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The choice of lipids and surfactants for injectable extravenous microspheres

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Suspensions of lipid microspheres sizing from 1 to 30 μm, whose fluidity and lipid/surfactant composition is suitable for parenteral administration were developed. None of the formulations prepared with Precirol® (palmitostearate), as the only lipid, was physically stable during storage, because liquid suspensions formed semisolid gels within one week. 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.

Biodegradable materials may be used for injectable suspensions or implants providing controlled drug release profiles. Biodegradable substances, like lipids are promising candidates as components of biodegradable matrices. 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 has been also considered. For this route of drug delivery, larger particles, up to 30–50 μm can be accepted. 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. Prolonged release of drugs from SLN was demonstrated (Müller et al. 2000; Gasco 2001). Further prolongation is possible if the particle diameter is increased and microparticles instead of nanoparticles are used (Masters and Domb 1998).

Since the published work on technology of lipid microspheres is very limited (Masters and Domb 1998), our aim was to produce lipid particles of sizes larger than 1 μm, using surfactants chosen from a very limited group of substances acceptable for parenteral use: phospholipids, poloxamer, polysorbates and sorbitan fatty acid esters. In contrast to SLN (Müller et al. 2000), lipid microparticles are less stable in an aqueous suspension and form easily semisolid gel-like structures. In our studies suspensions of lipid microspheres whose fluidity and lipid/surfactant composition is suitable for parenteral administration were developed.

The aqueous suspensions of lipospheres contained 10% (w/w) lipid and up to 5% (w/w) surfactant agents. Glycerol was present in the aqueous phase as a tonicity adjusting agent. Precirol was used as a matrix forming agent because of its appropriate melting temperature (55 °C) and biocompatibility.

The process developed for SLN involves high pressure homogenization which is a critical step allowing particle sizes below 1 μm (Müller et al. 2000), but this step should be omitted in preparation of the lipid microspheres. In our

Table: Characteristics of the suspensions of lipospheres prepared with different surfactants, with (+) or without (−) homogenization with ultrasounds and stored at 4 °C.

Nr	Surfactants	Ultrasounds	Consistency		Fraction (%) of particles below		
			After preparation	After 7 days	30 μm	10 μm	1 μm
Glyceryl palmitostearate 10% w/w							
1	Lecithin 3.0%	−	semisolid	−			
2	Lecithin 2.4%	−	liquid	semiliquid	87.8	56.2	4.0
	Polysorbate 1.0%	+	semisolid	−			
3	Lecithin 2.4%	−	liquid	semisolid	98.1	87.9	14.7
	Polysorbate 4.0%	+	semisolid/phase separation	−			
4	Lecithin 2.0%	−	liquid	semiliquid	95.0	82.5	13.4
	Polysorbate 1.0%	+	liquid	semisolid	100.0	99.7	32.1
	Poloxamer 1.5%						
5	Polysorbate 3.0%	−	liquid	semisolid	98.9	98.5	30.5
		+	liquid	semiliquid	92.6	92.3	53.5
6	Polysorbate 2.0%	−	semisolid	−			
	Span 1.0%	+	semisolid	−			
Glyceryl palmitostearate and Miglyol (4 : 1) 10% w/w							
7	Polysorbate 2.0%	−	liquid	liquid	98.2	97.1	16.5
		+	liquid	liquid	97.4	96.6	61.1
Glyceryl palmitostearate and Miglyol (4 : 1) 20% w/w							
8	Polysorbate 3.0%	+	semisolid	−			

studies ultrasonic dispersion was employed as an alternative method of homogenization.

Characteristics of the lipid microspheres prepared with different surfactants are presented in the Table. None of the formulations prepared with palmitostearate as the only lipid was physically stable during storage. Liquid suspensions stored either at room temperature or at 4 °C formed semisolid gels within one week and agglomerates of the microspheres were observed under the microscope. The type of the surfactant influenced the rate of this process – the fastest gel formation was observed with lecithin (formulation 1), while suspensions stabilized with polysorbate, alone or in the mixture with lecithin and poloxamer (formulations 4, 5) were more stable.

The gelation mechanism was studied by Westesen and Siekmann (1997) and Freitas and Müller (1999) but it is not completely understood yet. It was observed that in general dispersions with a highly recrystallized lipid phase showed such phenomena. Crystallization of bulk triglycerides from the melt after rapid cooling usually occurs in the metastable form which transforms into the stable form upon heating or storage (Bunjes et al. 1996). Storage temperature, light exposure, type of packing material, type of surfactant, concentration of lipid phase are factors, which influence this process (Freitas and Müller 1998; Bunjes et al. 2003). Gel formation in solid lipid nanoparticles can be circumvented by a proper choice of emulsifier blends, their concentrations and process parameters. Westesen and Siekmann (1997) demonstrated that use of phospholipids leads to the formation of semisolid systems but nonionic surfactants can prevent this phenomenon. However, the dispersions of lipid microspheres investigated in our study formed gel despite of the use of nonionic surfactants.

Different technological approaches were employed to avoid gel formation. It was found that the rate of cooling (at room temperature or in ice) did not significantly influence the process. The use of ultrasound resulted in an increasing fraction of the submicron particles from less than 20% to more than 50%. Surprisingly, the formulations subjected to ultrasound were even less stable than prepared without this homogenization step (formulations 2, 3 and 4). However, no relationship between the size of the particles and gelation rate was observed.

The above observations suggested that the unsatisfying stability may be related to the type of the lipid. Its crystallization, too fast or delayed, may be a reason for changes in the structure of the matrix and further agglomeration during storage.

The matrix of the lipid microspheres was modified by incorporating a lipid with a lower melting point – Miglyol, which is a pharmaceutical oil accepted for parenteral administration. Recently Müller et al. (2002) discovered that liquid lipids are able to change the internal structure of the SLN, eliminating the effect of drug expulsion from the particles. The new generation of the particles was called nanostructured lipid carriers (NLC). In our studies this type of structures was obtained by combining Miglyol with glyceryl palmitostearate in the ratio 1 : 4. The mixture of lipids allowed successful preparation of a liquid suspension of the microspheres (formulation 7). No gel was formed even during a long-term storage for 8 months. Moreover, a satisfying stability of the suspension was achieved using polysorbate in concentrations as low as 2% (w/w). However, it was not possible to produce the suspension containing the same concentration of the surfactant with increased content of the lipids to 20% (w/w) (formulation 8).

The lipid microspheres (formulation 7) were sterilized in an autoclave. After this process coalescence of oily droplets was observed on the surface, but a homogenous emulsion was easily obtained after short shaking of the vial while the product was still warm. Upon cooling a suspension of the lipid microparticles was formed. The analysis of this formulation showed that neither particle size distribution nor stability was influenced by the process of thermal sterilization.

Our study demonstrates that sterile solid lipid microspheres may be produced using saturated triglycerides in combination with medium chain unsaturated triglycerides. The nature of the lipid and lipid/surfactant ratio are the most important factors which determine the stability of the system.

Experimental

Precirol® (glyceryl palmitostearate) was a gift from Gattefossé, Lyon, France. Miglyol® 812 (medium chain triglycerides) was manufactured by Caelo Caesar and Loretz, Hilden, Germany. Polysorbate (Tween® 80) was purchased from Merck (Darmstadt, Germany), Span® 60 – sorbitan stearate from Fluka Chemie (Buchs, Switzerland) and poloxamer 188 – Pluronic® F68 from Boehringer Ingelheim (Heidelberg, Germany). Egg lecithin (Lipoid E-80) was obtained from Lipoid (Ludwigshafen, Germany).

The lipid microspheres were obtained in the following process. Lipids were melted at 80 °C, surfactants were dissolved and the lipid phase was mixed with the aqueous phase containing glycerol (2.3% w/w). The emulsification was performed at the same temperature using a high shear mixer Ultra-Turrax (IKA Labortechnik, Staufen, Germany) at 8000 rpm for 5 min. An ultrasound probe was transferred to the emulsion and the homogenization was performed for 5 min at 22 kHz. Crystallization of lipids was slow (at room temperature) or fast (in ice) with a constant stirring. The suspensions of microparticles were stored either at room temperature or in a refrigerator (4 °C) for at least 7 days. Stable formulations were sterilized in an autoclave (121 °C, 15 min).

Visual and microscopic observations (light microscope, magnification × 150 and × 600) were carried out. Particle size distribution was measured using a laser diffractometer Mastersizer E (Malvern Instr., Malvern, UK).

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