CHAPTER II

LITERATURE REVIEW

1. Definitions

In the literature on parenteral fat emulsions, there are many different terms that are used synonymously and sound alike but that describe different physical systems. The International Union of Pure and Applied Chemistry (IUPAC) generally defines emulsion as dispersion of liquids or liquid crystals in another liquid (Lucks et al, 2000; IUPAC, 1972). In general, depending on the outer phase, it is possible to distinguish between water-in-oil and oil-in-water emulsions. Parenteral fat emulsions (PFEs) suitable for intravenous application belong to the oil-in-water emulsions and have a mean droplet size of 200-500 nm. PFEs are milky in appearance. When diluted with water, they change by degrees to a clear solution with a blue shimmer caused by diffraction of light on the oil droplets (Mizushima, 1985; Mizushima, 1988, Mizushima, 1990). However, under certain conditions (high pressure, low amount of oil, high amount of lecithin), it is possible to produce emulsions with a droplet size under 100 nm and so called, nanoemulsion (Lucks et al, 2000). Due to the large interface and the high interface energy, PFEs and nanoemulsions are thermodynamically unstable systems.

A clear difference must be made between macro (emulsion) and the so-called microemulsions. The microemulsions are often wrongly described as parenteral fat emulsions (Panaggio, Rodes, and

Worthen, 1979) and represent, from the physical point of view, a completely different system. Microemulsions are thermodynamically stable, single phase, transparent or translucent, liquid, and in unsheared stable are optically isotropic systems consisting of several liquids that cannot be mixed together and are produced with the aid of surfactants or surfactant mixtures. The physical structure of these systems is the object of many investigations (Georges and Chen, 1986; Guest and Langevin, 1986; Hoffmann, Platz, and Ulbricht, 1986; Kahlweit et al., 1987; Lindman, Stilbs, and Moseley, 1981; Ruckenstein, 1986), in which they are described either as swollen micelles or as a critical solution (Lucks et al, 2000).

2. Selection of compositions

The major excipients in intravenous lipid emulsions are oil and emulsifier. In fact, the choice of oils and emulsifiers to be used for intravenous administration is severely limited. Vegetable oils are used almost exclusively for the intravenous route with natural materials such as the phosphatides (egg or soy) as the emulsifier.

For the oil phase, long-chain triglycerides (LCT) from vegetable sources (soybean, safflower, and cottonseed oils) are the classical use in emulsion formulation. They show the least incidence of toxic reactions and the greatest resistance to oxidation. Some can be used in large quantities for nutritional purposes. Soybean oil is the refined fixed oil obtained from the soy. It is composing of 50-57% linoleic acid, 5-10% linolenic acid, 17-26% oleic acid, 9-13% palmitic acid, and 3-6% stearic

acids (Wade and Weller, 1994). The oils need to be purified and winterized to allow the removal of precipitated materials after prolonged storage at $4^{\circ}C$ before use in formulation. Moreover, the known contaminants (hydrogenated oils and saturated fatty materials) should be minimized (Davis et al., 1985).

In addition, the use of medium-chain triglycerides (MCT) in fat emulsion formulations increased extensively during the 1970s (Bach et al., 1996). MCT are normally obtained from the hydrolysis of coconut oil and fractionation into free fatty acids that contain between 6-12 carbon atoms. MCT are found to increase the solubility of high concentrations of liposoluble drugs in the o/w emulsions. Recently, the oil phase of the emulsion is mainly based on a mixture of LCT and MCT. There is a series of investigation show that MCT/ LCT emulsions provide better stability in the preparations of total parenteral nutrition (TPN) than only LCT containing emulsions (Lucks et al, 2000).

Emulsions are thermodynamically unstable. The dispersed droplets intend to coalesce in order to reduce the interfacial area, thus reducing the interfacial free energy of the system. However, it can be kinetically stable by adding of emulsifying agent, which facilitates the preparation of the emulsion and slows down its inevitable destruction, coupled with input of large amount of energy, i.e., homogenization, sonication, etc (Davis et al., 1985).

The commonly used emulsifiers can be classified into:

i) Anionic surfactants include soaps, alkyl sulfates, and sacrosinates (medialans), sulfosuccinates, alkyl sulfonates and phosphated esters.

ii) Cationic surfactants include ethoxylated amines, ethoxylated quaternary compounds, pyridinium halides and fatty quaternary compounds.

It should be noted that for most ionic emulsifiers (anionic and cationic), the choice of counterion could have a great effect on the prepared emulsion properties (Weiner, 2000a).

iii) Nonionic surfactants include fatty acid alcohol ethoxylates, ethoxylated esters and ethoxylated or propylated block polymers.

iv) Amphoteric surfactants include amino-carboxy compounds, amino-sulfuric ester compounds and amino-sulfonic acid compounds.

v) Naturally occurring emulsifiers include lanolin, lecithin, and hydrocolloids such as acacia, carageenan and alginates. The hydrocolloids are generally not used alone and are considered to be auxiliary emulsifying agents.

vi) Finely divided solids include the various clays such as bentonite.

vii) Proteins are the excellent emulsifying agents used extensively in the food industry.

The single most important factor in the choice of any emulsifiers is toxicity. Emulsifying agents from the natural source are normally phsopholipids (PL). The natural phospholipds obtained from animal (egg) or vegetable (soybean) are considered to be safe compared to the synthetic emulsifiers. The most abundant glycerol phosphatides found in natural phospholipids are phosphatidylcholine (PC, lecithin), and phosphatidylethanolamine (PE), which referred to of cephalin. These two phosphatides are the major structural components of most biological membranes.

Phosphatidylcholine consists of two hydrocarbon chains linked to a phosphate-containing polar headgroup that is attached to choline polar entities. The positive charge on the choline headgroup counteracts the negative charge on the phosphate to give a neutral, which is a hydrophilic headgroup of lecithin. Compositions of egg and soybean lecithins are found to be slightly different (Table 1).

Table 1. Compositions (% w/w) of egg / soy lecithins (Modified from Othmer, 1995)

Phospholipids and derivatives	**Amount (%w/w)**	
	Egg lecithin	**Soy lecithin**
Phosphatidylcholine (PC)	69	21
Lysophosphatidylcholine (LPC)	2	1
Phosphatidylethanolamine (PE)	24	22
Lysophosphatidylethanolamine (LPE)	2	1
Phosphatidylinositol (PI)	trace	19
Phosphatidic acid (PA)	trace	10
Phosphatidylserine (PS)	3	1
Sphingomyelin (SP)	2	trace

Phospholipids can possess fatty acids of different chain length varying from C_{12} to C_{24} and unsaturation and may have different hydrophilic species linked to the phosphate such as choline or ethanolamine, according to which individual membranes of the phospholipid category are classified

(Table 1). Phospholipids which were high in PC were found to produce emulsion with poor stability as insufficient charge in stabilization of emulsion (Davis, 1974). Davis (1982) has recommended the necessity of incorporating the ionic lipids (such as phosphatidic acid, phosphatidylserine) into lecithins in order to produce more stable emulsions by changing the surface potential.

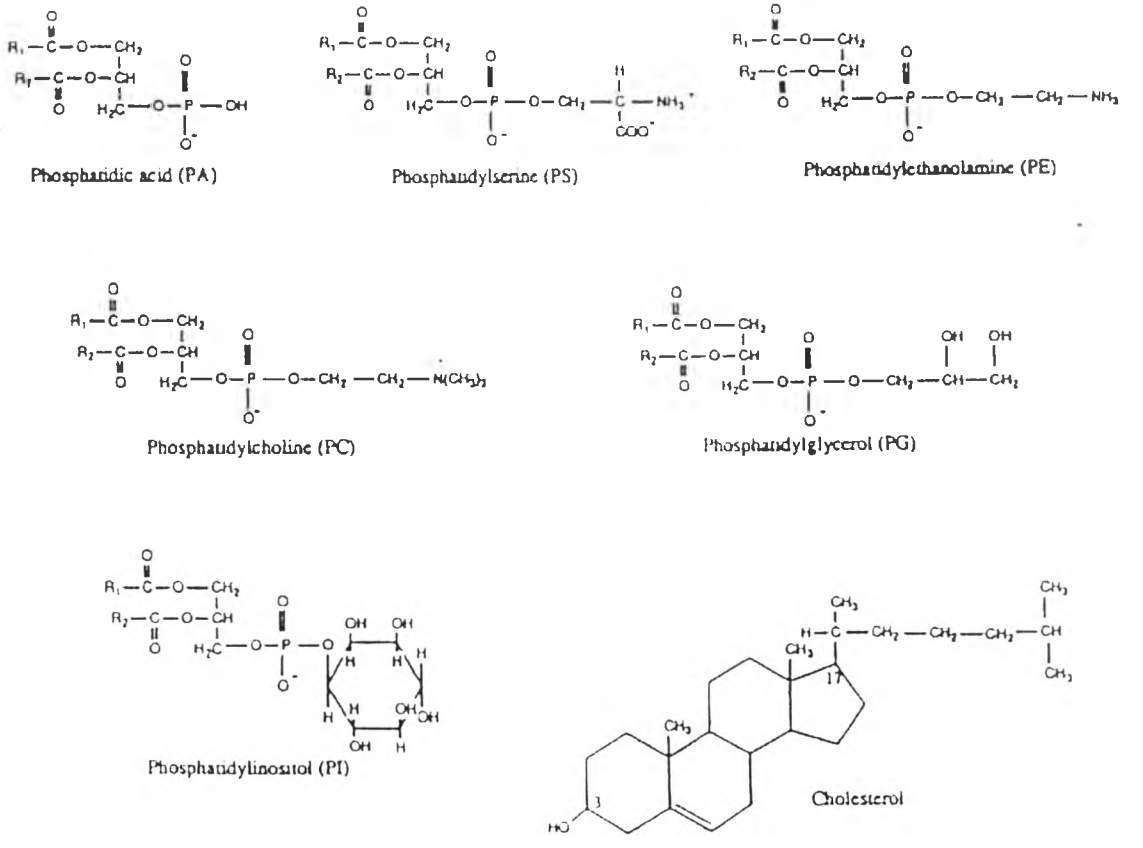


Figure 1. Structures of glycerophospholipids showing different headgroup (From Weiner, 2000b)

Charged lipids are used to increase the surface charge density to prevent close approach of the oil droplets, aggregation and fusion, etc. Anionic phosphoilipids have apparent pKa values between 3 and 4 (Arias and Rueda, 1992). Phosphatidylglycerol (PG) posses a negatively charge over the neutral

pH (pH 7.4) contributing to an electrostatic repulsive force within the pH range of intravenous lipid emulsions. The presence of negatively charged phosphatides enhances the stability by the formation of gel-like liquid crystalline interfacial films resulting in an increase in the electrostatic repulsive force (Muchtar et al., 1991; Rubino, 1990; Washington et al., 1989; Yamaguchi et al., 1995).

Cationic lipids bearing a net positive charge are almost unknown in nature. However, there are some pharmaceutical preparations using cationic lipid as part of emulsifiers, for example, liposomes can be prepared in which amphiphiles such as stearylamine or cetyl trimethyl ammonium bromide are incorporated (Philippot and Schuber, 1983). They can stabilize emulsion by increasing the charge density and repulsive force, as the positively charged phospholipids can bind negatively charged drugs and interact strongly with proteins and other anionic macromolecules. The complex interfacial films of PC and PE were produced by forming liquid-crystalline gel structure (Shchipunov and Kolpakov, 1993) resulting in more stability of emulsions. Liposomes composed of cationic lipids, dioleyl oxypropyl trimethyl ammonium chloride (DOTMA), and dioleyl phosphatidylethanolamine have been used to form a complex with DNA (Philippot and Schuber, 1983). They aid the entry of DNA into the cell, probably by virtue of increased cell binding and coupling with fusogenicity of the lipid themselves. It is likely that, after binding with DNA to form a complex, the lipids are no longer in liposomal form. However, the result from the study of Chansiri et al. (1999) indicated that positively charged egg phospholipids emulsions are not desirable because the positive zeta potential is neutralized during autoclaving.

The wide ranges of synthetic surfactants have been investigated as potential emulsifiers. From a toxicological point of view, nonionic surfactants are generally regarded as the most suitable for pharmaceutical formulation. Even so the range of non-ionic surfactants used is very limited (Davis et al., 1985). The nonionic surfactants considered are polyethylene glycol stearate, polyoxyethylene monostearate, polyethylene glycol, polyoxythylene sorbitan monoesters, etc (Davis et al., 1985). However, all of these classes of surfactant gave rise to toxic reactions at high concentration. Only one range of nonionic materials that was found to be safe for use was polyoxyethylene-polyoxypropylene compounds (Poloxamers), available commercially as Pluronics. They have been used widely for formulation of fat emulsion, either on their own or in combination with lecithin (Rosoff, 1988; Schuberth and Wretlind, 1961).

Polyoxyethylene (20) sorbitan monooleate (Tween 80) and polyoxyethylene (35) castor oil (Cremophor® EL) are probably the most commonly used (Lawrence, 1994). Other emulsifiers, such as bile salts and sodium cholate have also been considered (Benita and Levy, 1993; Davis et al., 1985).

The amount of emulsifier used in the formulation is important for the formation of interfacial film. Generally, all commercially parenteral emulsions are stabilized with 1.2 % lecithin regardless of the amount of oil used. While, theoretical calculations obtained from the study of Groves and his worker (1985) showed that the amount to achieve a monomolecular film covering the oil-water interface is only 50% of phospholipid that is really needed for the stabilization. The lesser amount of lecithin, 0.6-0.8%, is used in some studies (Carstensen et al., 1991; Friberg and Jansson, 1976; Lucks et al., 2000; Rydhag and Wilton, 1981). They found that the formation of liquid crystalline structures on

the interface was absolutely necessary for stabilization of emulsions (Friberg and Jansson, 1976; Levy et al., 1991; Lund, 1994; Rubino, 1990; Rydhag and Wilton, 1981; Weingarten et al., 1991). The crystalline structure can form especially in the presence of negatively charged phospholipids with soy lecithin.

The excess use of lecithin may result in free lecithin forming multilamellar structure, corresponding to multilamellar liposomes which promoting stability at high temperature, i.e. autoclaving. On the other hand, the free phospholipids in fat emulsions increase the level of serum cholesterol (Bach et al., 1996) and abnormal lipoproteins (Haumout et al., 1989; Messing et al., 1990). 10% emulsions are more pronounced for a higher amount of free phospholipid.

Using a mixture of emulsifiers can significantly reduce the amount of surfactant used and the emulsions can be prepared using lower energy method (Weiner, 2000a). The use of nonionic surfactant in combination with lecithin as emulsifiers in promoting stability of emulsion was studies by many workers. One of those was the study of Kan et al. (1999) which investigated the synergistic effect of egg phospholipid and Tween 80 in stabilizing the emulsion. The optimum weight ratio of egg phospholipid to Tween 80 was 1:1.

The dispersion medium of parenteral lipid emulsion may contain one or more of the following additives: isotonicity agents; electrolytes; buffer; anti-flocculants and preservative. Glycerol is usually recommended to use as tonicity adjustment (Benita and Levy, 1993). Sodium hydroxide and hydrochloric acid solutions are used to adjust the pH of emulsion to 7-8 to allow physiological

compatibility and maintain physical integrity of emulsion by minimizing fatty acid ester hydrolysis of oil and phospholipids from acidic conditions.

Moreover, emulsions are often needed to protect from oxidation or phase separation. Oxidation is prevented by the addition of antioxidants or reducing agents, such as 0.05-0.075% (w/v) tocopherols, 0.00116-0.03% (w/v) butylated hydroxytoluene, 0.00028-0.03% (w/v) butylated hydroxyanisole, and 0.01-0.1 % ascorbic acid (Nema et al., 1997; Wade and Weller, 1994). Phase separation can be prevented by the incorporation of stabilizer such as oleic acid or its sodium salt, cholic acid, deoxycholic acid and their respective salts. Stabilizer stabilizes the interfacial film by enhancing molecular interactions and increasing the electrostatic surface charge of oil droplets (Benita and Levy, 1993; and Levy and Benita, 1990).

3. Stability of emulsion

3.1 DLVO Theory

The interaction forces between droplets are important for their stability. DLVO theory is used to explain the stability of charged emulsions on the basis of the balance between the attractive forces (van der Waals) and repulsive forces (electrostatic forces, stearic forces, solvation forces and Born forces) (Lawrence, 2000).

For emulsion stabilized by ionic surfactants, van der Waals and electrostatic forces are most important (Lawrence, 2000). Van der Waals forces are the energy of attraction between pairs of atoms or molecules on neighboring particles. The energy of attraction varies with the distance between pairs of molecules. Electrostatic forces describe the repulsive forces arising from the interaction of the electrical double layers surrounding pairs of particles.

In contrary to ionic emulsions, uncharged emulsion stabilized by nonionic surfactants, van der Waals, stearic and solvation forces are most important. Stearic stabilization is one of the mechanisms to increase the stability of the emulsion. It arises from the present of the absorbed chains of emulsifier, which occurs as the chains approach and increase the local concentration of high molecular weight materials (Lawrence, 2000). Stearic stabilization consists of two forces corporating in emulsion stabilization, osmotic or solvation forces and entropic effects. For solvation force, it can be explained that when two droplets come in close contact, hydrophilic chains of surfactant start to overlap. It leads to an osmotic gradient in solution between concentrated polymer solution in overlap region and dilute solution in bulk solution. Then, water enters into the concentrated polymer region in an attempt to dilute it, resulting in force chains or droplets apart (Davis et al., 1985; Lawrence, 2000; Lucks et al, 2000). Another explanation is that the loss of freedom of motion of polymer chain and the loss of entropy when two droplets come together and polymer chains overlap. This is thermodynamically unfavorable, resulting in force droplets apart again or called entropic effect. Generally, stearic forces are dependent upon length of polymer chains. Longer the chains, greater the stabilization.

Stearic and hydration forces, which depend on adsorption, are inversely related to temperature. So, the forces are less effective at increasing temperature (Chansiri et al., 1999). The electrostatic repulsive force is inversely related to the dielectric constant of the medium (Everett, 1988). In case of lipid emulsions, the medium is water and its dielectric constant decreases as temperature increases. Therefore, the electrostatic repulsive force is larger at elevated temperatures than at room temperature (Chansiri et al., 1999).

3.2 Instability of emulsion

Changing in physicochemical properties of the emulsion results in emulsion instability. The most common physical stability problems occurred in the emulsion are creaming, flocculation, phase inversion, and coalescence or breaking of the emulsion (Weiner, 2000a)(Figure 2).

Creaming

Creaming is defined as the separation of an emulsion into two emulsions. One of which is more concentrated in disperse phase than the other according to the dispersed oil droplets move upwards and accumulate on the top under the influence of gravity. Creaming can usually be redispersed by simple agitation or shaking according to interfacial film of oil droplets is not destroyed.

Phase inversion

Phase inversion is defined as an emulsion changing from o/w to w/o emulsion, or vice versa. Phase inversion may occur by changing temperature, addition of a material that changes the solubility of the emulsifying agent (Lund, 1994; Swarbrick and Boylan, 1992)

Flocculation

Flocculation is defined as the aggregation of droplets to form clusters with each droplet maintaining its individuality. Flocculation is also usually reversed by shaking. The formation of these clusters can greatly accelerate the rate of creaming.

Coalescence

Coalescence or breaking represents the ultimate destruction of the emulsion leading to the phase separation.

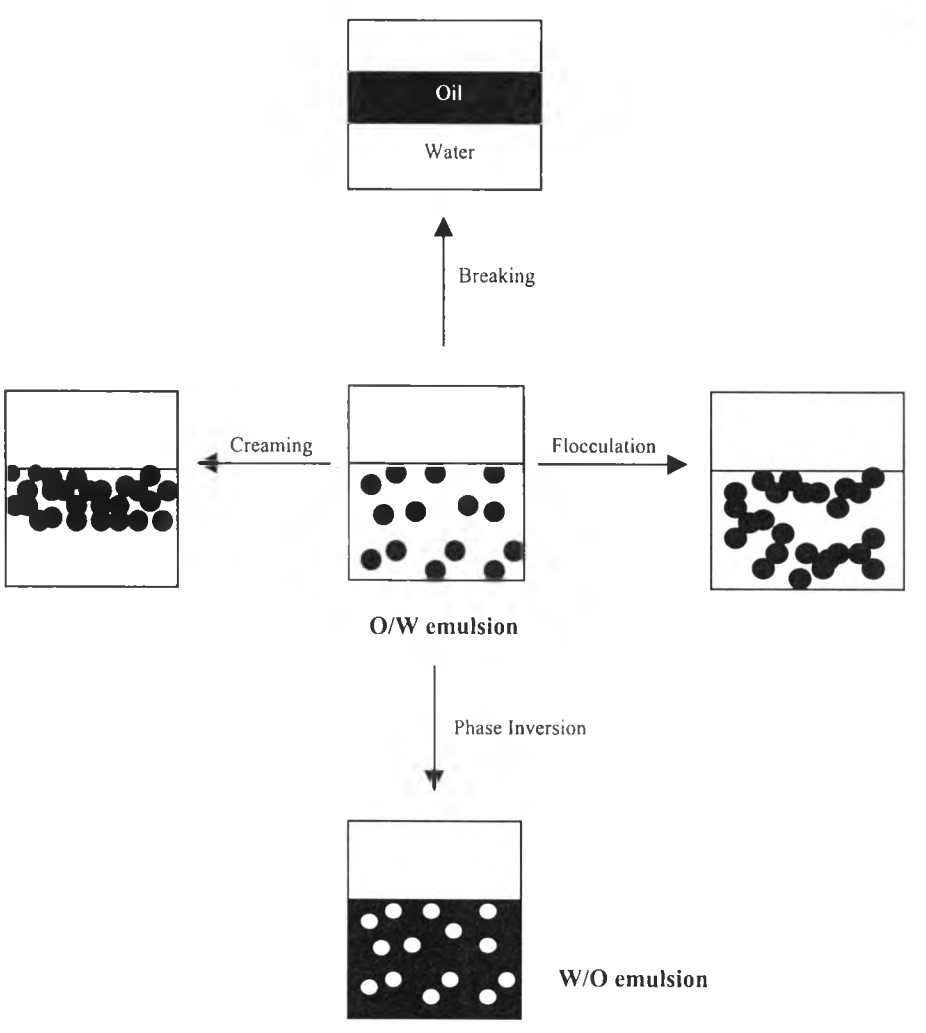


Figure 2. The physical instability of emulsion (Reproduced from Weiner, 2000a)

In addition, chemical instability may a result from hydrolysis of emulsifier, change in pH, release of free fatty acid, and rancidity of oil.

Phospholipids are susceptible to hydrolysis because most of the phospholipids contain unsaturated acyl chains as part of the molecular structure. The degradation process of egg phospholipids is lipid peroxidation and lipid hydrolysis. Lipid peroxidation is the chemical reaction

occurring on changing the unsaturated acyl chains of phospholipid molecules, diacylphosphatidylcholine and diacylphosphatidylethanolamine, to their corresponding monoacyl (lyso-) derivatives and free fatty acid moieties. The oxidative degradation involves in free radical generation and the formation of cyclic peroxides and hydroperoxides (Weiner, 2000b).

Lipid hydrolysis occurring from the hydrolysis of the ester bond of glycerol moiety results in lysophosphatidylcholine (lyso-PC) formation (Chansiri et al., 1999; Weiner, 2000b). The formation of lyso-PC could be used as a standard measurement for the chemical stability (Weiner, 2000b). Lyso-PC greatly enhances the permeability of cell membrane. Free fatty acid can also be formed by the hydrolysis of emulsified triglycerides of oils to the corresponding mono- and diglycerides. Although this reaction is believed to be relatively slow compared with the breakdown of diacylphosphatidyl derivatives in phospholipids, as far as long-chain triglycerides, such as soybean oil, are concerned (Herman and Groves, 1993).

Instability can occur during preparation, particularly upon autoclaving, under storage or through the addition of electrolytes and drugs.

The method of minimizing any instability problem is to use the proper phospholipids. They should be essentially free from any lyso-PC and lipases. In fact, other phospholipid components in lecithin can also be decomposed. For example, cholesterol has been shown to be oxidized rapidly.

As known that the incorporation of drugs and electrolytes can result in a drastically reduced stability and/or cracking of the emulsion. Undoubtedly, there are only a few emulsion containing drugs available on the market; namely, Diazemuls®, Liple® and Fluosol-DA®.

4. Pharmacopoeial requirement

An intravenous lipid emulsion, like all parenteral products, is required to meet pharmacopoeial requirements. The emulsion must be sterile, isotonic, non-pyrogenic, non-toxic, biodegradable, and both physically and chemically stable.

Two important constraints for parenteral emulsions are particle size and sterility.

4.1 Sterility

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). Actually, the condition used in steam sterilization is 121 ^{o}C with the pressure of 15 psi for 15 minutes, but Idson (1988) reported the use of autoclaving at 110 ^{o}C for 40 minutes.

4.2 Particle size

The intravenous emulsions systems usually have a mean size of the droplet of 0.2—0.5 μm with 90% or more particles below 1 μm (Groves, 1984). More recent limits for oil droplets in fat emulsions and total parenteral nutrition regimes permit an amount of 0.4% of the droplets to be larger than 5 μm (Lucks et al., 2000). As the smallest capillaries have a diameter of 5 μm (Chanana and Sheth, 1993; Collins-Gold et al., 1990; Mehta et al., 1992), so particles greater than 5 μm are clinically unacceptable and they must be excluded for intravenous use (British Pharmacopoeia, 1980). Larger oil droplets could block (emboli) in the capillaries (Singh and Ravin, 1986), particularly the fine capillaries of the lungs resulting in pulmonary emboli. An increase in droplet size sometimes presents because of inefficient homogenization or steam sterilization (Chaturvedi et al., 1992)

The stability of an emulsion can also be easily monitored by measuring the changes in the droplet size. Size reduction of the emulsion could minimize sedimentation and creaming of an emulsion. Small droplet size promotes good physical stability because droplet approach is prevented by Brownian movement. A small particle size can be achieved using a high–temperature, high- pressure techniques. The homogenized emulsion is recycled a number of times in order to achieve the necessary size reduction.

Osmolality is also important in parenteral administration. The variety of serum osmoalrity value was found in the literature. The osmolality of serum is in between 275-300 mOsmol/L (Reich et al, 2000). If the solutions have tonicity differ from the serum, they generally cause tissue

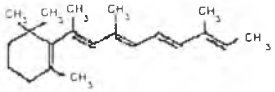
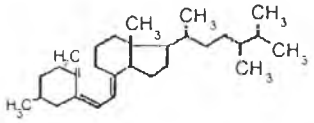
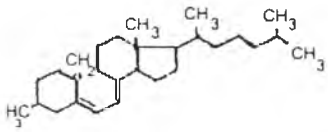
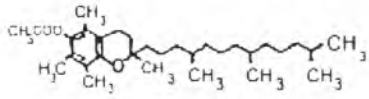
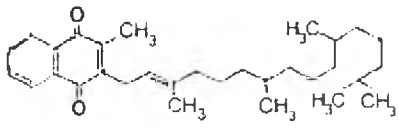
irritation, pain on injection, and electrolyte shifts. Osmolality of less than 900 mOsmol/L has been considered as safe for peripheral administration of parenteral nutrition solutions. Excessive infusion of hypotonic fluids may cause swelling of red blood cells, haemolysis, and water invasion of the cells in the body leading to convulsions and edema. On the other hand, excessive infusion of hypertonic fluids leads to a wide variety of complications such as hyperglycemia, glycosuria and intracellular dehydration, osmotic diuresis, loss of water and electrolytes, dehydration and coma.

5. Vitamins

Vitamins are one of the essential nutrients for normal metabolism and maintaining optimal health. But, some are not synthesized in the body or in inadequate quantities. Consequently, vitamins must be obtained from the diet. Most vitamins function as co-enzymes or co-factors. Deficiency states are recognized for all vitamins and in many cases excessive intake also leads to dispose. Vitamins could be divided into water-soluble and oil-soluble vitamins. Oil-soluble vitamins include vitamin A, vitamin D, vitamin E and vitamin K (Table 2).

Vitamin A or vitamin A alcohol, as called all-*trans* retinol, is necessary for a broad range of bodily function, including production of vision pigments, protection for the entire ectoderm and depelopment of the skin, supporting the growth especially the growth of cartilages and bones, and resistance to infectious agents and maintenance of health in many epithelial cells. Vitamin A is found

Table 2. Chemical name and structure of oil-soluble vitamins (Reproduced from Van Niekerk, 1988; USP 23)

Vitamins	Chemical names	Chemical structure	Chemical formula
Vitamin A	All-trans retinol		$C_{20}H_{30}O$
Vitamin D	Ergocalciferol (vitamin D_2) Cholecalciferol (vitamin D_3)	 	$C_{28}H_{44}O$ $C_{27}H_{44}O$
Vitamin E	Tocopherols, tocotrienols		$C_{29}H_{50}O_2$
Vitamin K	Phylloquinone (vitamin K_1) Menaquinone (vitamin K_2) Menadione (vitamin K_3)		$C_{31}H_{46}O_2$

only in animal organism. It presents in many animal tissues, especially fish and liver. Vegetables contain only the precursors of vitamin A, the carotenoid. Carotenoids in green plants serve can be converted to vitamin A following a digestion in the body (Van Niekerk, 1988; USP 23, 1995).

Vitamin D is synthesized in the skin when exposed to sunlight. It also presents at low concentration in some natural foods, and in many artificially- fortified food products. It is a steroid hormone, which has major effect on facilitates absorption of calcium from the intestine and assists in maintain calcium homeostasis and phosphate metabolism. The numerous compounds with vitamin D acitivity (calciferol), vitamins D_3 (cholecalciferol) and D_2 (ergocalciferol) have the greatest importance for the human. The provitamin, 7-dehydrocholesterol, is formed in the liver and intestinal mucosa and occurs in foods of animal origin. It is converted in the skin to vitamins D_3 under the influence of ultraviolet light. Vegetable contains ergosterol, which has a comparable function as a provitamin. After digestion with the food, it can also be converted to vitamins D_2 in the skin. Vitamins D_2 and D_3 demonstrate approximately the same activity in the human (Van Niekerk, 1988; USP 23, 1995).

Vitamin E obtains from vegetable oils, leafy green vegetables and whole grains. It functions as antioxidants, particularly preventing oxidation of unsaturated fatty acids and maintainance of the integrity of cell membranes. The term vitamin E consists of a group of eight substances that are chemically closely related, that are four tocopherols and four tocotrienols. The most effective of these substances is D- α- tocopherol (RRR- α- tocopherol), which occurs naturally. Vitamin E is essential for the metabolism processes of respiration and nucleic acid metabolism in all cells, particularly those

of the nervous system. Deficiency of vitamin E can lead to reproductive function, leading to the nickname of "antisterility vitamin" (Van Niekerk, 1988; USP 23, 1995).

Vitamin K occurs widely in nature, both in animals and in vegetables. Important sources of vitamin K_1 (phylloquinone) include green plants and algae. Vitamin K_2 (menaquinone) is found in animals and is produced by coli bacteria in the intestine of the animal. A further source of vitamin K_2 is the bacterial flora of the human large intestine. Vitamin K_3 (menadione) does not occur in nature. It is a purely synthetic product that should no longer be used for therapeutic purposes in humans because of its many side effects. Vitamin K is required for formation of several blood-clotting factors in the liver, and deficiency leads to bleeding disorders (Van Niekerk, 1988; USP 23, 1995).

Only a few of oil-soluble preparation are available in market. Some of those are Vitalipid® and OMVI®. The compositions of the formulations are in appendix C. Vitalipid® is prepared in the o/w emulsion dosage form while OMVI® is prepared in solution form. However, in administration, these preparations can be incorporated to lipid emulsion before use.

6. Manufacturing Process

The mean droplet size of intravenous emulsions must be smaller than the finest capillaries in the vascular system, otherwise, an oil embolism can occur. Emulsions prepared by use of conventional apparatus, e.g., electric mixers and mechanical stirrer, etc., show not only large droplet sizes but also a wide particle size distribution and are often unstable.

A submicron emulsion can be prepared by use of a two-stage pressure homogenizer in which the crude dispersion is forced, under high pressure, through the annular space between a spring-loaded valve and the valve seat. The second stage occurs in tandem with the first, so that the emulsion is subjected to two very rapid dispersion processes (Idson, 1988; Takamura et al., 1983). The influence of various mixers and homogenizers is depicted in Figure 3. As can be seen, course dispersion is characteristic of emulsions prepared with a magnetic stirrer or with a simple homogenizer of the stator-rotor type. The use of high shear mixers decreases the mean droplet size to the range of 0.65-1.1 μm. A monodispersed submicron emulsion can be prepared efficiently with a high-pressure homogenizer. High-shear mixers should be used for premixing before the homogenization process is conducted by repeatedly passing the course dispersion through the high-pressure homogenizer.

The emulsion preparation process involves the following steps, which are schematically illustrated in Figure 4.

(1) Three different approaches can be used to incorporate the drug and/or the emulsifiers in the aqueous or oil phase. The most common approach is to dissolve the water-soluble ingredients in the aqueous phase and the oil-soluble ingredients in the oil phase. The second approach, which was used in fat emulsion preparations (Idson, 1988), involves the dissolution of an aqueous-insoluble emulsifier in alcohol and then the dispersion of the alcohol solution in water, evaporation, and total removal of the alcohol until a fine dispersion of the emulsifier in the aqueous phase is reached. The third approach, which was mainly used for amphotericin B incorporation into an emulsion, involves the preparation of a liposome-like dispersion (Davis et al., 1987; Forster et al., 1988). The drug and

phospholipids are first dissolved in ethanol, dichloromethane, or chloroform or a combination of these organic solvents and then filtered into a round-bottomed flask. The drug-phospholipid complex is deposited into a thin film by evaporation of the organic solvent under reduced pressure. After sonication with the aqueous phase, a liposome-like dispersion is formed in the aqueous phase. The filtered oil phase and aqueous phase are heated separately to 70°C and then combined by magnetic stirring.

(2) The oil and aqueous phases are then emulsified with a high-shear mixer at 70-80°C.

(3) The resulting course emulsion (1-5 μm) is then rapidly cooled and homogenized into a fine monodispersed emulsion (0.1-0.5 μm) with a two-stage pressure homogenizer.

(4) Finally, the pH content of the emulsion is adjusted to the desired level, and the emulsion is filtered to discard course droplets and debris generated during the emulsification and homogenization processes.

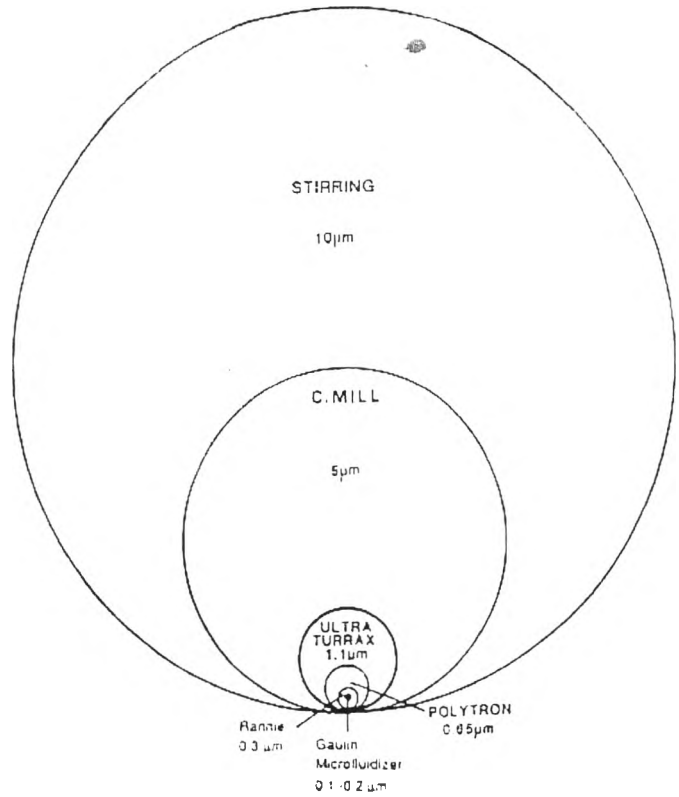


Figure 3. Effect of emulsification equipment on the mean droplet size of a submicron emulsion; C. Mill. Brogli Co., Appschwill, Switzerland: ULTRA TURAX (Janke Kunkel GmBh, Staufen, Germany): POLYTRON (Kinematica Ltd., Luzern, Switzerland) (From Benita and Levy, 1993)

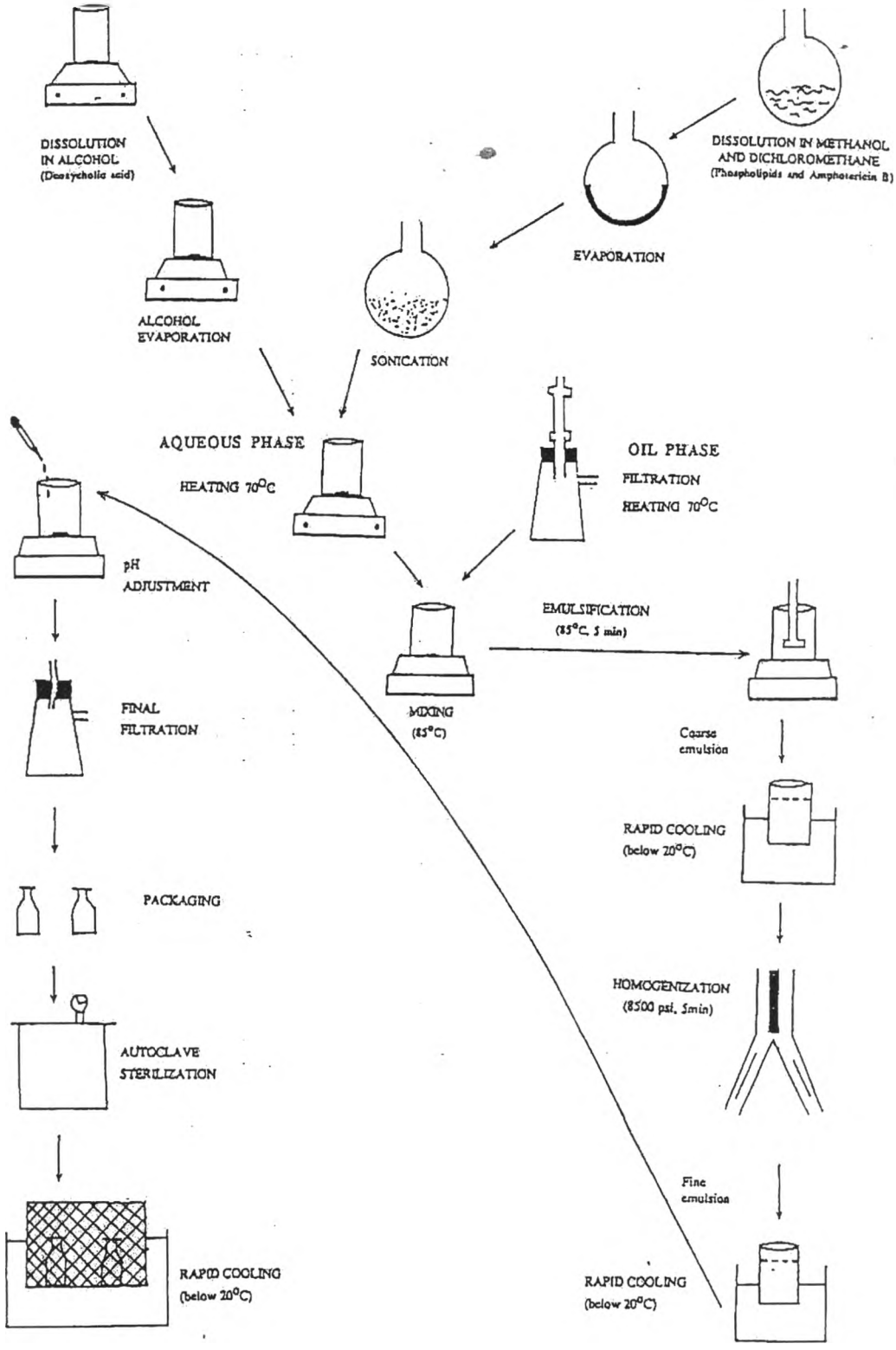


Figure 4. Schematic description of the submicron emulsion manufacturing process (From Benita and Levy, 1993)

Usually, the whole preparation process is conducted in a laminar flow hood under a nitrogen atmosphere in case excipients and drugs sensitive to oxidation are used. Sterilization is normally achieved either by the use of a standard steam procedure (autoclaving) or by the maintenance of aseptic conditions during the entire preparation process, depending on the sensitivity of the active ingredients to elevated temperatures.

High-pressure two-stage homogenizers of the Gaulin or Rannie type (APV Gaulin, Hilversum, The Netherlands, or APV Rannie, Inc., Albertsland, Denmark, respectively) or microfluidizers (Microfluidics Corp., Newtown, MA) based on an interaction chamber mechanism not disclosed by the manufacturer can be used to decrease the droplet size of the emulsion to the submicron size range (Levy and Benita, 1989).

7. Emulsion characterization

7.1 Particle size and size distribution

Particle size and size distribution of the oil droplet is one of the most important characteristics of an emulsion. Both the stability and the viscosity depend on the droplet size distribution. For example, the sedimentation and creaming tendency of an emulsion could be minimized by the reducing the mean particle size of the system. Measuring the change in droplet size can conveniently monitor the emulsion stability. In general, the intravenous fat emulsions should

contain particle in the range of 10 nm - 1 μm. The droplet size of emulsion which greater than 5 μm are clinically unacceptable because they can cause the formation of pulmonary emboli.

There are some commonly used methods to determine droplet size of the emulsion (Weiner, 2000a). These are:

1. Light Microscopy

This method is still the most commonly used to determine droplet size. It requires the dilution of the emulsion and the counting of 500 to 2000 globules. The use of photomicrographs and mechanical devices can reduce the tedium of counting.

2. Reflectance Measurements

The measurement bases on the observations that the color intensity imparted to an o/w emulsion decreases as the globule size decreases. Oil-soluble dyes can be added to colorless oils. However, this method is not suitable to determine particle size distribution.

3. Centrifugation

This method is based on the principle that sedimentation under the influence of centrifugation proceeds at a rate dependent on droplet size. A modified Stoke's Law equation can be used. This method is considered too complex for routine procedures.

4. Coulter Counter

This instrument enables large members of particles to be analyzed automatically and very rapidly. The method is based on the fact that the emulsion is a non-conductor as compared to an electrolyte solution. Conductivity of the cell decreases as a droplet of emulsion pass through an

aperture to displace an equal volume of electrolyte solution. The large the droplet, the greater the conductivity loss. The application is limited to o/w emulsions and requires dilution with electrolyte solution.

5. Microscopic studies

5.1 Transmission Electron Microscopic (TEM) examination

The simplest technique used for measuring emulsion particle size and evaluating emulsion stability through the detection of coalescence in emulsion containing droplets smaller than 500 nm in size involves an optical microscope. TEM method was developed by Du Plessis et al. (1986) for the determination of particle size in parenteral fat emulsion.

5.2 Scanning Electron Microscopic (SEM) examination

SEM method is also a valuable tool in the study of droplets in parenteral emulsions. SEM provides the three-dimensional appearance in shape and size of the fat droplets that could improve the interpretation of results.

6. Freeze Fracturing Techniques

The electron microscope which involves the rapid freezing of emulsions, fracturing of the frozen emulsions, and the preparation of carbon replicas provides a very accurate method for studying fine polydispersed suspensions and emulsions. This technique is capable of visualizing molecular dimensions and can identify the fine structure of large molecules, such as lipids or other structures, e.g., lamellar or monolayer structures, that are yielded by emulsifiers. The presence of vesicles, micelles, liquid crystals and other structure together with fat droplet is possible to detect by

this method (Buchheim, 1982; Groves et al., 1985; Rotenberg et al., 1982; Westesen and Wehler, 1992).

Clearly, it is difficult to use a single method for determination of all particle sizes in such wide range. For most intravenous emulsions, the mean diameter as well as particle size distribution can be accurately determined by the photon correlation spectroscopic method (Muller, and Muller, 1984).

7. Photon Correlation Spectroscopy

There are two complementary particle size analysis methods namely the photon correlation spectroscopy (PCS) method (Benita and Levy, 1993; Groves, 1984; Phillies, 1990; Westesen and Wehler, 1992;), which is considered most appropriate for studying droplets smaller than 1 μm in size. The other method is the computerized laser inspection system (Benita and Levy, 1993), which can measure droplet size greater than 0.6 μm, are needed to effectively cover the measured size range of 50 nm to 10 μm. The advantage of the laser inspection system over the widely used Coulter Counter system is that there is no need for an electrolyte solution, which can affect the stability of the emulsion.

The distribution frequency coming from the analyzer is generally in volume, i.e., the proportion of droplets included in a class is the volume proportion of these drops with respect to the whole internal phase volume. In some cases, the distribution in number or in surface is also required. It is worth remarking that if the distribution follows a log normal statistic, as is often the case, the Hatch-

Choate relationships may be used to rapidly translate volume distribution frequencies into equivalent number or surface distribution frequencies (Salager, 2000).

The notion of average size or distribution central tendency is a tricky one since there are many ways to calculate the mean, which are not at all equivalent, particularly with asymmetrical distributions. The mode is the most common size, while the median is the size so that half the internal phase volume is fragmented in smaller drops and half in larger ones. It is often symbolized as D (1/2) or D (v,0.5).

Other used mean values are the mean of the distribution in volume or mass, so-called D (4,3) because it is the ratio of the fourth moment of the distribution in number to the third one, or the mean of the distribution in surface, so-called Sauter mean diameter SMD or D (3,2), i.e., the ratio of the third moment to the second one. This last mean diameter is the diameter of the sphere that has the same surface/volume ratio as the whole population, the most significant value as far as the surfactant adsorption is concerned.

$$D(4,3) = \frac{\sum_{i=1}^{n} f_i x_i^4}{\sum_{i=1}^{n} f_i x_i^3} \qquad D(3,2) = \frac{\sum_{i=1}^{n} f_i x_i^3}{\sum_{i=1}^{n} f_i x_i^2}$$

Where f_i is the frequency in number in class i, characterized by its central diameter x_i. The arithmetic mean m or first moment is calculated as:

$$m = M_1 = \sum_{i=1}^{n} f_i x_i$$

The degree of polydispersity is given by the standard deviation, symbolized by σ, which is the square root of the second moment around the first moment value.

$$\sigma = \sqrt{\sum_{i=1}^{n} f_i (x_i - m)^2} = \sqrt{M_2 - M_1^2}$$

Where m and σ are the two parameters that appear in the theoretical expression of the normal distribution density:

$$f(x) = k \exp \left(\frac{(x - m)^2}{2\sigma^2} \right)$$

Where k is a numerical coefficient so that the integral below the frequency curve is the unity. If the distribution is log normal, then x, m, and σ are replaced by their natural logarithms.

Drop size distributions are often plotted as frequency against logarithm of diameter to test the symmetry around the first arithmetic mean value of first moment m of and assumed log normal distribution, which is a quite common test.

If the distribution is symmetrical (in the selected scale), the mode corresponds to the median, i.e., 50% of the calculated drop volume or number whatever the distribution is. Another way to estimate the asymmetry is to calculate the third moment of the distribution around the mean, which is zero for perfectly symmetrical distributions.

$$M_3 = \sum_{i=1}^{n} f_i (x_i - m)^3$$

The distribution shape and its changes are handy information on the emulsion formulation process or its evolution. For instance, the presence of two mode indicates two separate stirring processes, and could be related to the mixing of two emulsions or to improper stirring. An asymmetrical distribution with a long tail on the higher drop size particularly in logarithmic scale is indicative of incomplete mixing. The increase of the frequency in the same region as time elapses is a precursor signal of future stability problems. The disappearance of the left part of the main peak (smaller drop) is indicative of a slow ripening process. It is recommended that all practitioners practice extracting information from the drop size distribution.

7.2 Droplet surface charge

Emulsifiers can stabilize emulsion droplets, not only through the formulation of a mechanical barrier but also through the production of an electrical (electrostatic) barrier or surface charge. The electrical surface charge of droplets is produced by the ionization of interfacial film-forming components. The surface potential (zeta potential) of emulsion droplets dependent upon the extent of ionization of the emulsifying agent. The extent of ionization of some phospholipids present in lecithin is markedly pH dependent (Bangham, 1968; Davis, 1982).

The electrical charge on emulsion droplets is measured by use of either a ZetaPlus (Brookhaven Instruments, USA) or the moving-boundary electrophoresis technique, which has been shown to yield accurate electrophoretic mobility data.

ZetaPlus is an instrument for measuring the velocity of charged-colloidal particles in liquids. The electrophoretic mobility is determined by dividing the measured velocity by the electric field strength. Finally, the zata potential is calculated from the solution conditions and the mobility. Either mobility or zeta potential may be used as measures of dispersion stability, though zeta potential is more meaningful parameter.

High zeta potential (>-30 mv) should be achieved in most of prepared emulsions to ensure a high-energy barrier, which causes the repulsion of adjacent droplets and results in the formation of a stable emulsion (Benita and Levy, 1993).

7.3 pH

The pH of the emulsion should be monitored continuously over the entired shelf life of the emulsion to detect detrimental 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.

7.4 Osmolality

Osmolality, by difinition, is an expression of the total number of solute particles dissolved in one kilogram of solvent without regard for particle size, density, configuration, or electrical charge.

Traditionally, osmolality has been expressed as milliosmols per kilogram, with various abbreviations such as mOs/kg, mOsm/kg, and mOsmol/kg. The letters "Os" signify that osmolality is defined as the concentration, expresses on a molal basis, of the osmotically active particles in true solution.

It is very important for intravenous injections to have the osmolality of the product approximately equal to serum osmolality to avoid any toxicity occurring from the administration of hypotonic or hypertonic solution. The commonly accepted osmolality for peripheral administration of

parenteral nutrition solution is less than 900 mOsmol/L (Reich et al, 2000). However, the prepared formulation should have the osmoticity close to that of serum osmoticity (300mOsmol/L) or no greater than 900 mOsmol/L.

The commission of Clinical Chemistry of the International Union of Pure and Applied Chemistry (IUPAC) and the International Federation of Clinical Chemistry (IFCC) have recommended that the unit of osmolality is mmol/kg, and this has been adopted by the American Journal Chemical Chemistry as part of its general acceptance of Standard International units.

The easy way to determine whether the solution is isotonic, hypotonic, or hypertonic is by observing the change in the appearance of the erythrocytes when suspended in a solution. If the cells retain their normal characteristics, the solution is isotonic, but if haemolysis or marked change in the appearance of the erythrocytes occurs, the solution is not isotonic with the cells.

The osmolality of the preparation can also be determined by the use of osmometer. Vapro® 5520 osmometer is an electronic adaptation of the hygrometric method of vapor pressure determination. In direct means, the measurement of the osmolality is afforded by the fact that the addition of solute particles to a solvent changes the free energy of the solvent molecules. This results in a modification of the cardinal properties of the solvent, i.e., vapor pressure, freezing point, and boiling point. Compared with pure solvent, the vapor pressure and freezing point of a solution are lowered, while its boiling point is elevated, provided that a single solvent is present in the solution. Solutions containing more than one solvent generally behave in more complex ways.

In single-solvent solutions, the relative changes in solution properties are linearly related to the number of particles added to the solvent, although not necessarily linearly related to the weight of solute, since solute molecules may dissociate into two or more ionic components since this properties all change linearly in proportion to the concentration of solute particles, they are known as "colligative" properties.

Osmotic pressure is also a colligative property of a solution, but unlike the other three, it is not a cardinal property of the solvent. Solution osmotic pressure can be measured directly using a semi-permeable membrane apparatus, but only with respect to those solute particles that are impermeable, since smaller solute particles freely transude the membrane and do not directly contribute to osmotic pressure. Such a measurement is referred to as "colloid osmotic pressure" or "oncotic pressure". It is expressed in terms of pressure, in mmHg or kPa. Total osmotic pressure, i.e., that which can be calculated on the basis of total solute concentration is a theoretical concept only.

The measurement of total solution concentration, or osmolality, can only be made indirectly by comparing one of the solution colligative properties with the corresponding cardinal property of the pure solvent. The first practical lab laboratory instruments developed for routine measurement of osmolality were based upon depression of the freezing point and, until recently years, all osmometers for large-scale testing were based upon this methodology.

The Vapro osmometer embodies newer technology. It is based upon a measurement of vapro pressure depression made possible by thermocouple hygrometry. The vapor pressure method enjoys a significant intrinsic advantage over the measurement of either freezing point depression or

boiling point elevation in that it can be performed without the necessity for a change in the physical state of the specimen. It is thus a passive technique of measurement that is free from measurement artifacts that often occur when the specimen to be tested must be altered physically. This fundamental difference in methodology gives to the many advantages of the vapro pressure osmometer over the older method.

7.5 Viscosity or rheological properties

The primary purpose of rheological testing is as a quality control tool rather than as an instrument to gather theoretical insight. One of the factors affect the rheology of the emulsion is effect of emulsifier. Interfacial viscosity, droplet size, and viscosity of the external phase is influenced by the emulsifiers. Generally, an increase in the concentration of the emulsifier will increase the viscosity of the emulsion. Droplet size and size distribution on viscosity is complex since these effects depend on emulsion type, elasticity of the emulsion droplets, phase volume ratio and shear rates. However, when the size of the droplets is reduced, the emulsion is more crowded resulting in increasing of viscosity.

8. Safety

Not only the stability but the safe during until after lipid emulsion administration should be concerned. The ingredients used in emulsion formulation must be safe for human use. They must be metabolized in the body. So, the emulsion should be made up from the non-toxic, biocompatable and biodegradable materials to ensure safety and avoid any toxic reactions that might occur during intravenous use.

While administration of fat emulsion into the body, it will be distributed rapidly throughout the circulation and then cleared. Typically, 80-95% of the total injected particles is cleared by fixed macrophages of the recticuloendithelial system (RES) in the liver, spleen, lungs, and bone marrow (Poste and Kirsh, 1983; Seba, 1970).

However, Lucks et al. (2000) revealed that parenteral fat emulsions with a droplet size of approximately 0.2 to 0.5 μm are too big to pass through the capillary endothelium to liver and spleen. About 50% of the emulsion is taken by the muscle tissue, 25% by the spleen, 14% by the myocardium, and 13% by the subcutaneous tissue (Davis et al, 1985). Thus it may be concluded that the fat emulsion is treated as endogenous particles and is not trapped by the defense mechanism of the body, reticuloendothelial system.

If this was the case, large quantities of infused fat emulsion would be found in the liver and spleen and to a much lesser extent in the lungs (Wretlind, 1976). Davis et al (1985) suggested that

accumulation of fat in the reticuloendothelial cells could occur depending on the composition of the emulsion (emulsifier) and the size of the emulsion droplet.

Particle size, surface characteristics, and surface charge of the oil droplet, involve in the clearance of the emulsion from the blood stream by RES. In general, it has been shown that fine particles are cleared more slowly than coarse droplets. While charged particles, either positive or negative are cleared more quickly than neutral particle (Stossel et al., 1972). As well, emulsions stabilized by low molecular weight emulsifier are cleared more rapidly than those stabilized by high molecular weight emulsifiers (Geyer, 1967).

Davis et al. (1985) has examined the uptake of small lipid droplets by hepatic and Kupffer cells. They found that the uptake of small particles by hepatic cells through sinusoids in blood capillaries was dependent on the size and potency of the hepatic sinusoids, In addition, the size, composition, structure, and charge on the particles was considered to be relevant. A critical particle size of 100 nm was proposed to be suitable for uptake by hepatic cells.

The effect of lipid composition on subsequent activity of the reticuloendothelial system has been investigated by Davis et al. (1985). They found that different lipid emulsion could affect the reticuloendothelial system up to 72 hours after injection. DiLuZio and Riggi (1964) have also use lipid emulsions to assess reticuloendothelial function. An exponential clearance of emulsion particles from the blood was observed and the administration of colloidal carbon particles during elimination of the emulsion gave a competitive inhibitory effect. The test emulsion was cleared almost exclusively by the process of phagocytosis and therefore could not be used to study the activity of chylomicrons.

Interestingly, they found that a cottonseed oil emulsion stabilized by a mixture of nonionic emulsifiers (poloxamer), as well as the commercial emulsion, Intralipid®, was not removed to an appreciable extent by the process of phagocytosis. The nature of the emulsifying agent and size of the particles were thought to be of considerable importance.

Davis and Hansrani (1985) studied the phagocytosis of fat emulsion droplets *in vivo* by using mouse peritoneal macrophages. Their results indicated that the emulsion particles were readily taken up by the macrophages and the rate of uptake was influenced by the surface charge on the particles, and on their sizes. Thus, it appears that kinetically stable emulsion systems are taken up largely by the RES like other colloidal particles.

Accordingly, Gregoriadis and Neerunjum (1974) revealed that rate of removal of liposomes from the circulation was dependent on vesicle size and surface charge vehicle. Positive liposomes cleared less rapidly than negative ones. By this reason, it is believed that positively charged submicron emulsions may alter the pharmacokinetic profile of the incorporated selected drug resulting in enhanced localization of higher drug concentration in target organ. This hypothesis has already been proposed by other authors who investigated charge-reversed submicron emulsions (Davis et al., 1992). However, some investigators reported the toxicity of positively charged stearylamine liposomes either in cell culture systems or *in vivo* (Campbell, 1983; Magee and Miller, 1972; Mayhew et al., 1987). Adams et al. (1977) reported neurotoxicity of stearylamine liposomes after intracerebral injection to mice. However, it was believed that the dose that used in this study was too large. Kimelberg (1980) reported that one-thirtieth of the dose injected intracerebrally to a monkey did not produce any obvious toxic

effect. Likewise, Klang et al. (1994) observed no markedly acute toxicity effect occurred when intravenously injected positively charged submicron emulsion to mice. In fact, positively charged emulsified droplet did not penetrate through the blood-brain barrier and, therefore, did not reach the brain. However, no clear conclusion could be drawn from all cited publication on the toxic of stearylamine liposomes.

The emulsion would appear to be without an effect on red blood cells. The toxicity of surfactants contained in intravenous preparation was reported by many investigations. One of those was the study of Davis et al. (1985) which indicated that the emulsion could affect the rigidity of red blood cells and produce viscoelastic dilatency. Lysophosphatidylcholine and oleic acids which are the most important degradation products resulting from the hydrolysis of phosphatidylcholine can cause haemolysis. Oleic also has penetration enhancing properties.

9. Emulsion as drug delivery system

Because the close resemblance of fat emulsion profiles to chylomicrons, it is suggested that fat emulsion can be used as carrier for drug and possibly as targeted delivery system (Lucks et al, 2000).

Emulsions are useful for the delivery of therapeutic agents to the reticuloendothelisl system. Their potential applications range from antifungal agents to immunomodulators. These systems may also be used for localized therapy, or injection into other body cavities or joints. Emulsions hold great potential in the area of computer tomographic representation of reticuloendothelial organs. Although

they share the disadvantages common to all colloidal systems, some of the advantages of emulsion systems over other particulate carriers, such as liposomes and microparticles are:

(i) They are biodegradable.

(ii) Technologies exist for the large-scale production.

(iii) They have reasonable shelf life stability, and

(iv) They are clinically acceptable.

Lipid emulsion as drug carriers have been investigated in a number of studies. The use of soybean oil emulsion as carrier for lipid-soluble drug has been pioneered by Jeppsson (1975) who used an intralipid-like emulsion as vehicle for administration of four local anesthetics. In the formulation, the drug, as barbituric acids, cyclandelate, diazepam, or local anaesthetic, was dissolved in the oil phase of the emulsion. The routes used have been intravenous, intraarterial, subcutaneous, intramuscular, and interperitoneal. The lower acute toxicity values were found in the emulsion form. Another important indication from the pharmacological data of the emulsion dosage forms was the absence of the initial peaks associated with the administration of the aqueous solution of the drug. A delay in absorption of the drug from the emulsion was also observed which was not as pronounced as when soybean oil was used exclusively as the vehicle (Jeppsson, 1972).

Diazepam is used in anesthesia intravenously and in the treatment of epileptic convulsion. According to its low solubility in water, when the drug solution is diluted with blood, precipitation of diazepam in the vein occurred. Pain and vein infection is also often observed. Because of these, the

development of an emulsion formulation containing diazepam was carried out (Dardel, Mebius, and Mossberg, 1976; Jeppsson, Groves, and Yalabrik, 1976; Jeppsson and Ljungberg, 1975; Levy and Benita, 1989; Levy and Benita, 1990; Levy et al, 1989; Winsnes, Jeppsson, and SjÖberg, 1981). An emulsion dosage form of diazepam has been systematically investigated. It was found that incorporation of diazepam into the oil phase of a soybean oil-in-water emulsion considerably reduce the toxicity reactions of the drug that was found in the conventional solution dosage form. The therapeutic index of the drug in the emulsion was found to be about four times that of the solution form (Jeppsson and Ljungberg, 1975). von Dardel et al. (1983) indicated that a sharp decrease in the number of local side effects can be found compared with conventional aqueous solutions of diazepam.

For barbituric acids (Jeppsson, 1972), when the drug was administered in the oil phase of soybean emulsion, a prolongation of anesthesia was observed in comparison with the aqueous dosage form. The results were explained either by a slow release of the drug from the oil particles or the possibility of more specific delivery of the drug to the central nervous system (CNS).

Similar results were found in linocaine emulsion dosage form in the studies of Jeppsson (1975) on the intravenous administration of lignocaine to the cat in a fat emulsion. The prolongation of response was thought to be a combined mechanism of trapping lipid particles in the myocardium and the slow release of the drug from the particles.

The prolongation of sleep induced by the delivery of pentobarbital in a lipid emulsion was found to an alteration in the metabolism of the drug by the presence of large quantities of triglyceride (Buszello and Müller, 2000).

Other hypotic drugs, as propofol (Simons et al., 1988) and etomidate (Suttmann et al., 1989), were also investigated.

An emulsion dosage form of corticosteroids also found the superior over the aqueous solutions. Higher plasma concentration, higher accumulation and alteration of tissue distribution of the drug were observed in the emulsion form (Lucks et al, 2000; Buszello and Müller, 2000).

Prostaglandin-E1 was formulated using a soybean oil emulsion as a carrier. A lower incidence of irritation when given intranasally for inhibition of bronchoconstriction was reported in an emulsion formulation (Buszello and Müller, 2000).

The emulsion formulation of amphotericin B caused a decrease in toxicity results in an improvement in its therapeutic index. El-sayed and Repta (1983) reported that intravenously administered emulsion formulation was tolerated at the highest level tested, approximately 9 mg/kg, whereas, the maximum tolerated dose for similarly injected fungizone was 1 mg/kg. The lethal dose at 50% (LD_{50}) for both formulations were similar.

The use of emulsion as drug carrier systems was also applied in many areas such as in chemotherapy (mitomycin C or bleomycin), in adjuvants with cancer immunotherapy, or in computerized tomography as contrast agent hepatoma. Recently, parenteral fat emulsions have also been used as adjuvants for the application of vaccinations (Lidgate et al., 1989; and Lidgate et al., 1992).

The variety of drugs in emulsion formulations for various routes of administration under investigation is given in Table 3.

Table 3. List of drugs under investigation for incorporation in emulsion (From Busello and Müller, 2000)

Drug	Route of administration	Indication
Amphotericin B	IV	Systemic antifungal agent, use in neutropenic patients
Barbiturates	IV	Sedation, anaesthesia
Cyclosporin	IV	Immunosuppressant
Deoxycholate-amphotericin B	IV	AIDS-associates cryptococcal meningitis
Diazepam	Topical	Sedation
Epirubicin	Transfemoral cannulation, intrahepatic arterial infusion	Chemotherapy, hepatocellular carcinoma
Etomidate	IV	General anaesthesia
Isocarbacyclin methyl ester ($PG\text{-}1_2$)	IV	Thrombotic disorders
Micronazole	IV and topical	Antifungal
NSAID	Topical	Analgesia, anti-inflammatory
Palmitoylrhizoxin	IV	Cytotoxic
Perfluorooctyl bromide: oxygen	IV	Blood substitutes
Perfluorooctyl bromide: imagent	IV	Contrast agent
$PG\text{-}E_1$	IV	Antiplatelet and vasodilatory actions
Physostigmine	Oral	Anticholinesterase, Alzheimer's disease
Pilocarpine	Ocular	Glaucoma treatment
Pregnanolone	IV	General anesthesia
Tetrahydrocannabinol	Ocular	Glaucoma treatment
Vitamin E	Topical	Antioxidant

In spite of considerable research activities, only the parenteral fat emulsions incorporated drugs that are listed in Table 4 are commercially available.

Table 4. Registered emulsions containing drug (From Lucks et al., 2000)

Brand name	Manufacturer	Drug	Oil phase	Applications
Diazepam-Lipuro	B. Braun Mesungen	Diazepam	Soy oil + MCT	IV
Disoprivan	Zeneca	Propofol	Soy oil	IV
Etomidat-Lipuro	Braum Mesungen	Etomidate	Soy oil + MCT	IV
Lipotalon	Merck	Dexaanethasone palmitate	Soy oil + MCT	i. arthr
Stesolid	Dumex	Diazepam	Soy oil, acetylated monoglycerides	IV

However, the influence of particle structures and constituents on the pharmacokinetics of incorporated drugs and bioacceptance of the carrier is considered (Table 5). A wide variety of surface-active agents can be used to prepare emulsion formulations. This variety is tremendously narrowed in the case of parenteral formulations. One of those major obstacles is the haemolytic effect of the majority of emulsifiers, which excludes their use for intravenous application (Buszello and Müller, 2000).

Table 5. Factors that may affect the tolerability of drug emulsions (From Busello and Müller, 2000)

Variable	Potential effect	Comment
Lysophospholipids	Haemolysis	Only at very high non-therapeutic doses (25-30%)
Additional synthetic phospholipids	Unexpected toxicity	May vary depending on phospholipids
Degree of saturation of phospholipids/ fat	Increase in total and LDL-cholesterol effects on membrane proteins	May occur on continuous infusion
Content of MCT	Increase in total and LDL-cholesterol and CNS effects	May occur on continuous infusion and/or at high dose
Cationic phospholipids	Increase immunogenicity of transported drug	Most likely with liposomal forms

An obvious major consideration in the use of emulsions in drug delivery is that a given emulsion-drug formulation should exhibit clear benefits over and above those seen with the conventional formulation of the drug (Table 6).

Table 6. Advantages of emulsion formulations over comparison conventional formulations (From Busello and Müller, 2000)

Drug	Advantages
Amphotericin B in Intralipid 20%	Fewer side effects, including renal impairment or chills equivalent anticandidiasis activity
Cyclosporin	Reduced nephotoxicity
Diazepam (Diazemuls)	Reduce thrombophlebitis improved LD_{50}(mice)
Propofol in Intralipid 10% (Diprivan)	Reduced local pain on IV injection

In most cases, the incorporation of drugs drastically reduces the physical stability of the emulsion. Therefore, only a few products are on the market despite considerable endeavors. Table 4 shows the emulsion preparations available on the market.

However, the potential of emulsion as delivery system can be further research. Many areas still need the development.