CHAPTER II

LITERATURE REVIEW

1. Definitions

Emulsions can be defined as a mixture of at least two immiscible liquid or liquid crystral phases with the interface being stabilized by an adsorbed monomolecular or micellar layer of surface active agents as shown in Figure 1.
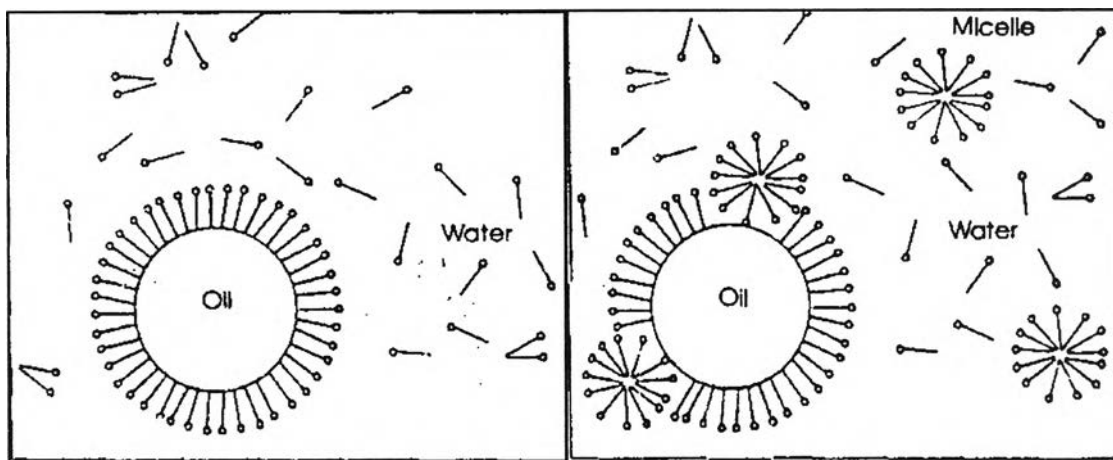

Figure 1. Schematic cross-sectional view of surfactant adsorption at oil/water interface. Oil particle stabilized by condensed monolayer of a surfactant molecule symbolized by circle for polar or ionic head and straight tail for hydrocarbon chain (left). Oil particle stabilized partly by adsorbed single surfactant molecules and partly by micellar aggregates (right) (Nielloud and Marti-Mestres, 2000).

Depending on the nature of the dispersed and continuous phases, different types of emulsions can be distinguished: oil-in-water (o/w) or water-in-oil (w/o) emulsions. If the dispersed phase represents an emulsion itself the systems formed are called multiple emulsions (Nieulloud and Marti-Mestres, 2000). 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. 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). 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, Müller and Klütsch, 2000). Due to the large interface and the high interface energy, PFEs and nanoemulsion 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 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; Gest and Langevin, 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, Müller and Klütsch, 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. The oil phase of the emulsion is normally based on long-chain triglycerides (LCT) containing fatty acid chain with 14-22 carbon atoms and sometimes with double bonds. Purified soybean or safflower oil contains LCT with a high proportion of n-6 polyunsaturated fatty acids whereas olive oil has LCT with n-9 monounsaturated fatty acids. Soybean oil composes of 50-57% linoleic acid, 5-10% linolenic acid, 17-26% oleic acid, 9-13% palmitic acid and 3-6% stearic acid (Wade and Weller, 1994). Fish oil includes LCT with 20 or more carbon atoms where the first double bond is located between the third and the fourth carbon (Tamilvanan, 2004). Apart from LCT, the medium-chain triglycerides (MCT) which are derived from coconut oil and compose predominantly of fatty acids containing 6-12 carbon atoms. MCT has been reported to differ from LCT as they are metabolized faster and undergo limited storage in tissues (Lai *et al.*, 2005). Both LCT and MCT either alone or MCT in combination with LCT have known for their long term commercial acceptability in parenteral lipid emulsions and are found in several FDA approved products. Driscoll *et al.* (2002) and Lai *et al.* (2005) demonstrated that lipid emulsions composed of MCT and LCT yield more stable formulations compared with those compounded with pure LCT lipid emulsions. Also in Europe lipid emulsion containing LCT/MCT enriched with fish oil became available for research. With MCT/LCT combinations in a specific ratio, lipid emulsion appears to provide a more readily metabolizable source of energy.

However, LCT lipid emulsion has been used in clinical practice for over 40 years. But for drug solubilization purpose, MCT is reported to be 100 times more soluble in water than LCT and thus to have and escalated solubilizing capability.

In addition, emulsions are thermodynamically unstable and subjected to various instability process like aggregation, flocculation, coalescence, creaming and hence eventually phase separation (Liu, 2000, Tamilvanan, 2004). However, the proper choice of emulsifier and preparation conditions such as input of large amount of energy.

The commonly used emulsifiers can be classified into:

i)   Anionic surfactants include soaps, alkyl sulfates and sarcosinates (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 counterion could have a great effect on the prepared emulsion properties.

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

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 be 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 phospholipids (PL). The natural phospholipids 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) and phosphatidylethanolamine (PE). 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).

Phospholipids can possess fatty acids of different chain length varying from C12 to C24 and unsaturation and may have different hydrophilic species linked to the phosphate such as choline or ethanolamine, according to which individual membranes of the phospholipids category are classified (Table 1). Phospholipids which are high in PC are found to produce emulsion with poor stability as insufficient charge in stabilization of emulsion. Emulsion stabilization by mixtures of charged or uncharged phosphatides may occur as a result of the formation of an interfacial lamellar or liquid crystalline phase. Such mixtures may be optimized by selection of a commercial lecithin that contains appropriate amounts of negatively charged phosphatides such as

phosphatidic acid (PA), phosphatidylserine (PS) or phosphatidylinositol (PI) (Hansrani, Davis and Groves, 1983). Nii and Ishii (2005) have revealed the difference in the PC ratio gives the difference in emulsion stability.

Table1. Compositions of egg and 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 (SM)	2	trace

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. The typical nonionic surfactants are polyethylene glycol stearate, polyoxyethylene monostearate, polyethylene glycol, polyoxyethylene sorbitan monoesters, etc. However, all of these classes of surfactant give rise to toxic reactions at high concentration (Jumaa, Kleinebudde and Müller, 1999). Only some nonionic materials that was found to be safe for use was polyoxyethylene-polyoxypropylene compounds (Poloxamers[®]), available commercially as Synperonics[®]. They have been widely used for formulation of fat emulsion, either on their own or in combination with lecithin.

Polyoxyethylene (20) sorbitan monooleate (Tween® 80), polyoxeyethylene (35) castor oil (Cremophor ® EL) and PEG-660-12-hydroxy-stearate (Solutol HS15) are also commonly used (Buszello *et al.*, 2000; Jumaa and Müller, 2002).

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. The excess use of lecithin may result in free lecithin forming multilamellar structure (i.e multilamellar liposomes) promoting stability at high temperature i.e. autoclaving. Moreover, the free phospholipids in fat emulsion increase the level of serum cholesterol.

It is usually observed that mixtures of surfactants form more stable emulsions than do single surfactants. The reason is the complex formation at the interface resulting in a more rigid stabilizing film. 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 phospholipids and Tween® 80 in stabilizing the emulsion. The optimum weight ratio of egg phospholipids to Tween® 80 was 1:1. The use of emulsifier is proposed to depend upon the hydrophilic-lipophilic balance (HLB)(Lund, 1994) (Table 2).

The HLB has been used as the basis for a more rational mean of selecting and classifying emulsifier. In spite of many advances in the theory of stability of lyophobic colloids, resort has still to be made to an empirical approach to the choice of emulsifier, devised in 1949 by Griffin. In this system, the hydrophile-lipophile balance (HLB) of surfactants in calculated based on a measure of the relative contributions of the hydrophilic and lipophilic regions of the molecule. Many oils and

waxy materials used in emulsions have required HLB value to form the stable emulsions. For example, castor oil has a required HLB value of 14 and soybean oil has a required HLB value of 8 (Krishna, Wood and Shet, 1998; Lund, 1994). HLB value can be adjusted to the required value by using a mixture of two surfactants, the lower HLB and the higher HLB value. A mixture of high HLB and low HLB surfactants give more stable emulsions than do single surfactants.

Table 2. The relationship between HLB numbers and surfactant properties (Lund, 1994).

HLB range	Properties
0-3	Antifoaming agents
4-6	Emulsifying agents (water-in-oil)
7-9	Wetting agent
8-18	Emulsifying agent (oil-in-water)
13-15	Detergents
10-18	Solubising agents

HLB values, however, may not be a precise parameter as the surfactants having the same HLB values may not produce emulsion in the same manner. The HLB values do not take into account the concentration of surfactants. It is often necessary to prepare a series of emulsions in order to determine the optimum concentration of emulsifiers used.

The dispersion medium of parenteral lipid emulsion may contain one or more of the following additives: isotonicity agents; electrolytes; buffer; anti-flocculants and preservative. Normally, parenteral emulsions have low osmotic pressure, therefore additives are needed to adjust the physiological tonicity. Glyerol is the most

recommended isotonic agent. The osmolality of emulsions should be in the range of 280-350 mOsm/L in order to prevent any hemolysis, pain, irritation and tissue damage at the site of administration. However, xylitol and sorbitol are also being used as isotonic agents in parenteral emulsion (Hansrani, Davis and Groves, 1983).

Oxidation can lead to unstable emulsions. Antioxidants are added in the formulation to minimize degradation. The suitable antioxidants are as follows: butylatedhydroxyl toluene (BHT), butylatedhydroxyl anisole (BHA) or tocopherol (Nema, Washkuhn and Brendel, 1997). Furthermore, the explosure to oxygen during the manufacturing process should be avoided. This can be accomplished by purging the solvent system with filtered nitrogen during the manufacturing process, blanketing the product with filtered nitrogen or argon during the filling operation and displacing oxygen from the head space of the filled container with filtered nitrogen (Hansrani Davis and Groves, 1983).

3. Stability of emulsion

3.1 DLVO Theory

From a theoretical standpoint, physical stability is probably the most complex property of emulsions. The key factor in determining the stability of an emulsion is believed to be the interfacial tension between the two phases. The lower interfacial tension (primarily governed by the surfactant) will increase the stability while very low interfacial tension is thought to be the primary factor leading to the stability of microemulsions. A number of models allowing a prediction of the stability of emulsions have been proposed. One of the most useful approaches is the

so-called DLVO theory, developed almost 50 years ago by Derjaguin and Landau (1941) and Verwey and Overbeek (1948). According to this theory, the stability of emulsion depends on a balance of electrostatic repulsive forces and London-type Van der Waals attractive forces (Liu, 2000).

For emulsion stabilized by ionic surfactants, Van der Waals and electrostatic forces are most important (Lawrence, 2004). 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, for uncharged emulsion stabilized by nonionic surfactants, the Van der Waals, steric and salvation forces are most important. Steric 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. Steric stabilization consists of two forces corporating in emulsion stabilization, osmotic or salvation forces and entropic effects. For salvation 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 an overlap region and a 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. 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 phenomenon is thermodynamically

unfavorable, resulting in force droplets apart again or called entropic effect. Generally, steric forces are dependent upon length of polymer chains. Longer the chains, greater the stabilization.

Steric 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. 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.

3.2 Instability of emulsion

Physical instability of emulsions usually begins with creaming i.e. slow floatation of lipid droplets on denser aqueous phase which is usually reversible by shaking. This leads to flocculation and coalescence i.e. aggregation of lipid droplets which is not reversible by agitation. The next stage is creaming of coalesced droplets which ultimately results in separation of oil and aqueous phases as shown in Figure 2 (Collins-Gold, Lyons and Bartholow, 1990; Liu, 2000). Due to this thermodynamic instability, almost all emulsions exhibit an increase in droplet size upon storage. The extent of this process depends upon the characteristics of formulation and its storage condition. Some of the factors that affect the physical stability of emulsions include the type and concentration of surfactant used to stabilize the emulsion, the phase volume ratio (i.e. ratio of oil to aqueous phase), compatibility of drug and excipients with the emulsion and storage condition of the emulsion.

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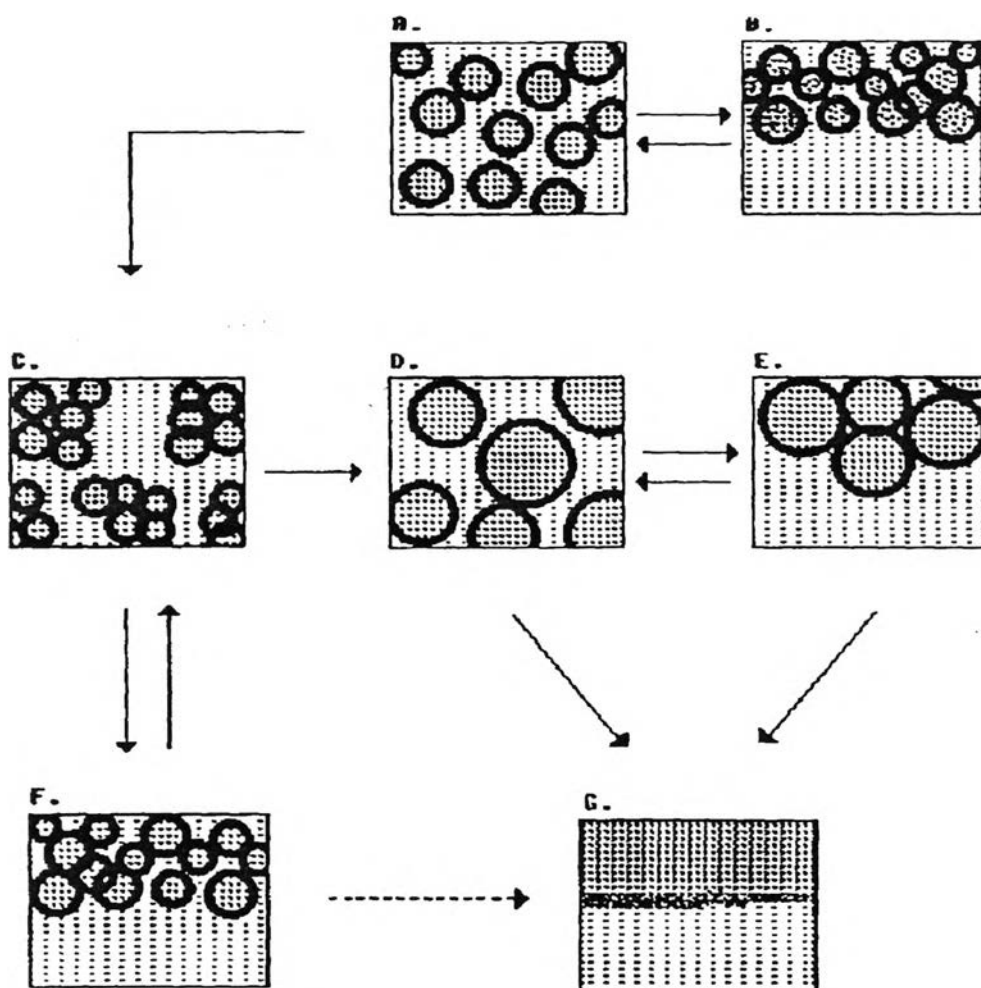

Figure 2. Schematic diagram of physical changes in a lipid emulsion. Solid arrows designate reversibility while the dashed arrow suggests uncertainty. (A) Freshly prepared lipid emulsion; (B) creaming-readily reversible, slow flotation of lipid droplets on more dense aqueous phase; (C) flocculation-aggregated droplets are not readily redispersed by agitation; (D) coalescence-irreversible merging of smaller droplets; (E) rapid creaming of coalesced emulsion; (F) rapid creaming of flocculated emulsion; (G) broken emulsion-separation of oil and water phases (Collins-Gold, Lyons and Bartholow, 1990).

4. Manufacturing Process

The typical preparation of parenteral emulsions consists of four major steps. In the first step, the oil and aqueous phases are conditioned by addition to both phases of components such as the surfactant or emulsifier, glycerol and other components like vitamins and drugs that are oil or water soluble. The emulsion is prepared in the second step that involves making oil-in-water coarse dispersions by combining the pre-heated aqueous and oil phases. Moreover, the rate of addition of the oil phase to the aqueous phase should be controlled and optimized. The rate can be controlled with an appropriately sized pump with the discharge directed towards the impeller blades. Typical equipment utilized for this process includes that manufactured by UltraTurrax (Janke&Kunlex GmbH, Germany) and Polytron (Kinematica, Luzern, Switzerland). The use of these medium shear mixers only decreases the mean globule size to approximately the 1 μm range. If the rate of addition is too rapid, incomplete dispersion of the oil into the aqueous phase can occur. Incomplete dispersion can be detected by direct visualization of small droplets of oil separation from the bulk, which leads to further instability. Optimization of the rate can be achieved by monitoring globule size and size distribution. The optimum size is formulation-specific, but it could be less than 20 μm for the coarse emulsion.

Once the coarse emulsion is formed, it is necessary to reduce the globule size even further by homogenization. An essential requirement for intravenous lipid emulsions is a small globule size, ideally not greater than 2 μm. A small globule size also promotes good physical stability because Brownian movement prevents creaming. The required globule size can be achieved by using various high-pressure

homogenizers or microfluidizers. Descriptions of the different technical approaches for manufacturing submicron emulsions have been reported in the literature (Hansrani, Davis and Groves, 1983). High pressure homogenization is routinely used for the production of emulsions for parenteral administration.

In the homogenizer devices, the crude emulsion is forced under pressure through the annular space between a spring-loaded valve and the valve seat. The emulsion may be homogenized in appropriate pressure and number of times in order to achieve the required particle size. One type of high pressure homogenizer is illustrated in Figure 3. However, a major concern of the homogenization process is the inevitable contamination produced from gasket materials, packing and metal parts. These contaminants also originate from pumps and other metal surfaces. The new method which can minimize the effect is microfluidization.
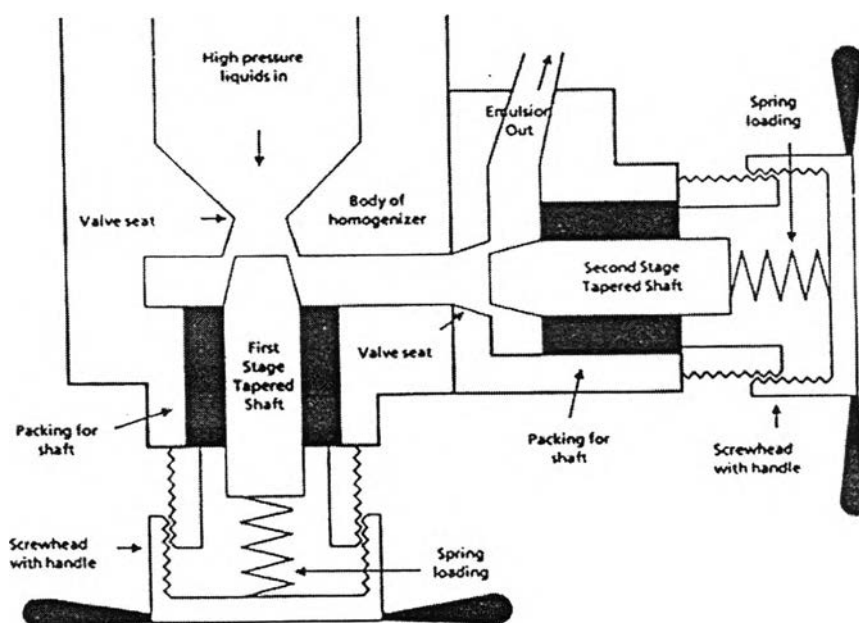

Figure 3. One type of single stage orifice for a high pressure homogenizer (Hansrani, Davis and Groves, 1983).

Microfluidization has been successfully used to produce parenteral emulsion (Block, 1996). Microfluidizer processing is based on a submerged jet principle in which two fluidized streams interact at ultrahigh velocities in precisely defined microchannels within an interaction chamber. A combination of shear, turbulence and cavitations forces results in the energy efficient production of consistently fine droplets with a narrow size distribution. Diagram of the microfluidizer is illustrated in Figure 4.
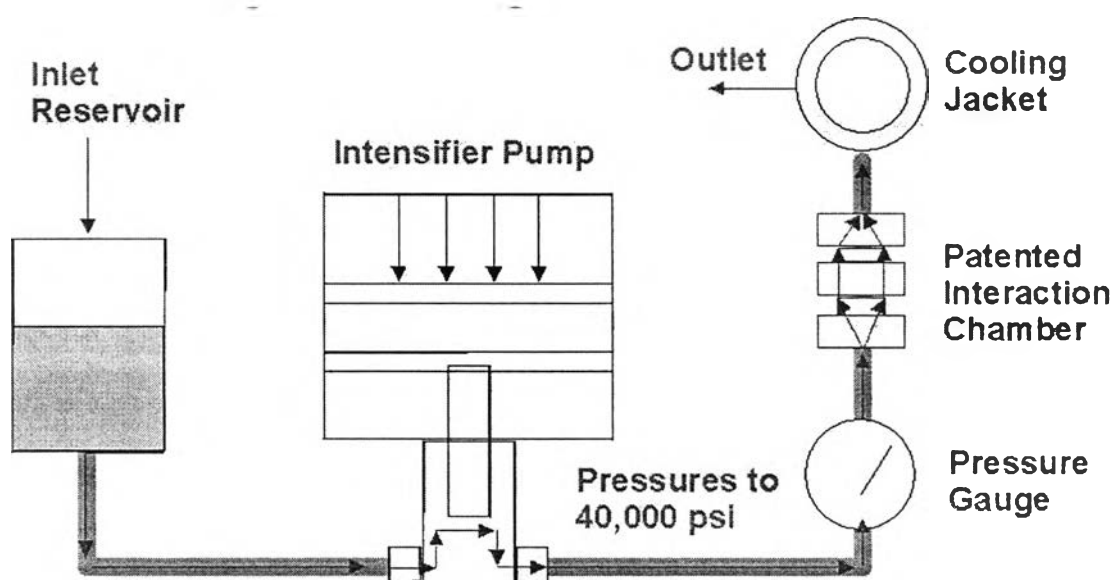

Figure 4. Diagram of microfluidizer (From Microfluidics).

Their utility on either a laboratory or industrial scale must be evaluated because equipment changes during scale-up could affect the physical and chemical stability of the emulsion and also its pharmacological efficacy. For example, Emulsiflex C-50 and Emulsiflex C-5, which are using the same principle but difference in capacity. The Emulsiflex C-50 has a capacity of 15-50 L/hr while the

Emulsiflex C-5 has a capacity of 1-5 L/hr (Avestin). Careful control of this step can be ensured and the globule size measurements are taken to determine the end-point of homogenization if sufficient in process.

Following homogenization, the complete emulsion requires pH adjustment before final filtration to remove large particles, but this cannot remove particles with dimension close to those of the oil droplets themselves. Consideration should be given to the flow rate through the final filter because the presence of small oil droplets will lower the throughput of the membrane filter.

Finally, emulsion should be sterile. Sterility is usually achieved by heat sterilization or filtration. Heat sterilization is preferred because of its advantages with respect to the ease of manufacturing and safety (Collins-Gold, Feichtinger and Wärnheim, 2000). Sterilization conditions must be selected carefully to ensure a sterile product while minimizing degradation of the thermolabile product (Alison, 1999; Cuéllar *et al.*, 2005).
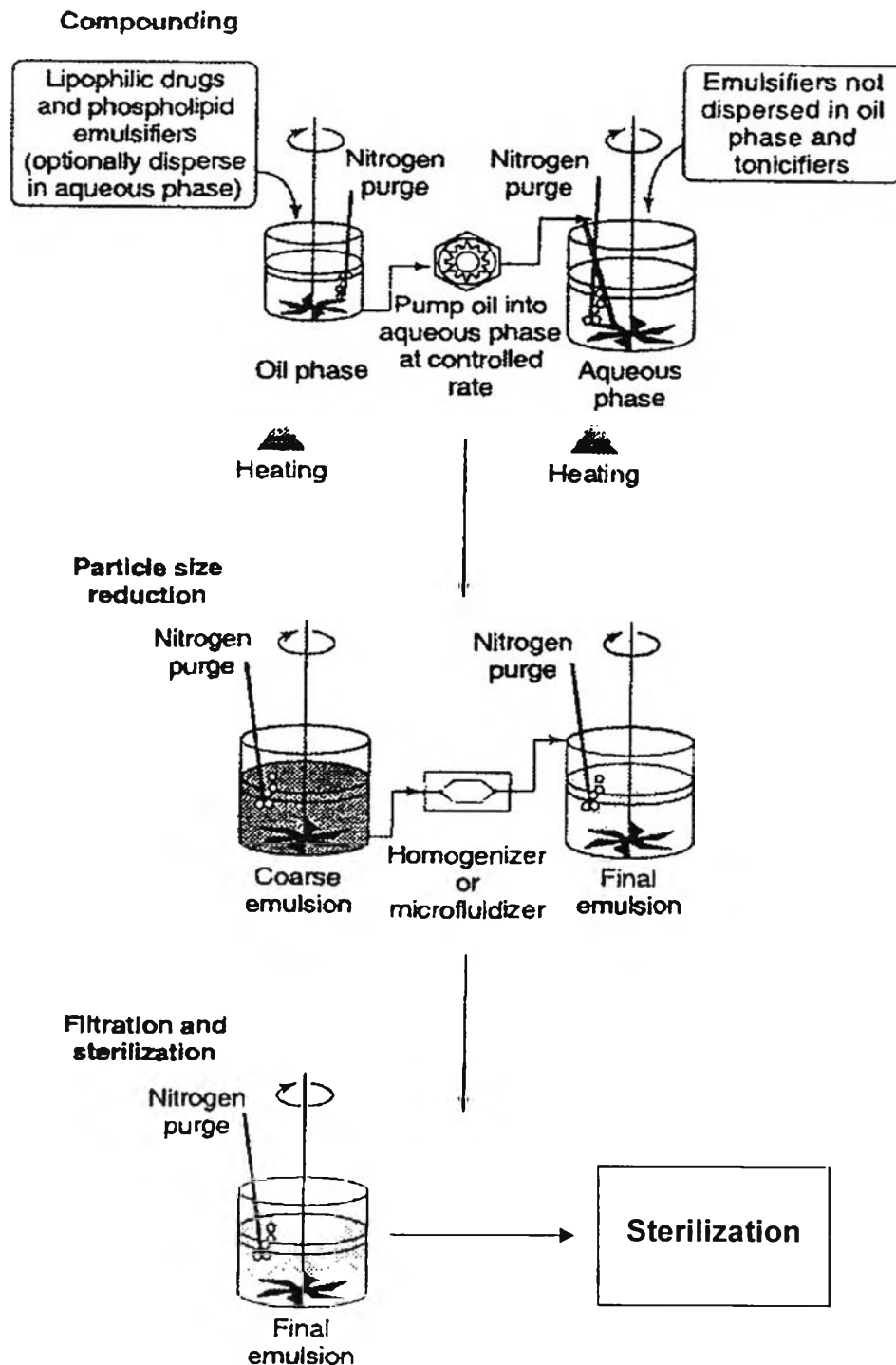
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Compounding

Lipophilic drugs
and phospholipid
emulsifiers
(optionally disperse
in aqueous phase)

Nitrogen
purge

Nitrogen
purge

Emulsifiers not
dispersed in oil
phase and
tonicifiers

Pump oil into
aqueous phase
at controlled
rate

Oil phase

Aqueous
phase

Heating

Heating

**Particle size
reduction**

Nitrogen
purge

Nitrogen
purge

Coarse
emulsion

Homogenizer
or
microfluidizer

Final
emulsion

**Filtration and
sterilization**

Nitrogen
purge

Sterilization

Final
emulsion

Figure 5. General flow diagram for the manufacture of a hypothetical oil-in-water parenteral emulsion (Alison, 1999).

5. Emulsion characterization

There are a number of physicochemical properties of emulsions that are important to consider when developing an emulsion for delivery of a drug. These include, but are not limited to, particle size, pH, osmolality and zeta potential which are used to monitor the physical stability of emulsions. Assay of potency and degradant levels are used to monitor the chemical stability of emulsions.

5.1 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. It is well established that the particle size distribution of intravenous emulsion is a critical factor for patient safety because larger particles (>5 μm) may cause pulmonary embolism (Cuéllar *et al.*, 2005; Jumaa, Kleinebudde and Müller, 1999).

A variety of instruments and techniques exist for monitoring particle size. The most widely used techniques rely on laser light scattering. These include Nicomp, Coulter and Malvern instruments. Electron microscopy has also been used but artifacts introduced by fixing techniques should be carefully controlled (Lui, 2000).

5.2 Droplet surface charge

Almost all particles in contact with a liquid acquire an electric charge on their surface. The electric potential at the shear plane is called the zeta potential. Zeta potential is an important and useful indicator of particle surface charge which can be used to predict and control the stability of colloidal suspensions or emulsions. The measurement of zeta potential is often the key to understand dispersion and aggregation processes in applications. The greater the zeta potential the more likely the suspension is to be stable because the charged particles repel one another and thus overcome the natural tendency to aggregate. The measurement of zeta potential allows predictions to be made about the storage stability of a colloidal dispersion. It is currently admitted that zeta potentials under 30 mV either negative or positive, optimum more than 60 mV are required for full electrostatic stabilization, potentials between 5 and 15 mV are in the region of limited flocculation and between 5 and 3 mV of maximum flocculation. Thus, particle aggregation is less likely to occur for charged particles (high zeta potential) due to electric repulsion. However, this rule cannot strictly be applied for systems which contain steric stabilizers because the adsorption of steric stabilizers will decrease the zeta potential due to the shift in the shear plane of the particle. Moreover, one has to take care about strong dependence of the zeta potential with ions present in the medium (Heurtault *et al.*, 2003).

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

Zetasizer is an instrument for measuring velocity of charged-colloidal particles in liquids. The electrophoretic mobility is determined by dividing the

measured velocity by the electric field strength. Finally, the zeta 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.

Yamaguchi *et al.* (1995) compared the properties of otherwise identical 10% soybean oil emulsions prepared from two different sources of lecithin. One containing 99% PC and the other contain only 70% PC with PE comprising much of the remaining phospholipids. The results showed that zeta potential is pH dependent and emulsions contained 70% PC with PE gave higher zeta potential than those contained 99% PC, reflecting the ionization properties of the free amino group of the PE. A charged drug molecule at the interface will also affect the zeta potential.

5.3   pH

The pH of the emulsion should be monitored continuously over the entire shelf-life of the emulsion to detect detrimental free fatty acid formation. Alkali fatty acids are commonly added to emulsions to adjust the pH of the formulations to physiological values. The initial pH of the emulsion may progressively decrease 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.

5.4 Osmolality

Osmolality, by definition, 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 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 osmolarity for peripheral administration of parenteral nutrition solution is less than 900 mOsm/L (Reich, Poon and Sugita, 2000). However, the prepared formulation should have the osmoticity close to that of serum osmolarity (300 mOsm/L) or no greater than 900 mOsm/L.

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 hemolysis 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, which 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 the properties change linearly in proportion to the concentration of solute particles are known as "colligative" properties.

Osmotic pressure is also a colligative property of a solution. 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 laboratory instruments developed for routine measurement of osmolality were based upon

depression of the freezing point and, until recently year, all osmometers for large-scale testing were based upon this methodology.

The Vapro® osmometer embodies newer technology. It is based upon a measurement of vapor pressure depression made possible by thermocouple hygrometry. The vapor pressure method provides 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 altered physically. This fundamental difference in methodology gives to the many advantages of the vapor® pressure osmometer over the older method.

6. Safety

Not only the stability but the safety upon 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, biocompatible and biodegradable materials to ensure safety and avoid any toxic reactions that might occur during intravenous use.

In general, the hemolytic test *in vitro* serves as a screening method for the toxicity of lytic agents contained in intravenous formulations. Using this test the erythrocyte damage induced by these agents *in vivo* can be estimated (Pape, Pfannenbecker and Hoppe, 1987). In the case of mixed micelles, for example, Martin and Marriott (1981) and Newbery *et al.* (1984) suggested that the formation of mixed

micelles between bile salt and phospholipids is important to protect the erythrocyte membrane against bile salt-induced damage. Lichtenberger *et al.* (1983) proposed that phospholipids protect membranes by being adsorbed as a monolayer on to the surface creating a hydrophobic barrier which results in reduced hemolysis. However, both proposed mechanisms do not sufficiently explain the protective mechanisms, especially in the case of lipid emulsions where it is well known that the addition of Pluronic F68 to the lipid emulsion formulation reduces the hemolytic activity of incorporated drugs (Bock and Müller, 1994).

Previously, the safety and stability of emulsions were studied by monitoring hemolysis caused by the interaction between erythrocytes and emulsions stabilized with various lecithins. Nagasaka and Ishii (2001) revealed that the hemolysis caused by the interaction between erythrocytes and emulsions was shown to be related to the phospholipids dispersed in the water phase, and was dependent on the PC contents in both the emulsifying agent used for preparation of the emulsion and in the erythrocyte membrane. The sphingomyelin in the erythrocyte membrane was shown to be an important component for the stabilization of erythrocytes against hemolysis induced by lipid emulsions.