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Emulsions containing partially water-miscible solvents for the preparation of drug nanosuspensions

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Abstract

The aim of this study was to investigate the feasibility of partially water-miscible solvents, such as benzyl alcohol, butyl lactate and triacetin, to prepare drug nanosuspensions by a solvent quenching technique. Mitotane, which possesses very poor water solubility and low bioavailability, was used as model drug. Preparation was by emulsifying an organic solution of the drug in an aqueous solution of a stabilising agent followed by rapid displacement of the solvent from the internal into the external phase, provoking solid particle formation. To verify the influence of emulsion droplet size on the drug particle size, 0.1 or 0.2% of different emulsifiers (Tween 80, caprylyl-capryl glucoside or lecithin) and different homogenisation conditions (Ultra Turrax or a high pressure homogenizer at 200 or 1000 bar for three cycles) were used. In general, emulsion droplet size decreased with high pressure homogenization and on increasing the number of cycles. The size of drug particles, obtained after adding water at a constant rate, was dependent on the droplet size in the emulsion. Drug particles of ~80 nm were obtained using butyl lactate, supporting the hypothesis that drug particle formation by the emulsification diffusion process involves generating regions of local supersaturation. Because of the increase in available surface area, the dissolution rate of diaultrafiltrated suspensions increased greatly compared to commercial product.

Keywords: Emulsion; Solvent quenching; Nanoparticles; Dissolution

1. Introduction

Oral administration of poorly water-soluble drugs in man and laboratory animals often leads to irregular and incomplete absorption from the gastrointestinal tract. This may be explained because the extremely low water solubility of the drug probably makes dissolution the rate-limiting step in the absorption process. One approach to the formulation of poorly soluble drugs is to administer them in a form having a high specific area. The most common method of subdividing solid substances is by ultrafine milling, but this process has several disadvantages, such as broad size distribution with only a very small fraction of particles in the nanometer size range, even if recently the use of a high pressure homogenizer has produced submicron particles [1,2].

Submicron particles may also be prepared directly by a precipitation process leading to hydrosols [3]. Limitations of hydrosols are that the drug must be soluble in at least one solvent which is miscible with

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a non-solvent to perform precipitation and that growth of the precipitated nanoparticles must be stopped to avoid formation of micrometer particles. A recently developed method is to dissolve the substance in a supercritical fluid which may be evaporated very rapidly [4].

A variety of methods that rely on two phases are reported extensively in the literature [5]. When an organic solvent is used as the disperse phase of an oil-in-water (O/W) emulsion, at least two methods can be used to fabricate drug suspensions: using low–medium boiling point solvents with negligible water solubility the precipitation of the solute can be achieved by solvent evaporation or by a quenching technique using partially water-miscible solvents. In the first method, the drug or the mixture of organic substances is dissolved in a non-polar solvent and the solution dispersed in an aqueous phase; when the non-polar solvent is evaporated the drug or the mixture of organic substances precipitates and hence a suspension is formed. One particle is formed in each emulsion droplet and thus it is possible to control the amount of organic substance in each particle and the final particle size by controlling the emulsion droplet size. The most commonly used technique employs halogenated alkanes, such as methylene chloride or chloroform, as disperse phase [6]. However, the use of these solvents raises environmental and human safety concerns over residual solvent, so they cannot be recommended for routine manufacturing process. A number of investigations have thus sought safer solvents as disperse phase. Among them, ethyl acetate and ethyl formate are considered preferable, and a number of studies have focused on developing a microencapsulation process utilising evaporation of these solvents [7,8].

One of the novel methods to prepare organic suspensions is to use a partially water miscible solvent and extract the solvent from an O/W emulsion by adding water. The solvent diffusion procedure using ethyl formate, ethyl acetate or propylene carbonate has led to successful fabrication of good quality drug-loaded microspheres [8,9]. The process is based on the water miscibility of these solvents. Upon transferring a transient O/W emulsion into water, polymeric droplets solidify instantly due to the almost complete diffusion of the organic solvent from the polymeric droplets to the continuous phase. By controlling the key parameter process, particles with different characteristics can be obtained.

The aim of this study was to investigate the feasibility of preparing drug nanosuspensions by the solvent diffusion process using other solvents accepted as having low toxicity, such as butyl lactate, triacetin or benzyl alcohol, and to study the influence of emulsion droplet size on the size distribution pattern of drug nanoparticles. Mitotane, an anticancer drug normally administered by the oral route with a very poor solubility and low bioavailability [10], was used as model drug.

2. Materials and methods

2.1. Materials

Mitotane (1,1-dichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane), benzyl alcohol, butyl lactate, triacetin, Tween 80 and bovine serum albumin (BSA) were provided by Fluka (Buchs, Switzerland). Soya lecithin (Epikuron 200) was from Lukas Meyer (Hamburg, Germany). Caprylyl-capryl glucoside (Oramix CG-110) was a gift from Seppic (Milan, Italy). All other chemicals were obtained from Sigma (Deisenhofen, Germany).

2.2. Preparation of nanosuspensions

The steps in the preparation of drug nanosuspensions were as follows.

2.2.1. Preparation of emulsions

The organic solvents, benzyl alcohol, triacetin or butyl lactate, were used as internal phase to prepare oil-in-water emulsions (O/W). Mitotane (250 mg) was dissolved in 5.8, 9.1 or 9.7 ml of benzyl alcohol, triacetin or butyl lactate, respectively. The solvent solutions were then poured, under magnetic stirring (500 rpm), into 94.2, 90.9 and 90.3 ml, respectively, of water containing 0.1–0.2 g emulsifier to produce coarse O/W emulsions. Tween 80, Oramix CG100 or lecithin were used as emulsifiers. After 5 min, the premix was homogenised using an Ultra Turrax at 12 000 rpm for 2 min followed by a high pressure

homogenisation (Niro Soavi, Italy) at a pressure of 200 or 1000 bar (one to three cycles).

Oil droplet size of the different formulations was monitored with a Leitz light microscope (magnification 756×) by sampling aliquots of emulsion immediately after each process step.

Physical stability was determined by subjecting each emulsion to various centrifugal forces (5000, 7500, 10 000 rpm) for 10 min and then visually checking for evidence of separation. Each experiment was performed in triplicate.

2.2.2. Formation of drug nanosuspensions

Additional distilled water (100 ml) was added at 100 ml/min into the initial O/W emulsion to extract the solvent of the internal phase into the continuous phase.

The average diameter, polydispersity index and Z-potential of mitotane suspensions were immediately determined by a laser light scattering technique (Brookeven, USA). The dispersions were diluted 1:50 with water for size determination or with KNO_3 0.005 M for Z-potential determination.

2.2.3. Purification of drug nanosuspensions

Suspensions were then washed by diaultrafiltration with a TCF2 system (Amicon, Danvers, USA) using a Diaflo YM 100 membrane (cut-off 100 000 Da). First 100 ml of suspension were concentrated to 20 ml, then 20 ml additional water was added and the suspension again concentrated to 20 ml. This procedure was repeated a further three times. The average diameter, polydispersity index and Z-potential of the diaultrafiltrated mitotane suspensions were determined as described above.

The residual solvent concentration in the suspensions was determined after each diaultrafiltration step for only the systems containing benzyl alcohol, by a HPLC method using a C_8 column (4.6 mm×15 cm, Merck) with a mobile phase consisting of methanol and water (10/90, v/v) at a flow rate of 0.8 ml/min.

2.3. Mitotane solubility

An excess of mitotane was added to each solvent used in the emulsion formulations, to water, to 2% BSA water solution and to water containing the same solvent percentage and emulsifiers as in the suspensions, the resulting suspensions were stirred at 25°C for 48 h and filtered through a 0.22-μm membrane. The solutions were then assayed for mitotane concentration by HPLC, using a C_{18} column (4.6 mm× 15 cm, Merck) with a mobile phase consisting of methanol and water (80/20, v/v) at a flow rate of 0.8 ml/min. Each mitotane batch was analysed in triplicate.

2.4. Differential scanning calorimetry (DSC)

DSC was performed with a Perkin-Elmer differential calorimeter. Commercial mitotane and mitotane obtained by ultracentrifugation from diaultrafiltrated nanosuspensions were placed in a conventional aluminium pan and a scan speed of 10°C/min was employed. The weight of the samples was in the 0.8–1-mg range.

2.5. Dissolution study

Diaultrafiltrated nanoparticles from Tween 0.05%–Oramix 0.05% emulsions containing a known amount of mitotane (0.5–1 mg) were resuspended in 100 ml of 2% BSA water solution to maintain sink conditions and incubated at 37°C under gentle magnetic stirring at 300 rpm. At appropriate intervals, 5-ml aliquots were removed and replaced by 5 ml fresh dissolution medium; these aliquots were filtrated (cut-off 100 nm, Millipore) and assayed for mitotane concentration by HPLC. Each mitotane batch was analysed in triplicate.

As reference systems for the dissolution tests, a concentrated water–mitotane suspension, obtained by dispersing 10 mg of commercial drug in 1 ml of water containing 0.01% Tween, and a suspension, obtained by adding 5.6 ml benzyl alcohol containing 250 mg mitotane to 200 ml 0.1% Tween and then diaultrafiltrated, were used.

3. Results and discussion

The first step in the production of drug nanoparticles by the solvent diffusion technique is to prepare solvent-in-water emulsions with partially water-miscible solvents, containing the drug, as disperse phase. Benzyl alcohol, triacetin and butyl lactate, solvents

possessing low toxicity, were used to prepare the primary emulsion.

A number of variables affect the solvent droplet size of the emulsions and probably the properties of the resulting solid particles; these include phase ratio, type and concentration of emulsifier agent, mixing technique, processing temperature, and technological conditions of manufacturing. To reduce the number of experiments, the emulsions were prepared at a fixed phase ratio using pure water as external phase and 0.1 or 0.2% emulsifier, while the influence of different emulsifiers and mixing equipment was investigated. Safe and efficient emulsifiers (Tween 80, lecithin and Oramix) [11,12] and an Ultra Turrax and high pressure homogenizer were used in different operative conditions.

The water solubilities of benzyl alcohol, triacetin and butyl lactate are 3.8, 7.1 and 7.7% w/w, respectively [13].

To prepare the solvent-in-water emulsions (O/W) 5.8, 9.1 or 9.7 ml of benzyl alcohol, triacetin or butyl lactate, and 94.2, 90.9 or 90.3 ml of water containing one of the emulsifiers were used. These produced about the same volume of dispersed phase in the O/W emulsions, because when the dispersed phase is emulsified in water, a proportion of organic solvent, depending on its water solubility, leaches to and saturates the water phase.

If a water insoluble drug is dissolved in the dispersed phase, the subsequent dilution of 100 ml of emulsion to 200 ml with additional distilled water extracts most of the dispersed phase, converting the organic solvent droplets into solid particles.

To prepare 100 ml of drug-containing emulsion, 250 mg mitotane, a very low water-soluble anticancer drug, were previously dissolved in the solvent. The drug's solubility in benzyl alcohol, butyl lactate and triacetin is 246, 339 and 518 mg/ml, respectively: the volume of the dispersed phase was thus sufficient to avoid the drug precipitating during preparation of the emulsion, as also confirmed by optical microscopy. Mitotane's solubility in water containing the same solvent percentage as after water addition to the primary emulsions, in the absence and in the presence of 0.1% Tween, is reported in Table 1. Even if mitotane's solubility increased markedly in the presence of emulsifiers, a remarkable amount of drug was available for precipitation on the addition of water.

Table 1
Mitotane solubility at 25°C

Water	2.7±0.5 μg/ml
2% BSA	38±2 μg/ml
Benzyl alcohol	246±14 mg/ml
Butyl lactate	339±16 mg/ml
Triacetin	518±20 mg/ml
2.90% Benzyl alcohol[a]	41±2 μg/ml
2.90% Benzyl alcohol–0.1% Tween[a]	54±3 μg/ml
4.85% Butyl lactate[a]	47±3 μg/ml
4.85% Butyl lactate–0.1% Tween[a]	64±3 μg/ml
4.55% Triacetin[a]	58±2 μg/ml
4.55% Triacetin–0.1% Tween[a]	76±4 μg/ml

[a] Aqueous solution.

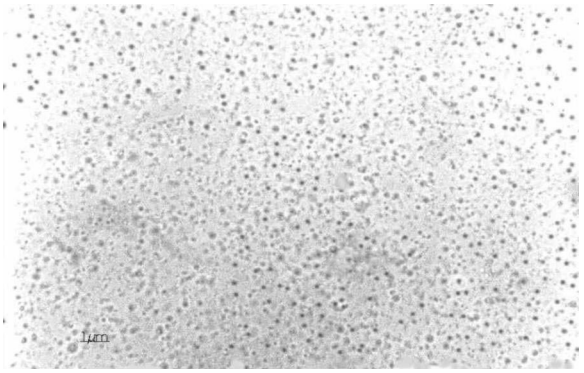

Fig. 1. Photomicrograph (magnification 756×) of the emulsion containing benzyl alcohol and 0.2% Tween, passed through a high pressure homogenizer (1×200 bar).

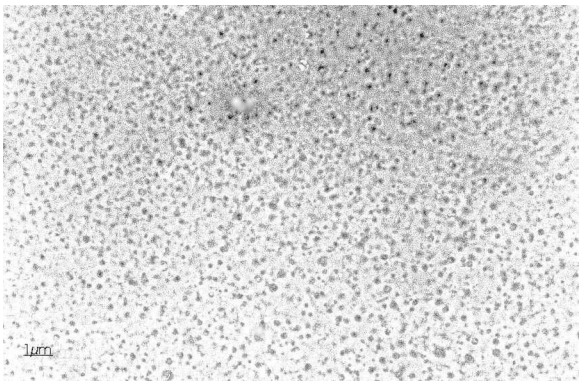

Fig. 2. Photomicrograph (magnification 756×) of the emulsion containing butyl lactate and 0.2% Tween, passed through a high pressure homogenizer (1×200 bar).

Optical microscopy was utilised to verify the influence of the emulsifier and of the different operating conditions on the emulsion droplet sizes. It was not possible to use the laser light scattering technique, because of the impossibility of diluting the emulsions, e.g. with solvent saturated water, without changing their original droplet size.

Figs 1–3 report as examples the photomicrographs of the emulsions containing benzyl alcohol, butyl lactate and triacetin and 0.2% Tween, passed through a high pressure homogenizer (1×200 bar). As can be seen, mean diameters of ~200–300 nm were obtained using benzyl alcohol or butyl lactate, while larger solvent particles were obtained using triacetin. Triacetin is a larger molecule than benzyl alcohol or butyl lactate, and its low penetration into the hydrocarbon tail of the surfactant could presumably have a negative effect on the packing parameter [14].

The same emulsions subjected to three cycles at 200 or 1000 bar could not be to examined by light microscopy due to the limits of this technique. Under visual observation, translucent systems were obtained by increasing the number of cycles and the operative pressure, indicating that the emulsions were finer.

These results were confirmed by visual examination of the effect of centrifugal force on the physical stability of the emulsions. No separation was observed at 10 000 rpm for the systems containing benzyl alcohol or butyl lactate passed through a high pressure homogenizer three times at a pressure of 200 or 1000 bar. In contrast, with triacetin both emulsions passed for three cycles at 200 bar, but those at 1000 bar started to segregate at 7500 rpm. This observation is in agreement with earlier reports substantiating that droplet sizes are a result of breakage and coalescence phenomena, and that for some systems increasing the operative pressure does not lead to a reduction of emulsion droplet size [15,16].

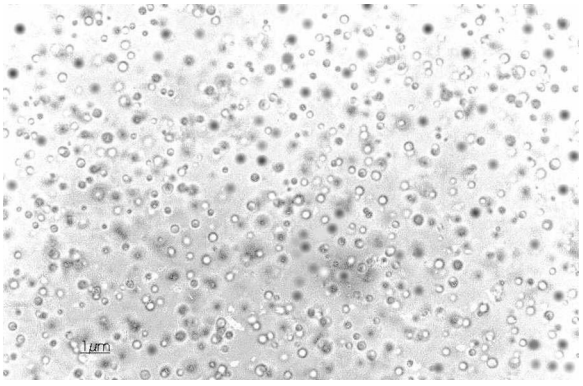

Fig. 3. Photomicrograph (magnification 756×) of the emulsion containing triacetin and 0.2% Tween, passed through a high pressure homogenizer (1×200 bar).

The subsequent dilution under a standard stirring rate of 100 ml emulsion with an additional 100 ml of distilled water converted the microdroplets into solid particles.

Another key parameter that probably affects the characteristics of the drug particles, including the size distribution pattern, is the time to quench the solvent from the disperse phase [8]. In this study a high constant rate of water addition (100 ml/min) was used for all emulsions. Considering the water solubility of the solvents and the composition of the emulsions, the amount of solvent that diffused from the internal phase to the external water phase may be expected to be about the same for all solvents and, even if other parameters such as molecular weight or water solubility of the solvents might affect the time of solvent quenching, the drug particle size obtained with different solvents was assumed not to be related to the duration of quenching.

Tables 2–4 report the mean particle diameter and polydispersity index determined by laser light scattering, of mitotane suspensions obtained from emulsions containing benzyl alcohol, butyl lactate or triacetin, different emulsifier agents and prepared under different operational conditions. A decrease in the mean diameters of the suspensions was observed for all systems on passing the emulsions for three cycles at 200 bar through the high pressure homogenizer, whereas setting the operative pressure at 1000 bar did not further decrease the drug particle size. The mechanism by which the diffusion of solvent from the droplets induces the aggregation into nanoparticles is not yet clear, and chemical instability has been proposed. In particular PLA nanoparticle formation by the emulsification–diffusion process has been attributed to the generation of new globules of the nanometer range size and phase transformation in these regions, with the original emulsion droplet size playing an important role [9,17].

In general, the mean diameters of the mitotane

Table 2
Photon correlation spectroscopy diameter (nm) and polydispersity index (PI) of mitotane suspensions obtained after water addition to the emulsions containing benzyl alcohol and prepared using different emulsifiers and homogenisation conditions

	U. Turrax (PI)	1×200 bar (PI)	2×200 bar (PI)	3×200 bar (PI)	3×1000 bar (PI)
Tween 0.1%	260±23 (0.21)	184±18 (0.14)	163±15 (0.12)	148±12 (0.09)	155±12 (0.10)
Tween 0.05%–Oramix 0.05%	214±20 (0.15)	164±16 (0.10)	123±14 (0.08)	119±12 (0.07)	117±11 (0.08)
Tween 0.05%–Lec. 0.05%	255±24 (0.19)	201±20 (0.14)	194±15 (0.12)	172±14 (0.11)	187±15 (0.12)
Tween 0.2%	215±22 (0.12)	142±12 (0.09)	126±12 (0.08)	111±12 (0.06)	117±12 (0.06)
Tween 0.1%–Lec. 0.1%	204±21 (0.17)	182±14 (0.15)	166±14 (0.13)	155±12 (0.12)	144±14 (0.12)
Tween 0.1%–Oramix 0.1%	174±18 (0.11)	129±11 (0.09)	103±10 (0.08)	98±10 (0.06)	105±11 (0.06)

Table 3
Photon correlation spectroscopy diameter (nm) and polydispersity index (PI) of mitotane suspensions obtained after water addition to the emulsions containing butyl lactate and prepared using different emulsifiers and homogenisation conditions

	U. Turrax (PI)	1×200 bar (PI)	2×200 bar (PI)	3×200 bar (PI)	3×1000 bar (PI)
Tween 0.1%	237±24 (0.22)	210±20 (0.16)	186±16 (0.12)	180±16 (0.10)	182±18 (0.09)
Tween 0.05%–Oramix 0.05%	156±14 (0.18)	120±12 (0.12)	128±12 (0.11)	106±10 (0.10)	94±10 (0.10)
Tween 0.2%	232±20 (0.18)	190±18 (0.2)	174±15 (0.08)	154±15 (0.06)	152±16 (0.07)
Tween 0.1%–Oramix 0.1%	145±13 (0.15)	112±14 (0.09)	82±8 (0.08)	80±8 (0.06)	84±10 (0.06)

Table 4
Photon correlation spectroscopy diameter (nm) and polydispersity index (PI) of mitotane suspensions obtained after water addition to the emulsions containing triacetin and prepared using different emulsifiers and homogenisation conditions

	U. Turrax (PI)	1×200 bar (PI)	2×200 bar (PI)	3×200 bar (PI)	3×1000 bar (PI)
Tween 0.1%	298±32 (0.25)	236±25 (0.18)	225±20 (0.15)	192±18 (0.12)	195±20 (0.12)
Tween 0.05%–Oramix 0.05%	230±25 (0.21)	222±24 (0.14)	190±20 (0.12)	202±22 (0.10)	210±20 (0.09)
Tween 0.2%	278±28 (0.18)	190±20 (0.14)	202±18 (0.10)	198±18 (0.08)	204±18 (0.09)
Tween 0.1%–Oramix 0.1%	215±24 (0.15)	190±20 (0.11)	164±15 (0.09)	155±16 (0.08)	160±15 (0.08)

Table 5
Photon correlation spectroscopy diameter (nm), polydispersity index (PI) and Z-potential (mV) of mitotane suspensions after, immediately and 30 days before diaultrafiltration

	Before		After	
	nm (PI)	mV	Day 0, nm (PI)	Day 30, nm (PI)
Benzyl alcohol (Tween 0.05%–Oramix 0.05%)	119±12 (0.07)	−21	135±15 (0.10)	141±15 (0.18)
Benzyl alcohol (Tween 0.1%–Oramix 0.1%)	98±10 (0.06)	−22	110±12 (0.08)	121±14 (0.14)
Butyl lactate (Tween 0.05%–Oramix 0.05%)	106±10 (0.10)	−19	124±14 (0.11)	132±14 (0.18)
Butyl lactate (Tween 0.1%–Oramix 0.1%)	80±8 (0.06)	−20	108±12 (0.08)	112±12 (0.16)
Triacetin (Tween 0.05%–Oramix 0.05%)	202±22 (0.10)	−20	213±24 (0.12)	220±25 (0.18)
Triacetin (Tween 0.1%–Oramix 0.1%)	155±16 (0.08)	−21	176±18 (0.10)	188±20 (0.16)

dispersions were considerably smaller than those of the original emulsion, and in particular, the dispersions obtained using the high pressure homogenizer, containing butyl lactate and the mixture Tween–Oramix, appeared transparent at visual observation.

It was not possible to correlate emulsion droplet diameter to drug suspension size because of the impossibility of correctly measuring the droplet size of the emulsions, but the results support the hypothesis of generation of new globules during the solvent diffusion step.

For all systems examined, the use of a mixture of Tween and Oramix produced finer particles than those obtained using Tween alone, and in the case of butyl lactate 0.1% of this mixture was more efficient than 0.2% of Tween. The combined use of Tween and lecithin produced moderate results only with benzyl alcohol and poor results with butyl lactate and

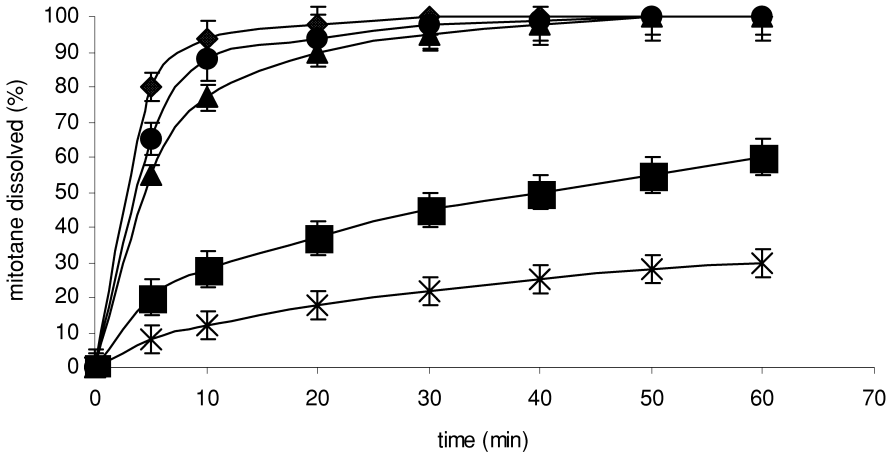

Fig. 4. Mitotane dissolution profiles of the commercial wetted product (*), reference solution (■), nanosuspension obtained from benzyl alcohol emulsion (●), butyl lactate emulsion (◆) and triacetin emulsion (▲).

triacetin (data not reported). This may be ascribed to the higher lipophilicity of lecithin than Oramix, leading to an inadequate hydrophile-lipophile balance (HLB) value of the mixture.

Table 5 reports the mean diameters, Z-potentials and polydispersity indices of mitotane suspensions obtained using Tween–Oramix and benzyl alcohol, butyl lactate or triacetin, before and immediately after the diaultrafiltration process and after 30 day's storage. The suspension sizes and Z-potential values did not change significantly; however, a significant increase of polydispersity index was found after 30 days.

The residual solvent content was determined after each diaultrafiltration step only for the suspensions prepared with benzyl alcohol. The alcohol content halved after each step reaching 0.2% at the end of the process. The concentration would obviously be reduced by increasing the number of steps in the diaultrafiltration process.

The dissolution profiles of mitotane from diaultrafiltrated suspensions obtained with benzyl alcohol, butyl lactate, and triacetin, using the mixture 0.05% Tween–0.05% Oramix as emulsifier, are reported in Fig. 4 together with those from reference systems. The dissolution rate of mitotane was markedly

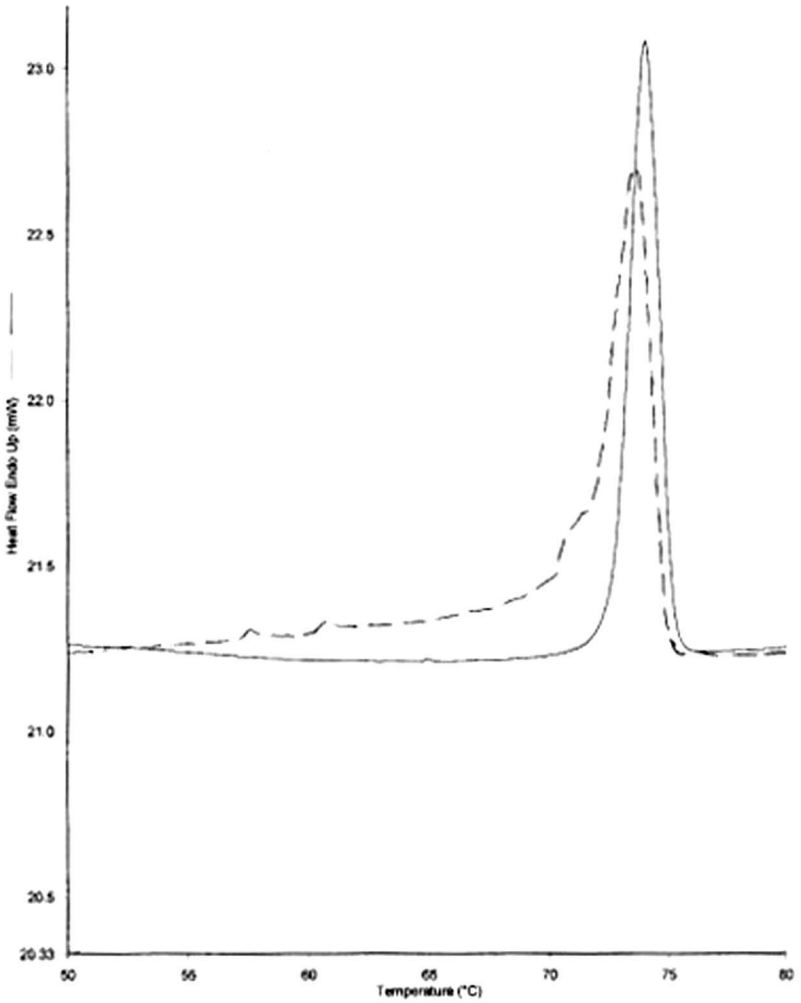

Fig. 5. DSC thermograms of mitotane commercial product (——) and mitotane obtained via solvent quenching technique (----).

enhanced in the systems obtained by the solvent quenching technique compared both to the dissolution rate of the drug from the commercial wetted product and that of the suspension obtained by direct precipitation from benzyl alcohol drug solution. The fastest dissolution rate was obtained for the nanosuspension obtained from the butyl lactate emulsion which showed the smallest size. To verify whether the fast dissolution of mitotane from the suspensions obtained via the quenching method was due to the formation of mitotane in an amorphous state [18], DSC measurements were carried out. DSC thermograms of the commercial product and of the product obtained after ultracentrifugation of the diaultrafiltrated suspension from the benzyl alcohol–Tween 0.1% system are reported in Fig. 5. The endothermic peaks show a slightly lower intensity from 85 ± 3 to 74 ± 8 J/g, and the peak location of mitotane is slightly shifted towards lower temperatures. However, the magnitude is not large enough to show a significant change in the crystallinity of mitotane and the increase in the dissolution rate was thus attributed to an increase in the available surface area of the particles obtained.

4. Conclusion

The emulsion–diffusion technique using pharmaceutically acceptable solvents, such as benzyl alcohol, butyl lactate or triacetin, led to successful fabrication of drug nanosuspensions. The formation of nanoparticles is highly dependent on the emulsion droplet size: using optimized formulations and homogenization parameters, mitotane nanoparticles below 100 nm with very low polydispersity were obtained.

A remarkable increase in the dissolution rates of drug particles obtained by the solvent diffusion technique compared to that from commercial product was obtained. Dissolution rate increased with smaller particle size, and this was attributed to an increase of the available surface area of the drug particles.

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