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Preparation of solid lipid nanoparticles loaded with garlic oil and evaluation of their *in vitro* and *in vivo* characteristics

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Abstract. – OBJECTIVE: Solid lipid nanoparticles (SLN) are colloidal drug carriers and may be suitable for delivery of garlic oil, a nutraceutical with medicinal properties, whose use has been limited by its poor solubility. We tested whether poor solubility of garlic oil would be overcome by complexing with SLN by high-pressure homogenization and ultrasound techniques. The effects of lipid phase, surfactant mixture and loading concentration of garlic oil on particle size and distribution were also investigated.

MATERIALS AND METHODS: High pressure homogenization technique was used to prepare SLN, using orthogonal experiment method to optimize entrapment efficiency, loading efficiency, and recovery of SLN. Pharmacokinetics of garlic oil loaded solid lipid nanoparticles after oral administration to rats was studied by using LC/MS/MS method.

RESULTS: Mean particle size and zeta potential of SLN were, respectively, 106.5 ± 40.3 nm and -30.2 mv. The majority of SLN had a less ordered arrangement of crystals at room temperature, which was beneficial for increasing the drug loading capacity. Drug entrapment efficiency was > 90 percent and showed a relatively long-term physical stability. It was feasible to prepare a lyophilized product with good long-term stability. When 10% trehalose and 5% sucrose were used as cryopreservants, SNL particle size increased from 106.5 nm prior to lyophilisation to 155.3 nm after reconstitution. The garlic oil content in SLN decreased to about 85% (respectively, 34.3 *vs.* 39.4 mg/mL prior to lyophilisation) due to volatility of garlic oil. Pharmacokinetic studies in rats demonstrated that distribution and elimination of diallyl trisulfide (DATS) and diallyl disulfide (DADS) in garlic oil were rapid. Additionally, elimination of garlic oil-SLN complex is faster than that of garlic oil alone, probably, due to phagocytosis.

CONCLUSIONS: An SLN complex with garlic oil exhibits characteristics similar to those of parenteral emulsions, even after lyophilization and reconstitution.

Key Words:
Solid lipid nanoparticles, High pressure homogenization, Ultrasound techniques, Garlic oil, Freeze-drying, Pharmacokinetics.

Introduction

Solid lipid nanoparticles (SLN) present the advantages but avoid the disadvantages of other colloidal carriers[1-3]. It is not surprising that SLN attracted attention in the recent years and are viewed as a carrier system alternative to traditional colloidal systems, such as emulsions, liposomes, polymeric microparticles or nanoparticles[4,5]. To prepare SLN, biocompatible compounds are used. This minimizes toxicity frequently associated with polymeric nanoparticles due to presence of toxic monomer residues or solvents and toxic degradation products. Additional advantages of SLN are amenability for large-scale industrial production by high-pressure homogenization, resistance against chemical degradation, controlled drug release, absence of organic solvents, wide range of lipid/surfactant combinations, and high drug payload[6-9].

Garlic (*Allium sativum*) is a widely used food product that exerts bioactivity in hypolipidemia, thrombosis, atherosclerosis, mutagenesis, carcinogenesis and antimicrobial defences[10]. Garlic contains a variety of volatile oils, collectively referred to as garlic oil, which are bioactive ingredients of

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this food product[11-16]. The major compounds of garlic oil are organosulfuric compounds, diallyl sulfide, diallyl disulfide (DADS), and diallyl trisulfide (DATS). Despite promising bioactive characteristics, the use of garlic oil in clinic practice is limited due to poor water solubility.

The aim of this study was to test whether it would be feasible to prepare glyceryl monostearate nanoparticles loaded with garlic oil by using high pressure homogenization. We also tested the impact of lipid phase, surfactant composition, drug loading, and freeze-drying on physicochemical characteristics of SLN. We further studied pharmacokinetics of the garlic oil-SLN complex to test its fate *in vivo*.

Materials and Methods

Reagents

The injection grade garlic oil and drug standard diallyl trisulfide (DATS; purity about 90% and > 97% after refinement) were purchased from Shangdong Jinxiang Food and Medicine (China). Diallyl disulfide (DADS, purity of 80%, with remainder being other allyl sulfides) was obtained from Sigma-Aldrich (Steinheim, Belgium). Glycerol monostearate was bought from Tianjin Chemical Reagent (China). Stearic acid was purchased from Tianjin Bodi Chemical Reagent (China). D-95 distilled monoglyceride was from Guangzhou Jialishi Food Technology (China). Compritol 888 ATO was a kind gift from Cedex (France). Poloxamer 188 was supplied by BASF (Ludwigshafen Rhine, Germany). Lecithin was purchased from Shanghai Taiwei Pharmaceutical (China). Methanol and acetonitrile (chromatographic grade), and other chemicals (analytical reagent grade) were purchased from Shangdong Jinxiang Food and Medicine.

Preparation of the Garlic Oil–SLN Complex

SLN were prepared by high pressure homogenization and modified ultrasound method. Lipid matrix was melted at about 80º C, and weighed garlic oil was added to obtain a clear melting solution in lipid phase. The aqueous phase was prepared by dissolving poloxamer 188 in double-distilled water under stirring and heating to the temperature of molten lipid phase. The hot-water surfactant solution was poured into molten lipid phase, and the mixture was vigorously stirred using FA25 Fluko high shear homogenizer (Shanghai, China) at 10,000 rpm for 3 min to form coarse emulsion. Then, pre-emulsion was either further homogenized 10 times at 1000 bar using a high pressure homogenizer (NS1001L PANDA 2K homogenizer, Niro Soavi, Parma, Italy) or ultrasonicated for 6 min (every 3 sec, 2-sec pulse) using a JY92-II probe-type ultrahomogenizer (Ningbo Kesheng Instruments, China). The resulting oil/water nanoemulsion was cooled down to room temperature. As lipids recrystallized, SLN were formed.

Preparation of Garlic Oil Solution

A garlic oil solution (20 mg/mL, w/v) was prepared in 10% Tween-80 solution. This solution was stored at 4º C.

Lyophilization of SLN Dispersion

The optimized GO-SLN dispersion was frozen at -80° C for 5 hours (Heto Ultra Freezer; Marietta, GA, USA). Afterwards, the samples were subjected to freeze-drying (FD-1B-55, Beijing Boyikang Laboratory Instruments, Beijing, China). The drying time was 24 hours until dry powder was formed.

Measurement of Size and Zeta Potential of SLN

Mean diameter and *z*-potential of SLN in suspension were determined by dynamic light scattering method using a Nicomp-380 submicron particle zetasizer (Particle Sizing Systems, Variations, Port Richey, FL, USA) at a fixed angle of 90º, at room temperature, after the suspension was diluted appropriately with the aqueous phase of the formulation. Measurements were made in triplicate with all prepared batches.

Transmission Electron Microscopy

Particle morphology was examined by transmission electron microscopy (CM 10 Philips, Eindhoven, Netherlands). The samples were properly diluted with tri-distilled water and placed on Cu grid coated with C film. After staining with 2% (w/v) phosphotungstic acid and drying at room temperature, the samples were examined by transmission electron microscopy.

Differential Scanning Calorimetry

Differential scanning calorimetry was performed using DSC60 (Shinadzu, Hokkaido, Japan). The instrument was calibrated with indium (calibration standard, purity of > 99.999%) for melting point and heat of fusion. A heating rate of 10º C/min was employed in the range of

30-100º C. Analysis was performed under nitrogen purge (50 ml/min). Standard aluminum sample pans (40 Al) were used. A 10-mg sample was used for the analysis. An empty pan was used as reference.

HPLC Analysis of Garlic Oil in SLN

DATS in garlic oil served as the marker. The amount of garlic oil incorporated in SLN was determined by HPLC using a 510 pump unit control with a 20-µl loop and rheodyne sample injector (N2000 Chromatopac integrator) and a 486 UV detector (both from Waters, Milford, MA, USA) set to 220 nm. A reverse-phase Kromasil C_{18} column (4.6 × 200 mm, 5 µm particle size) was used. Mobile phase consisted of acetonitrile-water-tetrahydrofuran (v/v/v: 65/34/1); flow rate was set to 1.0 ml/min. The data were recorded using N2000 chromatography data system (Zhejiang Zhida Information Engineering, Zhejiang, China).

Entrapment Efficiency

Entrapment efficiency was determined by measuring the concentrations of free and total drug in the dispersion medium. A freeze-thawing-aggregation-filtration method was used to separate the free drug from the suspension. First, SLN were frozen at -80º C without any cryoprotectant. This was followed by thawing to room temperature, when apparent aggregation has occurred. The flocculation was filtered through a 0.45 µm filter. The amount of free drug in the filtrate was determined by HPLC. The loading efficiency was calculated from the amount of free drug and the initial amount of the drug used to load SLN. Entrapment efficiency (EE%) was calculated using the following equation ("W" = weight):

$$EE\% = \frac{W_{\text{initial drug}} - W_{\text{free drug}}}{W_{\text{initial drug}}} \times 100\%$$

The In Vitro Release Kinetics of Garlic Oil in SLN

The *in vitro* release study was conducted within 24 hours after preparing SLN. The garlic oil-SLN solution (2 ml) was added to a dialysis bag with a molecular weight cut-off of 12,000-14,000 Da (Sino-American Biotechnology, USA). The dialysis bag was placed in a beaker that contained 100 ml of 10% hydroxypropyl- -cyclodextrin (HP- -CD) or 30% alcohol. The medium in the dialysis bag was stirred at 100 rpm and warmed up to 37º C. At predetermined time intervals, a 2-ml sample of the medium was obtained and replaced with the same amount of fresh medium. The amount of garlic oil released from SLN was measured by HPLC described above.

Pharmacokinetics of the Garlic Oil-SLN Complex

Six Wistar rats (260-300 g) were used in the experiments. The animals were housed in hanging wire cages with free access to food and water, and a 12 hour light-dark cycle (lights on at 6:00 am) at a temperature of 19-20º C. The experimental protocol was designed according to the guidelines of the Chinese Council on Animal Care and approved by the Animal Care Committee of the General Hospital of Shenyang Military Region. For experiments, 0.5 mL of garlic oil-SLN complex or garlic oil solution (10 mg garlic oil) were administered within 1 min via the jugular vein. Blood samples were taken from the rats at predetermined time intervals and processed immediately.

Blood Sample Treatment and Analysis

A 0.2 mL sample of rat blood was added to a 5 mL glass test tube followed by 20 µL of 10% (v/v) hydrochloric acid. The tube was briefly mixed on a YKH-II vortex-mixer (Jiangxi Medical Appliance, China). Then, 0.2 mL of acetonitrile containing interior label (100 ng/mL or 2 µg/mL) were added, and the tube was vortexed immediately. Hexane (0.4 or 1 mL) was added, and the tube was capped and shaken vigorously for 0.5 min. The tube was then centrifuged at 2000 g for 5 min in a bench-top centrifuge (TDL-5, Shanghai Anting Medical Appliance, Shanghai, China). Finally, 1 µL of the organic layer was used for gas chromatographic analysis.

Analyses were performed on gas chromatograph (Agilent 6890N, Tokyo, Japan) equipped with a ^{63}Ni electron-capture detector. A fused-silica capillary column (30 m 0.25 mm) was used coated with a 0.25 µm thick film of 5% phenyl methyl siloxane (Agilent, Santa Clara, CA, USA) as the stationary phase. The conditions for gas chromatographic separation were as follows. The oven temperature was set to 120° C and maintained for 15 min. Temperatures at the injection port and detector were, respectively, 180º C and 250° C. Ultra-pure nitrogen (purity > 99.999%, Shenyang Kerui Special Gases, Shenyang, China) was used as a carrier gas and make-up gas at

flow rates of, respectively, 1 ml/min and 60 ml/min. All injections were carried out in a split injection mode with a split ratio of 1:10.

Pharmacokinetic Analysis

The curves of blood concentration *vs.* time for DATS and DADS in rats were analyzed by non-compartmental estimations using DAS2.0 (Institute of Material Medica, Shanghai, China). The maximum blood concentration (C_{max}) and the time to reach C_{max} (T_{max}) were taken directly from the profile of observed concentration *vs.* time.

Statistical Analysis

SPSS17.0 software package (SPSS Inc., Chicago, IL, USA) was used to analyze the data. Measurement data were compared with *t*-test, expressed as the mean±standard deviation (x±s). A value of $p < 0.05$ was considered to be statistically significant.

Results

Preparation of SLN

SLN can be prepared using different methods[1,17,18]. High shear homogenization and ultrasound are dispersing techniques which were initially used for the production of SLN. Both methods are widespread and easy to handle. However, dispersion quality is often compromised by the presence of microparticles. Furthermore, metal contamination has to be respected if ultrasound is used. High pressure homogenization has emerged as a reliable and powerful technique for preparation of SLN, and includes hot and cold homogenization techniques. In contrast to other techniques, this technique is easily scalable. The advantage of solvent emulsification/evaporation over the cold homogenization process is in the avoidance of any thermal stress. However, a clear disadvantage of this technique is the use of organic solvents. With regard to microemulsion method, due to dilution step, the lipid yields are considerably lower compared with formulations obtained with high pressure homogenization.

Figure 1 demonstrates the impact of pressure and cycles on the size of the garlic oil-SLN complex. We chose 1000 bar and 10 cycles as ultimate conditions. To decrease the particle size, homogenization time was optimized to 6 min and ultrasound power to 400 watt. There was no decrease in particle size when homogenization time and ultrasound power were increased. The results showed that both methods were suitable to this investigation. The optimum average particle sizes were 106.5 ± 40.3 nm and 152.3 ± 52.5 nm, respectively.

The Effect of Lipid Phase

A clear advantage of SLN is that the lipid matrix is made from physiological lipids, which decreases their acute or chronic toxicity. The term "lipid" is used in a broader sense and includes triglycerides (e.g., tristearin), partial glycerides (e.g., Imwitor), fatty acids (e.g., stearic acid), steroids (e.g., cholesterol), and waxes (e.g., cetyl palmitate)[19]. To find a suitable lipid phase, stearic acid, glycerol monostearate, D-95 distilled monoglyceride and Compritol 888 ATO were selected as a lipid phase at a fixed concentration of 2.5%. The complex emulsifier was composed of 1% poloxamer 188 and 0.2% lecithin. The concentration of garlic oil was 1.0%.

SLN with stearic acid as the lipid phase rapidly aggregated during the preparation. When D-95 distilled monoglyceride was used as the lipid phase, SLN agglomerated on the next day when stored at 4° C. The particle size of SLN with glycerol monostearate and Compritol 888 ATO as lipid phases were, respectively, 95.3 ± 38.1 and 268.9 ± 139.5 nm. Therefore, glycerol monostearate was chosen as the solid lipid phase to constitute the core of SLN.

The lipid content of more than 5%-10% will increase homogenization efficiency and agglomeration of the particles increase. Figure 2 shows the effect of content of gelatin microspheres over 1-10% on the particle size of SLN. When concentration of gelatin microspheres was higher than 2.5%, particle sizes increased proportionally to the increase of concentration of gelatin microspheres.

The Effect of Surfactant Mixture

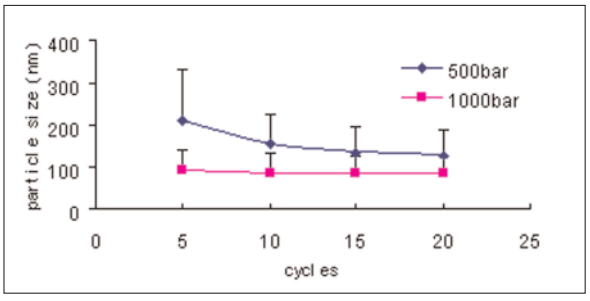

Figure 1. Effect of pressure and cycles on particle size of the garlic oil-SLN complex.

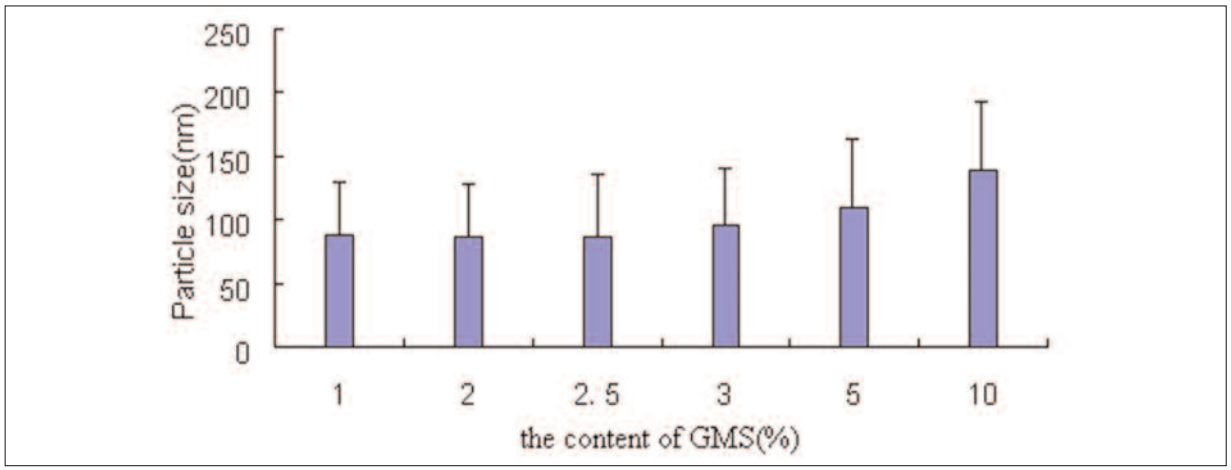

Figure 2. Effect of different concentrations of gelatin microspheres on particle size of SLN.

SLN prepared with combination of surfactants generally tend to have smaller particle sizes and higher storage stability. The latter effect is due to a more efficient prevention of particle agglomeration[7,20]. In addition, the increase in the surfactant amount in colloidal dispersions may contribute to a decrease of mean particle size because of the surface-active properties of surfactants[19]. We chose poloxamer 188 and lecithin as emulsifiers and examined whether the amount and composition of surfactants influenced those parameters in our SLN formulation. The results are presented in Figure 3. When either poloxamer 188 or lecithin was used as emulsifiers, the stability of SLN was less than that of SLN prepared with both emulsifiers. Mean particle sizes of SLN prepared with 1% poloxamer 188 or 1% lecithin were, respectively, 100.9 and 155.8 nm, and the corresponding z-potentials were, respectively, -17 mv and -33 mv. Mixing of poloxamer188 and lecithin reduced the particle size, resulting in 101.2 nm in SLN with 4:1 weight ratio of poloxamer188 and lecithin, and z-potential of -26 mv. However, particle size did not decrease linearly according to the increase in the total amount of poloxamer 188 from 0.8% to 3.0%, when lecithin was kept at a constant concentration of 0.2%.

The Effect of Loading Concentration of Garlic Oil

The impact of garlic oil loading on mean particle size of SLN was also studied. The results are presented in Table I.

The data described above demonstrate that lipid phase, amount and ratio of surfactant mixture, and concentration of garlic oil are the important modifiers of SLN particle size. Only when the appropriate balance of these is provid-

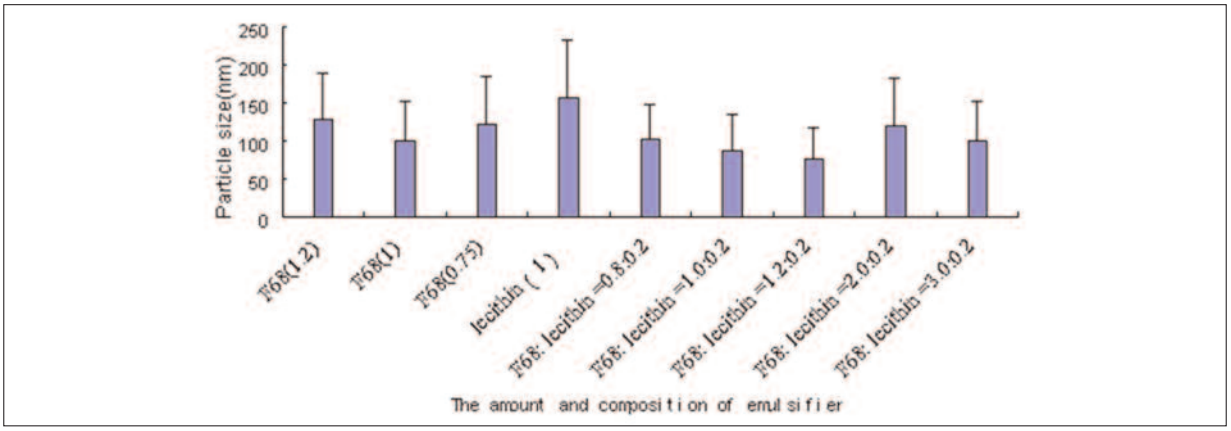

Figure 3. Effect of the amount and composition of surfactants on particle size of SLN.

Table I. Effect of garlic oil concentration on particle size.

Garlic oil (%)	Size (nm)	Encapsulation efficiency (%)
1	88.4 ± 42.3	96.78
2	78.4 ± 29.0	94.23
4	102.8 ± 39.5	93.95

Footnote: Data are shown as percentages or mean ± SD.

ed, can SLN be of smaller particle size and desired distribution uniformity.

The Effect of Cryoprotectants During Lyophilization

Long-term physical and chemical stability is still an important problem of SLN. In addition, SLN for intravenous use need to meet certain criteria of microparticle contamination and toxicity. Also the size is important, as SLN with a diameter of > 5 μm may cause death due to embolism. For that reason, aggregation and particle growth have to be avoided during storage. Chemical stability of hydrolizable drugs is another problem[21-23].

We tested eight different carbohydrates (mannite, fructose, lactose, glucose, sucrose, sorbitol, maltose, and trehalose) and two polymers (polyvinyl alcohol and polyvinyl pyrrolidone) at different concentrations. When lyophilized without a cryoprotectant, remarkable agglomeration of SLN was observed.

Reconstitution of SLN was done by addition of 2 ml of water and manual shaking. This was to mimic clinical settings since vortex mixing or ultrasonification are not accessible in the clinic. Both 10% trehalose and 5% sucrose were the most efficient cyroprotectants among the tested, as the mean particle size of SLN increased only by ~1.5 times (from 106.5 nm prior to lyophilization to 155.3 nm after redispersion). When total concentration of cryoprotectants was lower than 10% (w/v), SLN collapse occurred. However, the content of garlic oil in SLN decreased to about 85% from that prior to lyophilization (39.4 mg/mL *vs.* 34.3 mg/mL) due to volatility of garlic oil, suggesting that garlic oil could be stably retained in SLNs and that garlic oil was lost from the surface of SLN during lyophilization.

Characteristics of Optimized Formulation of the Garlic Oil-SLN Complex

Derived from optimization studies presented above, the garlic oil-SLN complex was prepared with 8 mg/g surfactant mixture composed of a 3:1 (w/w) mixture of poloxamer188 : lecithin and 40 mg/g of garlic oil. Particle size and zeta potential of the optimized complex garlic oil were, respectively, 106.5 ± 40.3 nm and -30.2 mv. Figure 4 shows transmission electron microscopy images of the complexes prepared by high pressure homogenisation. These images demonstrate that melt-high pressure homogenization process leads to formation of spherical microparticles with smooth surface.

The physical state of the particles is important from the technological and biopharmaceutical point of view. Differential scanning calorimetry gives an insight into melting and recrystallisation behaviour of crystalline materials, such as lipid nanoparticles. Breakdown of the crystal lattice by sample heating yields inside information on polymorphism, crystal ordering, eutectic mixtures, and glass transition processes[24]. The status of lipid nanoparticles stabilized with lecithin and poloxamer188 was investigated using differential scanning calorimetry. Figure 5 shows differential scanning calorimetry thermograms of poloxamer188, gelatin microspheres, mixture of gelatin microspheres with garlic oil, and gelatin microspheres and the garlic oil-SLN complex. The peak melting temperature of the garlic oil-SLN complex (57.10º C) tended to be lower compared with that of commercial lipids (61.42º C), and higher than that of a mixture of gelatin microspheres and garlic oil (45.70º C). It is possible that eutectic mixture formed after gelatin microspheres and garlic oil were mixed together placed the temperature above the melting temperature of

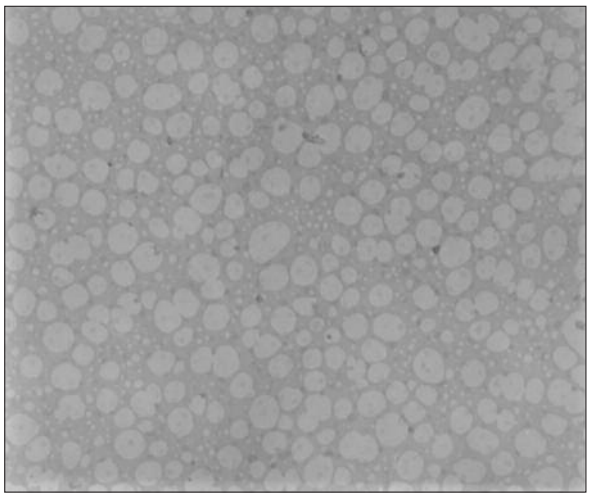

Figure 4. Appearance of garlic oil-SLN complexes under transmission electron microscopy. Magnification × 15,500.

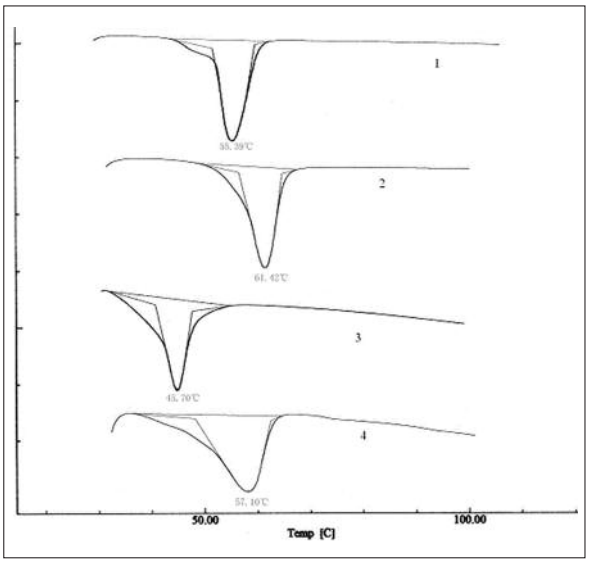

Figure 5. Differential scanning calorimetry thermograms of lyophilization of SLN dispersions heating from 30º to 100º C at a rate of 10º C. 1: Poloxamer 188; 2: gelatin microspheres; 3: physical mixture of GMS and garlic oil; 4: garlic oil-SLN complex.

the lipid, or because of less ordered crystal or amorphous state of gelatin microspheres due to the presence of garlic oil. According to differential scanning calorimetry results for considered systems, it can be assumed that crystallization of the garlic oil-SLN complex starts at room temperature.

Encapsulation Efficiency and Evaluation of the in vitro Release

The encapsulation efficiency in all formulations was higher than 90% due to high liposolubility of garlic oil. However, it is difficult to be released from SLN in general medium that cannot provide a sink condition. Many research groups used vertical or flow-through Franz diffusion cells, or dialysis bag/tubes to study of drug release from solid lipid and polymeric nanoparticles and niosomes[25-29]. Figure 6 shows the percentage release of garlic oil from the garlic oil-SLN complex in the medium. Burst release was observed during the first hour; this resulted in the release of about 1% of the drug from SLN. After that, a prolonged release was observed, with about 10% of drug released within the next 72 hours. Differential scanning calorimetry measurement of powdered SLN demonstrated that gelatin microspheres in SLN were still preserved in high crystal state and that garlic oil dissolved in gelatin microspheres. One per cent of garlic oil

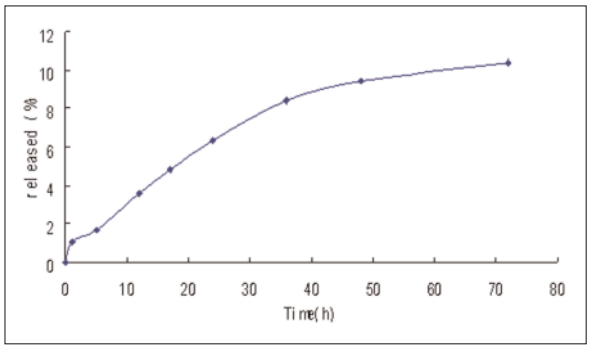

Figure 6. Release profiles of SLN in dissolution medium (composed of 10% HP-β-CD).

adsorbed on the surface of nanoparticles or precipitated in the superficial lipid matrix; thus, the dissolution profile of SLNs exhibited a burst of the drug during the initial stage. During the later stage, drug release was continuous and slow, indicating that the drug release rate was determined by the diffusion of the drug from the rigid matrix structure.

Pharmacokinetic Parameters

Since garlic oil is comprised of complex multi-constituents volatile oil, one indicator cannot entirely reflect its characteristics an *in vivo* study. Therefore, DATS and DADS, the major active components (combined content of > 70%), were selected as indicators in the pharmacokinetics study. The curves of blood concentration of DATS and DADS *vs.* time after intravenous administration of garlic oil solution or garlic oil-SLN are shown in Figure 7. We observed that both DATS and DADS in garlic oil solution and

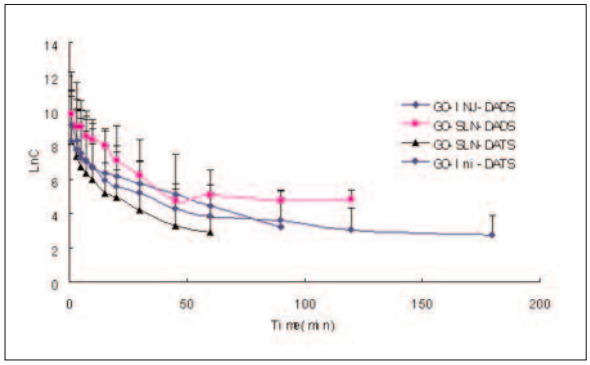

Figure 7. The curve of blood concentration vs. time after administration of a single dose of the garlic oil-SLN complex (10 mg of garlic oil) via the jugular vein. Data from 6 rats are shown.

Table II. Pharmacokinetics of garlic oil loaded into SLN.

Parameter	GO-SLN-DATS	GO-INJ-DATS	GO-SLN-DADS	GO-INJ-DADS
T_{max}, min	1.0 ± 0.01	1.0 ± 0.02	1.0 ± 0.02	1.0 ± 0.03
C_{max}, ng/ml	3574.3 ± 25.01	3692.8 ± 64.08	21416.7 ± 50.15	9335.8 ± 41.01
AUC_{0-t}, mg/L × min	18292.7 ± 261.01	31945.0 ± 196.12	182161.9 ± 174.11	91087.9 ± 258.14
$AUC_{0-\infty}$, mg/L × min	18654.1 ± 268.10	33644.0 ± 183.01	185208.1 ± 190.12	99039.4 ± 188.50
MRT_{0-t}, min	7.3 ± 0.05	19.4 ± 0.25	16.9 ± 0.41	19.5 ± 0.61
$MRT_{0-\infty}$, min	8.7 ± 0.25	33.6 ± 0.05	20.9 ± 0.07	34.4 ± 0.012
$AUMC_{0-t}$	134096.5 ± 145.36	619917.9 ± 159.24	3078840.0 ± 191.11	1778537.0 ± 216.02
$AUMC_{0-\infty}$	162947.0 ± 165.28	1112281.3 ± 190.15	3863416.1 ± 200.11	3409031.5 ± 301.54

Footnote: Nanoparticles were injected in jugular veins of 6 rats. Data are mean ± SD.

the complex of garlic oil-SLN rapidly degraded *in vivo*. Furthermore, DADS curve kinetics, which first descended, then underwent a secondary peak and then decreased again, showed that while DADS was degrading, DATS was transformed into DADS. This meant that the ultimate concentration of DADS resulted from the sum of degradation and DATS transformation into DADS.

Pharmacokinetic parameters are summarized in Table II. Compared with the solution of garlic oil, MRT_{0-t} (or $MRT_{0-\infty}$) of DATS (7.3 min) and DADS (16.9 min) in the garlic oil-SLN complex were shorter than those (19.4 and 19.5 min) in garlic oil solution. The main reason is that SLN are taken up by mononuclear phagocytes and a significant amount of SLN is trapped in organs with phagocytic activity, such as the liver[30]. The phagocytes express high levels of lipase that rapidly degrades the lipids, which leads to a loss of protection of garlic oil[7].

Conclusions

High pressure homogenization and ultrasound techniques were employed to prepare SLN. Garlic oil was incorporated in SLN. The mean particle size of optimized formulation was 106.5 ± 40.3 nm with a desired uniform distribution. The majority of SLN crystals were in a less ordered arrangement and still kept the solid lipid status at room temperature. The encapsulation efficiency of SLN was > 90% percent and showed a relative long-term stability at 4° C. Although about 15% of garlic oil was lost during lyophilization process, lyophilization was still a suitable method to improve the long-term physical and chemical stability of the garlic oil-SLN complex. Pharmacokinetics study showed that that this complex degrades *in vivo* more rapidly than the solution of garlic oil because the complex is targeted by phagocytes.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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