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The expulsion of lipophilic drugs from the cores of solid lipid microspheres in diluted suspensions and in concentrates

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Abstract

The aim of the study was to compare incorporation of bupivacaine base, bupivacaine stearate and indomethacin in diluted suspensions of lipospheres (10%, w/w of lipid) and in concentrates (50%, w/w of lipid). The lipid cores were composed of a mixture of solid and liquid triglycerides (Precirol and Miglyol 4:1). The lipospheres sizing between 0.5–10 μm (suspensions) and 0.5–20 μm (concentrates) were prepared using a hot emulsification with high-shear mixing and cold resolidification method. None of the studied drugs was successfully incorporated in the lipid core. The increased incorporation of drugs determined in the concentrated lipospheres was only apparent, since in fact all the dose was only attached to the surface of the lipid particles and was transferred to the aqueous phase in the course of an intensive agitation. The presence of hydrophilic polymers in the aqueous phase did not prevent the expulsion effect although drug precipitation was retarded. The expulsion effect did not correlate with the solubility of drugs determined in the bulk lipids.

Keywords: Lipospheres; Concentrated lipospheres; Bupivacaine; Bupivacaine stearate; Indomethacin; Expulsion; Incorporation

1. Introduction

Lipids are promising candidates as components of biodegradable matrices incorporating drugs, appropriate for parenteral delivery. Technology of solid lipid nanoparticles (SLN®), sizing below 1 μm (average 0.1–0.3 μm), was developed in order to obtain injectable nanospheres, which can be administered intravenously (Müller et al., 2000). Use of the lipid particles as drug carriers for extravenous injection (intramuscularly, subcutaneously, for nerve infiltration) has been also considered (Masters and Domb, 1998; Toongsuwan et al., 2004). For this route of drug delivery, larger particles, up to 30–50 μm (lipospheres, lipid microspheres or microparticles) are acceptable. Recently Müller et al. (2002) proposed concentrated SLN containing up to 80% of lipid phase as a new drug delivery system. The concentrate is semisolid and should be diluted before injection.

The release of a drug incorporated in the lipid matrix whose melting point is above body temperature occurs due to degradation of the particles by lipase present in the site of injection. This mechanism enables prolonged release of drugs from SLN (Müller et al., 2000; Gasco, 2001) and lipid microparticles (Masters and Domb, 1998). In the respect of the extended drug release micron-sized lipospheres are theoretically more effective than SLN.

In contrast to SLN the research on lipid microparticles is very limited. Similarly to SLN, physical instability of the dispersions of lipid microspheres, demonstrated by gelation of the system, can be a problem. In our previous studies (Pietkiewicz and Sznitowska, 2004) suspensions of lipid microspheres whose fluidity and lipid/surfactant composition is suitable for parenteral administration were developed. Precirol (palmitostearate) was used as a matrix forming agent because of its appropriate melting temperature (55 °C) and biocompatibility. Physically stable 10% (w/w) suspensions of lipid microspheres were produced using saturated triglycerides in combination with medium chain unsaturated triglycerides (Miglyol) as lipids and polysorbate 80 (2%, w/w) as a surfactant. The process developed for lipid microspheres does not involve high-pressure homogenization which is

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a critical step allowing particle sizes below 1 μm and is required for SLN (Müller et al., 2000).

Despite of numerous studies there is no clear evidence that SLN are good carriers for many drugs since incorporation of different drugs may be unpredictable. Good incorporation potential of SLN was demonstrated for coenzyme Q10 (Wissing et al., 2004a), cyclosporine A (Hu et al., 2004), paclitaxel (Chen et al., 2001), prednisolon (Mühlen et al., 1998), mifepristone (Hou et al., 2003) and several others presented in the recent review (Wissing et al., 2004b). On the other hand, problems with incorporation of other lipophilic molecules like gamma-cyhalothrin (Frederiksen et al., 2003), tetracaine and etomidate (Mühlen et al., 1998) as well as coenzyme Q10 (Bunjes et al., 2001) were reported. Moreover, majority of very lipophilic spin probes were found to be located not in the core of SLN but at the interphase between phospholipid layers and the core (Ahlin et al., 2000, 2003). The low incorporation capacities were explained by the crystalline structure of the solid lipid (Westesen et al., 1997).

The aim of the reported study was to compare incorporation of lipophilic compounds in diluted suspensions (10%, w/w of lipid) and in concentrates (50%, w/w of lipid) of lipospheres. Bupivacaine base and bupivacaine stearate as well as indomethacin were investigated as model drugs. The lipid cores were composed of a mixture of solid and liquid triglycerides in order to obtain less crystalline structure as proposed for SLN (Müller et al., 2002).

Suspensions of lipid microspheres with bupivacaine were already described in the literature but the results regarding drug loading capacity were not consistent, largely depending on types of the triglycerides and surfactants: Toongsuwan et al. (2004) demonstrated fast precipitation of the expulsed drug, while Masters and Domb (1998) succeeded in a high-drug loading.

2. Materials and methods

2.1. Materials

Bupivacaine free base was donated by Polfa Pharmaceutical Works (Warsaw, Poland) and indomethacin by Jelfa (Jelenia Góra, Poland). Precirol (glyceryl palmitostearate) was a gift from Gattefossé (Lyon, France). Miglyol 812 (medium chain triglycerides) was manufactured by Caelo Caesar and Loretz (Hilden, Germany). Egg lecithin (Lipoid E-80) was purchased from Lipoid (Ludvigshafen, Germany) and polysorbate (Tween 80) from Merck (Darmstadt, Germany). Stearic acid was manufactured by Loba Feinchemie (Fischamend, Austria).

Sodium carmellose was purchased from Sigma (Steinheim, Germany), hydroxyethylcellulose (Natrosol 250 HX) from Hercules (Wilmington, USA) and poloxamer 188 (Pluronic F68) from Boehringer Ingelheim (Heidelberg, Germany). Cethyl alcohol and cetostearyl alcohol were manufactured by Henkel (Hamburg, Germany).

2.2. Preparation and analysis of bupivacaine stearate

Bupivacaine stearate was prepared by melting at temperature 108 °C bupivacaine free base with stearic acid in molar ratio 1:1. Alternatively, both substances were dissolved in ethanol and the solvent was evaporated from the combined solutions (co-precipitation method). The resulting substances were subjected to DSC analysis and the thermograms were compared with those of bupivacaine base and stearic acid.

2.3. Solubility and partitioning of drugs in water and lipids

In order to determine the solubility of bupivacaine base and bupivacaine stearate in water or in a phosphate buffer (pH 7.0 and 8.0) the drugs in excess were suspended in a solvent and the suspension was stirred with a magnetic stirrer at temperature 20 ± 1 °C for 24 h. After centrifugation the supernatant was filtered and analysed for drug content.

The solubility of bupivacaine, bupivacaine stearate and indomethacin in solid bulk lipids or their mixtures with Miglyol was estimated by visual inspection of lipid films obtained after cooling the melted lipids containing drugs in different concentrations.

The partitioning of bupivacaine base and bupivacaine stearate between lipid and water was determined. Bupivacaine was dissolved in melted Precirol (200 mg per 1 g of the lipid) and warm water was added (80 °C). After 10 min of stirring the emulsion was cooled and after 24 h the separated aqueous phase was analysed for concentration of bupivacaine. For Miglyol/water partition coefficient saturated aqueous solution of bupivacaine was shaken with the oil and concentration of the drug in the aqueous phase was analysed. From the difference of concentrations before and after partitioning the partition coefficient was calculated.

All determinations were done at least in triplicates.

2.4. Preparation of lipospheres—in the form of suspension and concentrate

The content of lipids in suspensions was 10% (w/w) and in concentrates—50% (w/w). In all formulations polysorbate 80 was used as an emulsifying agent (2.0%, w/w) and glycerol was added for isotonicity (2.3%, w/w). Precirol in a mixture with Miglyol (4:1) constituted the lipid matrix of the lipospheres. The formulations were prepared as follows using a hot emulsification and cold resolidification method. Precirol was melted with Miglyol and polysorbate at 80 °C, bupivacaine base, bupivacaine stearate or indomethacin was added and the lipid phase was transferred to the aqueous phase (water and glycerol at 80 °C). Concentration of drugs in suspensions or concentrates are shown in Table 1. Hydrophilic polymer, if present (Table 1), was dissolved in the aqueous phase. The emulsification was performed at 80 °C using a high-shear mixer Ultra-Turrax (IKA Labortechnik, Staufen, Germany) at 8000 rpm for 5 min. Crystallization of lipids occurred at 4 °C, while for some formulations fast crystallisation by immersion in a liquid nitrogen was also performed. The suspensions or concentrates were stored in a refrigerator (4 °C). In all formulations containing bupivacaine, before cooling, pH was adjusted to 8.0 with NaOH.

Table 1
The effect of drug concentration and hydrophilic polymers on crystallisation of the drug and its content in the aqueous phase of suspensions and concentrates of lipid (Precirol and Miglyol 4:1) microspheres

Drug	Drug concentration (%, w/w)	Polymer in aqueous phase (%, w/w)	Crystallization of drug	Free drug content (% of total)
Suspensions containing 10% (w/w) lipid				
Bupivacaine free base	0.5	–	48 h	100.4
	0.5	CMC 1.0	3 days	87.0
	0.5	HEC 1.0	7 days	101.0
	0.5	Poloxamer 5.0	<24 h	n.s.
Bupivacaine stearate	1.0	–	3 days	93.4
Indomethacin	0.2	–	7 days	100.0
	0.2	Poloxamer 5.0	3 weeks	102.0
	0.5	Poloxamer 5.0	<24 h	n.s.
Concentrates containing 50% (w/w) lipid				
Bupivacaine free base	2.0	–	n.d.	82.5*
	10.0	–	n.d.	28.9*
Bupivacaine stearate	4.0	–	n.d.	44.4*
Indomethacin	1.0	–	n.d.	60.9*
	5.0	–	<24 h	n.s.

CMC: sodium carmellose; HEC: hydroxyethylcellulose; n.s.: not studied due to drug crystallisation before ultrafiltration; n.d.: not detected in the undiluted concentrates; asterisk (*): the concentrate diluted with hand shaking.

In the preliminary studies suspensions of lipospheres containing Precirol as a sole lipid were also prepared using the same method as described above.

For indomethacin additional formulation was prepared by dissolving the drug in ethanol (50% solutions) and introducing this solution into the melted lipid, Precirol. Further, the suspension of lipospheres (without Miglyol) containing 0.4% (w/w) indomethacin was prepared as described above. Besides, suspensions of lipospheres made of Precirol alone or its mixture with cethyl alcohol, cetostearyl alcohol and Miglyol were prepared and evaluated (Table 2).

2.5. Dilution of concentrated lipospheres

The concentrated lipospheres containing bupivacaine base or stearate were diluted with 0.015 mol/l HCl in the ratio 1:4. The concentration of HCl was chosen on the basis of the preliminary experiments to obtain pH 6.7 (±0.1) in the diluted formulation. Different procedures were employed for dilution: hand shaking for 3 min, ultrasounds for 15 min (22 Hz), Vortex for 5 min or Ultra-Turrax stirring (8000 rpm, for 5 min).

The dilutions were observed microscopically. Those, where crystals of the drug were not visible were subjected to the analysis of drug content in the aqueous phase.

The concentrates with indomethacin were diluted with water in the ratio 1:4.

2.6. Analysis

Visual and microscopic observations were carried out using an optical microscope. Particle size distribution was measured using a laser diffractometer Mastersizer E (Malvern Instr., Malvern, UK).

The total amount of bupivacaine (base or stearate) in the formulations and in the aqueous phase of the formulations (unincorporated dose) was studied by HPLC method using a mobile phase—0.01 M phosphate buffer pH 7.7:acetonitrile:methanol 35:40:25 and Lichrospher RP-18 column (250 mm, 5 μm; Merck) with detection at 220 nm. HPLC analysis of indomethacin was performed using methanol and 0.01 M sodium acetate buffer pH 3.6 (65:35) as the mobile phase and Lichrospher RP-18 column (125 mm, 5 μm) with detection at 260 nm.

Table 2
Solubility of indomethacin in bulk lipids and in suspensions of lipospheres (10% (w/w) of lipid; indomethacin content 20 mg/g of lipid)

Lipid	Drug solubility, X (%, w/w)	Precipitation of drug in suspension of lipospheres[a]	
		After preparation	After 7 days (4 °C)
Precirol	$2.0 < X < 5.0$	+	
Precirol:Miglyol, 4:1	$0.2 < X < 0.5$	–	+
Precirol:cetostearyl alcohol, 4:1	>10.0	Semisolid formulation	
Precirol:cetostearyl alcohol:Miglyol, 3:1:1	$0.5 < X < 1.0$	–	+
Precirol:cethyl alcohol, 4:1	>10.0	+	
Precirol:cethyl alcohol:Miglyol, 3:1:1	$0.5 < X < 1.0$	–	+

[a] Precipitation observed (+); no precipitation (–).

The free drug content was calculated on the basis of drug concentration in the aqueous phase, as a percentage of the total dose. In order to separate the aqueous phase, ultrafiltration was performed using centrifuge filtration units—Microcon YM-100 (cut-off 100 kDa, Millipore, Bedford, USA). Before filtration the concentrates with bupivacaine were diluted with water in ratio 1 + 4 adjusting pH to 1–2 with HCl in order to enable filtration and to dissolve crystals of bupivacaine, if present. The suspensions of lipospheres were also adjusted to acidic pH. For the analysis of the formulations with indomethacin dilution with water and 60% (v/v) methanol was done.

3. Results

Fig. 1 presents DSC thermograms of bupivacaine, stearic acid and bupivacaine stearate. A very small peak of stearic acid and no peak of bupivacaine are observed in a sample of bupivacaine stearate, but a new peak at 55 °C appears. It is concluded that a new chemical molecule was synthesized, with a small impurity of stearic acid. The same thermograms were recorded for the samples obtained by either melting or co-precipitation methods.

Solubility of bupivacaine and bupivacaine stearate in water at room temperature was 203 and 149 μg/ml, respectively. Concentrations of bupivacaine in saturated solutions in buffers pH 8.0 and 7.0 were 239 and 1035 μg/ml, respectively. Solubility of bupivacaine and bupivacaine stearate estimated semiquantitatively in bulk Precirol were 200 mg/g and above 250 mg/g, respectively. This was higher than solubility of bupivacaine base in two other triglycerides (glyceryl tristearate, 100 mg/g; glyceryl tripalmitate, 100 mg/g). Low solubility of the drug in a mixture of Precirol and Miglyol (4:1) was observed, i.e. aproximately 10 mg/g, although solubility in Miglyol alone was higher (more than 100 mg/g). For bupivacaine base the partition coefficients lipid/water were determined as 3741 for Precirol and 13.9 for Miglyol. The solubility of indomethacin determined in different lipid mixtures is presented in Table 2. In the presence of cethyl alcohol solubility of indomethacin in bulk Precirol increased, while added Miglyol decreased solubility of the drug significantly.

Figs. 2 and 3 demonstrate comparison of particle sizes and morphology in suspensions and in concentrates prepared with a mixture of Precirol and Miglyol as matrix component. The proposed method enables preparation of suspension of lipospheres with sizes ranging from 0.5 to 10 μm. Although laser diffractometer measurements revealed that the mean diameter of the

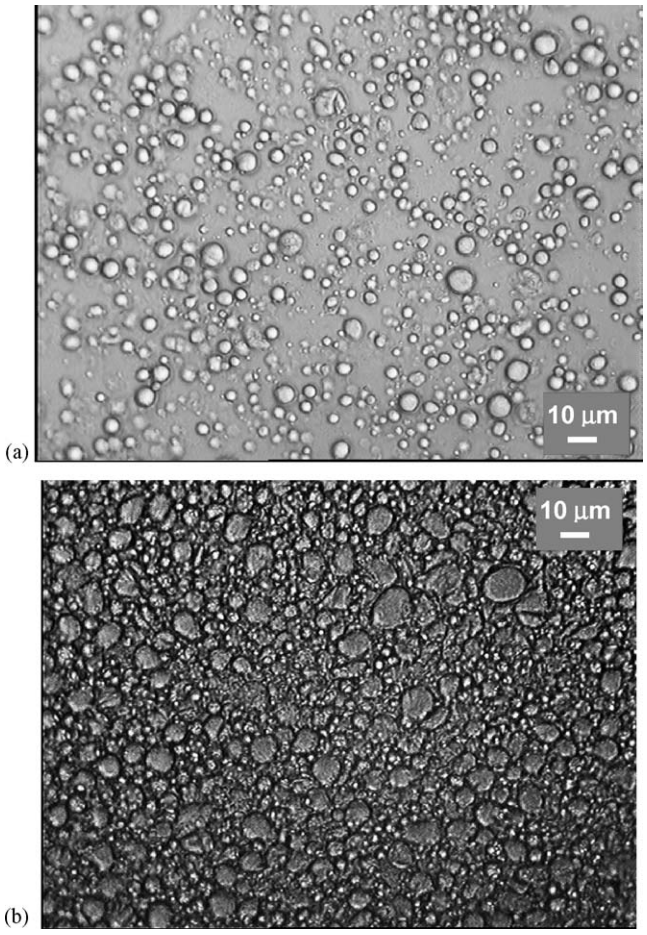

Fig. 2. Optical microscopic picture of suspensions of lipospheres (a) and concentrated lipospheres (b) with bupivacaine base.

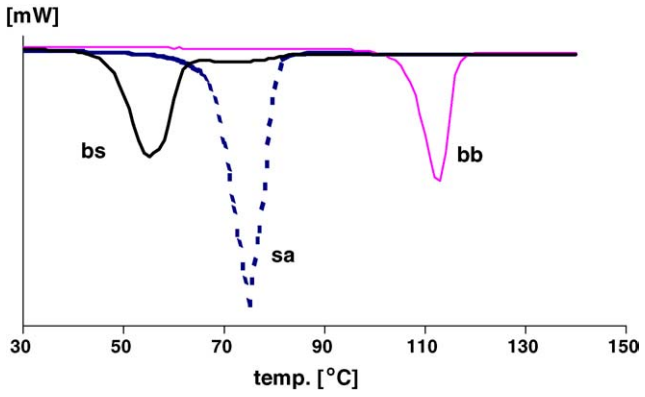

Fig. 1. Differential scanning calorimetry thermograms of bupivacaine stearate (bs), bupivacaine base (bb) and stearic acid (sa).

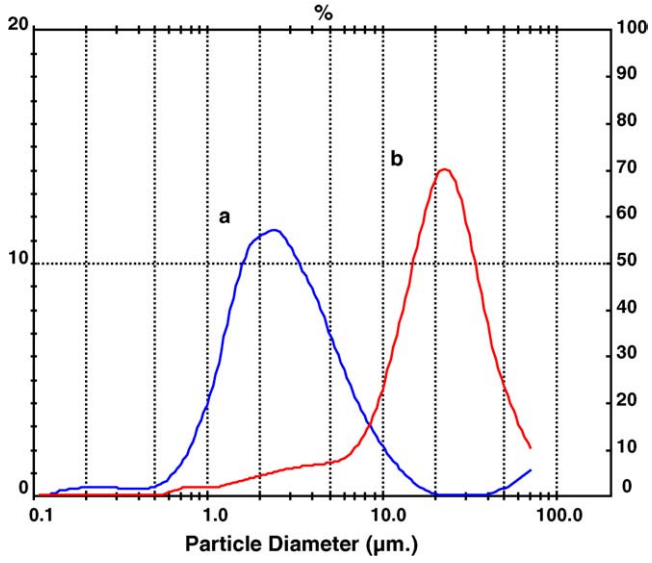

Fig. 3. Particle size distribution of lipospheres in suspension (a) and in diluted (hand shaking) concentrate (b) analysed with a laser diffractometry.

Table 3
The amount of bupivacaine determined in the aqueous phase (% of the total) of the concentrates during storage at 4 °C (mean values, $n = 3$)

Drug, %	Day 0	Day 1	Day 8
Bupivacaine base, 2.0%	82.6	–	95.4
Bupivacaine base, 10%	28.9	28.9	28.5
Bupivacaine stearate, 4%	44.4	53.3	41.9

particles in the concentrates is approximately 20 μm (1–60 μm), but probably agglomerated particles were measured, since under microscope no lipospheres larger than 20 μm were found. The shape of the lipid particles both in suspensions and in concentrates was spherical (Fig. 2). In the presence of bupivacaine or indomethacin neither shape nor size of the particles was changed. Similar size of the lipospheres was achieved when other lipids were used as matrix components.

The suspensions containing 10% (w/w) of lipid were fluid and did not gellify during storage at 4 °C for 12 months. The presence of the incorporated drugs did not change the fluidity, as observed visually during at least 6 months. The pH of the formulations with bupivacaine and indomethacin was 7.5–8.0 and 4.5–5.5, respectively.

In Table 1 suspensions and concentrates, containing either bupivacaine or indomethacin, are compared in respect of the amount of the free drug present in the aqueous phase and the kinetics of its crystallization. Within 48 h after preparation bupivacaine crystals (needles) were observed in suspensions of lipospheres (Precirol and Miglyol 4:1) with bupivacaine base used in concentration 0.5% (w/w). Suspensions of lipospheres incorporating bupivacaine stearate were more stable, but still crystallisation of the free drug was observed after 3 days. In contrast, in suspensions of lipospheres prepared with Precirol without Miglyol bupivacaine precipitated directly after preparation. Indomethacin, when introduced in concentration 0.2% (w/w) precipitated in the aqueous phase of the suspensions after 7 days, in a form of long needles. Precipitation was evident, irrespectively of the speed of cooling the formulations—at 4 °C or in liquid nitrogen. Needle-shaped crystals of the precipitated indomethacin were also detected shortly after cooling the suspension of lipospheres (mean size 3 μm) which were prepared by introducing the drug as ethanolic solution instead of dissolving it in melted lipid.

Precipitation was retarded if hydrophilic polymers were present in the formulations. Poloxamer was effective for indomethacin, prolonging crystallization time up to 3 weeks, however even in its presence crystallization occurred within 24 h after the suspension was subjected to ultrasounds for 20 min. When the concentration of indomethacin was increased to 0.5%, the polymer did not prevent fast crystallization which occurred within 24 h. In contrast, poloxamer did not retard crystallization of bupivacaine, however suspensions containing hydroxyethylcellulose and sodium carmellose were stable up to 3 and 7 days, respectively (Table 1).

The formulations where crystals were not visible under microscope were analysed for the content of free drug in the aqueous phase and the results are shown in Table 1. In suspensions of lipospheres containing bupivacaine or bupivacaine stearate 87–101% of the total drug was found in the aqueous phase. The analysis of the ultrafiltrate of the lipospheres containing indomethacin revealed that only 2–4% of the total dose was present in the aqueous phase. However, after dilution of the suspensions with 60% methanol all the dose was found in the ultrafiltrate (Table 1). This procedure did not dissolve the lipid particles what was proven by observations under microscope and additionally by measuring spectrophotometrically the absorbance of an extract obtained when lipospheres loaded with a lipophilic dye, Sudan III, had been treated in the same manner (data not shown).

Despite of the high concentrations of bupivacaine base or stearate (up to 10%, w/w) introduced to the concentrates (lipid content 50%, w/w), no crystals of bupivacaine were observed during storage of the undiluted preparations (Table 1). In the case of indomethacin such observation was true only for the concentrates containing 1.0% (w/w) of the drug. If the drug content was higher (5%) the undissolved drug was present in the form of square crystals as detected under microscope.

The free drug content in the aqueous phase was determined after ultrafiltration of the diluted concentrates. Dissolution of eventually present crystals of bupivacaine was ensured by adjusting pH to acidic. In the concentrates containing 2% of bupivacaine in the form of either base or stearate (4% of the stearate corresponds to 2% of bupivacaine), 82.5% (77.8–88.3%) and 44.4% (43.0–45.85), respectively, of the total drug was found in the aqueous phase (Table 1). When the amount of bupivacaine base was increased to 10% (w/w) in the concentrates, only 28.9 % was found in the aqueous phase. This allows for estimating the amount incorporated 142 mg/g of lipid. The amount of bupivacaine present in the aqueous phase was not changed during 8 days of storage at 4 °C as shown in Table 3.

Table 4
The amount of bupivacaine and indomethacin determined in the aqueous phase (% of the total) of the concentrates diluted using different procedures (mean values, $n = 3$)

Drug and concentration	Solvent (ratio)	Method of dilution	Drug in the aqueous phase (%)
Bupivacaine base, 10%	0.015 M HCl (1:5)	Hand shaking 3 min	28.9
		Vortex 5 min	93.2
		Ultrasounds 15 min	101.9
Indomethacin, 1%	Water (1:5)[a]	Hand shaking 3 min	60.9
	Water (1:5)[a]	Ultrasounds 15 min	75.8
	60% methanol (1:50)[a]	Hand shaking 3 min	97.5

[a] Diluted with methanol 60% directly before ultrafiltration.

Different procedures of diluting the concentrates resulted, however, in different recoveries of the drugs from the aqueous phase, what is demonstrated in Table 4. Using more intensive mechanical stress than with hand shaking (ultrasounds, Vortex, high-shear mixing) allowed for releasing practically 100% bupivacaine to the aqueous phase. In all diluted concentrates crystallisation of the drugs was observed within 24 h (4 °C).

The same was observed for the concentrates containing indomethacin as is shown in Table 4. The free drug content was related to the mode of agitation during dilution: when hand shaking with water was performed 60.9% (57.4–64.5%) of the drug was extracted but almost total dose was recovered if 60% methanol was used for dilution, in spite of the fact that the cores of the lipospheres were not soluble in this solvent.

4. Discussion

Lipospheres with the lipid cores composed of Precirol and Miglyol (4:1) and with polysorbate 80 as an emulsifying agents were prepared using a simple method employing high-shear mixing. The composition of the lipids and emulsifier enabled preparation of suspensions containing 10% (w/w) of lipid which remained fluid during a long-term storage. The lipid particles were spherical, above 1 μm in size, what allows for calling them lipid microspheres (lipospheres) in contrast to solid lipid nanoparticles (SLN) which are characterized by sizes below 0.5–1 μm.

Concentrated SLN were introduced by Müller et al. (2002) and following this idea concentrated lipid microspheres, containing 50% (w/w) of lipid, have been also prepared. In contrast to suspensions, these formulations were semisolid, but upon dilution with water (1 + 4) they formed fluid suspensions. The lipid particles in the concentrates were larger than in "de novo" prepared suspensions, but also spherical and, when diluted, in the size range acceptable for extravascular injection (Figs. 2 and 3). The advantage of the "ex tempore" diluted concentrates, in comparison to suspensions, was five times lower concentration of the surfactant–polysorbate.

Bupivacaine and indomethacin were chosen as model lipophilic active substances. Bupivacaine was used in a form of the free base and stearate. The stearate was synthesized from bupivacaine base and stearic acid and DSC thermograms (Fig. 1) prove that the stearate was formed, with only small amount of residual stearic acid. Both methods of preparation, melting or co-precipitation, were comparably suitable for synthesis of bupivacaine stearate. Low solubility in water and good solubility in lipids makes both forms of bupivacaine appropriate for incorporation in the lipospheres. Among three studied triglycerides Precirol alone offered the highest solubility for bupivacaine base and bupivacaine stearate. The solubility in lipids as high as 200 mg/g theoretically should result in suspensions and concentrates of lipospheres containing at least 2 and 10% (w/w) bupivacaine, respectively.

When suspensions of lipospheres with bupivacaine in concentration 0.5% were prepared with Precirol alone, practically all drug was found in the aqueous phase of the suspensions, and precipitation of the drug was observed directly after cooling the system. This was in contrast to a very good solubility of bupivacaine in Precirol. The conclusion was made that bupivacaine is excluded from the lipid matrix during crystallization. The "expulsion effect" was already described for SLN (Westesen et al., 1997) and incorporation of a liquid oil (8–16%) to the solid lipid core was proposed as a method to improve the loading capacity of the formulations, which were called nanostructured lipid carriers, NLC (Jenning et al., 2000; Müller et al., 2002). Considering this idea the attempt has been made to prepare lipid microspheres containing Miglyol in addition to Precirol (1:4 ratio).

In spite of the low solubility of bupivacaine observed in bulk Precirol with Miglyol significant retardation of drug precipitation was achieved in suspensions composed of the mixture of these lipids. However, no improve in incorporation of the drug in a modified matrix of lipospheres was observed (Table 1). This may support the theory that the liquid oily constituent is not actually building the structure of the lipid core but is also excluded from the solid matrix as demonstrated by Jores et al. (2003, 2004) in the case of lipid nanospheres. Fast crystallization of the lipid matrix by immersion in liquid nitrogen did not result in higher efficacy of drug incorporation in the lipospheres as evaluated on the basis of the free drug in the aqueous phase (data not shown).

It was expected that bupivacaine in the form of stearate can fit better into the crystalline structure of the solid lipid, and this should result in increased incorporation efficacy. This effect, however, was not observed and the "expulsion" of the total dose of bupivacaine stearate occurred, in spite of higher lipophilicity of this chemical form (Table 1).

The third drug, indomethacin was chosen due to its high log *P* 4.2, however its solubility in lipids was not as high as observed for bupivacaine (Table 2) and was very low in the presence of Miglyol. Suspensions and concentrates of lipospheres containing 0.2–0.5, and 1.0–5.0% of indomethacin, respectively, were examined.

As for bupivacaine, the expulsion was also observed in the case of indomethacin (Table 1). Introducing indomethacin in the form of ethanolic solution did not result in more efficient incorporation either.

To support a theory that expulsion of the drug from the lipid matrix depends on the type of the lipid, various combinations of the solid or solid and liquid lipids were studied as matrix formers for suspensions of lipospheres with indomethacin. The solubility of indomethacin in bulk lipids did not correlate with the rate of drug precipitation in suspensions of lipospheres (Table 2). Like in the case of bupivacaine, opposite relationship was even observed since in the presence of Miglyol, which decreased solubility of indomethacin significantly, precipitation of indomethacin was retarded. This indicates that even if optimal composition of lipids may offer a crystalline structure which enables efficient incorporation of drug molecules, it will not be easy to find such combination on the basis of drug solubility estimated in bulk lipids.

The presence of hydrophilic polymers in the aqueous phase of the suspensions of lipospheres may prevent crystallization of drugs (Frederiksen et al., 2003). Depending on the type of the

polymer retardation of this process was observed: poloxamer was effective in the systems containing indomethacin but did not show any effect for bupivacaine. For the latter sodium carmellose and hydroxyethylcellulose were more suitable. However, none of the polymers eliminated the expulsion effect as demonstrated by total dose of bupivacaine or indomethacin found in the aqueous phase (Table 1).

The above observations are in agreement with the recently published report on fast crystallization of bupivacaine free base in the dispersions of lipospheres (1–300 μm) composed of triglycerides and phospholipids, with this phenomenon retarded in the presence of sodium carmellose (Toongsuwan et al., 2004).

In comparison to suspensions of lipospheres increased content of lipids in the concentrates resulted in only partial recovery of drugs from the aqueous phase what suggested that incorporation of the drug in lipospheres was efficient (Table 1). It was observed that in the formulation where bupivacaine total content was 10% (w/w) the efficacy of drug incorporation in the lipid matrix was higher than in the concentrate containing 2% (w/w) of the drug and bupivacaine stearate was more suitable than bupivacaine base. During short-term storage (8 days) the amount of bupivacaine found in the aqueous phase of the concentrated lipospheres did not change (Table 3) what means that expulsion of the drug was not progressing even if crystallization of the lipids and polymorphic transition are time dependent (Bunjes et al., 1996, 2003; Freitas and Müller, 1999).

Further observations, however, demonstrated that the surface of the lipospheres, not the core, is the site were the drug is localized in the concentrates. During storage of the concentrates diluted with water precipitation of all drugs was observed within 24 h. However, the most important evidence for unsuccessful incorporation of bupivacaine in lipospheres comes from the fact that more intensive agitation during dilution – with Vortex, high-shear mixer or ultrasounds – resulted in a complete diffusion of bupivacaine to the aqueous phase (Table 4). The same was observed when 60% methanol was used for dilution of the concentrates containing indomethacin. These observations prove that also in the concentrated lipospheres incorporation of the lipophilic drugs was unsuccessful.

If the suspensions of lipospheres are compared with the concentrates it may be concluded that increased content of the lipid material, decreased ratio of surfactant/lipid content as well as the semisolid consistency are factors which enable quite strong adsorption of drugs at the surface of the lipospheres. Such systems, upon dilution with gentle agitation still provide prolonged release of the drug as was demonstrated in our preliminary studies (only half of the total content of bupivacaine was released in vitro during 24 h). However, good reproducibility of the liberation rate will be problematic, what makes these systems unsuitable as prolonged release formulations for clinical practice.

5. Conclusions

None of the studied lipophilic drugs, i.e. bupivacaine base, bupivacaine stearate and indomethacin, was successfully incorporated in the core of the micron-sized lipospheres composed of glyceryl palmitostearate (Precirol) and the strong drug expulsion was not eliminated by introducing liquid oil to the solid matrix. The results may support the theory that solid lipid does not embed the liquid oil which forms a separate compartment. The drug excluded from the core easily distributes to the aqueous phase, unless the system is viscous/semisolid as in the concentrated lipospheres. The increased incorporation of drugs determined in the concentrated lipospheres was only apparent, since in fact all the dose was concentrated on the surface of the lipid particles and was transferred to the aqueous phase in the course of an intensive agitation. The presence of hydrophilic polymers in the aqueous phase does not prevent the expulsion effect although drug precipitation may be retarded. The choice of the lipid forming the core of lipospheres, considered as the most important factor determining drug incorporation efficacy, cannot be based on the solubility of drug determined in bulk lipid films, since such correlation has not been observed.

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