CHARPTER I

INTRODUCTION

Since prehistoric times, *Centella asiatica* has been used as a medical plant. The therapeutic use of this herbal remedy with its wide range of applications has been known in South East Asia and India for centuries. This plant continues to be used within the framework of folk medicines as an effective remedy. *Centella asiatica* is now located at the interface between traditional and modern, scientifically oriented medicine (Brinkhaus et al., 2000). Different uses are claimed for the plant, the more commons being are its use as a wound healing agent, and constitutes of brain tonics for the mentally retarded.

The active ingredients of *Centella asiatica* have been determined to be triterpenoids, the constitutes of which include: asiaticoside, madecassic àcid and asistic acid. The chemical structure, the functional group of ester and alcohol, of these constitutes could be degraded by hydrolysis and oxidation, particularly. Moreover, *Centella asiatica* is a poorly water soluble, a bitter taste compound. Centella preparations used in conventional medicine are prepared in oral form (tablets and drops), topical form (ointment and powder) and in the form of injections (s.c., i.m., and i.v.) (Brinkhaus et al., 2000).

Solubility is an essential factor for drugs. It also poses a major challenge for pharmaceutical companies developing new pharmaceutical product, since nearly half of the active substances being identified through the new paradigm in high-through put screening are either insoluble or poorly soluble in water. A limiting factor for in vivo performance of poorly water soluble drugs, increasing the dissolution rate of poorly water soluble substance is thus important for optimizing bioavaibility.

Solid lipid nanoparticle (SLN) represents an alternative colloidal drug delivery system. The use of solid lipids as matrix materials for drug delivery is

well-known from lipid pellets for oral drug delivery. Nanoparticles made form solid lipids are attractively increasing attention during recent years. The idea to use solid lipids instead of liquid oils is a very attractive idea to achieve controlled drug release because drug mobility in a solid lipid is considerably low compared with in liquid oil (Mehnert and Mader, 2001).

The SLN can be employed for any purpose to incorporate drugs for control drug release. The system shows low toxicity due to its composition of physiological compound. Moreover, loading both lipophilic and hydrophilic drugs into solid matrix is possible. The solid matrix can also protect incorporated active ingredients against chemical degradation (Muller, Mehnert ey al ., 1995).

The preparation of SLN has been studied. Several techniques have been developed to obtain nanometer size range with narrow size distribution. Generally, the lipid nanopellets were prepared by dispersing a melted lipid in a surfactant solution by stirring or sonication. However, dispersion quality is often compromised by the present of microparticles (Mehnert and Mader 2001). Sjostrom and Bergenstahl (1992) described a production method to prepare nanoparticle dispersion by solvent diffusion method. The narrow size distribution in nanometer could be achieved by this technique. The lipid matrix is dissolved in a water-miscible-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. Depending on the fat load and emulsifier used, particles with average diameters of 30-100 nm can be obtained. An important advantage of this technique is the avoidance of any heat. On the other hand, solvent emulsification-evaporation suspension are fairly dilute, due to the limited solubility of the lipid in the organic solvents used. However, disadvantage is the need to use organic solvent (Trotta et al., 2003).

To overcome these problems, high pressure homogenizer (HPH) was used to prepare SLN. Under optimized production conditions, SLN can be produced with quality acceptable for parenteral administration. However, high temperature

may also increase the degradation rate of drug and the carrier.The homogenization step can be repeated several times. It should always be kept in mind of the high temperature of the sample (approximately 10 °C for 500 bar) (Jahnke 1998). In most cases, 3-5 homogenization cycles at 500-1500 bars are sufficient. Increasing the homogenization pressure or the number of cycles often results in an increase of the particle size due to particle coalescence which occurs as a result of the high kinetic energy of the particles.

An innovative and successful carrier system should allow a high loading capacity for incorporated drugs as well as long term incorporation. Many different drug have been incorporated in SLN for the parenteral application including the corresponding references. Yang et al (1999) determined the in vitro release of campotheccin from stearic acid SLN in conjunction with potential targeting to the brain using dialysis bag technique at 37 °C. The data revealed a sustained release and could be fitted to a Weibull distribution. Heiati et al (1997) have studied the in vitro release of AZT-P from triluarin SLN using bulk equilibrium reverse dialysis sac at 37 °C. The observed initial burst is attributed to partial AZT-P localization in phospholipids micells. Further, dependence of release profile on the type of phospholipids could be shown, i.e. phospholipids with phase transition temperatures (PTT) below 37 °C to fast release, PTT > 37 °C represented a stronger diffusional barrier causing slower release. Hu et al (2002) have studied in vitro release kinetics of clobetasol propionate from SLN prepared by solvent emulsification diffusion. The lyophilized product was dispersed in aqueous dissolution medium without dividing membrane. The authers observed a biphasic release profile following Higushi (45 % release after 3 h, followed by 5.9 % per day for 4 days).

The advantage of using nanoparticles for drug delivery results from their two basic properties. Firstly, due to their small size, nanoparticles penetrate into even small capillaries and are taken up within cell, allowing an efficient drug accumulation at the target sites in the body. Secondly, the use of biodegradable materials for nanoparticles preparation, allows sustained drug release at the

targeted over a period of day or even weeks after injection (Vinogradov et al., 2002).

The aims of this investigation were to develop new technological procedure for nanosuspension production and to evaluate the method in comparison solvent diffusion method with HPH method. Glycerol behenate and Gelucire® 44/14 were chosen to be lipid carriers due to its physiological nature. Poloxamer 188, Phospholipon 40, Phospholipon 90 and Tween 80 were used as single stabilizer and combined stabilizers which can be used in parenteral products (Nema, and Brendel., 1997).

Objectives

The aims of this study were as following:

1. To study the effect of preparation method, emulsion diffusion and high pressure homogenizer on the physicochemical characteristics of SLN using glycerol behenate and Gelucire® 44/14 as lipid matrix.

2. To study the effects of type and amount of single stabilizer, Poloxamer 188, Phospholipon 40, Phospholipon 90 and Tween 80 and combined stabilizers on the stability of blank SLN and asictic acid, asiaticoside loaded SLN.

3. To study the cellular viability and cellular uptake of asictic acid, asiaticoside loaded SLN by using ECV-304 cells.

4.To study the release of asictic acid, asiaticoside from drug loaded SLN.