Etoposide-Loaded Nanoparticles Made from Glyceride Lipids: Formulation, Characterization, in Vitro Drug Release, and Stability Evaluation

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ABSTRACT

The aim of the study was to prepare etoposide-loaded nanoparticles with glyceride lipids and then characterize and evaluate the in vitro steric stability and drug release characteristics and stability. The nanoparticles were prepared by melt emulsification and homogenization followed by spray drying of nanodispersion. Spray drying created powder nanoparticles with excellent redispersibility and a minimal increase in particle size (20-40 nm). Experimental variables, such as homogenization pressure, number of homogenization cycles, and surfactant concentration, showed a profound influence on the particle size and distribution. Spray drying of Poloxamer 407-stabilized nanodispersion lead to the formation of matrix-like structures surrounding the nanoparticles, resulting in particle growth. The in vitro steric stability test revealed that the lipid nanoparticles stabilized by sodium tauroglycocholate exhibit excellent steric stability compared with Poloxamer 407. All 3 glyceride nanoparticle formulations exhibited sustained release characteristics, and the release pattern followed the Higuchi equation. The spray-dried lipid nanoparticles stored in black polypropylene containers exhibited excellent long-term stability at 25°C and room light conditions. Such stable lipid nanoparticles with in vitro steric stability can be a beneficial delivery system for intravenous administration as long circulating carriers for controlled and targeted drug delivery.

KEYWORDS: lipid nanoparticles, high-pressure homogenization, spray drying, Poloxamer 407, steric stability

INTRODUCTION

Nanoparticles derived from solid lipids have received considerable attention in recent years and are proposed as an alternative drug delivery system to emulsions, liposomes, and polymeric nanoparticles.[1] Lipid nanoparticles are usually aqueous dispersions of solid lipids or dry powders obtained by lyophilization[2] or spray drying.[3] Lipid nanoparticles overcome the membrane stability and drug-leaching problems associated with liposomes and emulsions,[4] the biodegradation and toxicity problems of polymeric nanoparticles,[5] and also facilitate prolonged drug release.[6] Lipid nanoparticles are prepared from biocompatible lipids and possess excellent biodegradability and low toxicity.[5,7]

A striking advantage of lipid nanoparticles is the feasibility of large-scale production by a high-pressure homogenization technique.[8] Much research is available on formulation, characterization,[9] in vitro degradation, lipid recrystallization behavioral studies by techniques[10] such as differential scanning calorimetry,[11] small-angle and wide-angle radiograph diffractometry,[12] etc, and their in vitro drug release potential.[11] Extensive research by Bunjes and coworkers[13-15] reports on the crystalline properties of lipids and their recrystallization patterns during nanoparticle preparation and the influence of nanoparticle size on the recrystallization pattern.

Many articles appear in the literature on the formulation of nanodispersions and their stability studies in dispersion form.[16] To ensure prolonged stability of the drug delivery system, it may be necessary to convert the liquid dispersions into dry form for storage. Few articles contain research studying the preparation of dry-powdered lipid nanoparticles.[3,17] Lyophilization and spray drying are the 2 techniques converting the nanodispersions into dry particles. Of these techniques, spray drying is cost-effective and can be used beneficially for large-scale purposes. Spray drying of lipid nanoparticles requires great care because of the low melting temperatures of lipids used in the formulation. Some studies[18-20] used an organic solvent to reduce the processing temperature and facilitate the drying of heat-sensitive materials. The removal of organic solvents from the lipid nanoparticle matrix again requires exposure to high temperatures, which is not always advisable.

The biodistribution of these colloidal carriers and the delivery of incorporated drugs to the target sites after intravenous administration are mainly determined by their physicochemical properties, such as size and surface hydrophobicity, through their recognition or nonrecognition by

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the body's reticuloendothelial system (RES).[21,22] The rapid removal of colloidal particles by the macrophages of RES is a major obstacle in targeting tissues elsewhere in the body, such as in bone marrow and solid tumors.[23] To overcome the particle recognition by RES, surface modification of the colloidal carriers by coating with block copolymers[21] and synthesis of poly(ethylene glycol)ylated derivatives were reported.[24] For such colloidal carriers, it is advantageous to perform an in vitro steric stability test, before commencing the in vivo studies, to see whether these carriers possess steric repulsion activity in vitro.

The present study investigates the formation of lipid nanoparticles by melt emulsification and a high-pressure homogenization technique followed by spray drying of the nanodispersion. Factors influencing the nanoparticle formation and spray-drying process were determined and optimized. The nanoparticles were subjected to electrolyte-induced flocculation test to determine their steric repulsion properties. The stability study of nanoparticles was performed for 5 months, and parameters such as particle size and entrapment efficiency were determined.

MATERIALS AND METHODS

Chemicals

Etoposide was a gift sample obtained from the Dabur Research Center (Ghaziabad, Uttar Pradesh, India) and Cipla Ltd (Mumbai, India). Glycerol monostearate (GMS) was purchased from Loba Chemie Pvt Ltd (Mumbai, India). Glycerol distearate (GDS) was obtained from Gattefosse GmbH (St Priest, France). Tripalmitin (TP) was purchased from Sisco Laboratories Pvt Ltd (Mumbai, India). Hydrogenated soya phosphatidylcholine (HSPC) was purchased from Lipoid GmbH (Ludwigshafen, Germany). Sodium tauroglycocholate was purchased from Qualigens Fine Chemicals (Mumbai, India). Poloxamer 407 was obtained from BASF (Ludwigshafen, Germany). All other chemicals used in this study were of analytical grade.

Preparation of Etoposide-Loaded Lipid Nanoparticles

By using the 3 lipids GMS, GDS, and TP, we prepared the etoposide-loaded lipid nanoparticles by melt emulsification and a homogenization technique modified from the one reported by Olbrich et al[25] and Jenning et al.[26] The melting points of GMS, GDS, and TP are 56°C, 55°C, and 82°C, respectively. Briefly, etoposide was dissolved in a small quantity of methanol (0.2 mL), then HSPC was added, and the mixture was warmed slightly to form a clear melt. The methanol was then evaporated completely by heating the phase between 50°C and 55°C. This drug containing HSPC was added to a glyceride lipid and heated 5°C above the melting point of the respective lipid to obtain a clear melt. The hot melt was emulsified by stirring for 5 minutes at 5000 rpm (2124*g*) (Remi table top stirrer; Remi Instruments, Mumbai, India) into the aqueous phase containing sodium tauroglycocholate, which was preheated to 5°C above the temperature of the lipid phase. The hot emulsion was then homogenized in the high-pressure homogenizer (Emulsiflex C5; Avestin Inc, Ottawa, Canada) and maintained in a water bath at 90°C. The nanodispersion formed was spray dried using a spray drier (JISL Instruments, Mumbai, India) after the addition of lactose monohydrate to obtain the powder nanoparticles. The spray drying was performed at an inlet temperature of 70°C for etoposide-loaded TP (ETP) nanoparticles and 53°C for etoposide-loaded GMS (EGMS) and etoposide-loaded GDS (EGDS) nanoparticles and an outlet temperature of 40°C. Spraying was performed at an inlet air pressure of 2.5 kg/cm^2 and aspiration rate of 30 on scale. The flow rate was maintained at 3 mL/min.

Lipid nanoparticles were also prepared using Poloxamer 407 as a surfactant to study the influence of surfactants on the steric stabilization of nanoparticles. These nanoparticles were prepared as explained above by melt emulsification and a high-pressure homogenization technique.

Determination of Drug Content in Nanoparticles

The nanoparticles were aggregated by the addition of 0.1 mL of 10 mg/mL protamine sulfate solution, kept aside for 10 minutes and centrifuged (Sigma 3K30 refrigerated high speed centrifuge; Sigma Instruments, Germany, Osterode) at 8000 rpm (5438*g*) for 10 minutes. The supernatant was decanted, and the sediment was washed with distilled water to remove the surfactant and adsorbed drug (if any) and centrifuged. To the pellet obtained was added 2 parts by weight of sucrose. The total volume of dispersion of the pellet was maintained at 1 mL. The dispersion was then lyophilized. Twenty-five milligrams of lyophilized powder was weighed and dissolved in a mixture of methanol-chloroform (50:50). Required dilutions were performed with the same solvent mixture and analyzed for drug content using UV-visible spectrophotometer (Shimadzu UV 1601; Shimadzu, Kyoto, Japan) at 286 nm against the solvent blank containing same concentration of HSPC used in the nanoparticle formulation.

Characterization of Nanoparticles

Particle Size Analysis

The size analysis of nanoparticles was performed by laser scattering using a Malvern Hydro 2000SM particle size analyzer (Malvern Instruments Ltd, Worcestershire, UK). The aqueous nanoparticulate dispersion was added to the sample dispersion unit containing a stirrer and then stirred

to minimize the interparticle interactions, and the laser obscuration range was maintained between 10% and 20%. The analysis was performed 3 times, and the average values were taken.

Zeta Potential Measurement

Zeta potential of nanoparticles was measured in a Malvern Zetasizer 3000 HS_A (Malvern Instruments). The nanoparticles were dispersed in phosphate-buffered saline (pH 7.4), and the zeta potential was determined.

Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) analysis of the bulk lipids and nanoparticles was conducted using a differential scanning calorimeter (DSC 60; Shimadzu) set at a heating rate of 10°C/min.

Scanning Electron Microscopy

The powder nanoparticles were fastened onto a brass stub with double-sided adhesive tape. The stub was fixed into a sample holder and placed in the vacuum chamber of a JEOL JSM 1560 LV (JEOL, Tokyo, Japan) scanning electron microscope and observed under low vacuum (10^{-3} mm HG).

Determination of In Vitro Steric Stability by an Electrolyte-Induced Flocculation Technique

An in vitro steric stability test of nanoparticles was performed by slight modification of the earlier reported method.[24] Lipid nanoparticle dispersion (1 mg/mL) equivalent to 5 mg of lipid content was added to 5 mL of flocculation mixture. The flocculation mixture contained different concentrations (0 to 1.4 mol/L) of the flocculating agent sodium sulfate (Na_2SO_4) dissolved in 16.7% sucrose solution. Immediately after addition of the lipid nanoparticle, the optical turbidity of the resultant suspensions was measured at 600 nm using UV-visible spectrophotometer (Shimadzu UV 1604).

In Vitro Drug Release Studies

In vitro release of etoposide from lipid nanoparticles was evaluated by the dialysis bag diffusion technique reported by Yang et al.[27] The release studies of etoposide from EGMS, EGDS, and ETP nanoparticles were performed in phosphate buffer (pH 7.4). The aqueous nanoparticulate dispersion equivalent to 2 mg of etoposide was placed in a cellulose dialysis bag (cutoff 12 000; HIMEDIA, Mumbai, India) and sealed at both ends. The dialysis bag was immersed in the receptor compartment containing 50 mL of dissolution medium, which was stirred at 100 rpm and maintained at 37 ± 2°C. The receptor compartment was covered to prevent the evaporation of dissolution medium. Samples were withdrawn at regular time intervals, and the same volume was replaced by fresh dissolution medium. The samples were analyzed using a UV-visible spectrophotometer set at 286 nm. All the experiments were repeated 3 times, and the average values were taken.

Stability of Nanoparticles

The nanoparticle dispersions of EGMS, EGDS, and ETP were subjected to short-term stability studies in black and transparent polypropylene containers stored separately in a dark refrigerator with a temperature set at 4°C to 8°C (ie, the container in which the samples were stored was covered with thick black paper). After assessing the short-term stability of the dispersions in the containers, the containers responsible for greater stability were selected for the stability studies of the final spray-dried nanoparticle formulations. The spray-dried EGMS, EGDS, and ETP nanoparticles were subjected to stability studies for 5 months.

RESULTS AND DISCUSSION

After preliminary trials, the homogenization pressures (ranging from 70 to 105 MPa) that produced low-particle size were considered for the study. The homogenization pressure and cycle number were found to have a profound influence on the final size of EGMS, EGDS, and ETP nanoparticles in dispersion (Figure 1). The particle size decreased with an increase in homogenization pressure in the case of EGMS and EGDS nanoparticles, whereas the size of ETP nanoparticles increased with pressure. An increase in the number of homogenization cycles at 70 MPa resulted in a decrease in the size of EGMS nanoparticles after a maximum of 4 cycles. The size of EGDS and ETP nanoparticles at 70 MPa reduced for 3 homogenization cycles and increased thereafter. At 105 MPa, however, the size of EGMS and EGDS nanoparticles decreased for 2 homogenization cycles and increased thereafter, whereas the size of ETP nanoparticles increased after the second cycle. The tripalmitin, being a lipid with a high melting point, showed drastic differences in particle size with homogenization conditions. With fewer than 3 homogenization cycles at 70 MPa or 2 homogenization cycles at 105MPa, the size distribution was multimodal, indicating the presence of a microparticulate fraction in the dispersion. Homogenization leads to the development of cavitation forces, which breaks down the particle structure to the smaller ones. There is an optimum pressure and homogenization time until which the lipid nanoparticles undergo a

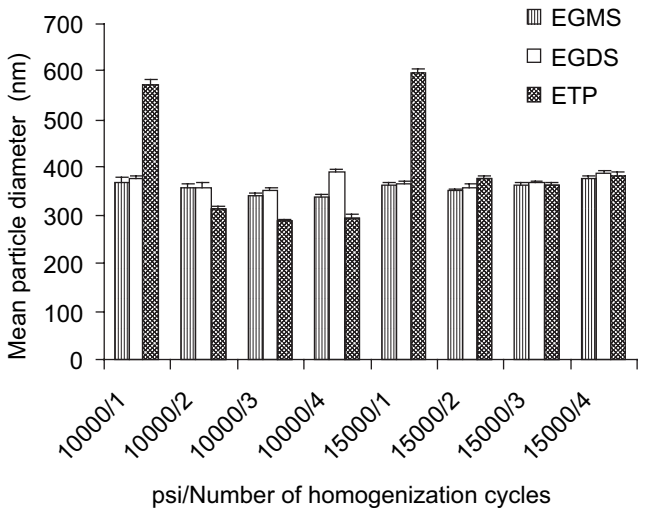

Figure 1. Influence of homogenization pressure and number of cycles on the mean diameter of lipid nanoparticles. The nanoparticles were stabilized by 3% wt/vol sodium tauroglycocholate. The lipid composition of the nanoparticles was 1:29 ratio of etoposide-lipid and 1:2 ratio of HSPC-glyceride. EGMS, etoposide-loaded glycerol monostearate nanoparticles; EGDS, etoposide-loaded glycerol distearate nanoparticles; ETP, etoposide-loaded tripalmitin nanoparticles. The data represent mean ± S.D. of 3 experiments.

decrease in size and above which the excess cavitation forces and exposure of particles to these conditions for a longer time leads to particle aggregation. At higher homogenization pressures, the kinetic energy of the system increases resulting in particle collision and, thereby, the coagulation. The high particle collisions also distort the surfactant film coating the particle surface and enhance the particle aggregation.[3] The increase in size of all 3 lipid nanoparticles after 3 homogenization cycles at 105 MPa can be attributed to the particle coagulation as a result of exposure of the nanoparticles to greater cavitation forces for a longer time and also due to increased kinetic energy of the system. The optimum parameters resulting in low particle size were found to be homogenization at 70 MPa for 4 cycles for GMS nanoparticles and 70 MPa for 3 cycles for GDS and TP nanoparticles.

The use of bile salts (cholate salts) in the preparation of nanoparticles has been widely reported.[28-30] Figure 2 describes the influence of the sodium tauroglycocholate (surfactant) concentration on the size of nanoparticles prepared by processing under optimized conditions mentioned above. An increase in the surfactant concentration up to 3% wt/vol resulted in a significant reduction in size of nanoparticles, and a further increase did not greatly influence the particle size. The decrease in size of nanoparticles at high surfactant concentrations is due to effective reduction in interfacial tension between the aqueous and lipid phases, leading to the formation of emulsion droplets of smaller size, which on cooling results in smaller nanoparticles. High surfactant concentrations effectively stabilize the particles created by forming a steric barrier on the particle surface, thereby protecting the particles from coagulation. The optimum concentration of sodium tauroglycocholate needed to produce smaller sized nanoparticles (350 to 380 nm) was 3% wt/vol in case of all 3 glyceride nanoparticles.

The effect of formulation variables, such as drug-lipid ratio and HSPC-glyceride ratio within the lipid composition, on the entrapment efficiency of nanoparticles is shown in Table 1. The entrapment efficiency of nanoparticles increased with an increase in the etoposide-lipid ratio, which was high at 1:29 ratio. An increase in the entrapment efficiency of the nanoparticles with an increase in the HSPC-glyceride ratio is due to the enhanced solubility of the etoposide in lipid fraction. HSPC, being phospholipids, possesses solubilization properties of lipophilic compounds and, hence, increases the solubility of etoposide in the respective lipids. An interesting trend was observed by varying the HSPC-lipid ratio within the lipid composition in all 3 nanoparticles. The entrapment efficiency of the nanoparticles was found to be reduced with a decrease in the HSPC quantity within the lipid composition. This can be attributed to the higher solubility of etoposide in HSPC than in the glyceride lipids. The highest entrapment efficiency was found at the composition of 1:29 (the etoposide-lipid ratio) and 1:2 (the HSPC-glyceride ratio). The entrapment efficiencies at this composition were found to be 97.63% for EGMS, 96.82% for EGDS, and 99.4% for ETP nanoparticles. The size of the nanoparticles was measured for all the compositions prepared to determine the entrapment efficiency (Table 1). The size of nanoparticles slightly increased with an increase in the drug-lipid ratio and decreased with the decrease in HSPC concentration. This can be attributed to the decrease in stress on the

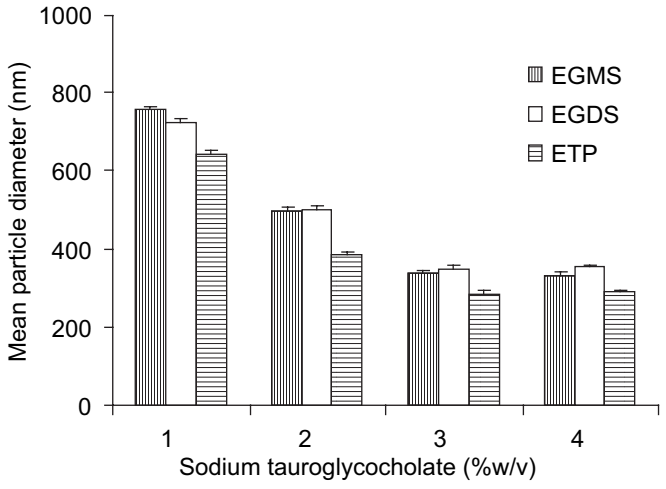

Figure 2. Influence of the concentration of sodium tauroglycocholate on the mean diameter of lipid nanoparticles.

Table 1. Influence of Formulation Composition on the Entrapment Efficiency of Lipid Nanoparticles*

Etoposide-Glyceride + HSPC Ratio	HSPC-Glyceride Ratio	Entrapment Efficiency of EGMS (%)	Mean Particle Diameter of EGMS (nm)	Entrapment Efficiency of EGDS (%)	Mean Particle Diameter of EGDS (nm)	Entrapment efficiency of ETP (%)	Mean Particle Diameter of ETP (nm)
1:19	1:2	80.30	371	81.40	378	80.82	348
1:24	1:2	94.18	377	86.60	382	87.44	353
1:29	1:2	97.63	383	96.82	387	99.40	354
1:19	1:3	70.71	374	76.90	380	72.41	352
1:24	1:3	87.90	380	81.70	387	78.74	357
1:29	1:3	93.93	387	93.60	394	97.96	362
1:19	1:4	63.04	383	67.41	388	61.00	361
1:24	1:4	70.94	388	77.46	395	72.40	364
1:29	1:4	89.20	395	90.14	402	93.75	372

*The values are the average of 3 determinations. EGMS, etoposide-loaded glycerol monostearate nanoparticles; EGDS, etoposide-loaded glycerol distearate nanoparticles; ETP, etoposide-loaded tripalmitin nanoparticles.

surface of emulsion droplets by HSPC, thereby controlling the droplet curvature due to its cosurfactant property. The average mean diameter of optimized formulations of EGMS, EGDS, and ETP nanoparticles was found to be 383, 387, and 354 nm, respectively.

Spray drying of native nanodispersions caused the dried particles to stick to the glass surface of the drying chamber, which resulted in particle aggregation and poor yield. The use of carbohydrates as film formers for preventing the particle aggregation in lyophilization has been studied by Bodmeie et al.[18] These carbohydrates are expected to form a film on the particle surface during spray drying and prevent the direct contact of the lipid layer of nanoparticles with the hot surface of drying chamber. In this study, lactose monohydrate (a disaccharide), 1 part, 2 parts, and 3 parts by weight of lipid content in the formulation, is used to facilitate better spray drying of nanoparticles, and the final particle size was determined. Of these 3 different batches, spray drying with 2 parts lactose gave relatively smaller particles, and a further increase in lactose quantity did not cause reduction in particle size. Lower concentration of lactose monohydrate (1 part) was insufficient to protect the particles from sticking to the walls of the drying chamber and resulted in a poor yield. A higher amount of lactose monohydrate ($\geq$2 parts) improved the yield of nanoparticles and prevented the particle aggregation. Spray drying of the nanodispersion yielded powder particles with a slight increase in size. But, the size increase is not significantly higher, unlike results reported by Freitas and Muller[3] with cetylpalmitate and Compritol 888 ATO nanoparticles. Spray drying resulted in an increase in the average mean diameter to 22 nm, 27 nm, and 33 nm of EGMS, EGDS, and ETP, respectively, with unimodal size distribution. This clearly indicates that the spray-drying conditions adopted hardly affected the nanoparticle size. The spray-dried nanoparticles possessed excellent redispersibility characteristics in aqueous medium and did not require any sort of energy, such as sonication. The redispersibility of nanoparticles is probably due to the formation of hydrosoluble matrix embedding the particles inside during the spray-drying process.

Poloxamer 407-stabilized GDS nanoparticles were found highly aggregated after spray drying (4.60 μm) compared with the nanodispersion (354 nm) and exhibited broader size distribution. Furthermore, the aggregates were found trapped in a matrix-like structure, which illustrated by their scanning electron micrograph (Figure 3). The results obtained are in agreement with that reported by Freitas and Muller.[3] This aggregation and formation of matrix-like structure is not properly understood and may be cased by the change in the physical nature of Poloxamer 407 and the dehydration of propylene oxide and ethylene oxide blocks[31] within the Poloxamer 407 surfactant molecule at the inlet drying temperature. Similar case of aggregation of Poloxamer-stabilized nanoparticles at higher temperatures has been reported earlier.[32] A significant increase in size of Poloxamer 188-stabilized Compritol SLN after steam sterilization was reported by Schwarz and co-workers.[33] The reported significant increase in size of Poloxamer 188-stabilized cetylpalmitate and Compritol 888 ATO nanoparticles by Freitas and Muller[3] may be caused by the similar effect we observed. Such particle behavior is not observed with the nanoparticles stabilized by sodium tauroglycocholate (Figure 3, B,C,D). The smaller particle size of Poloxamer 407-stabilized EGDS nanoparticles even after homogenization at higher temperatures may be due to the cavitation force-induced continuous particle breakdown resulting in size reduction of the nanoparticles, though they are in an aggregated form. This clearly indicates that the stabilizer used for nanoparticle preparation greatly influences the final particle properties recovered by the spray-drying process.

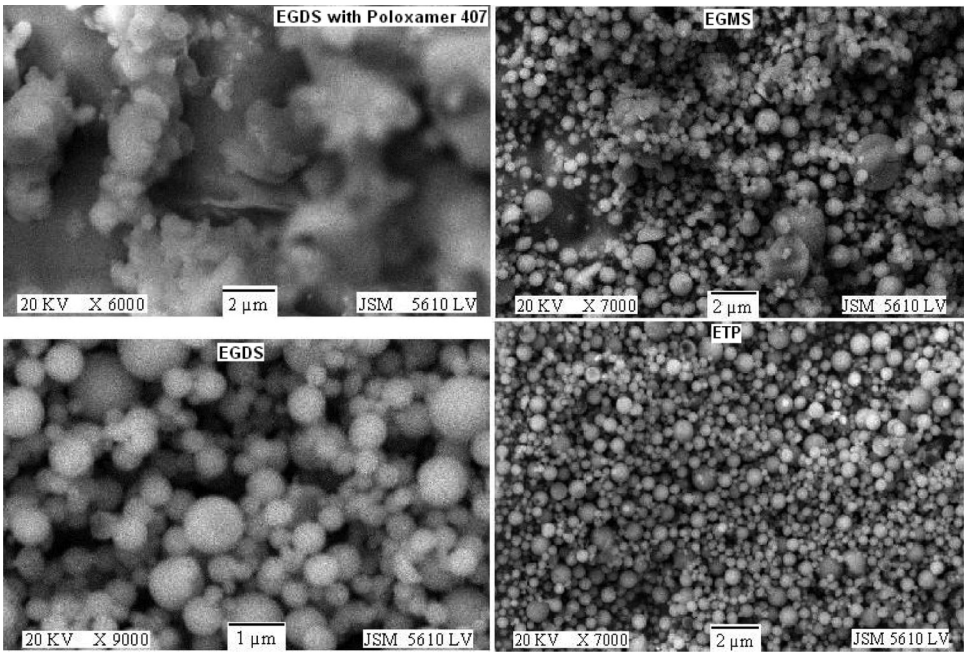

Figure 3. Scanning electron micrographs of EGMS, EGDS, and ETP nanoparticles.

Zeta Potential Measurement

Of the 3 spray-dried formulations of glyceride nanoparticles, the ETP (−46.6 mV) nanoparticles exhibited high zeta potential followed by EGDS (−30.0 mV) and EGMS (−24.3 mV) nanoparticles. The trend of increase in zeta potential from monoglyceride to triglyceride is probably due to the increase in the substitution number of carbon chains.

Differential Scanning Calorimetry

The thermal curves of GMS, GDS, and TP bulk lipids showed endothermic peaks at 65.6°C, 65.6°C, and 63°C, respectively (Figure 4). The melting endothermic peaks of the spray-dried nanoparticles appeared at slightly lower temperatures (58°C for EGMS, 61°C for EGDS, and 59°C for ETP nanoparticles, respectively) than the corresponding bulk lipids, and melting endotherms were broader. The results obtained are in good agreement with those reported by Bunjes et al.[34] GMS, GDS, and TP are able to form a crystalline lipid matrix after the melt homogenization and spray-drying process. Tripalmitin nanoparticles are reported to form solid colloidal dispersions, unlike other triglycerides such as trimyristin and trilaurin.[35] The decrease in melting temperature of nanoparticle formulated glyceride lipids compared with the bulk has been attributed to their small size and presence of surfactants.[34]

In Vitro Steric Stability by an Electrolyte-Induced Flocculation Technique

The electrolyte-induced flocculation technique helps in determining the resistance of nanoparticles to aggregation against the flocculating agents. The EGMS, EGDS, and ETP nanoparticles prepared using sodium tauroglycocholate exhibited stability in presence of the flocculating agent sodium sulfate (Figure 5). Of the 3glyceride nanoparticles, ETP nanoparticles exhibited excellent stability, followed by EGDS and EGMS nanoparticles. The absorbance curve of ETP nanoparticles is almost planar, and the nanoparticles remained almost stable even at 1.4 mol/L sodium sulfate concentration, indicated by the stable absorbance. The absorbance of EGMS and EGDS nanoparticles slightly increased, initially followed by stable absorbance later.

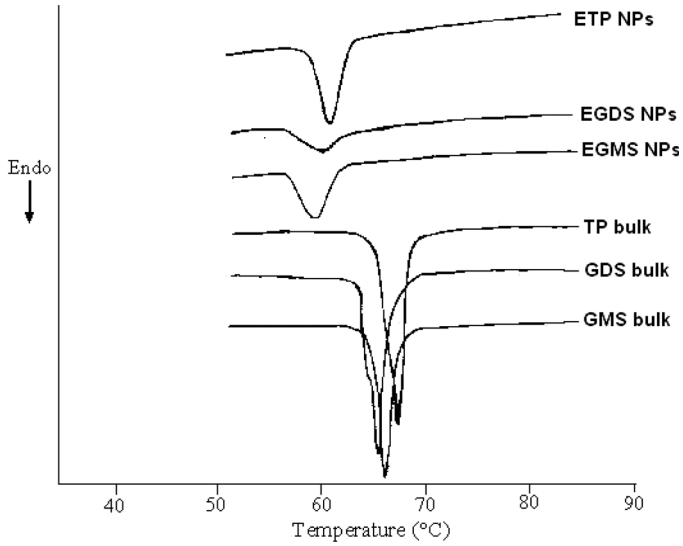

Figure 4. DSC curves of bulk glycerol monostearate (GMS), bulk glycerol distearate (GDS), bulk tripalmitin (TP), and etoposide-loaded glycerol monostearate, glycerol distearate, and tripalmitin spray-dried nanoparticles.

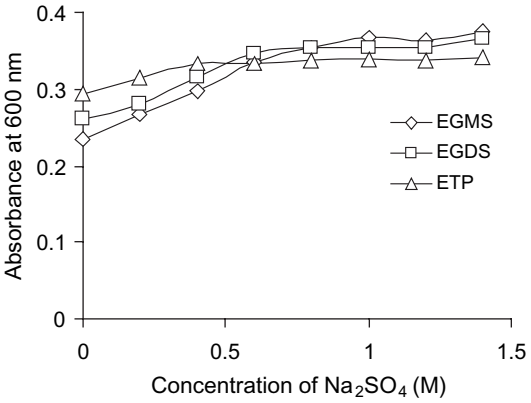

Figure 5. Steric stabilization effect of etoposide-loaded glycerol monostearate, glycerol distearate, and tripalmitin nanoparticles. The nanoparticles were stabilized by 3% wt/vol of Poloxamer 407.

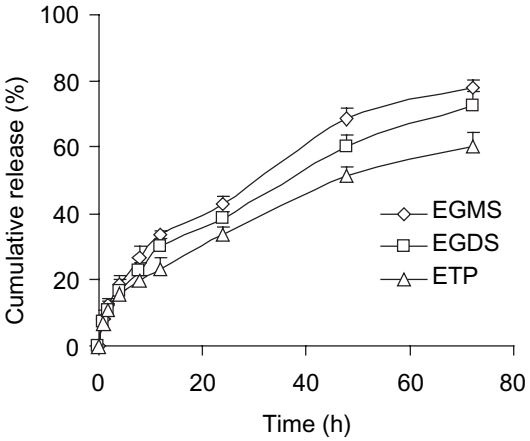

Figure 7. In vitro release profiles of etoposide from etoposide-loaded glycerol monostearate, glycerol distearate, and tripalmitin nanoparticles in phosphate buffer (pH 7.4).

Poloxamers are reported to possess excellent steric stabilization properties and are thought to prevent opsonization in blood and enhance the circulation half-life of polymeric nanoparticles after intravenous administration.[22,36] Also the lipid nanoparticles prepared using Poloxamers have been shown to inhibit phagocytosis.[37] But, in the in vitro study, the nanoparticles prepared using Poloxamer 407 did not exhibit a steric stabilization effect. To confirm concentration dependence of the steric stabilization effect of Poloxamer, the nanoparticles were prepared using different Poloxamer 407 concentrations (2% wt/vol, 4% wt/vol, and 6% wt/vol) (Figure 6). In this case, the nanoparticle aggregation started just after, 0.4 mol/L sodium sulfate concentration administration, indicating the poor steric stabilization of nanoparticles by Poloxamer 407. This is probably caused by the extensive dehydration of propylene oxide and ethylene oxide blocks within the Poloxamer molecule during emulsification and hot homogenization conditions, leading to the reduced steric repulsion activity. Hence, our studies reveal that sodium tauroglycocholate possesses the steric stabilization property relatively better than Poloxamer 407 for glyceride nanoparticles.

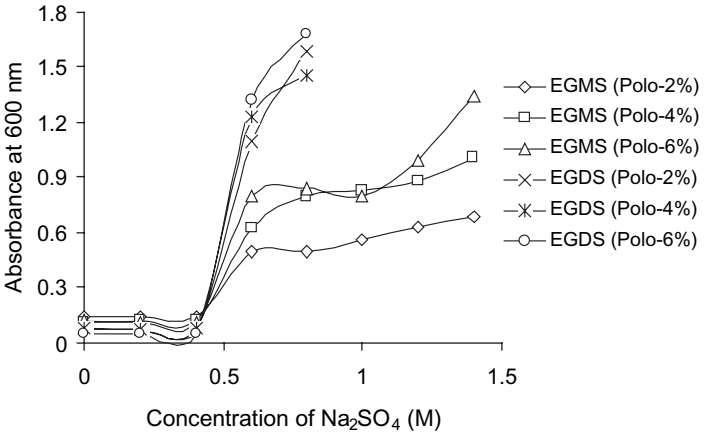

Figure 6. Steric stability test of etoposide-loaded glycerol monostearate and glycerol distearate nanoparticles prepared from different concentrations of Poloxamer 407 (Polo).

In Vitro Drug Release Studies

The EGMS, EGDS, and ETP nanoparticles exhibited sustained release properties (Figure 7) followed by a slight initial burst release. This is probably caused by the release of drug adsorbed on the nanoparticle surface or precipitated from the superficial lipid matrix. Prolonged release in the later stage can be attributed to the slow diffusion of drug from the lipid matrix. The esterification of glycerol by long-chain fatty acids is responsible for high hydrophobicity of these glycerides.[38] The drug release profiles from nanoparticles exhibited linear relationships by Higuchi plotting. The correlation coefficients of the equation for EGMS, EGDS, and ETP nanoparticles were 0.994, 0.996, and 0.996, respectively. The t_{50} (time taken for 50% drug release) was calculated as 30.5, 35, and 46.75 hours for EGMS, EGDS, and ETP nanoparticles, respectively. The t_{50} values clearly indicate that the ETP nanoparticles exhibited a high sustained effect compared with EGDS and EGMS nanoparticles. This is probably caused by the high lipophilicity of tripalmitin ($C_{51}H_{98}O_6$) compared with glyceride distearate (C_{16}–C_{18}) and glycerol monostearate ($C_{21}H_{42}O_4$) due to more carbon chains contributing to the more sustained release effect. The sustained release characteristics of glyceride nanoparticles coupled to the steric repulsion properties could be a beneficial delivery system as long circulating carriers in drug delivery applications.

Stability of Lipid Nanoparticles

The lipid nanoparticulate dispersions in transparent polypropylene containers stored at 4°C to 8°C in the dark showed rapid aggregation within 1 month of storage. The dispersions underwent sedimentation, and a significant

increase in particle size was observed (5.2, 6.7, and 4.8 μm for EGMS, EGDS, and ETP, respectively). In contrast, the dispersions stored in black containers remained stable, with only a very slight change in particle size after 5 months (397, 395, and 368 nm for EGMS, EGDS, and ETP, respectively). A similar finding of the improvement of stability of Compritol SLN when stored in brown glass containers was reported by Freitas and Muller.[16] Based on the above results, the stability of spray-dried nanoparticulate formulations was studied in black polypropylene containers at 4°C to 8°C in the dark and also at 25 ± 2°C at room light conditions. Storage of nanoparticle dispersions in the black polypropylene containers at 4°C to 8°C in the dark improved the stability compared with that stored in transparent containers at the same conditions. This indicates that the black polypropylene containers could block radiation from passing through, whereas the transparent containers could not. This clearly demonstrates the sensitivity of glyceride nanoparticle dispersions to light radiations. Storage of spray-dried nanoparticles in black polypropylene containers both at 4°C to 8°C in the dark and 25°C at room light conditions maintained the stability of the nanoparticles, which is due to the light resistance of black containers. The nanoparticles remained stable, which is indicated by their constant mean particle diameter (409, 421, and 385 nm for EGMS, EGDS, and ETP, respectively), entrapment efficiency, and excellent redispersibility. This further supports the advantage of spray drying in the formation of powder nanoparticles stable for a longer time.

CONCLUSION

The study demonstrates that the melt emulsification and homogenization technique followed by spray drying is an advantageous and viable technique to obtain stable powder particles, having a smaller mean diameter and excellent redispersibility characteristics. The size of nanoparticles can be controlled by the experimental variables, such as homogenization pressure and cycle number, and formulation variables, such as surfactant, cosurfactant, and carbohydrate concentrations. The aggregation and matrix-like structure formed by Poloxamer 407-stabilized, spray-dried nanoparticles suggests that caution has to be exercised while using the Poloxamers in the spray drying of formulations. The excellent in vitro steric stability of the sodium tauroglycocholate-stabilized nanoparticles with sustained release characteristics can form a foundation for further studies into the in vivo use of these nanoparticles as long circulating carriers in blood.

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