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Formulation and Evaluation of PEGylated GMS based Solid Lipid Nanoparticles

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Abstract—*Poor solubility is a major concern for bioavailability issue of certain anticancer drugs. Solid lipid nanoparticle is an efficient lipid based drug delivery system which can enhance the bioavailability of poorly water soluble drugs. . Solid lipid nanoparticles are at the forefront of the rapidly developing field of nanotechnology with several potential applications in drug delivery, clinical medicine and research as well as in other varied sciences. The aim of present work was to formulate PEGylated Solid Lipid Nanoparticles (SLNs) using glyceryl monostearate as a lipid. PEGylation increases the solubility, size, molecular mass and stability of the drugs. PEG coating imparts amphiphilic characteristics to the SLN. The SLNs were prepared by solvent diffusion method. The SLNs were characterized for particle size analysis, zeta potential, drug content, entrapment efficiency, Transmission Electron Microscopy (TEM) studies. Drug loaded SLNs were prepared thereafter to verify the drug loading capacity of the PEGylated SLNs. Drug release profile for the SLNs was performed to verify their drug release ability in a simulated in-vivo environment. Hemolysis study was also done to check their haemocompatibilty. All parameters were found to be in an acceptable range. Therefore, the drug loaded SLNs can potentially be utilized in an anticancer drug delivery system.*

1. INTRODUCTION

Nanoparticles are colloidal particles ranging from 10 to 1000 nm (1.0 µm), in which the active components (drug or biologically active material) are dissolved, entrapped, and/or to which the active principle is adsorbed or attached [1]. Nanoparticles can be used to provide targeted delivery of many drugs, to sustain the drug effect in target tissue, to improve oral bioavailability and to enhance the stability of therapeutic agents against enzymatic degradation [2]. Now-a-days nanotechnology, as applied to medicine, brought significant advances in the diagnosis and treatment of disease. The desired applications in medicine include drug delivery, nutraceuticals, both in vitro and in vivo diagnostics and production of improved biocompatible materials [3]. Nanoparticles are emerging as a class of therapeutics for cancer and can show improved efficacy, while simultaneously decreasing side effects, owing to properties such as greater targeted localization in tumors and active cellular uptake [4]. Solid lipid nanoparticles (SLN) introduced in 1991 represent an alternative carrier system to traditional colloidal carriers such as emulsions, liposomes and polymeric micro and nanoparticles [5]. They have potential to carry lipophilic or hydrophilic drugs for therapy or diagnostics [6].

Cancer is one of the leading causes of death worldwide, second only to heart diseases. Chemotherapy is considered an important treatment modality in cancer and will probably remain so far considerable time. However, systemic administration of most drugs for cancer therapy produces severe side-effects due to their cytotoxic effects on normal cells. The prospect of improved cancer chemotherapy using SLN as a drug delivery system is promising. SLN can be used to incorporate variety of lipophilic, hydrophilic and ionic compounds effectively.

Active tumor targeting may also be possible by altering Biodistribution of SLN through physicochemical properties of surface to minimize systemic toxicity and target drug to specific site. Excipients used in the preparation of SLN are biocompatible and approved by regulatory agencies [7]. The technique of covalently attaching polyethylene glycol (PEG) to a given molecule is known as “PEGylation” and is a well-established method in the field of targeted drug delivery systems. The objective of most PEG conjugation techniques aims at increasing the circulation half-life without affecting activity. PEG attaches covalently to the nanoparticle surface and provides a hydrophilic steric barrier around SLN, thereby resulting in formulations with increased stability during storage and application [8]. PEGylation increases the solubility, size, molecular mass and stability of the drugs. PEG-modified SLNs have been reported to enhance the biological half-life and efficacy of encapsulated drugs, enhancing their stability, and also their transport and absorption in the body.

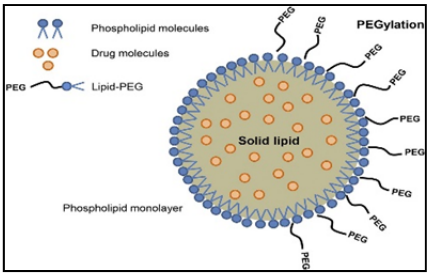

Fig. 1: Schematic representation of SLN coated with PEG [9]

2. MATERIALS AND METHODS

Materials

GMS was purchased from CDH Fine Chemicals, Delhi; Tween 80 (Polyoxyethylene sorbitan monooleate) and Drug were purchased from Fluka Analytical, Switzerland. Phosphate buffered saline (PBS) and Dimethyl Sulfoxide (DMSO) were procured from Sigma-Aldrich USA. PEG was obtained from Sigma-Aldrich USA. Ethanol and acetone were purchased from Merck, Mumbai. Other ingredients and solvents were from HPLC or chemical laboratory purity grades, as needed, and were purchased locally.

2.1 Preparation of SLNs

Drug loaded SLNs were prepared by the solvent diffusion method in aqueous system (Trotta et al). SLN formulation consists of 2 phases: Organic phase and aqueous phase. The lipid (organic) and aqueous phases were separately prepared in glass vials.

Firstly, Glyceryl monostearate (GMS) was heated to 70^0C in a water bath and added to ethanol along with specified amount of drug and PEG di-stearate to form organic phase. The aqueous phase was obtained by mixing surfactant 1% of Tween 80 and co-surfactant Soya- Lecithin in a 1:1 ratio. The aqueous phase was subjected to magnetic stirring until the Soya Lecithin was completely dissolved in Tween 80 and water. The lipid solution or organic phase was poured into aqueous phase, maintained at 700C under constant stirring. This emulsion was further kept into ice bath at 00C. The resulting o/w emulsion was further sonicated in a bath sonicator for 15 min and a probe sonicator for 5 min. The SLNs dispersions were purified by centrifugation at 40C at 9000 rpm for 30 minutes followed by water washings (x3). The SLN suspensions were solidified by evaporating the solvent in Rotary evaporator.

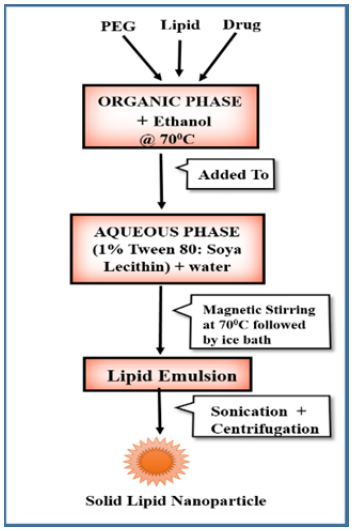

Fig. 2: Schematic for the preparation of SLN

2.2 Characterization Techniques of SLNs

Sufficient as well as efficient characterization of SLN dispersion exists as an essential pre-requisite for its quality monitoring [10]. Characterization of the SLNs is necessary for its quality control. Characterization of SLN is a serious challenge due to the colloidal size of the particles and the complexity and dynamic nature of the drug delivery system [11]. The synthesized SLNs were subjected to Physico-chemical characterization by DLS, Zeta Potential and TEM.

2.2.1 Particle Size Analysis

Average particle size of synthesized SLNs was determined using dynamic light scattering technique (Malvern Nano Zeta sizer 90S, UK). For DLS, the dry powder of the SLN formulation was re-suspended in distilled water to form a homogeneous solution. Dilution is done until Refractive Index (RI) becomes 1.4. All dynamic light scattering experiments were performed at 25^0 C and at a scattering angle of 90^0.

2.2.2 Zeta Potential Measurements

The zeta potential indicates the degree of repulsion between adjacent, similarly charged particles in dispersion. For zeta potential analysis, SLN dispersion are diluted in distilled water almost 50 fold and ultrasonicated for homogenisation. Small amount of suspension was taken in clear disposable zeta cell and then characterized by Zetasizer.

2.2.3 TEM Analysis

Particle morphology and size of PEGylated Drug loaded SLNs were confirmed by TEM. For sample preparation, one drop of the loaded SLN formulation was placed on a carbon coated copper grid of 200 mesh size and air dried for 45 min to evaluate the morphology by TEM. Observation under different magnification was performed.

2.3 Determination of Drug content in the SLNs

The drug loading (% DL) and entrapment efficiency (% EE) of Drug loaded SLNs was determined by UV-Vis Spectrophotometer. The sample was prepared by disrupting the drug loaded SLNs in DMSO (1 mg/ml) and used this for analysis in 1.4 mm well stoppered quartz cuvette in the range of 200-700 nm.

To calculate the entrapment efficiency (EE), 5 ml of SLNs dispersion was placed into Eppendorf tubes and centrifuged at 9000 rpm for 30 min at 4^0 C. After centrifugation, the dispersion separates into supernatant clear aqueous phase and precipitated pellets of SLNs.

The % EE and % DL were calculated as follows [12]:

$$\text{Drug loading}(\%\ \text{DL}) = \frac{\text{amount of drug encapsulated in SLNs}}{\text{amount of drug added}} \times 100$$

$$\text{Entrapment efficiency}(\%\ \text{EE}) = \frac{\text{amount of drug encapsulated in SLNs}}{\text{amount of SLNs}} \times 100$$

2.4 In vitro drug release profile study

The dialysis method was used to study the release of the chemotherapeutic drug from the SLNs. To quantify their release, suspensions of Drug loaded SLNs in PBS and acetate buffer were analyzed by UV-Vis spectrophotometry. The release studies were examined at two different pH values, 7.4 (0.1 M phosphate buffer saline) and 5.5 (acetate buffer), using a Spectra-Por® Float-A-Lyzer (MWCO 8kDa). The Float-A-Lyzer was soaked overnight in distilled water before use. 5 mL of each drug loaded nanocarriers was transferred to the Float-A-Lyzer, which was then placed in the respective buffer solution (dialysis medium). The system was placed in water bath on continuous magnetic stirring at 120 rpm.

At pre-determined time intervals, 2 mL sample was withdrawn from dialysis medium to record its UV absorbance and was replaced with 2 mL of fresh buffer to maintain its sink condition (constant volume throughout the study). Calibration curves of the drug in acetate buffer and PBS were used to calculate the drug concentration in the SLNs. The following equation was used to calculate the cumulative percentage of drug released from the SLNs [13]:

$$\%\ \text{drug release} = \left[1 - \left\{\frac{\text{absorbance}_t}{\text{absorbance}_{t_0}}\right\}\right] \times 100$$

2.5 Hemolysis studies: In vitro Evaluation

Interaction of PEGylated Drug loaded SLNs with red blood cells (RBCs) was examined by hemolysis studies. To evaluate the safety of the nanoparticles, the hemolytic activity was performed. For this, blood was drawn from healthy human volunteers in heparinized tubes and then centrifuged at 3000rpm for 10 min to separate the RBCs from the plasma. The RBC pellet obtained was washed thrice with PBS (pH 7.4) to remove any protein remaining and other debris and suspended in 50 mL of PBS 7.4 to form RBC suspension. After that, 0.4 mL of sample (Drug solution and Drug loaded SLNs) was mixed with the 1.6 mL of the RBC suspension. These were incubated at 37^0 C with gentle shaking to evaluate the time dependent hemolytic character of the compound. At different time points ranging from 15 min to 4 h, 2 mL of the mixture was centrifuged for 10 min at 3000 rpm to precipitate the erythrocytes.

The optical density of the supernatant was measured at 540 nm using UV-Vis spectrophotometer, and the % of cells undergoing hemolysis was calculated using the following formula [14]:

$$\%\ \text{hemolysis} = \frac{\text{absorbance}_{(\text{compound})} - \text{absorbance}_{(\text{-ve control})}}{\text{absorbance}_{(\text{+ve control})} - \text{absorbance}_{(\text{-ve control})}} \times 100$$

3. RESULTS AND DISCUSSIONS

3.1 Preparation of Drug loaded SLNs

The solvent diffusion method was applied in the current study to prepare SLNs. Ethanol is highly soluble in water with minimal interfacial tension, so it diffuses very quickly in aqueous phase thereby resulting in the precipitating of lipids which in the presence of stabilizer leads to the formation of nanoparticles. Increase in the concentration of surfactant in SLN formulations could reduce the interfacial tension between lipid matrix and aqueous medium, consequently favor the formation of SLN with smaller particle size [15]. PEG (Polyethylene Glycol) modified SLNs ameliorates the biological life and efficacy of encapsulated drugs, enhancing their stability, and also their transport and absorption through the GI tract [16-20]. The prepared SLN dispersion was found to be uniform and homogeneous in appearance.

Fig. 3: Nanoemulsion

3.2 Characterization of SLNs

3.2.1 Particle Size Analysis

The particle size of Drug loaded SLNs synthesized by solvent diffusion method was evaluated using Zeta sizer and average

particle size was found to be 101.2 nm. It was inferred from the obtained results that particle size of the synthesized SLNs was within the favorable size range.

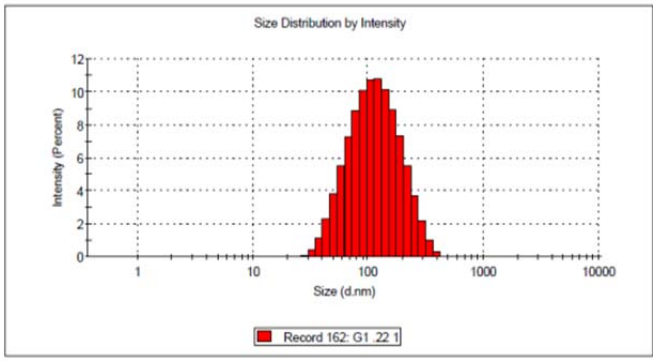

Fig. 4: Particle Size of SLNs

3.2.2 Zeta Potential of SLNs

It determines the stability of synthesized SLNs. The zeta potential was found to be -24.8mV. It was inferred from the obtained results that high negative value of zeta potential are known to favor storage stability. The negative charge was likely caused by the slightly ionized fatty acids from the glycerides used (GMS).

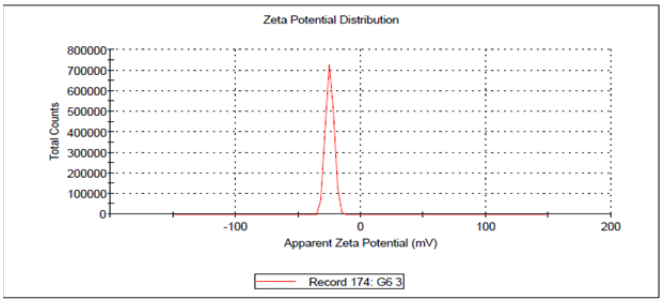

Fig. 5: Zeta Potential of SLNs

3.2.3 TEM Analysis

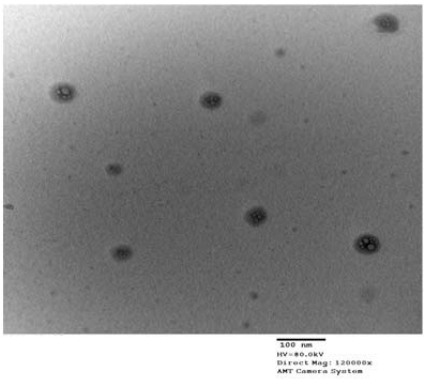

Fig. 6: TEM Micrograph

The TEM micrographs revealed the spherical surface morphology of the nanoformulations. The TEM images show a dark core of the particles, clearly depicting the drug loading in the core of the SLNs.

3.3 Drug Loading and Encapsulation Efficiency

The test for drug content was carried out to ascertain whether the drug is uniformly distributed in the formulation. The loading efficiency of the drug depends on their physical properties, especially hydrophobicity and hydrophilicity (HLB system) [21]. The EE and DL of PEGylated SLNs were determined by separation of NPs from the aqueous medium containing free drug by centrifugation at 9000 rpm at 4^0C for 30 minutes.

Entrapment efficiency for the Drug was 70% where as Drug Loading was found to be 33.63%.

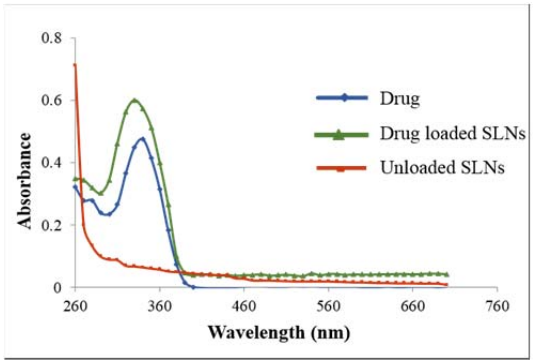

Fig. 7: UV Spectra of Drug loaded GMS SLNs

3.4 In vitro Release Studies

The dialysis bag method was used to determine the release profile of the drug from PEGylated Drug loaded SLNs. This is an efficient method to study the drug release from nanocarriers. In vitro release studies under simulated physiological conditions are essential to comprehend the effect of a drug [22]. Consequently, it is essential to realize in what form and to what extent the drug is encapsulated in a carrier system. The In vitro release profile was constructed in the form of the percentage of drug released from the SLNs cumulatively as a function of time.

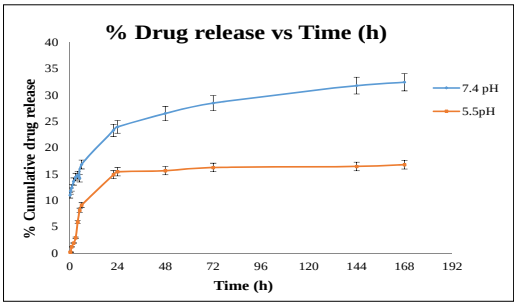

Fig. 8: In vitro drug release study at pH 7.4 and 5.5

Fig. illustrates the drug release at two pH values: pH 7.4 to simulate the physiological environment, and pH 5.5 to simulate the acidic tumor microenvironment.

As expected, the solid state of the lipids in the SLNs prolonged the release of the entrapped drugs from the nanoparticles. These results validate the possibility of a controlled release from SLNs.

3.5 In vitro Hemolysis studies

For any formulation intended to be administered by the intravenous route, it is important to determine their toxicity in blood, evaluation of hemolytic activity is the best method to accomplish this. The basic objective of this study was to quantify the damaging effects of Drug loaded SLNs on the RBC membrane. 2% Triton X-100 and Phosphate Buffer Saline 7.4(PBS 7.4) were used as the positive and negative controls for this study. Any RBC lysis was estimated from the absorbance of the sample measured at 540nm. It was found that even upto 4 h, Drug loaded SLNs showed less than 1% hemolytic activity. These values were well within the biologically acceptable range of 5% as per ASTM standards [23]. This study showed that Drug loaded SLNs is completely safe to erythrocytes and are suitable for the intravenous mode of injection.

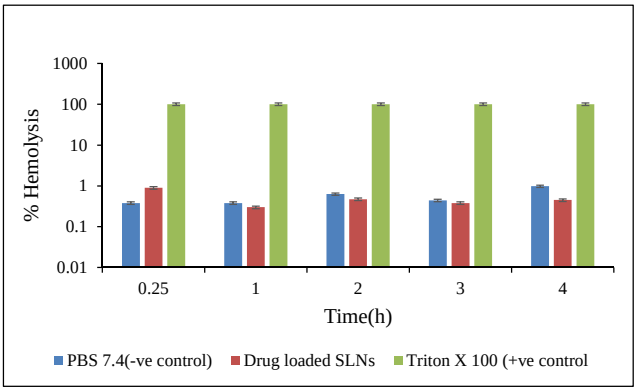

Fig. 9: Hemolysis Plot of drug loaded SLNs

4. CONCLUSIONS

Solid lipid nanoparticles can be utilized to improve the therapeutic efficacy of drugs with low bioavailability, and reduce their effective dose requirements. These characteristics make them a highly sustained drug release system. In terms of preparation methods, SLN systems were introduced to overcome limitations associated with traditional drug carrier systems such as polymeric systems. Surface modification of SLN by PEG coating helps in improving drug bioavailability. This study is based on the ability of the SLNs to effectively entrap chemotherapeutic drug which normally have low bioavailability in the body. The optimized formulations were characterized by TEM, DLS and UV-Vis Spectrometry for various parameters. The average particle size of SLNs was found to be 101.2 nm with poly dispersity index (PDI) of 0.169 which indicated homogeneous and unimodal size distribution. The zeta potential of SLNs was found to be -24.8mV which indicates the stability of the nanoparticles as absolute zeta potential values around 25 mV.

TEM images showed spherical surface morphology with size between 80-100nm. Drug loading and entrapment efficiency has been found to be highly significant and drug release studies have also been done at physiological pH and acidic tumor pH. Hemolytic activity of the drug loaded SLNs was studied to determine its toxicity in blood.

5. FUTURE SCOPE

This prepared formulation can be used as a carrier system for any kind of drugs since it is an optimized system in terms of particle size, zeta potential, drug release potential and hemolysis study. The future scope of the present study includes development of such drug loaded SLNs for further in vitro and in vivo studies so as to give more insights into their anti-proliferative studies. These can serve as potent alternatives for cancer treatment with minimum side-effects and maximum benefits.

REFERENCES

[1]. Chowdary KPR, Rao AS (1997). Indian Drugs, 34, 549-56.
[2]. Rao GCS, Kumar SM, Mathivanan N, Rao MEB. (2004) Indian Drugs, 41, 389-95.
[3]. Duncan R. (2003) Nat Rev Drug Disc, 2, 347-60.
[4]. Mark E. Davis, Zhuo (Georgia) Chen, Dong M. Shin (2008).Nature reviews-Drug Discovery, 7, 771-82.
[5]. P. Ekambaram, A. Abdul hasansathali K. Priyanka. (2012). solid lipid nanoparticles: a review, Scientific Reviews Chemical Communications, 2(1), 80-102.
[6]. C. Shah, V. Shah, U. (2011). Upadhyay, solid lipid nanoparticles: a review, current pharma research, 1(4), 351-368.
[7]. Jessy Shaji*, Vinay Jain. (2010) Solid lipid nanoparticles: a novel carrier for chemotherapy, International Journal of Pharmacy and pharmaceutical sciences, 2 (3).
[8]. Melike Üner, Gülgün Yener. (2007).Importance of solid lipid nanoparticles (SLN) in various administration routes and future perspectives, International Journal of Nanomedicine, 2(3) 289–300.
[9]. Phatsapong Yingchoncharoen, Danuta S. Kalinowski and Des R. Richardson. (2016). Lipid-Based Drug Delivery Systems in Cancer Therapy: What Is Available and What Is Yet to Come, Pharmacological Reviews, 68 (3) 701-787.
[10]. Gohla SH, Dingler A. (2001).Scaling up feasibility of the production of solid lipid nanoparticles (SLN), Pharmazie, 56, 61–3.
[11]. Muller RH, Mäder K, Gohla S.(2000) Solid lipid nanoparticles (SLN) for controlled drug delivery - a review of the state of the art; Eur. J. Pharm. Biopharm, 50,161–77.
[12]. Fundaro A, Cavalli R, Bagoni A, Vighetto D, Zara GP, Gasco MR.(2000). Non-stealth and stealth solid lipid nanoparticles (SLN) carrying doxorubicin: pharmacokinetics and tissue distribution after i.v. administration to rats; Pharm. Res., 42 (4), 337–43.

[13]. Chen D, Yang T, Liang W, Zhang Q. (2001). In Vitro and in Vivo Study of Two Types of Long-Circulating Solid Lipid Nanoparticles Containing Paclitaxel; Chem. Pharm. Bull. 49, 1444–47.
[14]. Reddy JS, Venkateshwarlu V. (2004). Novel delivery systems for drug targeting to the brain. Drugs Future, 29(1), 63–83.
[15]. Siekmann, B. and Westesan, K. (1992).Submicron-sized parenteral carrier systems based on solid lipids. Pharm. Pharmacol Lett., 1, 123-126.
[16]. Yang S, Zhu J, Lu Y, Liang B, Yang C. (1999). Body distribution of camptothecin solid lipid nanoparticles after oral administration Pharm. Res., 16, 751–57.
[17]. Tabatt KSM, Olbrich C, Muller RH. (2004). Effect of cationic lipid and matrix lipid composition on solid lipid nanoparticles by solvent emulsification–diffusion technique. Eur J Pharm Biopharm, 57:155–162.
[18]. Mehnert, W. and Mäder, K. (2001). Solid lipid nanoparticles: production, characterization and applications. Advanced Drug Delivery Reviews 47: 165–196, 2001
[19]. Drummond, D.C., Meyer, O., Hong, K., Kirpotin, D.B. and Papahadjopoulos, D. (1999). Optimizing liposomes for delivery of chemotherapeutic agents to solid tumors, Pharmacology Reviews, 51: 691–744, 1999.
[20].Muller R.H., et al., (2000). Solid- liquid (semi-solid) lipid particles and method of producing highly concentrated lipid particle dispersions, German patent application 199 45 203.2.
[21]. Manjunath K, Reddy JS and Venkateswarlu V (2005). Solid lipid nanoparticles as Drug Delivery Systems, Exp Clin Pharmacol 27(2): 1-20.
[22]. Siekmann B., Westesen K.,(1994). Thermoanalysis of the recrystallization process of melt-homogenized glyceride nanoparticles, Coll. Surf. B: Biointerf. , 3 (3): 159-175.
[23] Wong, H. L.; Li, Y.; Bendayan, R.; Rauth, M. A.; Wu, X. Y. , (2007a).Solid lipid nanoparticles for anti-tumor drug delivery. In Nanotechnology for Cancer Therapy, Amiji, M. M., Ed. CRC Press: Boca Raton, 741 -776.