Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 257 (2003) 153–160
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Preparation of solid lipid nanoparticles by a solvent emulsification–diffusion technique

Michele Trotta*, Francesca Debernardi, Otto Caputo

Dipartimento di Scienza e Tecnologia del Farmaco, via P. Giuria 9, 10125 Torino, Italy

Received 14 November 2002; received in revised form 5 February 2003; accepted 6 February 2003

Abstract

A preparation method for nanoparticles based on the emulsification of a butyl lactate or benzyl alcohol solution of a solid lipid in an aqueous solution of different emulsifiers, followed by dilution of the emulsion with water, was used to prepare glyceryl monostearate nanodispersions with narrow size distribution. To increase the lipid load the process was conducted at 47±2 °C and in order to reach submicron size a high-shear homogenizer was used. Particle size of the solid lipid nanoparticles (SLN) was affected by using different emulsifiers and different lipid loads. By using lecithin and taurodeoxycholic acid sodium salt, on increasing the GMS percentage from 2.5 to 10% an increase of the mean diameter from 205 to 695 nm and from 320 to 368 nm was observed for the SLN prepared using benzyl alcohol and butyl lactate, respectively. Transmission electron micrographs of SLN reveal nanospheres with a smooth surface.

Keywords: Solid lipid nanoparticles; Glyceryl monostearate; Solvent diffusion

1. Introduction

The use of nanoparticles as drug-carrier system is a very attractive possibility to achieve controlled drug release. A clear advantage of solid lipid nanoparticles (SLN) over polymeric nanoparticles is the fact that the lipid matrix is made from physiological lipids, which decreases the danger of acute and chronic toxicity (Mehnert and Mader, 2001).

The techniques commonly used to produce SLN are based on high-pressure homogenization, dilution of microemulsions or solvent removal from oil-in-water emulsions. There are two general approaches within the homogenization technique, hot and cold homogenization: in both cases, a preliminary step involves drug incorporation into the lipid melt. Hot homogenization (Müller and Lucks, 1996; Muller and Runge, 1998) can therefore be regarded as homogenization of an emulsion, while cold homogenization (Jahnke, 1998) is effectively high-pressure milling of a suspension. Most SLN produced by hot homogenization are characterized by an average particle size below 500 nm and low microparticle content. In general, compared to hot homogenization, larger particle size and broader size distribution are observed in cold homogenized samples (Mehnert and Mader, 2001). Cold homogenization minimizes thermal exposure of the drug but does not avoid it completely, due to the melting of the drug–lipid mixture in the initial step. Other problems of hot homogenization, such as drug entrapment and crystallization, can however, be overcome.

Gasco (1993) developed an SLN preparation technique based on dilution in cold water of an oil-in-water

* Corresponding author. Fax: +39-0-11-6707687.
E-mail address: michele.trotta@unito.it (M. Trotta).

0378-5173/03/$ – see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0378-5173(03)00135-2

microemulsion. For a microemulsion to be formed with a solid lipid at room temperature, it must be heated above the melting point of the lipid. Surfactants and co-surfactants include lecithin and bile salts, but also alcohols such as butanol, which is less favorable in regulatory terms. Subsequent addition of the microemulsion to water leads to precipitation of the lipid phase forming fine particles. Large-scale production of SLN by the microemulsion technique also appears feasible (Carli, 1999).

Another proposed technique to produce SLN is the solvent emulsification–evaporation method (Sjöström and Bergenstahl, 1992; Siekmann and Westesen, 1996). The lipid matrix is dissolved in a water-immiscible organic solvent (e.g. chloroform) that is emulsified in an aqueous phase. Upon evaporation of the solvent under reduced pressure, a nanoparticle dispersion is formed by precipitation of the lipid in the aqueous medium. Depending on the fat load and emulsifier used, particles with average diameters of 30–100 nm can be obtained. An important advantage of this technique is the avoidance of any heat. On the other hand, solvent emulsification–evaporation suspensions are fairly dilute, due to the limited solubility of the lipids in the organic solvents used. Furthermore, in contrast to hot homogenization, the solvent-evaporation method may create toxicological problems arising from solvent residues.

In a previous study (Trotta et al., 2001) drug nanosuspensions were prepared from emulsions containing partially water-miscible solvents with low toxicity, such as benzyl alcohol or butyl lactate, by a solvent diffusion technique. The process is based on the water miscibility of these solvents. Upon transferring a transient oil-in-water emulsion into water, the drug dissolved in the organic solvent solidifies instantly due to diffusion of the organic solvent from the droplets to the continuous phase. Using optimized formulations, drug nanoparticles below 100 nm with very low polydispersity were obtained.

Recent studies, however, have corroborated that water immiscibility of a disperse solvent is not a prerequisite for making emulsions for preparing microspheres; the solvent diffusion technique using ethyl formate, methylethyl ketone or benzyl acohol (Sah, 2000; Sah et al., 1996; Leroux et al., 1995) lead to successful fabrication of good quality drug-loaded PLGA microspheres.

The aim of this study was to investigate the feasibility of preparing glyceryl monostearate nanoparticles from solvent-in-water emulsions by the diffusion technique, using solvents and surfactants accepted as having low toxicity.

2. Material and methods

2.1. Materials

Glyceryl monostearate (GMS) was from Goldschmidt (Essen, Germany). Soya lecithin (Epikuron 200) was from Lukas Meyer (Hamburg, Germany). Caprylyl-capryl glucoside (Oramix CG-110) was a gift from Seppic (Milan, Italy). Tween 80, taurodeoxycholic acid sodium salt (TDC), cholic acid sodium salt (CA), benzyl alcohol and butyl lactate were from Fluka (Buchs, Switzerland). Distilled water was purified using a Milli-Q system (Millipore, Bedford, MO). All other chemicals were of analytical grade and used without further purification.

2.2. GMS solubility

Benzyl alcohol and butyl lactate were used as solvents and saturated with water at 40, 45, 50 and 55 °C. A series of samples was prepared by adding increasing amounts of GMS to 1 ml of water-saturated solvents. The samples were sealed and stirred at 40, 45, 50 or 55 °C for 12 h. GMS solubility was considered to be that of the sample at highest GMS content that appeared transparent on visual observation.

2.3. SLN preparation

Benzyl alcohol or butyl lactate and water were mutually saturated at 47±2 °C for 10 min in order to ensure initial thermodynamic equilibrium of both liquids. Typically, 250 mg GMS were dissolved in 1000 μl of water-saturated solvent and this organic solution was emulsified at 47±2 °C with 9.0 ml of solvent-saturated aqueous solution containing 100 mg emulsifier mixture, using an Ultra Turrax (IKA, Staufen, Germany) at 12,000 rpm for 1 min. Various pairs of selected from Tween 80, Oramix CG-110, Epikuron 200, TDC or CA were used, in (1:1 w/w) mixture, as emulsifier system. The lipid nanoparticles

were precipitated by quickly adding water (50 ml) into the initial emulsion (10 ml) to extract the solvent into the continuous phase. After continual stirring for 60 min, the suspensions were purified.

A series of experiments in which the amount of GMS was progressively increased, maintaining the same percentages of the other components of the emulsion, were also performed. The percentage (w/w) of GMS in the emulsion ranged from 2.5 to 10%.

2.4. Purification of SLN dispersions

SLN dispersions were washed by utrafiltration with a TCF2 system (Amicon, Danvers, USA) using a Diaflo YM 100 membrane (cut-off 100,000 Da). The first 100 ml of suspension were concentrated to 20 ml, then 20 ml additional water was added and the suspension reconcentrated to 20 ml. This procedure was repeated a further three times. The residual solvent concentration in the dispersions was determined only for the systems containing benzyl alcohol, by an HPLC method at 254 nm using a C 8 column (4.6 mm × 25 cm, Merck) with a mobile phase consisting of methanol and water (10/90 v/v) at a flow rate of 0.8 ml/min.

2.5. SLN characterization

2.5.1. Particle size and Z-potential analysis

The average diameter, polydispersity index and *Z*-potential of the ultrafiltrated dispersions were determined by the laser light scattering technique (Brookhaven, New York, USA). Measurements were obtained at an angle of 90°. Scattering intensity data were analyzed by a digital correlator and fitted by the method of inverse Laplace transformation. The dispersions were diluted with water for size determination or with 0.005 M KNO_3 for *Z*-potential determination. The pH of the samples ranged from 6.0 to 6.2. Measurements were made in triplicate for all the batches prepared.

2.5.2. Transmission electron microscopy (TEM)

The morphology of the particles was examined by TEM (CM 10 Philips, Netherlands). Samples were obtained from emulsions containing 8% GMS, lecithin, TDC and benzyl alcohol or butyl lactate, stained with a solution of osmium tetroxide at 2% and finely spread over a slab.

2.5.3. Differential scanning calorimetry (DSC)

DSC was performed with a Perkin-Elmer differential calorimeter (Norwalk, Conn, USA). Suspensions obtained by dispersing in water at room temperature a weighed amount of commercial or water-recrystallized GMS, and GMS nanoparticles obtained from 8% GMS, lecithin, TDC and benzyl alcohol or butyl lactate emulsions were placed in conventional aluminium pans and a scan speed of 5 °C/min was employed. The weight of GMS in the samples was in the 1–1.2 mg range.

3. Results and discussion

The first step in the production of lipid nanoparticles by the solvent diffusion technique is to prepare a solvent-in-water emulsion with a partially water-miscible solvent, containing the lipid in rational amounts, as disperse phase.

After preliminary experiments, benzyl alcohol or butyl lactate, both solvents possessing low toxicity, was used to prepare the primary emulsion. The water solubility (w/w) of benzyl alcohol and butyl lactate are 3.8 and 7.7%, respectively. Table 1 reports the solubility of GMS in the water-saturated solvents at different temperatures. The results show that GMS solubility is acceptable for preparing the primary emulsions only above 45 °C.

A number of variables affect solvent droplet size, and probably also the properties of the resulting solid particles (Sah, 2000). Thus, in order to reduce the number of experiments, in this first part of the study the emulsions were prepared at fixed phase ratio (10% w/w) using water-saturated solvent as external phase, 1% emulsifier and an Ultra Turrax at 12,000 rpm for 1 min, while investigating the influence on lipid particle size of different emulsifiers and lipid loading.

Table 1
Glyceryl monostearate solubility at different temperatures

Temperature (°C)	Solubility (mg/ml)	
	Butyl lactate	Benzyl alcohol
40	54.0 ± 2	46.5 ± 1.8
45	1025 ± 22	1370 ± 25
50	1450 ± 35	1920 ± 40
55	2025 ± 52	2830 ± 60

To verify the influence of emulsifiers on SLN size, emulsions (10 ml) containing 250 mg GSM were prepared using mixtures of emulsifiers (Tween 80: Oramix CG-110, Tween 80:Epikuron 200, Epikuron 200:Oramix CG-110, Epikuron 200:TDC and Epikuron 200:CA) in 1:1 weight ratio. The use of 1% of a single emulsifier gave, by visual observation, coarse emulsions with high coalescence rate of the solvent droplets. Emulsifying agents are used to slow down this inevitable separation. In some rare cases a single emulsifier can yield the desired emulsion. More often, though, in the case of oil-in-water emulsions, mixed surfactants have been reported to have a synergistic effect on emulsion stability in term of coalescence rate. The combined use of two or more emulsifying agents appear to produce mixed surfactant films at the interface having high surfactant coverage as well as sufficient viscosity to promote stability (Tadros, 1983). However, the importance of this concept in industrial emulsions, where it is the rule rather than the exception to use mixed surfactants, would seem to justify further research in this area.

Fig. 1 reports the mean particle diameters of GMS suspensions obtained by adding water to emulsions containing 2.5% GMS, butyl lactate or benzyl alcohol and 1% different emulsifiers.

Particle size of SLN dispersions from emulsions containing non-ionic surfactants is generally larger than those obtained in the presence of ionic surfactants. The combination of non-ionic surfactants with lecithin also increases the particle size. From these preliminary experiments, it appears that the addition of TDC or CA to lecithin as co-emulsifying agent decreases the particle size of the SLN dispersions.

The smallest SNL were obtained from benzyl alcohol, Epikuron and CA emulsions. The emulsion containing butyl lactate, lecithin and CA was impossible to prepare because of the low solubility of the bile salt in this system.

The importance of the emulsifier on the size of a lipid dispersion has also been demonstrated on microemulsion-based SLN dispersions. Cavalli et al. (1998) used stearic acid as lipid phase and compared an ionic surfactant system composed of Epikuron and TDC with a non-ionic system composed of Tween 80. The particle size of the SLN dispersion produced with ionic surfactant was considerably smaller than that of the non-ionic formulation.

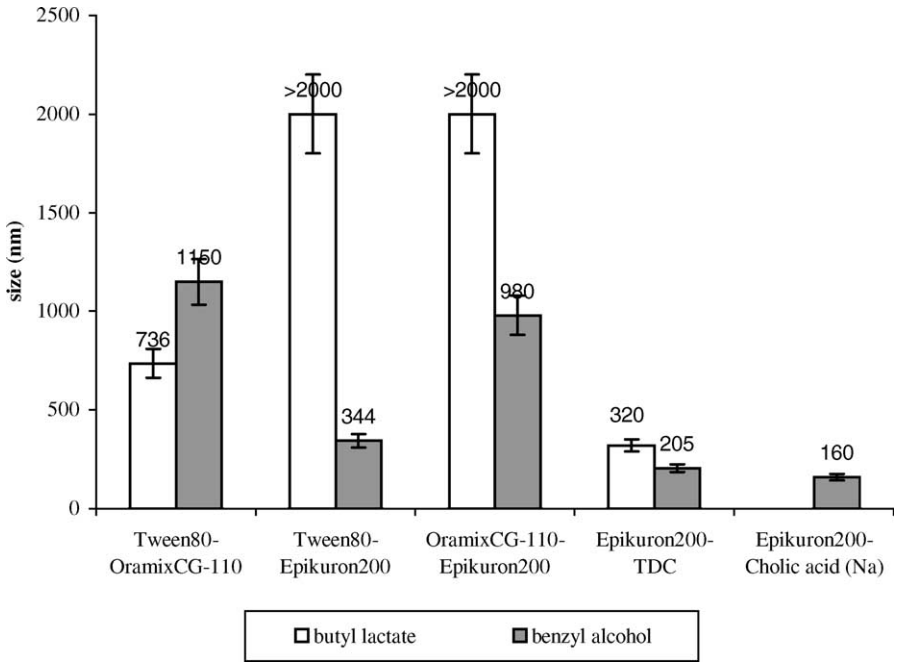

Fig. 1. Influence of different emulsifiers (1:1 w/w) on the mean size of the GMS particles.

Table 2
Mean particle size (nm), polydispersity index (PI) and *Z*-potential (mV) of dispersions containing different percentage of GMS (±S.D., $n = 3$)

Percentage (w/w)	Butyl lactate		Benzyl alcohol	
	nm	mV	nm	mV
2.5	320 ± 13 (0.09)	−22 ± 2	205 ± 15 (0.08)	−35 ± 4
5	333 ± 15 (0.1)	−20 ± 2	277 ± 22 (0.14)	−33 ± 3
8	342 ± 14 (0.12)	−24 ± 3	452 ± 21 (0.14)	−36 ± 3
10	363 ± 20 (0.15)	−24 ± 3	695 ± 19 (0.11)	−37 ± 3

To compare the influence of different GMS loading on SLN size, emulsions containing benzyl alcohol or butyl lactate, 2.5–10% GMS, Epikuron and TDC were prepared: the results are in Table 2. The smallest sizes were obtained using benzyl alcohol and the lowest GMS loading. Mean particle size of the GMS dispersions increased on increasing GMS loading; whereas the increase in particle size obtained using butyl lactate was relatively small (about 16% on increasing from 2.5 to 10% GMS) in the case of benzyl alcohol the increase was very considerable (from 205 to 695 nm). The influence of lipid loading on particle size has also been reported for SLN produced via homogenization (Mehnert and Mader, 2001; Siekmann and Westesen, 1994). Increasing the lipid content above 5–10% in most cases results in larger particles, including microparticles, and broader particle size distribution. This is caused both by a decrease of homogenization efficiency and an increase in particle agglomeration, and has also been observed for lipid nanoemulsions. The nanoparticle size was also found to change as a function of the percentage of matrix dissolved when preparing polymeric nanoparticles; the nanoparticle size was partly attributed, according to Stoke's law, to density variations of the internal phase of the emulsion (Leroux et al., 1995).

Table 2 also reports the *Z*-potential value of ultrafiltrated GMS suspensions obtained using benzyl alcohol or butyl lactate. The values did not change significantly by increasing the lipid load, however, a significant increase in the absolute *Z*-potential values was found using benzyl alcohol.

During the purification process, 99.8% of the benzyl alcohol was eliminated after purification of the particles using three washings; the residual amount of benzyl alcohol was 30 ppm and about the same residual solvent content should be present in the GMS suspensions prepared with butyl lactate. The differences in *Z*-potential were attributed to the different influence of the solvents on the composition of the stabilizing surfactant layer. Depending on the nature of the solvent, the ratio of Epikuron and TDC might change; the different ratio of these surfactants to each other in the interface could cause differences in *Z*-potential. The measurement of *Z*-potential allows predictions about the storage stability of colloidal dispersions. As particle aggregation is less to occur for charged particles (high *Z*-potential) because of electric repulsions, the use of benzyl alcohol should be more convenient.

Figs. 2 and 3 show the transmission electron micrographs of 8% GMS nanoparticles prepared using benzyl alcohol or butyl lactate as disperse phase, lecithin and TDC as emulsifier mixture. The TEM micrographs revealed that the solvent dilution process lead to the formation of spherical microparticles with a smooth surface, even if the use of different emulsifiers, different solvents or the lipid load might influence the surface morphology or shape of the particles.

The physical state of the particles is very important from the technological as well as from the biopharmaceutical point of view. By the use of particles with a solid lipid matrix, stability problems, e.g. drug leakage or coalescence, often observed for lipid dispersions such as emulsions or liposomes may be overcome. Moreover, drug release from solid matrix is supposed to be degradation-controlled and thus slower than diffusion-controlled release from emulsions.

Supercooled melts are not unusual in SLN systems (Bunjes et al., 1998); they describe the phenomenon that lipid crystallization may not occur although the sample is stored at a temperature below the melting point of the lipid. As the advantage for SLN drug-carrier system is essentially based on the solid

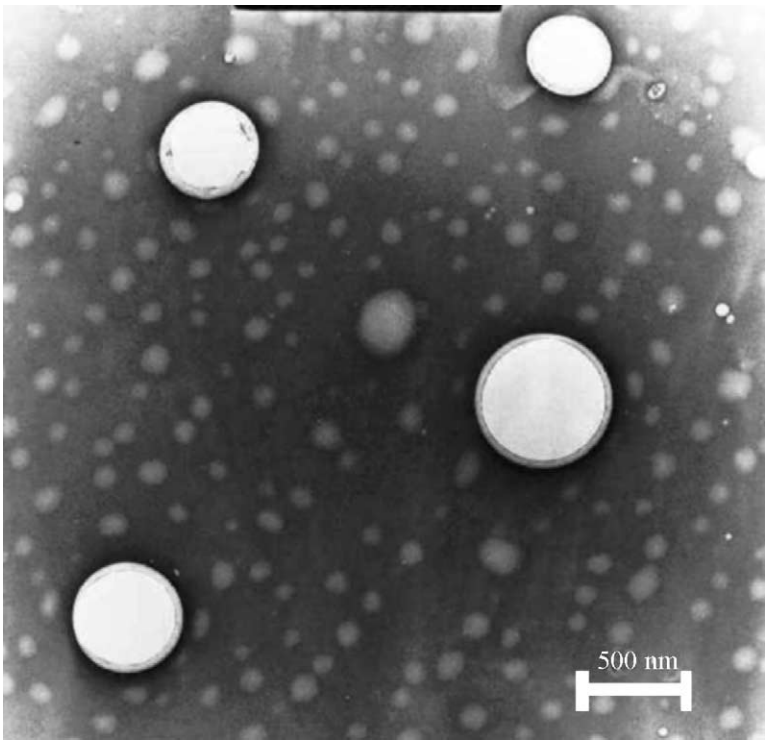

Fig. 2. Transmission electron microscopy (TEM) micrograph of 8% GMS nanospheres (bar = 500 nm) from benzyl alcohol, lecithin, and TDC emulsions.

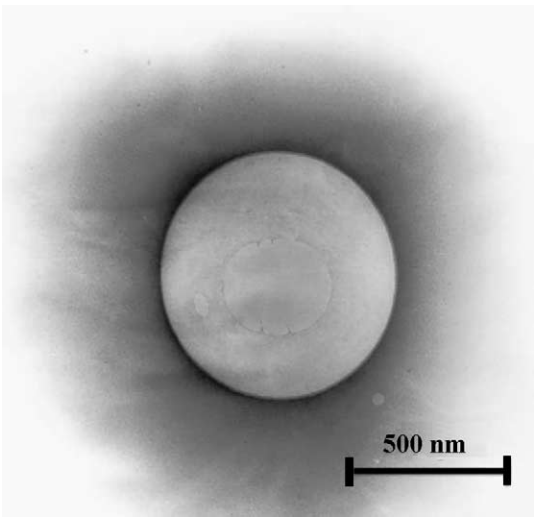

Fig. 3. Transmission electron microscopy (TEM) micrograph of a 8% GMS nanosphere (bar = 500 nm) from butyl lactate, lecithin, and TDC emulsions.

state of the particles, the solidification of the particles after the dilution of the emulsions has to be ensured.

The status of the lipid nanoparticles obtained from butyl lactate or benzyl alcohol emulsions stabilized with lecithin and TDC was investigated using DSC. Fig. 4 shows the DSC thermograms of GMS commercial and recrystallized product, and of the 8% GMS nanoparticles. The peak location of recrystallized product and GMS nanospheres are slightly shifted towards lower temperatures compared to that of commercial lipid, and there is a disappearance of the shoulder at the right side from the curve of commercial GMS.

It is quite difficult to explain this disappearance; it may be possible that both recrystallization from water of the commercial product and crystallization of the lipid after dilution of the emulsions lead to different chemical composition or/and different lipid modifications and several analytical methods (NMR, EPR, conventional or synchrotron X-ray diffraction etc.) are necessary to support any hypothesis. Anyway,

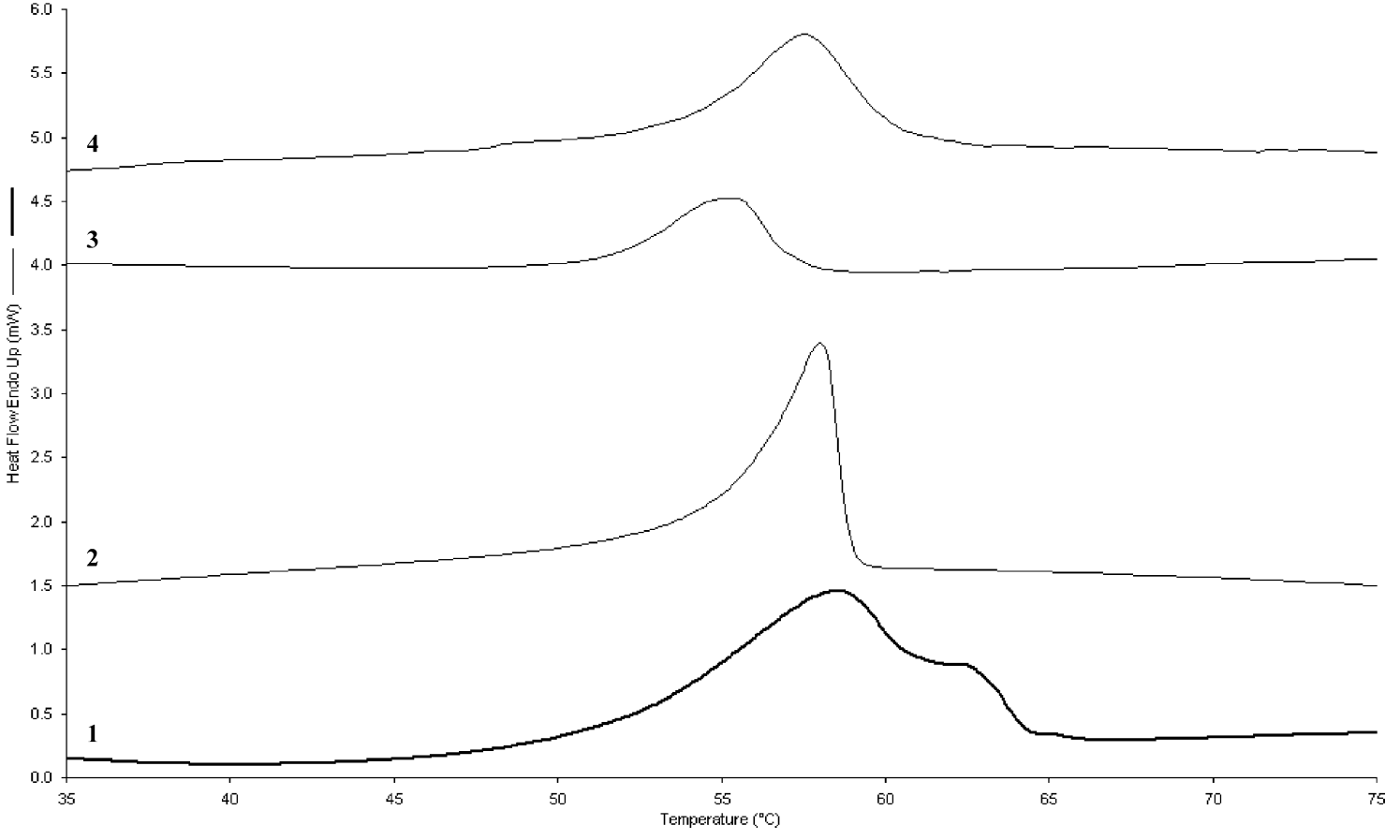

Fig. 4. Differential scanning calorimetry thermograms of commercial (1) and water-recrystallized GMS (2) dispersions, and GMS dispersions via solvent diffusion technique from benzyl alcohol, lecithin, and TDC emulsions (3) and from butyl lactate, lecithin, and TDC emulsions (4).

according to DSC results for the considered systems, it must be assumed that crystallization of the GMS particles started at room temperature after dilution of the emulsions.

4. Conclusions

The present study has shown that it is possible to produce solid lipid nanospheres with the emulsification–diffusion process using benzyl alcohol or butyl lactate. The use of these solvents should be useful to prepare drug-loaded nanospheres as carrier systems. A relatively high lipid load could be obtained increasing the temperature process. Furthermore, the GMS nanospheres are attractive for different applications because of their submicron-sized structure, narrow size distribution and their biodegradability.

Acknowledgements

This work was supported by a grant from the Ministero dell'Istrizione dell'Università e della Ricerca.

References

Bunjes, H., Siekmann, B., Westesen, K., 1998. Emulsion of supercooled melts, a novel drug delivery system. In: Benita, S. (Ed.), Submicron Emulsions in Drug Targeting and Delivery. Harwood Academic Publishers, Amsterdam, pp. 175–205.

Carli, F., 1999. Physical Chemistry and Oral Absorpion of the Nanoparticulate System, 5 Recentre Pharmapeptides.

Cavalli, R., Caputo, O., Marengo, E., Pattarino, F., Gasco, M.R., 1998. The effect of the components of microemulsions on both size and crystalline structure of solid lipid nanoparticles (SLN) containing a series of model molecules. Pharmazie 53, 392–396.

Gasco, M.R., 1993. Method for producing solid lipid microspheres having a narrow size distribution. United States Patent, USS 188837.

Jahnke, S., 1998. The theory of high pressure homogenization. In: Muller, R.H., Benita, S., Bohm, B. (Eds.), Emulsion and Nanosuspensions for the Formulation of Poorly Soluble Drugs. Medpharm Scientific Publishers, Stuttgart, pp. 177–200.

Leroux, J.C., Allemann, E., Doelker, E., Gurny, R., 1995. New approach for the preparation of nanoparticles by an emulsification–diffusion method. Eur. Pharm. Biopharm. 41, 14–18.

Mehnert, W., Mader, K., 2001. Solid lipid nanoparticles. Production, characterization and applications. Advanced Drug Delivery Reviews 47, 165–196.

Müller, R.H., Lucks, J.S., 1996. Arzneistofftrager aus festen Lipidteilchen, Feste Lipidnanospharen (SLN). European Patent No. 0605497.

Muller, R.H., Runge, S.A., 1998. Solid lipid nanoparticles (SLN) for controlled drug delivery. In: Benita, S. (Ed.), Submicron Emulsions in Drug Targeting and Delivery. Harwood Academic Publishers, Amsterdam, pp. 219–234.

Sah, H., 2000. Ethyl fomate—alternative dispersed solvent useful in preparing PLGA microspheres. Int. J. Pharm. 195, 103–113.

Sah, H., Smith, M.S., Chern, R.T., 1996. A novel method of preparing PLGA microcapsules utilizing methylethyl ketone. Pharm. Res. 13, 360–367.

Siekmann, B., Westesen, K., 1994. Melt-homogenized solid lipid nanoparticles stabilized by the nonionic surfactant tyloxapol. I. Preparation and particle size determination. Pharm. Pharmacol. Lett. 3, 194–197.

Siekmann, B., Westesen, K., 1996. Investigation on solid lipid nanoparticles prepared by precipitation in o/w emulsion. Eur. J. Pharm. Biopharm. 43, 104–109.

Sjöström, B., Bergenstahl, B., 1992. Preparation of submicron drug particles in lecithin-stabilized o/w emulsions I. Model studies of the precipitation of cholestreryl acetate. Int. J. Pharm. 88, 53–62.

Tadros, T.F., 1983. Emulsion stability. In: Becher, P. (Ed.), Encyclopaedia of Emulsion Technology, vol. 1. Marcel Dekker, New York, pp. 129–285.

Trotta, M., Gallarate, M., Pattarino, F., Morel, S., 2001. Emulsions containing partially water-miscible solvents for the preparation of drug nanosuspensions. J. Controlled Release 76, 119–128.