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Preparation and evaluation of nanosuspensions for enhancing the dissolution of poorly soluble drugs

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Abstract

Poorly water-soluble compounds are difficult to develop as drug products using conventional formulation techniques and are frequently abandoned early in discovery. In the present study, the melt emulsification method traditionally used to prepare solid lipid nanoparticles was adapted to produce drug nanosuspensions. The method was evaluated in comparison with the well known solvent diffusion process for ibuprofen as a model drug. Control of the preparation variables (stabilizers, drug content, homogenization procedure and cooling conditions) allowed formation of nanosuspensions with diameters less than 100 nm. The major advantage of the melt emulsification method over the solvent diffusion method is the avoidance of organic solvents during production, although the mean particle size is slightly greater. The combination of Tween 80 and PVP K25 as stabilizers yields nanosuspensions with the smallest average particle size. The formulation of ibuprofen as a nanosuspension, either in the form of lyophilized powder or granules, was very successful in enhancing dissolution rate, more than 65% of the drug being dissolved in the first 10 min compared to less than 15% of the micronized drug. The increase in in vitro dissolution rate may favourably affect bioavailability and improve safety for the patient by decreasing gastric irritancy.

Keywords: Nanoparticles; Ibuprofen; Melt emulsification method; Solvent diffusion method; Granules; Dissolution rate

1. Introduction

Solubility is an essential factor for drug effectiveness, independent of administration route. It also poses a major challenge for pharmaceutical companies developing new pharmaceutical products, since nearly half the active substances being identified through the new paradigm in high-throughput screening are either insoluble or poorly soluble in water (Patravale et al., 2004). A limiting factor for in vivo performance of poorly water-soluble drugs, following oral administration, is their resistance to being wetted by and dissolved into the fluid in the gastrointestinal tract. Increasing the dissolution rate of poorly water-soluble drugs is thus important for optimizing bioavailability. Over the last 10 years, nanoparticle engineering processes have been developed and reported for pharmaceutical applications. In this approach, poorly water-soluble compounds are formulated as nanometre-sized drug particles.

Nanosuspensions are sub-micron colloidal dispersions of pure drug particles in an outer liquid phase (Möschwitzer et al., 2004). The nanosuspensions can be used to formulate compounds that are insoluble in both water and oils and to reformulate existing drugs to remove toxicologically less favourable excipients. Such compounds have high crystal energy, indicated by a high melting point, which reduces the tendency of the crystal to dissolve, regardless of the solvent (Rabinow, 2004).

Nanoparticle engineering enables poorly soluble drugs to be formulated as nanosuspensions alone, or with a combination of pharmaceutical excipients. Nanosuspension engineering processes currently used are precipitation (Trotta et al., 2001; Debuigne et al., 2001), pearl milling (Liversidge and Conzentino, 1995) and high pressure homogenization, either in water or in mixtures of water and water-miscible liquids or non-aqueous media (Peters et al., 2000; Jacobs et al., 2001; Akkar and Müller, 2003; Kayser et al., 2003; Möschwitzer et al., 2004; Hecq et al., 2005). Different hydrophobic drugs have already been formulated successfully in this way, for instance naproxen (Liversidge and Conzentino, 1995), clofazamine (Peters et al., 2000), bupravaquone (Jacobs et al., 2001), nimesulid (Debuigne

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et al., 2001), mitotane (Trotta et al., 2001), amphotericin B (Kayser et al., 2003), omeprazole (Möschwitzer et al., 2004), nifedipine (Hecq et al., 2005) and spironolactone (Langguth et al., 2005).

An outstanding feature of nanosuspensions is the increase in saturation solubility, and consequently an increase in dissolution rate of the compound. This increase in the dissolution rate is additional to the increase caused by the greater surface area. In general, saturation solubility is a compound-specific constant, which is temperature dependent. However, due to an increased dissolution pressure, as described by Kelvin's equation, the saturation solubility increases below a particle size of approximately 1 μm (Müller et al., 2000). Similar to other nanoparticles (Ponchel et al., 1997), nanosuspensions show an increased adhesiveness to tissue. An additional feature of nanosuspensions is that they may induce changes in the crystalline structure, increasing the amorphous fraction in the particle or even creating completely amorphous particles. Considering these general advantages, the poorly soluble model drug ibuprofen was formulated as nanosuspensions.

Ibuprofen is a nonsteroidal anti-inflammatory drug (NSAID) belonging to the biopharmaceutical classification system (BCS) class II (Lindenberg et al., 2004). NSAIDs have some surface active properties (Fini et al., 1995). These molecules can insert into membranes, which could improve the absorption rate of the drug, but also could damage the gastrointestinal membranes following oral administration. Because of the dilution by gastrointestinal fluids, NSAIDs are never found in vivo above their critical micellar concentrations, but could accumulate at sites where dissolution occurs, starting from a solid dosage form, and there is the possibility that locally high concentrations can be achieved. High local concentrations of the NSAIDs should be avoided during oral administration, by diluting them with food or administering them as a dilute aqueous solution. In this respect, it is important to have the drug available in a highly soluble chemical form or a rapidly dissolving solid dosage form. For this reason, we decided firstly to investigate the feasibility of preparing nanosuspensions with ibuprofen, to use the nanosuspensions as a liquid for wet granulation, and finally to investigate the dissolution rate of the model drug.

The aim of our investigation was to develop a new technological procedure for nanosuspension production and to evaluate the method in comparison with the current solvent diffusion process. The melt emulsification method is well known for the production of solid lipid nanoparticles, but it has not yet been used for nanosuspension formulation.

2. Materials and methods

2.1. Materials

Ibuprofen was obtained from Sigma–Aldrich Chemie, Deisenhofen, Germany. Lutrol F68 (poloxamer 188), Lutrol F127 (poloxamer 407) and polyvinylpyrrolidone (PVP) K25 were from BASF (Germany), Tween 80 (polysorbate 80), sodium dodecyl sulphate (SDS) and benzyl alcohol from Fluka (Switzerland), and polyvinylalcohol (PVA) (Mowiol 4-98) from Clarient GmbH (Germany). Butyl lactate, ethyl acetate, hydrochloric acid and triacetin were from Merck (Darmstadt, Germany), cetylpyridinium chloride and dioctyl sulfosuccinate, sodium salt from Sigma–Aldrich Chemie (Deisenhofen, Germany), microcrystalline cellulose (Avicel PH101) from FMC (USA) and lactose from Lex d.o.o. (Slovenia). Water was purified by reverse osmosis.

2.2. Determination of ibuprofen solubility

The solubility of ibuprofen in water and in the partially water-miscible organic solvents benzyl alcohol, butyl lactate, ethyl acetate and triacetin was determined by adding an excess of the drug into the solvent. The suspensions were stirred on a magnetic stirrer at 25 °C for 48 h, filtered (cut-off 0.2 μm, Minisart SRP 25, Sartorius, Germany) and the content of dissolved ibuprofen was analysed by HPLC. Each sample was analysed in triplicate.

2.3. HPLC analysis of ibuprofen

Ibuprofen concentration was determined by HPLC (Agilent 1100 Series, Hewlett Packard, Waldbron, Germany) using a Nucleosil C_8 column (5 μm, 250 mm × 4 mm; Bia Separations, Ljubljana, Slovenia) at 35 °C. The mobile phase was a mixture of acetonitrile and phosphate buffer (pH 7.5), 28.5:71.5 and the flow rate 1.2 ml/min. The eluant was monitored by a diode array detector at 225 nm. Each sample was analysed in triplicate.

2.4. Preparation of nanosuspensions

2.4.1. Solvent diffusion method

Nanosuspensions were produced with different organic solvents using Ultra Turrax T25 (Janke & Kunkel, IKA Labortechnik, Germany). Ethyl acetate proved to be the most promising organic solvent for production of ibuprofen nanosuspensions. Ibuprofen (300 mg), dissolved in 18 ml of ethyl acetate, was poured into 132 ml 0.2% (w/w) water solution of various stabilizers stirred with Ultra Turrax T25 (UT) for 5 min at 8000 rpm. The emulsion was further homogenized by high pressure homogenization (APV-2000, Invensys, Denmark) at 200 bar for five cycles. After five homogenization cycles the emulsion was diluted with 150 ml of water, homogenized by APV to diffuse the organic solvent and convert the droplets into solid particles.

2.4.2. Melt emulsification method

Ibuprofen (2.5 g) was added to 500 ml of 0.5% (w/w) aqueous solution of stabilizer. The suspension was heated above the melting point of ibuprofen (approximately 75 °C) and homogenized with UT at 8000 rpm for 10 min to give an emulsion. It was transferred to a high pressure homogenizer and homogenized at 1000 bar for five cycles. During this process, the high pressure homogenizer sample holder was wrapped with a heating tape equipped with temperature controller (Digi-Sense Temperature

Controller, Eutech Instruments Ptc Ltd., Singapore) and temperature of emulsion was maintained above the melting point of the drug. The emulsion was then cooled either slowly to room temperature or on an ice-bath.

2.5. Lyophilization of nanosuspensions

To study the effectiveness of the nanosuspension formulation on ibuprofen dissolution rate, the nanosuspension was freeze-dried immediately after preparation. Nanosuspensions stabilized with poloxamer 188 were chosen for the freeze-drying process. Tween 80 cannot be present because it is in the liquid state at room temperature.

Twenty millilitre aliquot of nanosuspension was placed in 100 ml glass flask, rapidly frozen in liquid nitrogen and freeze-dried (Christ Beta 1–8 K, Germany) at a pressure of 0.120 mbar and at temperature 24 °C for 24 h to yield dry sample.

2.6. Preparation of granules

Three types of granules were prepared as follows: a nanosuspension prepared by melt emulsification was used as a granulating liquid. It was sprayed in the top spray chamber of fluid bed granulator (Glatt GmbH, Binzen, Germany) on 200 g of a mixture of 70% lactose and 30% microcrystalline cellulose and dried until the product temperature reached 30 °C.

Two types of reference granules were prepared as follows: 2.5 g of micronized ibuprofen was added to 500 ml of 0.5% solution of poloxamer 188 in water, stirred with UT at 8000 rpm for 10 min at room temperature, and sprayed on 200 g of powder mixture as described above.

The second reference granules were prepared in a mixer. Ibuprofen (2.0 g) was mixed with 198 g of powder mixture as described above and placed in the mixing holder; while stirring, 50 g 4% poloxamer 188 solution was slowly added. The granules were then air dried at room temperature. The relative humidity of all three types of granules was less than 2%, determined thermogravimetrically by Büchi B-302 (Switzerland).

2.7. Particle size analysis of nanosuspensions

Particle size was determined by photon correlation spectroscopy (PCS) using a Zetasizer 3000 (Malvern Instruments, Worcestershire, UK). PCS yields the mean particle diameter and the width of the particle size distribution (polydispersity index, PI). All the data presented are the mean values of three independent samples produced under identical production conditions.

2.8. Determination of ibuprofen content in granules

The ibuprofen content in granules was analysed by dissolving 130–160 mg of granules in 100 ml of 0.1 M HCl. The samples were stirred on a magnetic stirrer at 400 rpm at room temperature for 24 h, filtered and the amount of drug was determined spectrophotometrically at 222 nm (Spectrophotometer Perkin-Elmer—Lambda 20, Germany).

2.9. Dissolution studies

2.9.1. Dissolution studies of lyophilized ibuprofen from nanosuspensions

Three milligrams of lyophilized samples containing ibuprofen and poloxamer 188 (1:1) were suspended in 100 ml of 0.1 M HCl to maintain sink conditions and stirred on a magnetic stirrer at 50 rpm (Rotamix 560MMH, Tehnica, Slovenia) at room temperature. At predetermined time intervals, 2.5 ml aliquots were withdrawn filtered and the amount of dissolved ibuprofen was determined spectrophotometrically.

A suspension containing 1.5 mg of micronized ibuprofen and 1.5 mg of surfactant (poloxamer 188) in 100 ml of 0.1 M HCl was used as reference dispersion for the dissolution test.

2.9.2. Dissolution studies of ibuprofen from granules

Dissolution testing of different samples of granules containing a known amount of ibuprofen (8–10 mg) was performed using the USP XXV Type II (paddle method) (Erweka DT 6, Germany). The rotation speed of the paddles was set to 50 rpm. Nine hundred millilitres of 0.1 M HCl at 37 ± 0.5 °C was used as dissolution medium. At predetermined time intervals 3 ml samples were withdrawn, filtered immediately and the amount of dissolved drug was determined spectrophotometrically.

2.10. Differential scanning calorimetry

Accurately weighed samples (5–8 mg) were placed in non-hermetically sealed aluminium pans. An empty aluminium pan served as a reference. Samples were heated on a Pyris 1 DSC (Perkin-Elmer, USA) equipped with an Intracooler 2P cooling accessory. Heating rate was 10 K/min and nitrogen purge 20 ml/min.

2.11. Statistical analysis

Particle size data are reported as arithmetic means ± standard deviation. Statistically significant differences of the influence of stabilizers and methods used on mean particle size were determined using two-way analysis of variance (ANOVA). Significance was tested at the 0.05 level of probability. Statistical analysis was performed with the software package SPSS®.

3. Results and discussion

3.1. Solvent diffusion method

The first step in the production of nanosuspensions by the solvent diffusion method is to prepare a solvent-in-water emulsion with partially water-miscible solvents, with the dissolved drug in the dispersed phase. The selection of partially water-miscible solvent and the stabilizers is critical to obtain drug particles in the nanometre range. In general, solvents with high water miscibility and stabilizers able to form stable emulsions are preferred (Quintanar-Guerrero et al., 2005).

For nanosuspension production with the solvent diffusion method the drug should be very poor soluble in water

Table 1
Solubility of ibuprofen in water and organic solvents at 25 °C and solubility of organic solvents in water

Solvent	Ibuprofen solubility in organic solvents (mg/ml)[a]	Solubility of solvents in water (%, w/w)
Benzyl alcohol	377 ± 12	3.5[b]
Butyl lactate	242 ± 15	7.7[c]
Ethyl acetate	372 ± 22	8.0[b]
Triacetin	91 ± 7	7.1[c]
Water	0.056 ± 0.004	–
0.1 M HCl	0.036 ± 0.003	–

[a] Experimental data.
[b] Data from Yeo et al. (2003).
[c] Data from Trotta et al. (2001).

(<10^{-3}–10^{-4} mol/l) and highly soluble in the organic solvent (Sucker, 1998). The solubility of ibuprofen was determined in water and in four different organic solvents. According to experimental data shown in Table 1 the solubility of ibuprofen in water is very low, in triacetin low, while solubility in butyl lactate and ethyl acetate is higher and highest in benzyl alcohol. Solubilities in benzyl alcohol and ethyl acetate are not significantly different.

The selection of the partially water-miscible solvent for oil-in-water emulsion was performed with respect to its solubility in water and solubility of ibuprofen in it (Table 1). In the conducted experiments, nanosuspensions were formed when ibuprofen was dissolved in ethyl acetate and butyl lactate but, because of too low solubility of ibuprofen in triacetin, the later did not enable nanosuspension formulation.

The water miscibility of the solvent is determining factor on the process efficiency. In this sense, when benzyl alcohol as a solvent with low water miscibility was used, ibuprofen precipitated in particles larger than the nanometre range. The relatively high solubility of ethyl acetate and butyl lactate in water enables their fast diffusion from dispersed droplets into aqueous phase. Thus, as soon as the dispersed phase comes in contact with a large amount of aqueous phase during the emulsion dilution, fast diffusion of organic solvent occurs, leading to fast drug precipitation and particle formation. Ethyl acetate was therefore chosen for production of nanosuspensions by solvent diffusion. Further, ethyl acetate and butyl lactate are much more soluble in water than benzyl alcohol and consequently the volume of water that is added in the dilution step is smaller and the resulting nanosuspension can be more concentrated. Additionally, to dissolve the same amount of ibuprofen in ethyl acetate smaller volume of ethyl acetate was needed compared to butyl lactate.

The important step in preparation of nanosuspension is also the manner of diluting the emulsion. Dilution of emulsions in high pressure homogenizer sample holder and subsequent homogenization is much more efficient than dilution on a magnetic stirrer. Obviously, intense mixing of the water phase outside the interface of the droplets accelerates the diffusion of organic solvent into water, resulting in a rapid precipitation of the drug. The mechanical stress of magnetic stirrer is milder and consequently precipitation of large particles occurs, what was observed in our studies. Additionally, Peukert et al. (2005) reported that the intensity of mixing has a strong influence on the resulting particle size and size distribution in nanometre range. The more intense is the mixing smaller are the particles. The reason for this behaviour lies in the different dependencies of nucleation and growth rates on supersaturation, the thermodynamic driving force of solid formation. With increasing supersaturation, the nucleation rate increases much stronger than the growth rate. As a consequence, considerably more particles are formed at higher supersaturation, and are consequently smaller, as the local solution is depleted of drug by the precipitation process.

In general, subsequent homogenization of the precipitated particle suspension preserves the size of the particles obtained after precipitation step. Kipp et al. (2003) has reported, and we observed, that precipitated particles continue crystal growth if they do not undergo the high energy processing step.

A decrease in the mean particle diameter of nanosuspensions was observed when passing the emulsion at 200 bar through a high pressure homogenizer compared to homogenizing only with UT. For example, the size of particles in nanosuspensions stabilized with combination of Tween 80 and PVP K25 (1:1) prepared with APV homogenizer was 62.4 ± 0.5 nm in comparison to 226.6 ± 14.4 nm for nanosuspensions homogenized only with UT. Increasing the pressure to 1000 bar did not further decrease the drug particle size. This observation is in agreement with earlier report from Trotta et al. (2001).

A number of variables affect solvent droplet size, and also the properties of the resulting solid particles. The key parameters affecting the characteristics of the drug particles, including the size distribution pattern, are the drug solubility in the organic solvent, the diffusion rate of the solvent from the disperse phase and the homogenization process.

3.2. Melt emulsification method

The first step in the production of nanosuspensions by melt emulsification method is preparation of a hot emulsion with melted drug as the dispersed phase. The primary emulsion was homogenized in high pressure homogenizer and cooled to solidify the droplets of melted drug. It was found out that solvent diffusion method produces smaller particles than melt emulsification method (Fig. 1). This is explained by completely different mechanisms of the drug particle formation. In melt emulsification method particle formation is the consequence of the transformation of the melted drug into the solid state. In the solvent diffusion method, the particle formation is the consequence of drug precipitation from solution into the water. The size of the drug particles produced by melt emulsification method is dependent mostly on the size of dispersed droplets. Even if the emulsions prepared by different methods have the same average droplet size, the size of resulting particles from melt emulsion will always be larger. This is due the lower relative concentration of drug in solvent droplet in comparison to pure drug droplet in hot emulsion. Additionally, the effect of shear thinning is lower in the melt emulsification method, due to higher viscosity of melted drug compared to droplets in solvent diffusion method.

To prepare smaller drug particles from hot emulsion, the collision of droplets can be prevented by fast cooling. Comparison of the particle size in nanosuspensions produced with solidification under different cooling conditions shows that this step has

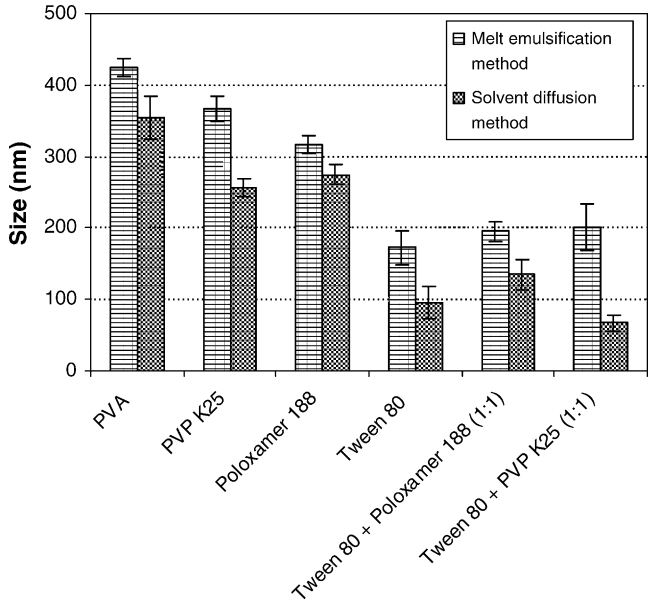

Fig. 1. Influence of different formulation methods and stabilizers on particle size of ibuprofen in nanosuspensions (mean ± S.D., $n = 3$).

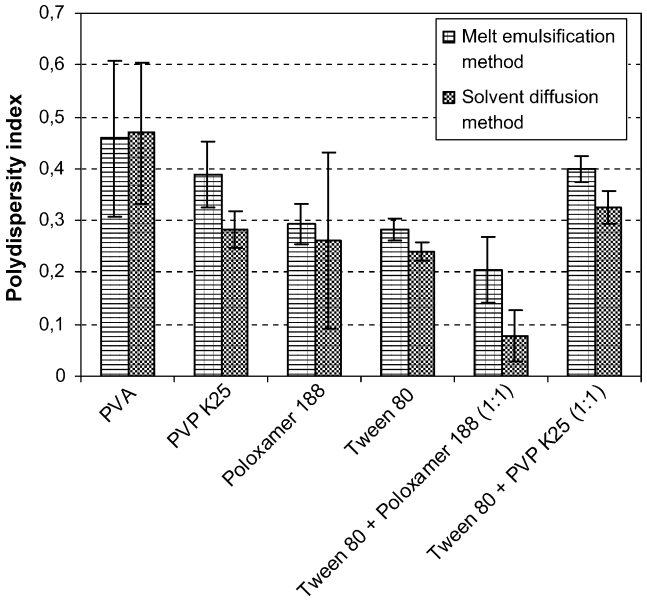

Fig. 2. Influence of different formulation methods and stabilizers on polydispersity index of ibuprofen in nanosuspensions (mean ± S.D., $n = 3$).

a significant effect on particle size. The average particle size in nanosuspension cooled on an ice-bath is smaller 147 ± 6.6 nm than that cooled at room temperature 173 ± 3.6 nm. Fast cooling causes very fast solidification of melted drug droplets, resulting in smaller drug particles.

The mean particle size and polydispersity index of ibuprofen suspensions obtained from emulsions containing different amounts of melted drug as dispersed phase and fixed concentration of stabilizers were determined by PCS (Table 2). An increase in the mean diameter of the suspensions was observed with increased drug concentration. If the amount of the inner phase is increasing the higher number of droplets can be formed leading into larger interfacial area. Such system is less stable because of higher interfacial energy. To convert the system into energetically more stable state droplets tend to coalescence resulting in bigger drug particle formation. The result of further increase in drug content was a formation of unstable emulsion with big drug droplets that could be seen with the naked eye on the surface of the sample.

3.3. Effect of stabilizers

The choice of stabilizer is specific to each drug candidate and each formulation procedure. The stabilizer (or mixture of stabilizers) should exhibit sufficient affinity for the droplet surface to enable preparation of emulsion and for the particle surface in order to stabilize the nanosuspension. In preliminary experiments by solvent diffusion method we used different concentrations of stabilizers from 0.05 to 1.00% (w/w) to stabilize the nanosuspension during the production process. Our results show that for short term stabilisation a concentration of 0.1% (w/w) was sufficient for nanosuspensions produced by solvent diffusion method and 0.5% (w/w) for nanosuspensions produced by melt emulsification method.

When the influence of different stabilizers was investigated the emulsions obtained by both methods were prepared with a fixed concentration of the drug. The type of compound employed for stabilization has a pronounced effect on particle size and polydispersity index (Figs. 1 and 2). Four different stabilizers were tested. The particle size in nanosuspensions stabilized with PVA, PVP K25 or poloxamer 188 is significantly greater than that stabilized with Tween 80. The main reason for efficient formation of droplets and stabilization of the nanosuspension appears to be the surfactant nature. The effectiveness of polymeric materials such as PVA, PVP K25 and poloxamer 188 is significantly smaller than Tween 80 in terms of particle size. Important function of polymers is that they can form a substantial mechanical and thermodynamic barrier at the interface

Table 2
Effect of drug concentration and stabilizers on particle size and polydispersity index of nanosuspensions produced by melt emulsification method

Stabilizer (0.5%, w/w)	Parameter	Concentration of ibuprofen		
		0.25%	0.5%	1%
Tween 80	Size	158.1 ± 5.5	198.8 ± 21.0	263.2 ± 22.3
	PI	0.378 ± 0.042	0.284 ± 0.021	0.401 ± 0.151
Poloxamer 188	Size	317.2 ± 12.9	275 ± 14.2	339.5 ± 18.6
	PI	0.491 ± 0.012	0.315 ± 0.121	0.294 ± 0.040

that retards the approach and coallescence of individual emulsion droplets (Myers, 1999). Nonionic nonpolymeric surfactants offer an advantage over polymers in that they have a higher adsorption potential than an equal-chain-length polymer (Palla and Shah, 2002). It seems that interactions between lypophilic area of ibuprofen and lypophilic part of Tween 80 are substantial for small droplets and consequently the small particles formation in melt emulsification method. Similar kinetically favourable interactions can be considered in solvent diffusion method during emulsification and homogenization.

A combination of Tween 80 and PVP K25 or combination of Tween 80 and poloxamer 188 yields nanosuspensions with particle sizes not significantly different than Tween 80 alone (Fig. 1). However, PVP and poloxamer are polymeric molecules, which can by adsorption on the droplet surface act as a steric barrier, preventing close contact of the droplets and later particles. Combination of stabilizers is also preferred for long-term stabilization as reported by Müller and Keck (2004) and Rabinow (2004).

Besides the results presented in Figs. 1 and 2, the effect of different stabilizers on the stability of produced nanosuspensions with Tween 80 was tested (Table 3). The effects of additional electrostatic repulsion, due to added negatively and positively charged surfactants, and higher concentration of steric stabilizers did not influence the particle size significantly. Furthermore, the results show that addition of poloxamer 407 and SDS increases the crystallisation rate of the drug in 0.5% concentration and higher.

3.4. Dissolution studies of lyophilized nanosuspensions

Ibuprofen is a weak organic acid, with pK_a 4.55 (Fini et al., 1995). Its solubility is pH dependent, increasing with pH. HCl (0.1 M) was selected for dissolution studies to simulate gastric condition, to slow the dissolution rate of the drug, and so allow greater discrimination of our processing effects.

For the characterization of lyophilized material, we determined particle size of redispersed lyophilized sample. The average particle size was 849.4 ± 10.5 nm, what was almost 3 times greater then before lyophilization (317.2 ± 12.9 nm), but more than 60 times smaller than micronized ibuprofen (D (v, 0.9) = 46.22 ± 4.35 μm; Mastersizer, Malvern Instruments, Worcestershire, UK).

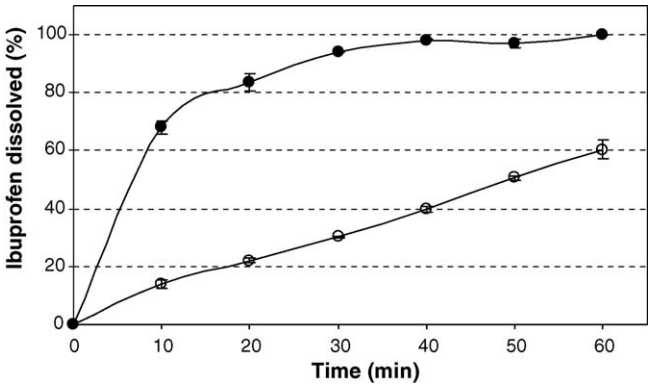

Fig. 3. Dissolution profiles of lyophilized nanosuspension stabilised with poloxamer 188 (●) and a mixture of micronized ibuprofen with poloxamer 188 (○) in 0.1 M HCl (n = 3).

The dissolution profiles of freeze-dried ibuprofen nanosuspensions, in comparison with a reference mixture of micronized ibuprofen with poloxamer 188, are shown in Fig. 3. The dissolution rate was markedly enhanced in the nanometre-sized system, as more than 65% of the drug dissolved in 10 min, as opposed to only 15% of micronized drug. This could be due to the increased surface area of the drug and possible better contact between nanosuspensions and dissolution medium. According to Noyes–Whitney equation, an increase in saturation solubility and decrease in particle size lead to an increased dissolution rate. The size of drug particles after lyophilization was not preserved, but still remained in nanometre scale. It is reported that the saturation solubility increases with decreasing particle size. However, this effect is only pronounced for particle below approximately 2 μm, especially below 1 μm (Müller et al., 2000).

So formulation of poorly water-soluble drugs as nanometre-sized drug particles has a dramatic effect on dissolution rate, drug solubility and consequently bioavailability. The bioavailability of ibuprofen is truly dissolution rate limited, so particle size reduction can significantly improve the performance of the drug (Lindenberg et al., 2004).

3.5. Differential scanning calorimetry studies

To characterize the freeze-dried nanosuspension DSC studies of ibuprofen, poloxamer 188 and freeze-dried nanosuspension

Table 3
Effect of different stabilizers in combination with Tween 80 on particle size and polydispersity index of nanosuspension prepared by melt emulsification method

Type of stabilizer	Stabilizer Tween 80 and	Concentration (%)	Size (nm)	PI
Steric	PVA	0.5	170.0 ± 2.1	0.176 ± 0.018
		0.8	170.4 ± 2.2	0.249 ± 0.057
	Poloxamer 407	0.5	Crystallization	–
	Poloxamer 188	0.5	182.6 ± 0.6	0.351 ± 0.044
Negatively charged	DOSS	0.5	175.0 ± 4.7	0.294 ± 0.018
		0.8	166.0 ± 0.9	0.242 ± 0.018
	SDS	0.5	Crystallization	–
Positively charged	CP-Cl	0.5	191.5 ± 0.4	0.260 ± 0.030

SDS: sodium dodecyl sulphate; DOSS: dioctyl sulfosuccinate, sodium salt; CP-Cl: cetylpyridinium chloride.

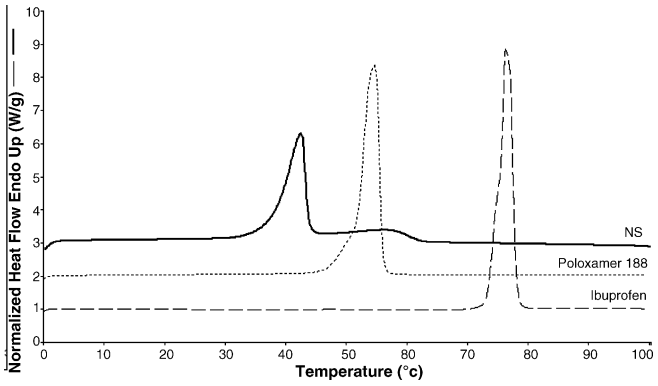

Fig. 4. Differential scanning calorimetry curves of ibuprofen, poloxamer 188 and lyophilized nanosuspension (NS) composed of ibuprofen and poloxamer 188 in ratio 1:1.

were performed. The DSC curve of pure ibuprofen exhibits a single endothermic peak at an onset temperature of 74.8 °C, due to its melting (Fig. 4). Poloxamer 188 has also a single melting peak with onset temperature at 51.4 °C. There are two distinguishable endothermic changes in the DSC curve of freeze-dried nanosuspension of ibuprofen and poloxamer 188 in the ratio of 1:1. The first endothermic change appears as a large narrow peak with onset temperature at 39.4 °C and the second as low broad peak with temperature of maximum at 56.8 °C, where is not possible to analyse the onset temperature. These results suggest the formation of a eutectic system between the drug and poloxamer 188. In this case, the peak at the lower temperature would belong to the melting of the eutectic mixture, and the second, change at the higher temperature, would indicate the melting of the surplus component. From the position of the second peak one could assume, that ibuprofen is the component left over after the eutectic has being formed. The melting peak of the clean poloxamer 188 appears at a lower temperature than the endothermal change of the remainder in the nanosuspension sample. To confirm this assumption a physical mixture composed of ibuprofen and poloxamer 188 in ratio 2:3 was scanned (data not shown). The peak of the eutectic appeared larger and the second peak was smaller than on the DSC curve of lyophilized nanosuspension with ratio of components 1:1. These results demonstrate that the composition of the lyophilized sample is not in a eutectic ratio, but contains some excess of ibuprofen. This is in accordance with literature data. A eutectic system between ibuprofen and poloxamer 188 is composed of 30% ibuprofen and shows an endothermic peak at 38.7 ± 1.2 °C (Passerini et al., 2002). The ibuprofen/poloxamer mixture in the freeze-dried nanosuspension forms the eutectic composition; therefore the increased dissolution rate of the drug can be explained by formation of the eutectic system and sub-micron sized drug particles during the production of nanosuspensions.

3.6. Comparison of the methods for nanosuspension preparation

In both methods, the intermediate step in production of nanosuspensions is an emulsion, which is homogenized and turned into nanosuspension. The size of the drug powder used for producing nanosuspensions by both methods is not important because, in the first case, the drug is dissolved in an organic solvent and, in the second, it is melted and then homogenized. The solvent diffusion process is less energy consuming than the hot homogenization process.

The requirements limiting the applicability of the precipitation technique are that the drug needs to be soluble, at least in one solvent, and that this solvent needs to be miscible with a non-solvent. These prerequisites exclude the processing of drugs which are simultaneously poorly soluble in aqueous and non-aqueous media.

The use of organic solvents raises environmental and human safety concerns over residual solvent, so they cannot be recommended for a routine manufacturing process. That is one important disadvantage of the solvent diffusion method. On the other hand, the melt emulsification method is useful only for drugs with melting points below water boiling point.

3.7. Dissolution of ibuprofen from granules

In order to assess whether the goal of improving the dissolution rate of ibuprofen in solid dosage forms was achieved, in vitro dissolution profiles of different ibuprofen samples were determined (Fig. 5). The dissolution rate of pure drug is very low: only 6% of the drug dissolved in the first 10 min, and the formation of a physical mixture with poloxamer 188 does not improve this value. On the contrary, the granules prepared using nanosuspension and by dispersion of ibuprofen in a solution of stabilizer both show a great increase in the dissolution of the drug over pure ibuprofen, 79 and 56%, respectively, of the drug are dissolved in the first 10 min. These results show that granulation with nanosuspension is a useful method to formulate a solid dosage form with higher dissolution rate of ibuprofen. Moreover, the fact that the simple mixture of the components does not improve the dissolution of the drug suggests that the

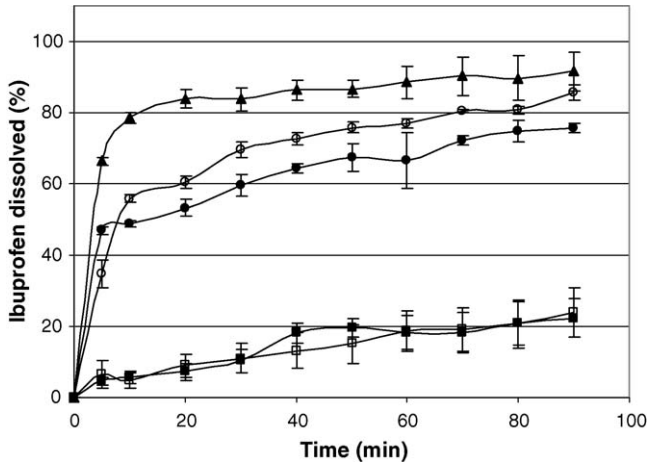

Fig. 5. Dissolution profiles of micronized ibuprofen (□), physical mixture of ibuprofen and poloxamer 188 (1:1) (■), granules composed of micronized ibuprofen and powder mixture granulated with poloxamer 188 solution in the mixer (●), and granules prepared in top-spray chamber either with dispersion of micronized ibuprofen in poloxamer 188 solution (○) or nanosuspension (▲) as granulating liquid.

enhancement in nanosuspension is not correlated with solubilisation or to a wetting effect of the stabilizer (poloxamer 188) on the drug, but due to decrease of particle size to nanometre range.

4. Conclusions

The results obtained in this study demonstrate that the melt emulsification method constitutes a viable alternative for preparing ibuprofen nanosuspensions. The particle size can be influenced by varying the process and its parameters, such as drug concentration, type and concentration of stabilizers, cooling conditions, and homogenization procedure. The major advantage of the melt emulsification technique relative to the solvent diffusion method is total avoidance of organic solvents during the production process. Further, it has been shown that formulating ibuprofen as a nanosuspension is highly successful in enhancing dissolution rate. The rate proved to be higher for smaller drug particles, thus influencing the bioavailability of the drug and improving safety for the patient by decreasing gastric irritancy.

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