CHAPTER II

LITERATURE REVIEW

Submicron emulsions for intravenous administration are heterogeneous system in which oil is dispersed as droplets in aqueous phase and stabilized by phospholipids. Emulsion droplets have a size and lipid compositions similar to chylomicron which is natural fat globules circulating in the blood stream after oral intake of dietary fats. Moreover, submicron emulsion would be expected to be metabolized via the same pathway as natural chylomicron. The advantage of submicron lipid emulsion is that a source of essential fatty acids and calories for patients unable to ingest food, they therefore have been widely used in clinical medicine for parenteral nutrition. Intralipid®, the first approved intravenous emulsion was developed for parenteral nutrition. It is oil in water emulsion and consists of 10 or 20% soybean oil droplets (70-400 nm in size) stabilized by monolayer of egg yolk phospholipids (1.2%) and glycerol (2.25%) as an osmotic agent. For the past decade and based on the basis knowledge including formula of Intralipid®, the intensive researches of drug delivery system for poorly water soluble drugs have been concentrated on the design of intravenous submicron emulsion formulations that led to successful marketed products such as the emulsion of 1% Propofol (Diprivan®, Zeneca, UK) (Prankerd and Stella, 1990; Washington, 1996). A recent study, the well-tolerate application of Amphotericin B, an anti-fungal drug, using a liposome formulation, which is commercially available as Ambisome® has invented. Several attempts undertook to incorporate this drug into commercial parenteral fat emulsions and lower nephro-toxicity compared with Fungisone® was reported (Egito et al., 1996; Washington et al., 1988). In addition, their biodegradable including biocompatible, physical stable and relatively easy to produce on large scale, have led to used of submicron lipid emulsions as drug carriers for lipophilic drugs. There are several products currently available on the market using an oil in water submicron emulsion for drug delivery e.g. Diazepam (Diazemuls®, Kabi-Pharmacia, Scandinavia and Diazepam-Lipuro®, Braun, Germany), Alprostadil (PEG_1) (Liple®, Green Cross, Japan), Perfluorodecalin and Perfluorotripropylamine (Fluosol-DA®, Green Cross and Alpha Therapeutics, Japan), Vitamin A, D_2, E and K_1 (Vitalipid®, Kabi-Pharmacia, Sweden), Propofol

(Diprivan®, Zeneca Pharmaceuticals, UK), Dexamethasone palmitate (Limethason®, Green Cross, Japan), Flurbiprofen axetil (Lipo-NSAID® and Ropion®, Kaken Pharmaceuticals, Japan), Etomidate (Etomidat Lipuro®, Braun, Germany). However, there are many drugs containing submicron emulsion under clinical and preclinical evaluation e.g. antifungal agents (amphotericin B, miconazole), anaesthetic agents (pregnanolone, halothane, isoflurane), cytotoxic agents (rhizoxin, taxol, penclomedine, nitrosourea). The potential pharmaceutical applications were described as following:

1. Submicron emulsions for parenteral nutrition

Submicron sized emulsions in which used for parenteral administration, usually named as fat emulsion or lipid emulsion. They are oil in water emulsions and used for nutrient therapy when patients are unable intake food or have undergone major surgery. These emulsions based on vegetable oils and stabilized by lecithin provide an adequate source of calories and essential fatty acids. Lipid is the most caloric substrate having the density of calorie more than twice of carbohydrate and protein. Lipid emulsions, therefore, have a practical advantage of providing more calories per volume in a small volume of isotonic fluid via a peripheral vein. As a source of essential fatty acids, lipid emulsions provide vary amount of linoleic and linolenic acid sufficient to prevent or treat essential fatty acid deficiency. Arachidonic acid which is also essential in humans can be synthesized from linoleic acid. Moreover, fatty acids cooperate in numerous metabolic processes besides energy production. They act as precursors for many important biologically active compounds such as prostaglandins and corticosteroids and are the structural integrity of cell membranes and lipoproteins. Lipid emulsions can treat in essential fatty acid deficiency, especially critical for the pediatric patient in whom essential fatty acids are needed during growth and development. Lipid emulsions also are indicated as a source of fat calories for achieving energy requirements. Lipid has a particular metabolic advantage over carbohydrate in patients with glucose intolerance such as the patient with diabetes mellitus or stress-induced glucose intolerance. In addition, for the ventilator-dependent patient in whom carbon dioxide retention is a problem, lipid emulsion in which fat contributes to the caloric intake will be potential benefit because carbon dioxide produced upon oxidation of fat is less than upon oxidation of

glucose. However, there are exceptions to the dairy use of lipid as a caloric source, in the patients with altered lipid metabolism or in whom an adverse reaction to lipid emulsions has occurred. The alteration of lipid metabolism has been found in patients with severe hepatic failure, severe renal failure and sepsis. In general, most of fat adsorbed from the gastrointestinal tract is incorporated in chylomicron and transported in this forms into the lymph and blood circulation. The chylomicron are stable emulsion (diameter about 0.5-1 μm) consisting of triglyceride (96%), phospholipids (0.8%) and small amounts of cholesterol (1.7%) and proteins (1.7%). The triglycerides are located in the centre, the phospholipids are covered on the surface acting as an emulsifier. Lipid emulsions for parenteral nutrition mimic the natural chylomicron, by mean of the emulsion droplets possess a core of triglyceride (soybean oil) which is stabilized by phospholipids layer (egg or soybean lecithin). Metabolism of lipid emulsion is similar to that of chylomicron.

2. Enhance solubility of poorly aqueous soluble drugs

Solubilization of poorly aqueous soluble drugs for intravenous administration is frequently performed by pH control (in the case of ionizable drugs), by the utilization of co-solvents such as ethanol, glycerol, dimethylacetamide (DMA), dimethylsulfoxide (DMSO) or by the complexation with β-cyclodextrins or cyclodextrin derivatives such as hydroxypropyl-β-cyclodextrin. However, the feasible limitation of these approaches include the precipitation of drug during storage period, pain on injection site and thrombophlebitis due to the presence of co-solvent. Moreover, drug precipitation at the injection site caused bioavailability reduction. The usefulness of oil in water submicron sized emulsions as carriers system was widely investigates due to their ability to incorporate hydrophobic drugs within their inner oil phase. The examples of drug containing submicron emulsion preparation for improving their solubility are following described.

Penclomedine (Prankerd et al., 1988), a practically insoluble (approximately 1 μg/ml) anti-tumor agent which has shown activity in several tumor model systems when screened as an aqueous suspension by the National Cancer Instituted. An intravenous formulation of this compound with the concentration of 1-5 mg/ml was

required for further biological testing. Several attempts to incorporate penclomedine into Intralipid® using extemporaneous addition approach failed. Precipitation of this drug was frequently occurred after dilution in both 10% and 20% Intralipid®. So that, Prankerd and co-worker (Prankerd et al., 1988) performed de novo emulsification technique in which penclomedine was dissolved in soybean oil and then emulsified with water using various lecithins as an emulsifier. The concentration of penclomedine in soybean oil was 183 mg/ml at room temperature, thus emulsions containing 1 or 5 mg/ml were easily obtained. All the emulsions had hydrodynamic particle sizes less than 500 nm. The emulsion prepared with the 99% α-phosphatidylcholine had initially the smallest particles, with an average of approximately 250 nm. The physical or chemical stability after 12 months was no significant change. Preliminary cytotoxicity results indicated that penclomedine emulsions showed better cytotoxic activity after both intraperitoneal and intravenous administration than a comparable dose from a suspension dosage form.

Taxol is an example of an anticancer agent which is a poorly water soluble and is formulated in a 1:1 mixture of dehydrated alcohol: cremophore® EL (Paclitaxel®, 6 mg/ml Taxol) (Tarr et al., 1987). This co-solvent mixture is diluted before administration in isotonic saline and remains stable for only 3 hours. The solubility of Taxol in soybean oil was only 0.3 mg/ml, therefore, extemporaneous preparations using commercial lipid emulsions were not attainable. Subsequently, they prepared an emulsion consisting of triacetin, soybean lecithin, pluronic F68, polysorbate 80 and ethyl oleate. Glycerol can be added to the emulsion at a concentration of 10% to prevent creaming. A stable emulsion was found at Taxol concentration of 10 and 15 mg/ml of emulsion. The average particle size of this emulsion was ranging from 0.5 to 5 μm. The average particle size grew to 2 μm and 4 μm after keeping for 1 week and 2 months, respectively. Although chemical stability remained more than 96% after 6 months at 22 °C, physical instability such as phase separation was observed. Comparative hemolysis testing of 10 mg/ml Taxol emulsion proved that it is less hemolytic than 20% Intralipid® emulsion. The acute toxicity of the emulsion excipients alone showed no toxicity when administered at a dose five times greater than that of the concentration in the Taxol emulsion. The acute toxicity of the Taxol emulsion included lethargy, ataxia and respiratory depression.

Rhizoxin is a cytotoxic and anti-fungal drug that inhibits tubulin polymerization (Takahashi et al., 1987). The aqueous solubility of rhizoxin is 12 μg/ml while the solubility in soybean oil was 2 mg/ml. Dilution of the reconstituted powder with 10% Intralipid® did not result in precipitation. Therefore, this approach could be used for slow intravenous infusion of this drug. In 1996, Kurihara and co-worker (Kurihara et al., 1996a) prepared emulsion containing 13-O-palmitoyl-rhizoxin by de novo emulsification technique. The formulation consisted of soybean oil or dioctanoyldecanoylglycerol as the oil phase, polyoxyethylene-(60)-hydrogenated castor oil as nonionic surfactant, egg yolk lecithin and water. This emulsions showed high concentration of palmitoyl rhizoxin in tumors of mice bearing the solid tumor M 5076 sarcoma (Kurihara et al., 1996b). Their result indicated that this emulsion effectively retarded the tumor growth and increased survival time.

Amphotericin B, is a polyene macrocyclic antibiotic derived from *Sreptomyces nodosus* which is used for the treatment of fungal infections. Renal disfunction is the adverse effect of this drug. Most patients receiving this drug at daily dose in excess of 0.5 mg/kg/day, suffer some degree of kidney damage. This drug is highly lipophilic and poorly absorbed orally, thus parenteral administration as a mixed micellar dispersion with the surfactant deoxycholate was investigated. However, the relatively high rate of clinical failures of amphotericin B with the commercialmicellar product (Fungizone®) in granulo-cytopenic and other immunocompromised patients could be the result of inadequate local concentrations of the active drug in tissue (Janknegt et al., 1992). Consequently, liposome encapsulation and other colloidal lipid vesicles have been developed to improve the therapeutic index of Amphotercin B and reduce its adverse effects (Gates and Pinney, 1993). These colloidal lipid based delivery systems are reported to reduced the systemic toxicity of Amphotericin B and show increased clinical efficacy as compared to the marketed micellar preparation (Cleary, 1996; Janknegt et al., 1992). Nevertheless, there are many different colloidal lipid preparations are currently marketed and available in clinical practice, a major drawback is that these new formulations are very expensive. Thus, several attempts have been made to develop alternative and less expensive lipid formulations based either on the extemporaneous addition of this drug to the commercial available lipid emulsions or de novo emulsification. In 1988, Kirsh and co-worker incorporated

Amphotericin B to Intralipid® by extemporaneous addition. At first, Amphotericin B was dissolved in sodium deoxycholate/dimethylacetamide solution and filtered to remove undissolved drug. Then, added to a vial containing 20% Intralipid® under aseptic conditions to a final concentration of 1 mg/ml of amphotericin B. The extemporaneous drug emulsion remained stable for 1 year at 4 °C when protected from light. This formulation exhibited the same MIC for *Candida albicans* as Fungizone® formulation. Numerous studies clearly showed that Amphotericin B containing lipid emulsion reduced nephrotoxicity while maintaining its antifungal activity. An alterative emulsion-based delivery system was developed using De novo emulsification. Davis and his colleagues (Davis et al., 1985) described De novo emulsification in which amphotericin B (final concentration 1 or 2 mg/ml) is dissolved in methanol and then mixed with water containing egg lecithin (emulsifier). After evaporation of the methanol, the oily phase (20% w/w) was added and the mixture was emulsified using sonic probe. This emulsion was reported to be stable for 7 months regarding to drug content and droplet size distribution. In vivo experiment in murine candidiasis indicated ED_{50} of 0.78 mg/kg for Fungizone® and less than 0.5 mg/kg for this emulsion preparation. According to the reduced nephrotoxicity of the emulsion formulation, it allowed the use of higher doses of Amphotericin B with pronounced therapeutic benefit. The comparison between the pharmacokinetics of conventional formulations and lipid emulsion formulations of Amphotericin B in a group of patients with neutropenia were studied (Ayestaran et al., 1996). It found that the pharmacokinetic behaviour was different. Amphotericin B containing lipid emulsion formulation showed the reduced toxicity and concentration in serum. It is possible that the higher doses of Amphotericin B could be used in certain clinical situations. Furthermore, the comparison of the efficacy and tolerance of Amphotericin B in 5% dextrose or administered in 20% Intralipid® was studied (Sorkine et al., 1996). The results of this study indicated that Amphotericin B in lipid emulsion compared with regular Amphotericin B was as effective in eradicating Candida infections due to the diminished frequency and severity of side effects causing significantly less renal impairment.

Propofol, a potent CNS depressant, is used intravenously for the induction and maintenance of anesthesia or sedation. Propofol exists as an oil of low water solubility

and therefore must be formulated as an emulsion for parenteral administration. The marketed emulsion product is isotonic and has a pH of 7.0 to 8.5 (adjusted with sodium hydroxide), and contains propofol (10 mg/ml), soybean oil (100 mg/ml), glycerol (22.5 mg/ml) and egg lecithin (12 mg/ml). Propofol emulsion can be diluted in dextrose solution before administration. Propofol readily crosses the blood brain barrier with a mean equilibrium half-life of 2.9 minutes because of its lipophilicity (octanol/water partition coefficient is 5012:1). The potential advantage of propofol is rapid recovery and low incidence of postoperative nausea and vomiting.

3. Stabilization of hydrolytically susceptible compounds

Drugs which are utilized for intravenous administration are commonly unstable in aqueous or partially aqueous solution. The method often used for stabilization of such a drug is by lyophilization technique. Reconstitution of the freeze-dried powder with an appropriate sterile solvent (immediately before administration) results in a product which is chemically stable for the period of administration requirement. However, lyophilization technique provides a greater cost and complexity than other methods for the preparation of parenteral products. The previous study has been shown that the rate of drug hydrolysis can be remarkably reduced by formulation in o/w emulsion.

Carbamic acid (1-methylethyl)-, [5-(3, 4-dichlorophenyl)-2, 3-dihydro-1H-pyrrolizine-6, 7-diyl] bis (methylene) ester (NSC no.278214) is an unstable cytotoxic agent. El-Sayed and Repta (A.El-Sayed and Repta, 1983) attempted to improve its stability by incorporating in Intralipid®. It was found that the dissolving of drug in DMA-Cremophore solution prior to dilution with commercial fat emulsion results in a suitable parenteral formulation in which the drug is approximately 100 fold more stable than in simple aqueous solution. Semustine [1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea methyl-CCNU] is unstable cytotoxic nitrosourea which has been formulated in parenteral emulsion (Fortner et al., 1975). The results of this study found that this drug was stable for 8 hours at room temperature. In 1986, Benita and co-worker investigated the incorporation of physostigmine salicylate in a soybean oil emulsion (Benita et al., 1986) resulting in a physically stable emulsion.

Rhizoxin (Takahashi et al., 1987) is a subject to degradation in aqueous solution, giving a narrow V-shaped pH rate profile with maximum stability at pH about 5-6. Based on this basis, the incorporation of hydrolytically susceptible drug into an emulsion could reduce hydrolysis. Despite the oil solubility of rhizoxin over the aqueous solubility, part of rhizoxin still remained localized in the aqueous phase where it could be easily degraded. Thus, to favor the localization of this cytotoxic drug in the inner oil phase of an emulsion, a palmitoyl derivative, 3-O-palmitoyl-rhizoxin, which is much more lipophilic than rhizoxin was synthesized (log P=14 instead of log P=2 for the free drug). This form was incorporated in lipid emulsion and more localized in the inner oil phase which demonstrated improving drug stability.

4. Reduction of irritation or toxicity

Conventional preparations of diazepam for intravenous use contain solvents which cause pain on injection site and thrombophelebitis in a high percentage of cases (Dardel et al., 1981). However, diazepam can be dissolved with advantage in o/w emulsion. Diazepam containing lipid emulsion has been injected to 9492 patients without serious side effects. Intramuscular injection of diazepam containing lipid emulsion resulted in a significantly smaller frequency of pain than the injection of Valium (7% and 43%, respectively). The pharmacokinetic behaviour of diazepam containing emulsion is similar to that of diazepam solution after i.v and i.m injection. Therefore, diazepam probably quickly separated from the oil droplets of emulsion after injection.

Limethason® is an intravenous injectable lipid emulsion in which dexamethasone-21-palmitate is dissolved as an active ingredient. Limethason® showed 2 to 5 times as potent anti-inflammatory activity as the water-soluble dexamethasone phosphate on chronic inflammatory disease (Yokoyama and Watanabe, 1996). The strong anti-inflammatory activity of this drug was based on a high distribution in the inflammatory lesion, a high uptake by macrophage and a suppressive effect on the macrophage function. These results indicated that Limethason® is more useful for chronic rheumatoid arthritis. Limethason® is a

prodrug and slowly hydrolyzed by esterases into its bioactive metabolite, dexamethasone, which demonstrated sustain anti-inflammatory effect. Therefore, Limethason® may be used at lower doses than conventional water-soluble dexamethasone drugs and hence this drug is expected to have reduced risk of steroidal inherent adverse effects.

5. Submicron emulsion as drug carriers

In the recent year, colloidal particles such as microparticles, nanoparticles, nanospheres, emulsions, liposomes and mixed micelles, have been investigated as potential carrier systems for the drug delivery or the targeting of drugs to specific sites in the body. Among them emulsion formulations have profited particular interest as a carrier of lipophilic drugs due to their biocompatibility and long term stability and they can be easily manufactured on an industrial scale (Takino et al., 1994). One approach to increasing the efficacy of drug and decreasing systemic adverse effects is to deliver the necessary amount of drug to the target diseased site. Many potent biologically active substances, with advance in medicine and pharmaceutical sciences, have been introduced into clinical practice. These substances show strong physiological activities, cause a local effect in the body and are rapidly metabolized. Therefore, a large dose of drug must be administered, resulting in their pharmacological actions effect throughout the entire body as well as various adverse reaction occasions. Moreover, the local adverse reactions such as pain and inflammation at the administration site are also observed. In order to solve these problems, pharmaceutical strategy such as drug delivery system (DDS), particularly targeted delivery, need to be developed. These pharmaceutical strategies have been studies on delivery including liposomes (Sharma and Sharma, 1997), micro- or nanoparticles, solid lipid nanoparticles (SLN) (Muller et al., 2000) and self-assembly surface active agent (Drummond and Fong, 2000). Although, liposomes are excellent drug carrier for drug delivery systems, they are relatively unstable and are not easy for large scale production. Furthermore, the safety of the raw materials used for drug carrier has to be considered, especially for toxicity problems such as the hemolytic property. According to these problems, lipid emulsions were invented to be a safe and excellent drug carrier. They are very stable for more than two years at room temperature and also easily mass-produced. Prostaglandin E_1 (PGE_1), dexamethasone

palmitate (corticosteroid) and flurbiprofen axetil (non-steroidal sedative) (Mizushima et al., 1983a; Mizushima et al., 1983b; Shoji et al., 1986) are presently available as drugs containing lipid emulsions on the market.

In general, the most common ophthalmic dosage forms are solutions, ointments and suspensions. The limitation of ophthalmic solutions is rapid elimination from the precorneal area resulting in poor bioavailability. However, they are still well known for manufacturers because of their simple preparing, filtration and sterilization. Most of drugs used in ophthalmic preparations are poorly water soluble or lipid soluble drugs e.g. steroidal and non-steroidal anti-inflammatory drugs giving a failure of accessible formulation. Although, petrolatum-based ointments represent a traditional solution to this problem, these vehicles are also given of drawbacks on accounting of their greasiness and vision blurring effect. Another possibility is a preparation of drug containing aqueous suspension but the coarse particle in this preparation causing eye irritation has to be considered. In recent year, submicron emulsions were exploited in drug carriers for ophthalmic formulations containing water insoluble drugs. The study of Muchtar and co-worker (1994) incorporated pilocarpine base in submicron emulsion which was prepared with the amphoteric surfactants e.g. lauroamphodiacetate and sodium tridecethsulfate (Miranol MHT), cocoamphodiacetate (Miranol C_2M) and egg yolk phospholipids. This formulation was stable to steam autoclaving and long term storage, and complying with all requirements for ocular application. The pilocarpine submicron emulsion served as a long acting antiglaucoma preparation and giving a single daily use. After that, the positive charged submicron emulsion was designed as a vehicle for intravenous or ocular administration (Klang et al., 1994). This vehicle was formulated with a combination of emulsifiers comprising egg yolk phospholipids, poloxamer 188 and stearylamine. The results of an ocular tolerance study in rabbit eyes as well as scanning electron microscopy (SEM) studies of the treated corneas indicated that this preparation was well tolerated and lacking of toxic corneal effects. After intravenous injections in mice and rats and no acute toxicity, hepatotoxic or nephrotoxic effects were observed. In addition, the physicochemical studies on the same submicron emulsion containing piroxicam (Klang et al., 1996) and miconazole (Wehrle et al., 1996) were examined. These investigations were carried out in an attempt to identify

the formulation and process parameter providing optimal experimental conditions for invention.

6. Potential for sustained release dosage forms

In emulsion system, drug action is prolonged because the drug has to diffuse from the oil dispersed phase through the aqueous continuous phase to reach the tissue fluids. The possibility of using emulsions for prolonged release of barbiturate injections has been reported (Jeppsson, 1972; Ljungberg and Jeppsson, 1970). Barbituric acids is dissolved in soybean oil and emulsified with egg phospholipids and pluronic copolymer. These emulsions acted as sustained release systems which prolonged the duration of action of barbiturates. The oil droplets acted as a reservoir in which the drug was released either to the blood or to the cell membranes of the brain capillaries by direct contact. In simple emulsion systems, the fraction of the drug for absorption depends on the phase volume ratio and the partition coefficient of the drug between aqueous and lipid phases in the delivery system (Madan, 1985). A larger value for the partition coefficient will delay absorption of the total dose from the emulsion. The phase volume ratio affects the way in which the partition coefficient relates to the fraction of the dose available for absorption. If the aqueous phase is much larger than the oil phase, then large partition coefficient values will result in a small fraction available for absorption. If the oil phase is much larger than the aqueous phase, then the fraction available for absorption available for the absorption is inversely proportional to the partition coefficient. In addition, protein binding and hydrolytic degradation of barbiturates do not occur when the drug is in oil phase, thus contributing to higher efficacy of the emulsion formulations compared to aqueous solutions.

Submicron emulsion compositions

In order to comply with the requirements of parenteral emulsion, the careful selection of the emulsion excipients needs to be considered. Two major excipients in the emulsion formulation, the oil and the emulsifier, should be especial attention. The detailed description of the excipients specifications for parenteral emulsion was described below.

1. Oil

The oil phases of submicron emulsion were based mainly on long chain triglyceride (LCT) from vegetable sources such as soybean, safflower, and cottonseed oils (Davis et al., 1983). The oils need to be purified and winterized to remove of precipitated wax materials after prolonged storage at 4 °C. Moreover, addition of antioxidants such as α-tocopherol may be employed to minimize oxidation during processing of the oil and subsequent emulsion. In the early of 70's, the use of the medium chain triglyceride (MCT) in lipid emulsion formulation was extensively increased. These MCTs are obtained from hydrolysis of coconut oil and fractionation into free fatty acids (mainly caprylic and capric acids) that contain between 6 and 12 carbon atoms. The MCTs are more soluble in water than LCTs so that they were used in medicated emulsion resulting in increased ability to dissolve large concentration of hydrophobic drug (Levy and Benita, 1990). The other oil phases e.g. triacetin, squalane and castor oil were investigated to solubilize Taxol and lipophilic anticancer drugs. These emulsions were monodispersed with a small average particle diameter. Furthermore, they are expected to dissolve rapidly in the body, thus, preventing phagocytosis and accumulation in the RES (Lundberg, 1994; Rubin et al., 1991; Tarr et al., 1987).

2. Emulsifier

Since emulsions are thermodynamically unstable systems, a mixture of surfactants should be added for improving stability (Benita and Levy, 1993). The main functions of the surfactants are to form a thin film at the interface, lower the surface tension, therefore, preventing flocculation and coalescence of the dispersed oil phase. Consideration of parenteral toxicity mainly as a result of hemolytic reaction has eliminated many emulsifying agents that might be used in parenteral emulsion. More recently, emulsifiers such as natural phospholipids, block copolymers of polyoxyethylene polyoxypropylene (poloxamer), polyoxyethylene castor oil derivatives (Cremophors®) and polyoxyethylene sorbitans (Tweens®) are now approved by the various pharmacopoeias for parenteral emulsion formulations (Benita and Levy, 1993). However, natural phospholipids are the most commonly used in

parenteral emulsion formulation. They have been obtained from both animal (egg yolk) and vegetable (soybean) sources. An advantage of using natural phospholipids is that they are metabolized in the same way as fat and are not excreted via the kidney. Another advantage is that some phospholipids stabilized emulsions are stable, resisting hydrolysis and oxidation if processed under inert atmosphere (Floyd and Jain, 1996). However, the toxicological effects of natural phospholipids were studied. These showed that soybean phospholipids was the principle cause of granulomatous lesions in rats and high concentration of soybean lecithin greater than 1% was increased blood pressure. These lecithin-associated adverse reactions were ascribed to impurities in the phospholipids (Floyd and Jain, 1996).

3. Additives

Additives are needed to adjust to physiological pH and tonicity. Glycerol is the most recommended as an isotonic agent. However, xylitol and sorbitol are also used as osmotic agents in some lipid emulsion preparation. The pH is adjusted to the desired value with an aqueous solution of sodium hydroxide or hydrochloric acid, depending on the value that should be reached. Furthermore, emulsion stabilizer such as antioxidants (α-tocopherol, deferoxamine mesylate, ascorbic acid etc.) and preservatives (p-hydroxybenzoic acid e.g. methyl and butyl derivatives) are often needed to prevent emulsions from oxidation and micro-organism growth, respectively (Benita and Levy, 1993; Levy and Benita, 1991).

Method of preparation of submicron emulsions

1. mixing

The suitable method of preparing submicron sized oil in water emulsion is firstly prepared a water in oil system and then invert it by adding more water. This technique is well known in manufacturing practices. However, the inversion method may not be used on a large scale. The phospholipids are mixed in a hot glycerol-aqueous solution and the alcohol itself would be flushed off under nitrogen atmosphere. Phospholipids are aqueous dispersion and require to be adequate dispersed before adding the oil phase. The crude emulsion is obtained by stirred

vigorously under nitrogen condition and then homogenized to reduce the large droplets to the required submicron sized level.

2. Homogenization

There are two main types of homogenizer in which general used in the manufacturer. They are low shear and high shear devices. Low shear devices include impellers and are the mixers necessary at the initial stage of an emulsification process. High shear devices are widely used for the emulsification of a variety of materials. The first one of this type is a colloid mills in which consist of a high speed rotating disc closely set to a static wall, a crude emulsion is sheared and passed between the gap. These devices are effective at reducing the average droplet size to around 5 μm. However, for intravenous application the droplet size must be smaller than 1 μm and this may only be achieved by repeated passing through a two stage high pressure homogenizer. In this machine, the crude emulsion is forced under pressure through the annular space between a spring loaded valve and valve seat. The second stage is in tandem which the emulsion is rapidly dispersed. Pressure between 2000 to 4000 lbs/sq.inch (150-280 kg/cm^2) and temperature of 40-80 °C are required and repeated passage through the machine to obtained satisfactory size reduction (Hansrani et al., 1983). However, pre-agitation with ordinary electrical agitators has been found to be very useful before homogenization step (Takamura et al., 1983). The whole manufacturing process is carried out under nitrogen atmosphere whenever possible. A major concern of the homogenization process is the unavoidable contamination produced from gasket materials, packing and metal parts. These contaminants also originate from pumps and metal surfaces wetted by the emulsion.

3. Filtration

Every components of the emulsion should be filtered to ensure low bioburdens before homogenization. Hydrophilic membrane is suitable for the aqueous components and hydrophobic filters for the oil components and also used for ethanol solution of phospholipids. However, after homogenization the emulsion is required final filtration to remove trash acquired during the homogenization process. This

implied a problem since the filter pore size is limited to removing large particles but cannot remove particles with dimensions close to the oil droplets.

4. Packing

Since the plastic containers are generally permeable to oxygen and this would limit application to a labile oxidized lipid emulsions. For this reason stopper glass bottles are general use. Type I or II glass may be employed and some manufacturers coated the inner surface of these bottles with silicone to provide a hydrophobic surface contacting with the emulsion. The composition of the stopper is critically important since it must not be permeable to oxygen or become softened by contact with the oil phase of the emulsion. Teflon-coated stoppers are available and prove to be suitable for the intravenous application. Final packing of the finished emulsion is likely to do under nitrogen.

5. Sterilization

Sterilization conditions for the thermo-labile have to be carefully selected to ensure sterilization and avoid excessive product degradation. A low bioburden prior to sterilization is therefore an essential requirement in order to allow minimal heat application. In general, it is known that an applied heat cause to breaking an emulsion. However, phospholipids stabilized emulsions may be sterilized by heat and the mean droplets size appears to decrease. The mechanism of this appearance is unclear but may be related to the formation of interfacial mesophases and also the presence of excess emulsifier in the aqueous phase acting as a reservoir. In addition, the alternative sterilization process of the emulsions is to sterilize individual components and then mix and homogenize by aseptic technique.

6. Storage

The final product of emulsions should be kept under nitrogen and not exposed to direct sunlight oxidative degradation. As noted, the physical stability in term of particle size does not change although the droplet charge (zeta potential) does change with time. This effect may be due to the simultaneous production of free fatty acid by

hydrolysis of interfacial phospholipids. However, the final products should not be exposed to temperature exceeding 30 °C. The oscillatory movement may cause separation of emulsions, and this has been observed with some phospholipids stabilized emulsions, possibly due to rupture of the stabilizing interfacial film around the oil droplets. So, it is reveal that the transportation could be considered.

Characterization of physical properties of submicron emulsions

Physical properties of emulsions which can be readily examined enclose particle mean diameter, size distribution, surface zeta potential, pH and drug content. All of these properties are important to predict emulsion stability. Emulsions must be sufficiently stable throughout manufacturing, terminal sterilization, transportation, storage, and also clinical administration. The physical properties of submicron emulsions are described as following

1. Size and droplet size distributions

Particle size distribution is one of the most important characteristics of submicron emulsion. For example, the stability of submicron emulsion during long term storage and accelerated tests such as sedimentation and creaming can be conveniently monitored by measuring the changes in droplet size distribution. Moreover, the in vivo fate of the emulsion droplets is also depended upon their sizes and size distribution as reported by several studies (Burnham et al., 1983; Grimes et al., 1979; Ishii et al., 1990). Size distributions are influenced by the emulsifier characteristics as well as by the method of manufacture. Parenteral emulsions should be formulated the dispersed droplet sizes correspond to the size of chylomicron. A wide range of particle sizes is found in emulsion systems, for the intravenous lipid emulsion should contain particle in the range of 50 nm to 1 μm. Particles about 5 μm are clinically unacceptable because they cause the formation of pulmonary emboli (Burnham et al., 1983). There is also evidence that particle size of emulsion directly effects on the rate at which an emulsion is utilized by the body (Laval-Jeantet et al., 1982). Emulsions with particle size ranging from 0.5 to 1 μm are utilized more rapidly by the body than that with 3 to 5 μm particle size. Embolism should occur in

renal, pulmonary, cerebral or myocardial capillary. Manufacture of parenteral products containing dispersed oil must be subject to strict control procedures to ensure safety. It has been recommended that the maximum size limit for such dispersed phase could not be greater than 1 μm. The mean particle size has been shown to decrease to approximately 250 nm upon an increase an egg phospholipids emulsifier concentration up to 1.2 %w/w (Ishii et al., 1990). The particle size is also affected by the oil concentrations; however, increase in oil concentration greater than 10% may significantly increase the particle size.

2. Droplet surface charge

The electrical charge on submicron emulsion droplets is measured using the moving boundary electrophoresis technique. Emulsifiers can stabilize the emulsion droplet not only by formation of a mechanical barrier but also by producing an electrical barrier or surface charge (Rubino, 1990a). The electrical surface charge of the droplets is produced by the ionization of interfacial film forming components. The zeta potential of an emulsion droplet will be dependent upon the extent of ionization of the emulsifying agent. The ionization extent of some phospholipids comprised in lecithin is markedly pH dependent (Rubino, 1990b). Commercial lecithins are a mixture of phospholipids which vary in composition. They may comprise phosphatidylcholine (PC) as the major component, zwitterionic in form, neutral over a wide pH range, together with negatively charged phospholipids such as phosphatidylethanolamine. In addition, other components such as cholesterol are presented and may affect the interfacial film-charge extent. Surface charge, therefore, could be optimized by choosing phospholipids with varying amounts of negatively charged phosphatides such as phosphatidic acid, phosphatidylserine or phosphatidylinositol (Hansrani et al., 1983; Rubino, 1990b). Furthermore, the addition of various additives could be effected on the electrokinetic properties of phospholipids-stabilized lipid emulsions (Washington, 1990a; Washington, 1992; Washington et al., 1989). In lipid emulsion consisting of phosphatidylglycerol (PG) cause an increase in the droplet charge. Based on this basis, high zeta potential values should be achieved in most of the emulsion preparations in order to ensure a high energy barrier which causes repulsion of droplets resulting in the formation of stabilized emulsions.

3. pH

It has already been shown that the main degradation pathway of lipid emulsions led to the formation of the fatty acids which gradually reduce the pH of the emulsion (Hansrani et al., 1983; Herman and Groves, 1993). The initial pH of the emulsion might decrease during storage period. However, this pH decrease can be controlled by adjusting the initial pH of the emulsion in order to minimized hydrolysis rate of the phospholipids and triglycerides. Therefore, the pH of the emulsion should be monitored continuously over the entire shelf life of the emulsion to detect free fatty acid formation.

Emulsion instability

For the utilization of emulsion as parenetral drug delivery system, the maximum diameter of oil droplets should be less than 2 μm. However, the oil droplets tend to coalesce with time at ambient or stressed conditions. Thus, the physical stability of the emulsions is critical in their use as drug delivery systems. The physical instability may proceed from creaming, flocculation and coalescence to complete phase separation (Walstra, 1996).

Creaming occurs as a result of external force such as gravitation, centrifugation and electricity. There is no change in droplet size or size distribution during creaming. Creaming takes place if the two phases of emulsion are unequal density. The droplets either rise to the top of emulsion (cream) or they settle to the bottom (sediment). A cream can be re-dispersed by shaking. Since the droplets are closed to each other, there is the possibility of flocculation and/or coalescence of the creamed droplets. Flocculated systems are easy to re-disperse. The occurrence of coalescence probably depends on the rupture of interfacial layer. The creaming rate can be reduced by decreasing the emulsion droplet size or increasing the viscosity of continuous aqueous phase.

Flocculation is the process in which emulsion droplets aggregate causing the incident of collisions and adhesion of inter droplet forces without rupture of emulsifier layer. In flocculation, two droplets are associated but still separated by the

interfacial layer. They can be re-dispersed by shaking. Flocculation may lead to coalescence if the interfacial film is ruptured.

Coalescence is the process in which the interfacial liquid film between two droplets is ruptured and they merge to form one large droplet. The coalescence process begins when two droplets undergo adhesive contact. The liquid film between droplets is discharged and the rate of discharge is related to the rigidity or micro-viscosity of the interfacial layer. After that, the interfacial layers rupture and the two droplets merge into one, thereby increasing droplet size.

Incorporation of drugs in submicron emulsions

Drug containing emulsion formulations may be employed to overcome solubility or stability limitations that are not otherwise dissolved by pH control or co-solvents such as ethanol, glycols, dimethylacetamide (DMA) or dimethylsulfoxide (DMSO). The limitations of these solvents include precipitation of the drug on injection, pain or phlebitis, and inherent toxicities due to the solvent itself. Application of submicron emulsion for parenteral administration as a vehicle for poorly water soluble drug has been increasing over the years. Two approaches have been used to incorporate drugs into an emulsion (Benita and Levy, 1993; Hansrani et al., 1983; Prankerd and Stella, 1990). They are de novo preparation and extemporaneous addition of drugs to formulated emulsion base.

1. De novo emulsification

This method is desirable to incorporate drugs, particularly poorly water soluble drugs, in the oil phase of the emulsion prior to emulsification process. Inclusion of hydrophobic drugs in the oil phase usually presents special problems due to their solubility limitation. However, these problems can generally be overcome by technique such as elevation of temperature and the use of surfactants. The whole preparation process should be conducted in a laminar flow hood under a nitrogen atmosphere in case the excipients and drugs sensitive to oxidation are used. The examples of drug containing submicron emulsion formulations prepared by de novo

emulsification technique are penclomedine (Prankerd et al., 1988), Taxol (Tarr et al., 1987), diazepam (Levy and Benita, 1989), propofol (Deegan, 1992) and so on.

2. Extemporaneous addition of drugs to formulated emulsion base

The second approach used to incorporate a concentrated solution of drug in a solvent such as dimethylacetamide or ethanol to submicron emulsion base or a commercial intravenous lipid emulsion such as Intralipid® or Liposyn® by aseptic technique addition. The addition procedure must be performed with great care due to the prevention of precipitation of the drug in aqueous phase of the emulsion and also prevent cracking of the emulsion. This approach has been used successfully in preliminary animal and clinical studies with cytotoxic agents but is not a suitable procedure for routine use because of emulsion stability problems.

Physical structure of parenteral lipid emulsions

Emulsions have been generally defined as dispersions of one liquid in another liquid. There are two common types of emulsions in which are generally well known in pharmaceutical field, they are oil in water (o/w) and water in oil (w/o) emulsion. In addition, multiple emulsions (w/o/w or o/w/o) are also interesting to be studied. In order to disperse one liquid in another which it is immiscible, the addition of the third component is necessary. The third component, namely emulsifier, is capable of adsorbing at the interface between the two liquid phases resulting in stabilized the dispersed oil droplets in emulsion system. For the past 40 years, lipid emulsions have been successfully used in parenteral nutrition. More recently, such emulsions have been used as delivery vehicles for poorly water soluble drugs. The parenteral lipid emulsions consist of oil phase, interface or phospholipids monolayer and aqueous phase. In fact, almost commercial emulsions are stabilized with 1.2% lecithin irrespective of amount of oil used. Theoretical amount of emulsifier calculation showed that the amount to achieve a monomolecular film covering the oil-water interfaces is only 50% phospholipids that is really needed for the stabilization (Groves et al., 1985). Therefore, it is noticeable that an excess of phospholipids is used to stabilize the parenteral lipid emulsion. The excess amount of phospholipids formed the complexity structures in this emulsion system. Nevertheless, the information

regarding the possible structures and their properties is limited. Several attempts have been investigated to indicate the infrastructures of parenteral lipid emulsion. Friberg and Jansson (1976) and Rydhag and Wilton (1981) proved that for stabilization of emulsions with phospholipids as emulsifiers, the formation of liquid crystalline structures on the interface is necessary. The lamellar liquid crystalline structures can uptake water, especially in the presence of negatively charge. According to this, these structures can be clearly distinguished from the oil and water phases, so that they have their own emulsifier phase consisted of several phospholipids layers.

In 1985, Groves and co-worker employed a negative staining technique in transmission electron microscopy to study the possible size and structure of Intralipid® emulsion. They suggested that Intralipid® droplets may consist of multiple layer structures and also multilamellar vesicles. According with the existence of these phospholipid reservoirs, the commercial fat emulsions seem to be good heat stability during autoclaving. As early 1991, Rotenberg and co-workers used a ^{31}P-NMR technique to quantify the relative amounts of phospholipids inside the droplet and on its surface and determined based on the original Intralipid® emulsion and the fractions collected after ultracentrifugation. This study suggested that the droplets in Intralipid® are mostly monolayer structures (unilamellar) and presumed that the results of Groves et al (1985) may have been due to straining artifacts.

In 1992, Westesen and Wehler performed an extensive study of the inner structure of intralipid emulsions. They employed the PCS, freeze-fracture electron microscopy and small angle X-ray diffraction techniques and suggested that small amount of bi-and/or oligolayer structure may exist in this emulsion system and these complex structures were confirmed by the study of Li and Caldwell (1994). They determined the infrastructure of commercial lipid emulsions by employing a combination of sedimentation field-flow fractionation (sedFFF) and other characterization techniques such as PCS and freeze-fracture electron microscopy. They found that the commercial lipid emulsions which contained similar composition exhibited the differences in both size distribution and densities. It was implied that these emulsion droplets may have a multilayered surfactant arrangement as well as an inclusion of water vesicles in the oil phase of the emulsion.

In the late of 1994, Ferezou and co-worker studied the influence of phospholipids/triacylglycerol (PL/TG) ratio of parenteral emulsions on the distribution and the physicochemical properties of the commercial 10, 20 and 30 %lipid emulsions. These emulsions were fractioned by using ultracentrifugation technique which can be separated oil phase from a subnatant, named mesophase (Jakoi and Quarfordt, 1974; Robinson and Quarfordt, 1979). Mesophase contained residual phospholipids which was a remaining of phospholipids monolayer covering the oil droplets. They used lipid analysis and ^{31}P-NMR technique to indicate that there were at least two types of fat particles coexisted in parenteral emulsions. They were named triacylglycerol rich particles and phospholipids bilayer particles or liposomes. The mean particle size of triacylglycerol rich phase of 10, 20 and 30% emulsion were 330, 400 and 470 nm, respectively while that of liposomes are in the range of 80-100 nm. The triacylglycerol rich particles contained a major of triacylglycerol and esterified phytosterols of native particles and a minor of fraction of phospholipids, unesterified cholesterol, phytosterols and other lipids. The phospholipids bilayer particles were constituted with the remaining phospholipids, little amounts of triacylglycerol and other lipids. In addition, it was found that the higher oil content of emulsion, the lower amount of phospholipids rich particles was observed, so that the residual concentration of phospholipids in 10% emulsion was higher than in 20% emulsion (Hajri et al., 1990). Considered as phospholipids excess, the mesophase related phospholipids was the first speculation to form vesicles, probably made up a single phospholipids bilayer, also called unilamellar liposomes (Groves et al., 1985; Williams and Scanu, 1986). However, Rotenberg and co-worker found that these mesophase could also contain small triacylglycerol rich particles (Rotenberg et al., 1991).

The structure and/or composition of parenteral lipid emulsions to be used as vehicles for drug delivery have effects on the release rate of their drug load and on the metabolic fate. When an emulsion is used as a slow release, drug penetration across the surfactant layer and diffusion away from this layer are likely to form the two barriers for drug transport. It has been proposed that emulsions should have a structure of a true monolayer coated droplet and micelle (Hiemenz, 1986). The complex structures such as emulsion droplets with multiple emulsifier layer or

multiple w/o/w emulsion could be formed and likely to affect the physical properties of emulsion, specifically , on their ability to release drug (Weiner, 1990).

The environment of drug localization has been effected on the efficiency and stability of drug in emulsion system. Although the knowledge of drug localization in various phases of phospholipids stabilized submicron emulsions is limit. Several studies of antioxidants activity within lipid system have been investigated. The relative effectiveness of antioxidants is depend upon the lipid substrates, test system, concentration, oxidation time and method used to determine lipid oxidation (Frankel et al., 1994; Huang et al., 1994). Antioxidant behavior is more complex when evaluated in emulsion systems than in bulk oil systems because more variable lipid oxidation including emulsifier, pH and buffer system (Barclay and Vinqvist, 1994; Pryor et al., 1988). Huang and co-worker (1996) demonstrates that the effectiveness of α-tocopherol and Trolox is very dependent on their physical properties and the physical states of lipid systems used as substrates. Polarity and solubility of antioxidants determine their concentrations in different locations in various multiphase systems. Their antioxidant activity is affected by their diffusion rates, stability and degree of dissociation, which may change with their location in this system.