CHAPTER I

INTRODUCTION

Intravenous lipid emulsions are used to feed patients whose medical condition makes necessary to provide parenteral nutrition. Intravenous emulsions are also used as potential carriers or controlled delivery systems for poorly soluble drugs. They can enhance solubilization or stabilization of the entrapped drug to sustained release and site-specific delivery. Nowadays, many researchers have tried to develop the gene therapy by using lipid emulsion (Tamilvanan, 2004).

Emulsion can be defined as a mixture of two immiscible phases, water and oil, with an emulsifier added to stabilize the dispersed droplets by forming a mono- or multi-layered coating film around the dispersed droplets in such a way to reduce interfacial tension or to increase droplet-droplet repulsion. In addition, they are thermodynamically unstable and subjected to various instability process like aggregation, flocculation, coalescence, creaming and hence eventually phase separation (Liu, 2000; Tamilvanan, 2004). However, the proper choice of emulsifier and preparation conditions such as input of large amount of energy e.g. homogenization, sonication, etc. can delay the instability and thus extend the shelf-life of the products. Emulsions can be characterized as oil-in-water (o/w) or water-in-oil (w/o) emulsions depending on the identity of the dispersed and continuous phases. Moreover, another type of emulsion is multiple emulsions which are water-in-oil-in-water (w/o/w) and oil-in-water-in-oil (o/w/o) (Nieulloud and Marti-Mestres, 2000).

Preparation of an emulsion requires mixing the two immiscible phases with the surfactant and applying mechanical energy in order to create shear forces to deform the interface and form droplets. There are a variety of devices that will accomplish this in either batch or continuous modes of operation. Lipid emulsion can be prepared by adding oil phase into the water phase at the same temperature. The mixture is pre-emulsified using a high speed homogenizer. Final emulsification is carried out by passing the coarse emulsion through a high pressure homogenizer (Liu, 2000). Cuéllar *et al.* (2005) and Liedtke *et al.* (2000) have studied the influence of high pressure homogenization on the emulsion characteristics. The homogenizing equipment supplied by different manufacturers has an influence on the quality of the lipid emulsions. The number of homogenization cycles necessary to decrease the polydispersity and to get a small particle population are also important parameters. Unfortunately, high oil concentrations often lead to an increase in particle size and viscosity of the system.

Intravenous lipid emulsions are required to meet pharmacopoeial requirements. There are a number of physicochemical properties of emulsions that are important to consider when developing an emulsion formulation for parenteral use. These include particle (droplet) size, osmolality, zeta potential and pH which are used to monitor the physical stability of emulsions (Liu, 2000). It is well established that the particle size distribution of intravenous emulsion is a critical factor for patient safety because larger particles (> 5 μm) may cause pulmonary embolism (Cuéllar *et al.*, 2005; Jumaa and Müller, 1999). As intravenous emulsion should be sterile, sterility is usually achieved by heat sterilization or filtration. Heat sterilization is preferred because of its advantages with respect to the ease of manufacturing and

safety (Collins-Gold, Feichtinger and Wärnheim, 2000). However, autoclaving may affect the stability of emulsions. Buszello *et al.* (2000) and Jumma and Müller (1998b) show an increase in particle size of emulsion after autoclaving.

Osmolarity is also important in parenteral formulation and should be similar to the osmolarity of serum, 275-300 mOsmol/L (Reich, Poon and Sugita, 2000). The high tonicity can cause tissue irritation, pain on injection, phlebitis and electrolyte shifts (Timmer and Schipper, 1991). The osmolarity of less than 900 mOsmol/L has been considered as safe for peripheral administration of parenteral nutrition solutions. In addition, the pH of emulsion should be monitored continuously over the period of time to detect free fatty acid formation. The initial pH of the emulsion may decrease progressively with time according to the degradation of phospholipids resulting in the formation of fatty acids, which gradually reduce the pH of the emulsion. Therefore adjusting the initial pH of the emulsion can be minimized the rate of hydrolysis of phospholipids and triglycerides (Rabinovich-Guilatt *et al.*, 2005).

The zeta potential or electrokinetic potential is related to the surface charge of the emulsion droplets and it is generally measured by electrophoretic techniques. The zeta potential is highly dependent on the surfactants used. Emulsion can be stabilized by lowering the interfacial tension at the oil/water interface or by increasing the interfacial charge of surfactant films surrounded droplets. Emulsion stability has usually been evaluated by flocculation rate or separation using interfacial tension and/or by zeta potential. An increase in the absolute value of zeta potential is correlated with a lesser tendency to flocculate. Generally, high negative or positive zeta potential values may stabilize the emulsion by preventing droplet coalescence and increasing electrostatic repulsion between the emulsion droplet surfaces. The

stabilization of intravenous emulsions can be achieved by the addition of charge surfactant to the phospholipid (emulsifier) which are capable of lowering the interfacial tension of the system and of forming an electric interfacial film around the oil globules (Jeong, Oh and Kim, 2001). It is currently admitted that the zeta potential of at least -30 mV is required for full electrostatic stabilization (Heurtault *et al.*, 2003).

Jumaa and Müller (1998b) have found the effect of components used in the formulation on the physicochemical properties and stability of intravenous lipid emulsions. The components of emulsions mainly are oil, emulsifier and other excipients. The oil phase of the emulsion is normally based on long-chain triglycerides (LCT) such as soybean and safflower oil. Apart from LCT, the medium-chain triglycerides (MCT) which are derived from coconut oil. MCT has been reported to differ from LCT as they are metabolized faster and undergo limited storage in tissues (Lai *et al.*, 2005). LCT and MCT or the combination of each have been approved by FDA for pharmaceutical products for intravenous route (Driscoll *et al.*, 2002; Jumaa and Müller, 1998a).

The most frequently used emulsifier in commercial intravenous emulsion is egg phospholipids as it is less toxic upon administration compared with the synthetic emulsifier (Hansrani, Davis and Groves, 1983; Nagasaka and Ishii, 2001; Nielloud and Marti-Mestres, 2000). Phospholipids can be hydrolyzed to lysophospholipids and free fatty acids during the autoclaving process and subsequent storing at 50° C. Although, there have been a few definite reports on emulsion toxicity, it is believed that lysophospholipids formed are potentially toxic and capable of inducing lysis of blood cells. In addition to these biological effects, the release of lysophospholipids

and free fatty acids may affect the physical stability of the emulsion (Rabinovich-Guilatt *et al.*, 2005). Nii and Ishii (2005) showed that the combination of primary emulsifier, phospholipids, and cosurfactant can produce more interfacial film necessary to stabilize emulsion upon autoclaving (Trotta, Pattarino and Ignoni, 2002).

Several types of cosurfactant which are pharmaceutical acceptable for intravenous formulation have been previously reported. The anionic surfactant, sodium oleate, at a concentration of 0.05% w/w has been used in intravenous emulsion with no effect on haemolysis (Driscoll *et al.*, 2001; Jumaa and Müller, 2000). The cationic surfactant e.g. oleylamine is used to increase the electrostatic properties of the emulsion (Rabinovich-Guilatt *et al.*, 2004). The nonionic surfactants which are widely used in parenteral formulation are polyoxyethylene-20-sorbitan monooleate(Tween®80), Polyethyleneglycol-660-12-hydroxy-stearate (Solutol®HS15) and Polyethyleneglycol-35-ricinoleate (Cremophore®EL) (Jumaa and Müller, 1998b; Jumaa and Müller, 2002). Tween®80 can be used in parenteral preparation in the range of 0.01 to 12% w/w (Nema, Washkuhn and Brendel, 1997). Buszello *et al.* (2000) revealed that the addition of Solutol®HS15 to the soy phosphatidylcholine leading to a significant decrease of oil droplet size. Cremophore®EL shows no teratogenic or embryotoxic effects after administration to pregnant rats up to 10% solution (Wade and Weller, 1994). The use of nonionic surfactant in combination with lecithin in promoting stability of emulsion has been studied by other workers. One of those was studied by Kan *et al.* (1999) who investigated the synergistic effect of egg phospholipids and Tween®80 on emulsion stability.

Other excipients used in intraveonus emulsion formulations are tonicity agents and antioxidants. Glyerol is usually recommended as an isotonic agent and can be

found in most parenteral emulsions. The addition of antioxidants such as tocopherols, butylated hydroxytoluene or ascorbic acid is necessary to protect the oils from oxidation. Moreover, a small amount of sodium hydroxide solution should also be added to adjust the emulsion pH to approximately 8 in order to prevent a dramatic drop in pH during storage.

The aim of this study is to find out the suitable formulation of intravenous emulsion stabilized by a combination of phospholipid and cosurfactant. Three types of cosurfactant are studied, sodium oleate, Tween®80 and *d*-α-tocopheryl polyethyleneglycol-1000-succinate (Vitamin E-TPGS). Sodium oleate and Tween®80 are used in pharmaceutical preparations including in parenteral applications (Jumaa, Kleinebudde and Müller, 1999). In addition, a derivative of natural-source vitamin E, Vitamin E-TPGS, is suggested to provide better stability of emulsion. Vitamin E-TPGS is a water-soluble derivative of natural vitamin which is formed by esterification of vitamin E succinate with polyethylene glycol 1000 (Eastman Chemical Company, USA). The advantages of Vitamin E-TPGS include a prevention of vitamin E deficiency and improvement of the oral bioavailability of vitamin E. Vitamin E-TPGS could be absorbed intact readily in the gastrointestinal tracts. The effect of preparation process, homogenization and autoclaving, on the physical stability and physicochemical properties are also investigated.

The objectives of the study

The purposes of this study were as follows:

1. To prepare intravenous lipid emulsions containing cosurfactants.
2. To investigate the effect of composition and preparation process on the physical stability and physicochemical properties of lipid emulsions.