (all w/w). All components were weighted into sealed containers and heated to 80°C. Thereafter a preemulsion was produced using an ultra turrax (Ika/Staufen, Germany) at 10,000 rpm for 10 min. The hot preemulsions were homogenised at a pressure of 1000 bar and a temperature of about 70°C with an EmulsiFlex^{w}-C5 (Avestin, Canada) high pressure homogeniser for 10 cycles. Subsequently, the dispersions were allowed to recrystallise at room temperature[19].

Tamoxifen loaded with Solid Lipid Nanoparticles (SLN) preparation: SLN were prepared with 300 mg tamoxifen and phospholipon 90 H (150 mg) was kept constant while the amount of hydrogenated palm oil softisan 154 was varied (600 and 900 mg). All components were weighted into sealed containers and heated to 80°C. Tamoxifen was added to aqueous solution of phospholipon 90 H, warmed to 98°C and added to Hydrogenated palm oil Softisan 154 heated to the same temperature. The procedure to evaluate the amount of tamoxifen loaded in the nanoparticles was as follows 5 mg of freeze-dried nanoparticles were solubilized in 20 mL of CH3OH. The organic solutions were filtered through Millex SR 0.45 μm filter unit (Millipore Corporation, MA, USA) and analyzed by HPLC to determine the amount of drug. No interaction was observed between drug molecules and filter membrane. Results were expressed as the percentage of the drug amount contained in 100 mg of dried material.

High Performance Liquid Chromatography (HPLC): Drug loading was evaluated by isolating the SLNs, dissolving in methanol and conducting HPLC (using a C18 column and acetonitrile 50 mM^{-1} phosphate buffer (pH 3) (58/42, v/v) as a mobile phase at the flow rate of 2 mL min^{-1}. UV detection for tamoxifen was conducted at 254 nm. Drug loading and cytotoxcity was calculated based on the amount of tamoxifen identified in a measured amount of SLN.

Determination cytotoxicity of tamoxifen loaded nanoparticles against MCF-7 cells: According to[6] cytotoxicity determination have been done with nor modification. MCF-7 cells were maintained in DMEM supplemented with 10% Fetal Calf Serum at 37°C in a humidified incubator containing 5% CO_2. Confluent cell monolayers were trypsinized and cells in exponentially growing phase were used in cytotoxicity experiments. The cytotoxicity of tamoxifen loaded nanoparticles against MCF-7 cells was assessed using MTT assay. MTT assay measures the ability of viable cells to reduce a water-soluble, yellow tetrazolium salt (MTT) into a purple, insoluble formazan product. The color reaction is used as a measure of cell viability and proliferation. MCF-7 cells in culture medium were seeded in 96-well plates and following incubation for adherence at 37°C in 5% CO_2, 20 μL of drug solution

or formulations was added into the wells. After 48 h incubation, 20 μL MTT solutions (5 mg mL^{-1}) was added to each well and the plates were incubated for further 2 h. The solution in each well containing media, unbound MTT and dead cells was removed by suction and 100 μL of DMSO was added to each well. The plates were then shaken and the Optical Density (OD) was read on ∑960 ELISA reader at test wavelength of 570 nm. Cells incubated in culture medium alone served as a control for cell viability (untreated wells).

Physiochemical properties:

Transmission Electron Microscopy (TEM): The morphology of the SLN was examined by TEM (Hitachi, Japan). The samples were stained with 2% (w/v) phosphotungstic acid for 30 s and placed on copper grids with films for viewing.

Particle size measurement: The average diameter and Polydispersity Index (PI) of SLN were determined by Photon Correlation Spectroscopy (PCS) using a Zetasizer 3 (Malvern, UK) at a fixed angle of 90°C and at 25°C. The aqueous SLN dispersions were diluted with distilled water before analysis. Each value is the average of 3 measurements.

Zeta potential measurement: The particle charge was quantified as Zeta Potential (ZP) using a Zetasizer 4 at 25°C. Before measuring, each sample had to be diluted with demineralized particle free water to an adequate intensity. Each measurement was performed at least in triplicate. The pH values of the samples were always between 6.2±0.9.

Differential scanning calorimetry: Differential Scanning Calorimetry (DSC) was performed with a Mettler DSC 822e (Mettler Toledo, Greifensee, Switzerland). Samples containing ~10 mg nanoparticle dispersions (identical to 1-2 mg of solid lipid) were weighed accurately into standard aluminum pans using an empty pan as a reference. DSC scans were recorded at a heating and cooling rate of 5°C min^{-1}. The samples were heated from 25-85°C and cooled from 85-20°C under liquid nitrogen. Enthalpies were calculated using the Mettler Star software.

RESULTS

Cytotoxicity activity and drug loading capacity: Cytotoxicity of tamoxifen from nanoparticles was assessed against MCF-7 cells. It is shown in Fig. 1 that loaded is non-cytotoxic Loaded of SLN displayed a low cytotoxic activity for tamoxifen solution in acetone. Table 1 and Fig 1 shown the drug EE% of SLNs. The data showed EE% high 90.27%. SLNs produced by this production method could also achieve higher drug incorporation for lipophilic drugs like tamoxife. The evaluation of the drug loaded into nanoparticles was performed dissolving known amount of drug-loaded SLNs into methanol and analyzing obtained solution by HPLC.

Transmission electron microscopy: Figure 2 shows the shape of the nanoparticles entrapping with the model drug. It was evident that the particles investigated revealed round and homogeneous shading, the particle size ranging approximately from 50-250 nm.

Table 1: Drug Loading (DL) and Entrapment Efficiency (EE) of different ratios of tamoxifen loaded SLN

Concentration of SLN	DL (%)	EE (%)
4 mg	18.0	90.005
8 mg	9.0	90.060
16 mg	4.5	90.270

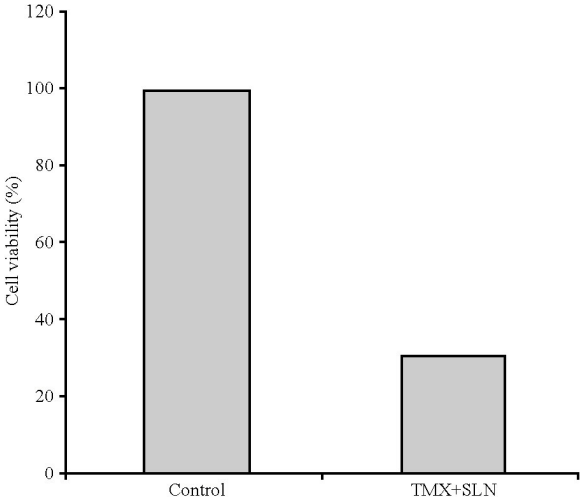

Fig. 1: Cytotoxixcity of tamoxifen loaded SLN against MCF-7 human breast cancer cell line

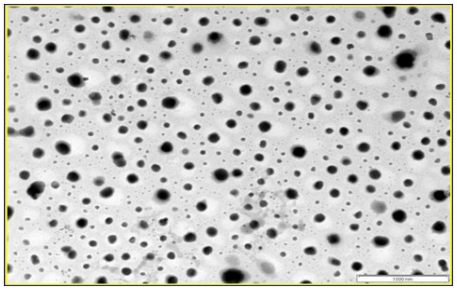

Fig. 2: TEM micrograph of Tamoxifen drug loaded SLN

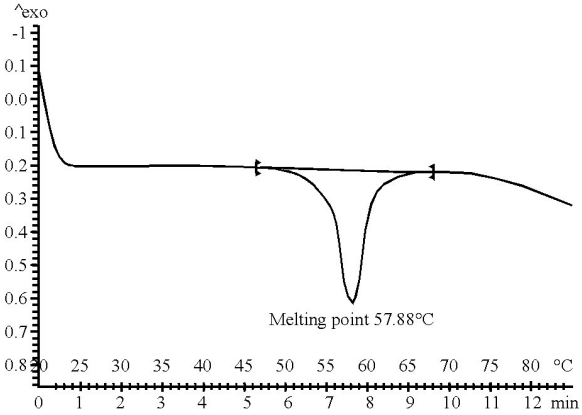

Fig. 3: Differential scanning calorimetry scans of SLN

Table 2: Particle size and zeta potential with Polydispersity Index (PI) of SLN

SLN: No.	Particle size (mm)	PI	Zeta potential (mV)
SLN (1)	142.70	0.274	-14.4
SLN (2)	107.30	0.219	-18.7
SLN (3)	95.50	0.255	-14.8
SLN (4)	66.20	0.182	-16.9
SLN (5)	128.50	0.200	-12.2
	108.48		-15.4

Differential scanning calorimeter: Melting points of colloidal systems were distinctly decreased by about 10-15°C as shown in the Fig. 3.

Average diameter and zeta potential: The zeta potential distribution of SLN as shown in Table 2. An adequate characterization of the solid lipid particles is a necessity for the control of the quality of the product. The average diameter of SLN measured by PCS Zetasizer 4 was 108.48 nm (n = 5) was showed in Table 2.

DISCUSSION

Cytotoxicity activity and drug loading capacity: It is believed that formulating tamoxifen with SLN could be advantageous to other colloidal or polymeric material due to the lack of toxicity of amphiphilic tamoxifen against fibroblasts and polymorphonuclear cells. Formulation with tamoxifen does not require the use of surfactants which may add to the overall toxicity of the nanoparticulate systems. Containing aliphatic chains linked with ester bonds to glucopyranose unit, tamoxifen is considered biodegradable by esterases. Finally, it is believed that formulating tamoxifen by SLN will help increase the drug's solubility by inclusion in CD cavity, facilitate the entrapment of high amounts of drug on different sites of solid lipid nanoparticles. Many different drugs had been incorporated in SLNs. According to[13] the prerequisite to obtain a sufficient loading capacity was a sufficiently high solubility of the drug in the lipid melts. Relative higher drug EE% was one of the major advantages of SLNs. The lipid crystalline structure related to the chemical nature of the lipid was a key factor to determine whether a drug would be expelled or firmly incorporated into the carrier systems. In the nanoparticle structure, the lipid forming highly crystalline state with a perfect lattice would lead to drug expulsion. On the other hand, the imperfection (lattice defects) of the lipid structure could offer space to accommodate the drugs. As a result, the structure of less ordered arrangement in the nanoparticles would be beneficial to the drug loading capacity like the samples in this study.

Transmission electron microscopy: Nanoparticles emulsified by a mixture of Lipid matrix and surfactant were produced at 1000 bar using the hot homogenisation technique. So in practice, samples were taken after 10 homogenisation cycles lead to reproducible and satisfying results. The number of homogenisation cycles necessary to decrease the polydispersity and to get a small particle population was slightly different. Solid Lipid Nanoparticles (SLN) are the new generation of nanoparticulate active-substance vehicles and are attracting major attention as novel colloidal drug carriers for topical use[16]. SLN are commonly produced by the hot homogenisation technique[3]. Alternatively a cold homogenisation process can be used to incorporate temperature sensitive drugs or to increase drug encapsulation. Nanoparticles were produced by either hot or cold homogenisation technique as described by[18].

Differential scanning calorimeter: Using DSC analysis, cooling scans are the most sensitive method to detect polymorphic forms. Freitas and Muller[5] and zur Mühlen[2] used this method to investigate the different crystallization of SLN. The cooling curves obtained 1 day after production showed that the formulation recrystallized in different polymorphic forms. SLN cooling curve shows a main peak at 57.8°C which can be attributed to the beta modification. But if the SLN cooling curve of the peak higher than 62°C suggested the presence of alpha modifications[2]. For the less ordered crystal or amorphous state; the melt of the substance did not require or just required less energy than the perfect crystalline. So; the melting point was depressed no matter whether it was drug-free or not. According to[16] the melting point decrease of colloidal

systems can be assigned to the colloidal dimensions of the particles in particular to their large surface to volume ratio and not to recrystallisation of the lipid matrices in a metastable polymorph possessing a lower melting point.

Average diameter and zeta potential: The measurement of the zeta potential allows predictions about the storage stability of colloidal dispersion. In general, particle aggregation is less likely to occur for charged particles (high zeta potential) due to electric repulsion[13]. The mean zeta potential was -15.4 mV (n = 5). Therefore, this method had gained a relative good stability and dispersion quality.

CONCLUSION

We concluded that tamoxifen loaded Solid lipid nanoparticles of appropriate particle size are obtained by hot high pressure homogenizer technique. Tamoxifen loaded SLN display significant cytotoxicity against MCF-7 cells and may be considered as an alternative formulation for this anti-estrogen drug.

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