CHAPTER I
INTRODUCTION

In recent years, considerable energy has been devoted to the formulation of colloidal drug delivery system acceptable for general systemic use and capable of carrying a drug to its target at the cellular level (Santhi et al., 1999). They are one approach for the controlled delivery of drugs by the intravenous route. Each of the currently investigated particulate carriers (polymeric nanoparticles, liposomes, fat emulsions) possesses specific advantages and disadvantages. Disadvantages of polymeric nanoparticles are: the relatively slow degradation of up to 4 weeks; cytotoxic effects observed in vitro after phagocytosis; toxic residues from the production; the lack of a method for production on large industrial scale and autoclaving is not possible. The degradation of liposomes is faster but there are still problems with the production (e.g., drug incorporation, physical and chemical stability, partial lack of large scale production methods). For fat emulsions, large scale production methods are available. However, the release of most drugs from fat emulsion is very fast due to the distribution of the drug between oil droplets and the large volume of the blood. The advantages of polymeric nanoparticles and fat emulsions can be combined by producing particles from solid lipids. They possess a solid matrix allowing controlled drug release (Schwarz et al., 1994).

Many researchers have studied the preparation of solid lipid nanoparticles (SLN) in nanometer size range with narrow size distribution. The techniques commonly used to produce SLN are based on high-pressure homogenization, dilution of microemulsions or solvent removal from oil-in-water emulsions. There are two general approaches within the homogenization technique, hot and cold homogenization: in both cases, a preliminary step involves drug incorporation into the lipid melt. Hot homogenization described by Müller and Lucks (1996) while cold homogenization (Jahnke, 1998) is effectively high-pressure milling of a suspension. Gasco and co-workers (Gasco, 1993; Cavalli et al., 1995a; 1995b; 1997; 2000a; 2003) developed an SLN preparation technique based on dilution in cold water of an oil-in-water microemulsion. Subsequent addition of the hot microemulsion to cold water leads to precipitation of the lipid phase forming fine particles. Another proposed

technique to produced SLN is the solvent emulsification-evaporation method (Sjöström and Bergenstahl, 1992; Siekmann and Westesen, 1996). The lipid matrix is dissolved in a water-immiscible organic solvent that is emulsified in an aqueous phase. Upon evaporation of the solvent under reduced pressure, a nanoparticle dispersion is formed by precipitation of the lipid in the aqueous medium. An important advantage of this technique is the avoidance of any heat. On the other hand, it may create toxicological problems arising from solvent residues when compared to the former techniques. In addition, large scale production of SLN by the high pressure homogenization and microemulsion techniques also appear feasible.

The variety of lipid matrices used in the formulation of SLN include fatty acid, partial glycerides, triglycerides, steroids, waxes which are solid at room temperature. Glyceryl palmitostearate and glyceryl behenate are partial glycerides which commonly used in SLN production (Müller et al., 1996; Schwarz and Mehnert 1997; Freitas and Müller, 1998a; 1998b; 1999; Maia et al., 2002; Videira et al., 2005). A clear advantage of SLN is the fact that the lipid matrix is made from physiological lipids which decreases the danger of acute and chronic toxicity. The choice of the emulsifier depends on the administration route and is more limited for parenteral administration. Polyoxyethylene stearates, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters, phospholipids and poloxamers are excipients used in commercially available solubilized injectable formulations (Kibbe, 2000; Strickley, 2004). Particularly in three latter groups, they widely used in SLN for parenteral products (Westesen et al., 1997; Yang et al., 1999; Heydenreich et al; 2003).

The major advantage of SLN is the possibility of production on large industrial scale. However, depending on the drug some potential problems can occur, such as drug leakage during storage and insufficient total drug loaded. To overcome the limitations of SLN, nanostructured lipid carriers (NLC) have been developed (Müller et al., 2000a; Souto et al., 2004a; 2004b). The latter produced by controlled mixing of solid lipids with spatially incompatible liquid lipids leading to special nanostructures with improved drug incorporation and release properties (Müller et al., 2002a; 2002b).

The most effective clinically available treatment for systemic fungal infections is Fungizone®, an intravenously administered colloidal dispersion of amphotericin B (AmB) with sodium deoxycholate. However, the clinical efficacy of Fungizone® is limited both by severe toxic side effects, such as fever, chills, hemolysis and vomiting, and by the symptoms of nephrotoxicity, which develop after several weeks of therapy (Fukui et al., 2003). Thus, several lipid formulations (Ambisome®, Amphocil®, Abelcet®) have been developed and commercialized. Although they have been proven to reduce AmB toxicity, their toxic effects and pharmacokinetic properties all differ and their use has been limited by their expense. Moreover, it has been recognized that the reduction in AmB toxicity was associated with substantial reduction in AmB activity (Espuelas et al., 2003). Their extremely high cost was due to the technology involved on their manufacture and the stability problems associated to the dosage forms, have made them unaffordable to less developed countries (Moreno et al., 2001). Often the patients have to make a choice between the expensive Ambisome® being US$ 1300 per day and the nephrotoxic effects of Fungizone® (being US$ 24 per day). Therefore, a cheap formulation alternative with reduced nephrotoxicity is needed for AmB (Müller et al., 2004b).

It has been firmly established that the toxicity of AmB is related to its aggregate state. Aggregates of AmB may directly cause membrane damage and leakage of both mammalian and fungal cells. On the other hand, monomer or small aggregates of AmB selectivity bind ergosterol, causing leakage of fungal cells (Caillet et al., 1995; Aramwit et al., 2000; Larabi et al., 2004). The equilibrium between monomers and aggregates appears to play a key role in drug activity (Gaborian et al., 1997). The slow release of the drug from lipid formulations ensures that the drug remains as a monomer and is unable to form aggregates, responsible for drug toxicity and increased activity. In the last decades, many studies have addressed the interactions between AmB and lipids. Different techniques have been employed, the most frequently used being UV-visible absorbance, NMR, X-ray diffraction and differential scanning calorimetry (DSC).

In this study, the use of solid lipid nanoparticles (SLN) and nanostructured lipid carrier (NLC) as new and less toxicity delivery systems for AmB was developed.

Microemulsion and high pressure homogenization techniques were investigated. Also, physicochemical characterization, toxicity, in vitro release, physical and chemical stability, and antifungal activity were evaluated.

Objectives of the study

The aims of this study were as the following:

1. To study the effect of process and composition of SLN and NLC preparations containing AmB via microemulsion and high pressure homogenization techniques on their physicochemical characteristics and release profiles.

2. To study the toxicity of AmB loaded SLN and NLC formulation compared with Fungizone®, commercial product.

3. To study the stability of AmB in colloidal dispersion and lyophilized preparations.

4. To examine and compare the in vitro antifungal activity of its AmB, Fungizone®, and AmB formulations.