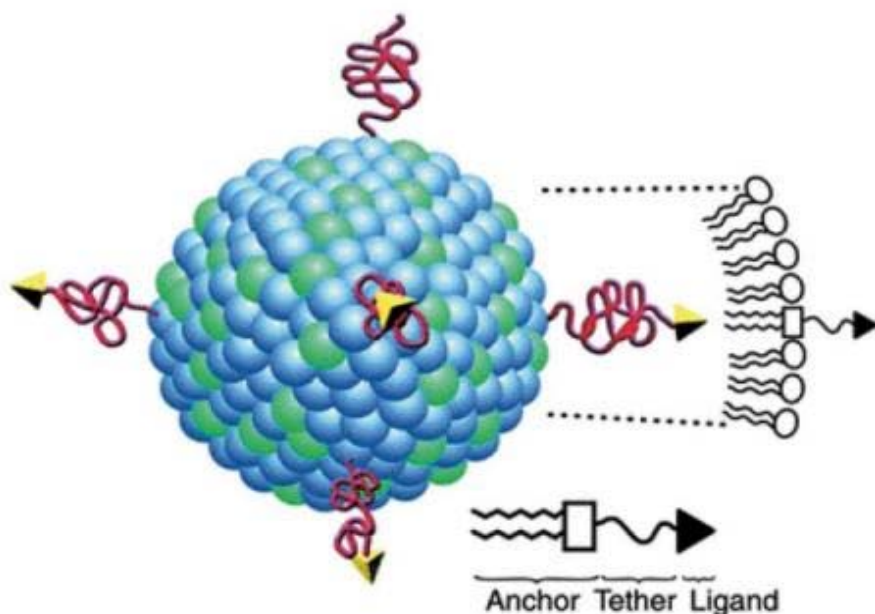


Role of Lipid Excipients in Modifying Oral and Parenteral Drug Delivery

Basic Principles and Biological Examples



Edited by
KISHOR M. WASAN, PhD

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*ROLE OF LIPID
EXCIPIENTS IN
MODIFYING ORAL
AND PARENTERAL
DRUG DELIVERY*



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BASIC PRINCIPLES AND BIOLOGICAL EXAMPLES

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WILEY-INTERSCIENCE

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PRINCIPLES IN THE DEVELOPMENT OF INTRAVENOUS LIPID EMULSIONS

Joanna Rossi and Jean-Christophe Leroux

- 4.1 INTRODUCTION
- 4.2 EMULSION STABILITY
- 4.3 ELIMINATION MECHANISMS FOR LIPID EMULSIONS
- 4.4 BIODISTRIBUTION OF LIPID EMULSIONS
- 4.5 PREPARATION OF EMULSIONS FOR INTRAVENOUS ADMINISTRATION
- 4.6 LIPID EMULSIONS FOR THE DELIVERY OF NUCLEIC ACID-BASED DRUGS
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4.1 INTRODUCTION

Emulsions can be defined as heterogeneous mixtures of two immiscible liquids, in which one phase is dispersed as fine droplets in the other. Small oil droplets dispersed in a continuous water phase is termed an ‘oil-in-water’ (o/w) emulsion. The opposite of this system is a ‘water-in-oil’ (w/o) emulsion, whereby the water phase is dispersed in an oily external medium. Among these types, only o/w emulsions can be used for intravenous administration [1]. Emulsions are thermodynamically unstable systems that will eventually destabilize into two separate phases. A third component, the surfactant or emulsifier, is added to stabilize the preparation by reducing the interfacial tension and increasing droplet–droplet repulsion through electrostatic and/or steric repulsive forces [2]. The addition of an emulsifying agent, however,

provides only kinetic stability. Even though emulsions are unstable systems, surface active agents may provide stability for several years, making the system useful for practical application [2].

Lipid emulsions have traditionally been used for parenteral nutrition to deliver essential fatty acids to patients unable to acquire them in food. As a result of the successful induction of lipid emulsions in parenteral nutrition, there has been increasing interest in developing emulsions as carriers for lipophilic drugs. Many intravenous lipid emulsion formulations are commercially available (Table 4.1) and a number of others are in clinical phase or in preclinical development (Table 4.2). Lipid emulsions are promising carriers for drug delivery as a result of their biocompatibility, reasonable stability, ability to solubilize high quantities of hydrophobic compounds, and relative ease of manufacture on an industrial scale [3, 4]. In addition, emulsions can protect the encapsulated drug against hydrolysis and enzymatic degradation in the blood compartment, reduce drug loss in infusion sets, lower the toxicity of cytotoxic compounds, and reduce the incidence of irritation and pain on injection [1, 4]. They can also provide a certain level of selectivity toward target tissues, increasing the therapeutic index of many drugs [5]. However, after intravenous injection, lipid emulsions can acquire apolipoproteins and be metabolized as natural fats or be recognized as foreign bodies and taken up by the cells of the mononuclear phagocyte system (MPS – also known as the reticuloendothelial system [RES]) [6]. Evading the MPS or natural fat metabolism is necessary when the encapsulated drug is to be delivered to non-MPS organs or liver parenchymal cells, respectively. The in vivo fate of lipid emulsions can be controlled to a certain extent by altering the physicochemical properties of the carrier, such as droplet size, composition and surface properties. This chapter discusses the main factors to consider when developing emulsions for intravenous injection.

TABLE 4.1 Several commercially available emulsions for intravenous injection

Product	Drug	Manufacturer	Indications
Diazemuls	Diazepam	Pfizer and Pharmacia	Anticonvulsive, sedative, muscle relaxant
Diazepam-Lipuro	Diazepam	B. Braun	General anesthesia
Diprivan ^a	Propofol	AstraZeneca	General anesthesia
Etomidate-Lipuro	Etomidate	B. Braun	General anesthesia
Limethason ^a	Dexamethasone palmitate	Mitsubishi Pharmaceutical	Rheumatoid arthritis
Liple (Lipo-PGE1)	Prostaglandin E1 (PGE1)	Mitsubishi Pharmaceutical	Vasodilator, platelet inhibitor
Propofol-Lipuro	Propofol	B. Braun	General anesthesia
Lipo-NSAID – Ropion ^a	Flurbiprofen axetil	Kaken Pharmaceuticals	Pain reliever
Vitalipid ^a	Vitamins A, D ₂ , E, K ₁	Fresenius Kabi	Parenteral nutrition

^aBased on the formulation of Intralipid (10 or 20% soybean oil; 1.2% egg lecithin; 2.5% glycerol).

TABLE 4.2 Some intravenously injectable emulsions in development and in clinical trials

Drug	Product name	Indications	Clinical ^a	Reference
Aclacinomycin A	–	Cancer chemotherapy	–	[110]
Amphotericin B	–	Treatment of fungal infections	–	[97, 98]
β-Elemene	SDP-111	Cancer chemotherapy	–	[111]
Cyclosporine A	–	Immunosuppressant	–	[112]
Docetaxel	SDP-014	Cancer chemotherapy	–	[111]
Perfluorooctyl bromide	Imavist	Ultrasound contrast agent	Phase III	[113]
Paclitaxel	TOCOSOL/S-8184	Cancer chemotherapy	Phase III	[72]
Paclitaxel	SDP-013	Cancer chemotherapy	–	[111]
Perfluorooctyl bromide	Oxygent	Artificial blood substitute	Phase III	[78]
Propofol	Ampofol	General anesthesia Sedation	Phase III	[114]
Propofol	IDD-D propofol	General anesthesia Sedation	Phase III	[115]
α-Tocopherol succinate	SDP-112	Cancer chemotherapy	–	[111]
Vincristine	–	Cancer chemotherapy	–	[116]

^aStatus as of March 2006.

4.2 EMULSION STABILITY

Emulsions are thermodynamically unstable systems and will inevitably break apart into separate oil and water phases. Emulsion instability is caused by the increase in surface free energy (ΔG) as small droplets are formed as a result of the enhanced surface area (ΔA). Adding a surfactant to the mixture reduces the interfacial tension (γ_{ow}) at the oil–water interface facilitating globule rupture during emulsification and stabilizes the preparation (Eqn 1).

$$\Delta G = \gamma_{ow} \Delta A \quad (1)$$

It is important to state that surfactants provide the emulsions only with kinetic stability, which delays the destabilization process. Nevertheless, surface-active agents can provide stability for several years, which is long enough for the system to be useful for practical purposes [2]. Emulsions that are thermodynamically stable are known as microemulsions. They are clear or translucent systems and do not require much energy input during emulsification. In contrast, emulsions are cloudy and require a greater amount of energy for emulsification [7]. The theory behind the formation of microemulsions is beyond the scope of this chapter.

4.2.1 Destabilization Processes

Emulsion destabilization can be characterized by three separate processes: flocculation, coalescence, and Ostwald ripening. Coalescence and Ostwald ripening are irreversible processes that lead to an increase in droplet size, requiring a large energy input to re-disperse the droplets. Flocculation, on the other hand, is reversible and occurs when droplets aggregate to form a clump of many individual droplets. The aggregated droplets move together as a cluster but each droplet still retains its separate identity. The interactions holding the droplets together are weak and can be broken by mild agitation. Even though floccules can be easily re-dispersed, they may eventually fuse together to form single, larger globules. The fusion of droplets is irreversible and is termed 'coalescence'. Ostwald ripening, which also increases droplet size, occurs in polydisperse formulations, wherein the smaller droplets are more soluble in the continuous phase than the larger ones. In this process, the oil from the smaller droplets dissolves in the aqueous phase and diffuses towards the larger droplets. This transfer of oil causes the big droplets to grow, while the smaller ones decrease in size. As the small droplets continue to shrink, the Ostwald ripening effect is enhanced. The progressive increase in droplet size over time will eventually lead to complete phase separation. Adding too much surfactant may promote Ostwald ripening because the excess surfactant will form micelles that enhance the solubility of the oil in the aqueous phase. Ostwald ripening can be reduced by increasing the viscosity of the continuous phase, decreasing polydispersity, or adding a third component that has a lower solubility in the continuous phase than the oil [8, 9].

Depending on the density differences between the dispersed and continuous phases, individual droplets or floccules can cream or sediment. If the dispersed phase is lower in density than the continuous phase, the droplets or floccules will rise to the surface, producing a highly concentrated layer of dispersed phase, which is known as a cream. In the case where the dispersed phase is higher in density than the continuous phase, a sediment will form at the bottom of the formulation. For o/w emulsions, creaming usually occurs because the oil phase is typically less dense than the aqueous phase. The rate of creaming or sedimentation can be linked to the size of the droplet by Stokes' equation (Eqn 2). According to this equation the limiting velocity of a falling sphere (v) is:

$$v = \frac{2}{9} \frac{a^2 \Delta \rho}{\nu} g \quad (2)$$

where a is the radius of the droplet, $\Delta \rho$ is the density difference between the dispersed and continuous phases, ν the viscosity of the continuous phase, and g the acceleration caused by gravity. Stokes' equation implies that droplets will rise or settle faster if the droplet size or the density difference between the dispersed and continuous phases is large, whereas an increase in continuous phase viscosity will slow down the separation process. As a result, creaming or sedimentation can be delayed by reducing droplet size, decreasing the density differences between the two phases and increasing the viscosity of the continuous phase. Not much emphasis is,

however, being placed on density adjustments to produce stable emulsions because there are a limited number of oils approved for intravenous administration and these oils have similar densities.

Submicrometer emulsions have colloidal properties and as a result are less susceptible than coarse emulsions to the gravitational forces in Stokes' equation [10]. Nano-sized droplets are subjected to random brownian motion and consequently are less inclined to cream or sediment. Brownian motion does not, however, provide complete protection against instability because droplets may aggregate or coalesce on random collisions. Stability against these collisions depends on the attractive and repulsive forces acting on the droplets. Typically, emulsions are stabilized by either electrostatic or steric repulsive forces (or a combination of the two).

4.2.2 Electrostatic Stabilization

The balance between attractive van der Waals' forces and electrostatic repulsive forces is described in the theory of colloidal stability, termed 'DLVO' after its developers Derjaguin, Landau, Vervy, and Overbeek. If the net force is attractive, the droplets will either flocculate or coalesce. In contrast, if the net force is repulsive, the particles will repel each other and the system is stable. The attractive interaction between particles arises from van der Waals' forces and is experienced by all particles. Van der Waals' forces dominate at short separation distances and the strength of this attractive force can be determined from the magnitude of the Hamaker constant (A). Emulsions can overcome the attractive van der Waals' forces through electrostatic repulsion with charged emulsifying agents. Electrostatic repulsion is provided by the electric double-layer surrounding the droplet. The electric double-layer is characterized by an adsorbed layer of fixed counter-ions and a diffuse layer of ions that move freely with the fluid. Two approaching particles will experience a repulsive force as the electric double-layers overlap. The total potential of interaction between two droplets is the sum of the attractive van der Waals' forces and the electrostatic repulsive forces (Eqn 3);

$$V_T = V_A + V_R \quad (3)$$

where V_T is the total interaction potential, V_A represents the attractive van der Waals' forces, and V_R signifies the electrostatic repulsive forces. The potential energy of interaction between two droplets as a function of separation distance is illustrated in Figure 4.1. The repulsive barrier generated by the electric double-layer corresponds to the maximum in the curve. The height of the energy barrier determines the stability of the emulsion and depends on the ionization of the surfactants.

For the system to be stable, the energy barrier must be high enough such that the droplets do not have enough kinetic energy to surpass it and reach the primary minimum. At the primary minimum (maximum attractive potential), droplet coalescence readily occurs. Flocculation takes place at the secondary minimum and, contrary to coalescence, is reversible by providing a small amount of kinetic energy to overcome the weak attractive forces holding the droplets together. Flocculated

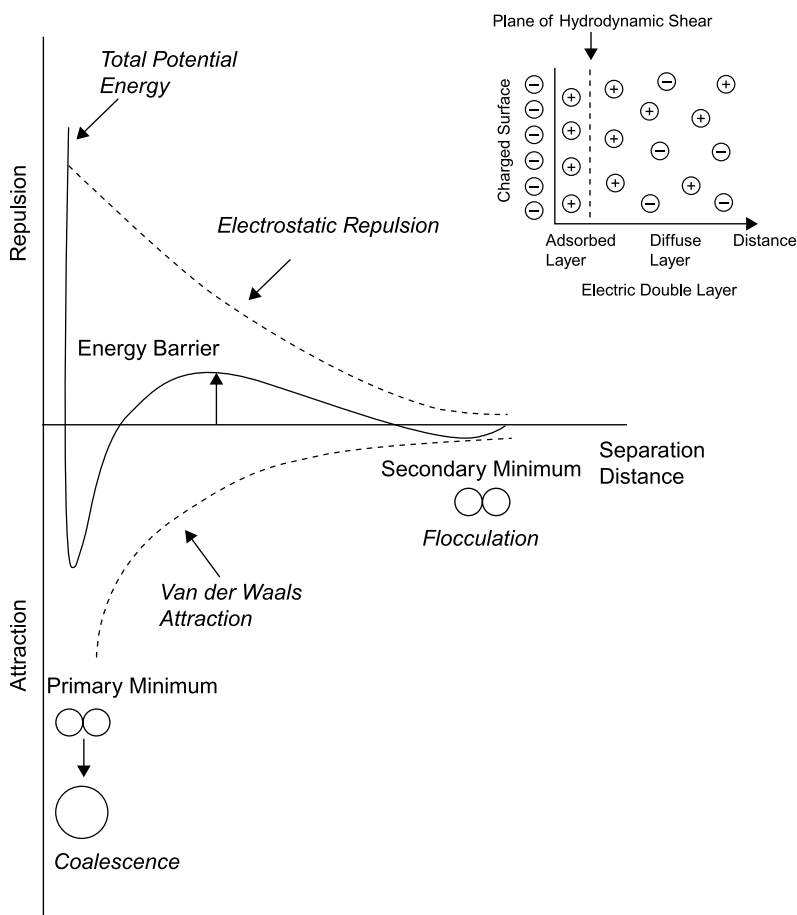


Figure 4.1 The total potential energy of interaction between two droplets as a function of separation distance (electric double-layer repulsion and van der Waals' attraction).

droplets are prevented from coalescing as a result of this repulsive energy barrier. If the flocculated droplets have enough energy to surpass the energy barrier, they will easily reach the primary minimum and coalesce. The strength of the electrostatic forces can be quantified by measuring the ζ (zeta) potential, which is the potential at the plane of hydrodynamic shear. Generally, emulsions are stabilized by electrostatic repulsive forces if the ζ potential is greater than ± 30 mV [2, 8, 11]. An emulsion stabilized by electric double-layer repulsion can be destabilized if the concentration of electrolytes is increased above a critical value. Adding electrolytes to an emulsion decreases the electric double-layer repulsion potential, whereas the van der Waals' attractive potential remains unchanged. As electrolyte concentration increases, the repulsive forces stabilizing the colloid become weaker until the net force is attractive and stability is lost.

4.2.3 Steric Stabilization

Emulsions can also be stabilized by steric repulsion through the grafting of long-chain polymers at the emulsion interface. Steric repulsion is a non-DLVO interaction that occurs as a result of the unfavorable overlap of the polymer chains as two particles approach each other [8, 12]. Steric stabilization occurs at short interdroplet separation distances and can provide a strong barrier against coalescence [8]. Optimal steric repulsion can be achieved at high polymer surface density as desorption and chain rearrangement are minimized [8].

4.3 ELIMINATION MECHANISMS FOR LIPID EMULSIONS

After intravenous injection, lipid emulsions may be metabolized in a manner similar to chylomicrons, or might be recognized as foreign bodies and removed by the cells of the MPS. The mechanism of elimination from the body depends on the physico-chemical properties of the emulsion. Both mechanisms of elimination can occur for a given lipid emulsion; however, one process may be favored over another. This section describes the two primary pathways of lipid emulsion elimination from the body.

4.3.1 Lipid Emulsions Metabolized as Endogenous Chylomicrons

Depending on the composition and surface properties, lipid emulsions may be recognized as chylomicrons and eliminated via the fat metabolism pathway. Chylomicrons are endogenous emulsions produced by the enterocytes of the small intestine after dietary lipids have been ingested. They are rich in triglycerides and possess apolipoproteins A-I, A-IV, and B-48 before entering the blood circulation (Figure 4.2) [13]. Chylomicrons are secreted into the lymph and enter the systemic circulation through the thoracic duct. After entering the blood, chylomicrons obtain the apolipoproteins Apo-C-II and Apo-E from the high-density lipoproteins (HDLs) and release Apo-A-IV. In the capillaries of adipose tissues and muscle, lipoprotein lipase (LPL) located on endothelial cells adsorbs on to the mature chylomicron and hydrolyzes the triglycerides to fatty acids [14]. The fatty acids are then absorbed mainly by adipose tissues and muscle. During lipolysis, a substantial amount of phospholipid, Apo-A and Apo-C is transferred to the HDLs and the size of the chylomicron is reduced considerably. The remnant chylomicrons, composed of mainly Apo-B-48, Apo-E, and cholesterol, are quickly removed from the blood by the liver. The uptake of remnant chylomicrons by the liver occurs via two Apo-E-specific recognition sites on parenchymal cells, which are the low-density lipoprotein receptor (LDLr) and the remnant receptor [15–17].

Injectable lipid emulsions differ from chylomicrons in that they do not have apolipoproteins on the surface before entering the bloodstream, although they may acquire them after systemic injection. Emulsions rich in triglycerides are known to acquire apolipoproteins (Apo-C-I, -C-II, -C-III, -E and possibly -A-IV), mainly from

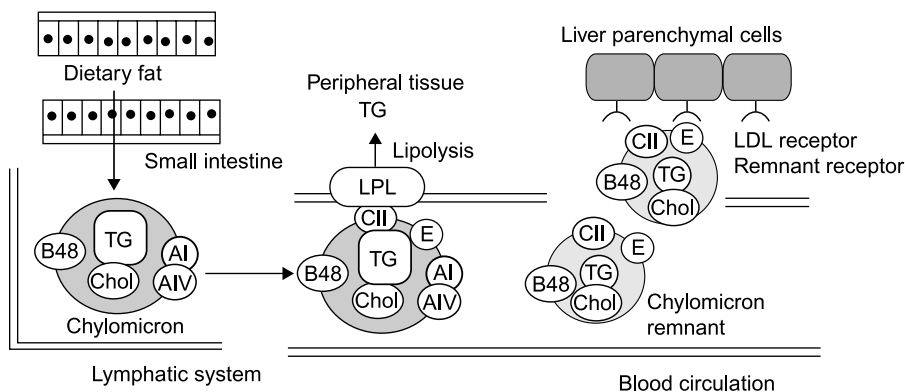


Figure 4.2 Absorption and metabolism of dietary fat. Dietary fats are metabolized and incorporated into chylomicrons in the small intestine. Then chylomicrons enter the blood circulation via the thoracic duct. During circulation, the triglycerides of chylomicrons are rapidly hydrolyzed via lipoprotein lipase (LPL) on the endothelial surfaces, and chylomicron remnants are produced. Finally, chylomicron remnants are cleared via the liver by the low-density lipoprotein (LDL) or remnant receptors. TG, triglyceride; Chol, cholesterol; AI, apolipoprotein AI; AIV, apolipoprotein AIV; B48, apolipoprotein B48; CII, apolipoprotein CII; E, apolipoprotein E. (Reprinted with permission from Elsevier Ref. [13] Copyright 2000.)

HDLs, soon after injection into the systemic circulation, and are metabolized in a pathway comparable to that described for chylomicrons [15, 18, 19]. Among the apolipoproteins acquired, Apo-C-II and Apo-E are essential for LPL activation and uptake of remnant emulsions by the liver, respectively [19].

Elimination of the lipid emulsion via the pathway of natural fat metabolism may be desirable when the liver parenchymal cells are the target site. On the other hand, if the target site is not the liver, apolipoprotein adsorption on to the emulsion should be avoided. The metabolism of lipid emulsions as natural fats depends strongly on the type of emulsifier [20, 21], presence of cholesterol [22], and chain length of the triglyceride oil [23].

4.3.2 Elimination by the Mononuclear Phagocyte System

If the body recognizes the lipid emulsions as foreign, they will be captured by the cells of the MPS, mainly the Kupffer cells of the liver and the macrophages of the spleen, and removed from the systemic circulation. The MPS takes up the emulsions via endocytosis and localizes them in the lysosomal compartment, where they are degraded by enzymes [24]. The extent of emulsion clearance from the systemic circulation is enhanced by the adsorption of opsonins (proteins) on to the colloid surface. The bound proteins then interact with the receptors on monocytes and macrophages, facilitating endocytosis. Carriers that become bound to opsonins will be rapidly cleared from the blood and prevented from reaching the target site(s) [24]. Immunoglobulins and complement components such as C1q and C3 fragments (C3b, iC3b) are well-known opsonins.

A major challenge in drug delivery using colloidal nano-carriers is to avoid clearance by the cells of the MPS when the target sites are non-MPS tissues. Overloading or saturating the MPS with large injection volumes has been shown to enhance the circulation time of lipid emulsions [25]. However, temporary impairment of the MPS may pose a health hazard to the patient [26]. Alternatively, the clearance rate of carriers from the blood can be altered by modifying the physicochemical properties of the emulsion, such as droplet size [27, 28] and surface characteristics [29]. This is discussed in detail in the next section.

4.4 BIODISTRIBUTION OF LIPID EMULSIONS

The biodistribution of an emulsion after systemic injection depends primarily on the droplet size, composition, and surface properties. A certain specificity toward the target site can be achieved by controlling the physicochemical properties of the emulsion. The principal factors that influence the biodistribution of emulsions has already been very thoroughly reviewed by Nishikawa [6]. This section provides a brief overview of these factors and has been updated with some recent work.

4.4.1 Effect of Lipid Emulsion Size

It is well known that droplet size greatly influences the uptake of the emulsions by the MPS [27, 28]. In general, larger particles are more susceptible to uptake by the MPS and are cleared more quickly from the systemic circulation. The influence of droplet size on the *in vivo* biodistribution of lipid emulsions was explored by Takino et al. [27]. The authors compared the biodistribution of large (250 nm) and small (100 nm) lipid emulsions composed of egg phosphatidylcholine (PC):soybean oil (1 : 1). [^{14}C]Cholesteryl oleate ([^{14}C]CO), a highly lipophilic compound ($\log P = 18.3$) that does not undergo lipolysis by LPL and remains associated with the emulsion, was incorporated into each emulsion to track the elimination of the whole droplet [30]. The large PC emulsion was rapidly eliminated from the blood, with 60% of the injected emulsion being recovered in the liver within 10 min. The small PC emulsion, however, remained in the blood for longer and accumulated less in the liver.

Similarly, Lundberg et al. [28] reported that droplet size influenced emulsion clearance rate from plasma. They observed that the smallest emulsion (50 nm) survived the longest in plasma, whereas the larger emulsions (100 and 175 nm) were cleared more rapidly (Figure 4.3). The influence of emulsion-like lipid nanocapsule size (20, 50, and 100 nm) on the extent of complement activation and macrophage uptake was evaluated by Vonarbourg et al. [31]. Similar to emulsions, lipid nanocapsules are core-shell structures with an oily internal phase that is stabilized by a monolayer of emulsifiers. They differ from lipid emulsions in the physicochemical properties of the hydrophilic/hydrophobic interface. In lipid nanocapsules, the emulsifiers form a semi-rigid shell, whereas the interface is more fluid in emulsions. The authors observed that larger lipid nanocapsules were stronger activators of the complement and taken up more by macrophages than the smaller ones.

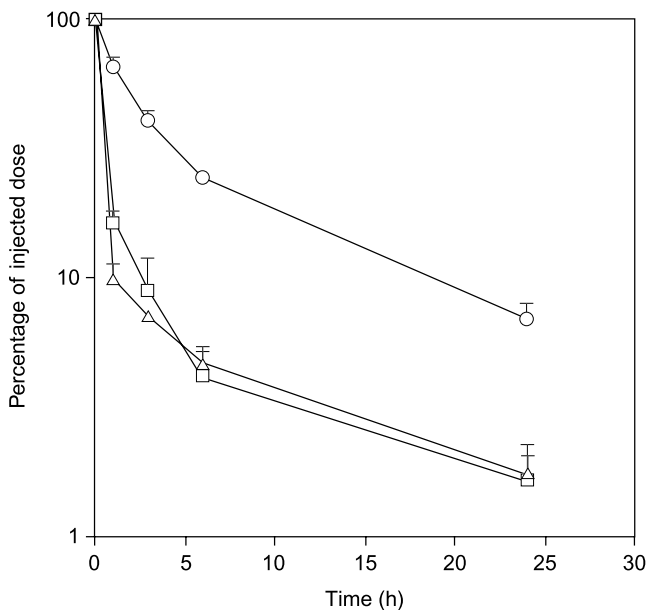


Figure 4.3 Effect of particle size on the clearance of cholesterol oleate (CO) label from plasma as a function of time after intravenous administration into mice. The emulsions were composed of triolein (TO):1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC):Polysorbate 80:polyethyleneglycol-modified 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylethanolamine (PEG₂₀₀₀-DPPE) (2:1:0.4:0.1, w/w). The droplet sizes of the emulsions injected were 50 (○), 100 (□), and 175 nm (Δ). (Adapted with permission from Elsevier Ref. [28] Copyright 1996.)

The size of the lipid emulsion was also shown to influence lipolysis. Kurihara et al. [32] found that the rate of lipolysis was much faster for the small-sized emulsions (about 100 nm) *in vitro* compared with the larger ones (225–416 nm). However, after intravenous injection of these formulations in rats, they observed that the small-sized emulsions remained in the plasma longer than the larger ones, which is consistent with the studies of Takino [27] and Lundberg [28]. Consequently, even though small emulsions were better substrates for LPL, large emulsions were cleared from the blood faster, which suggests a greater uptake of by the MPS.

Droplet size also determines the ability of the emulsion to escape the systemic circulation through the blood capillaries and reach the extravascular space. Capillary walls are composed of a single layer of endothelial cells surrounded by a basement membrane. They are classified into three types, based on their wall structure: continuous (intact), fenestrated, or discontinuous (sinusoidal) [33]. Both fenestrated and discontinuous capillaries have pores in the endothelium, whereas continuous ones have tight junctions between adjacent endothelial cells [34]. Continuous capillaries have an intact subendothelial basement membrane and can be found in most regions of the body, such as the skin, connective tissue, skeletal and cardiac muscle, alveolar capillaries of the lung, and brain [33]. In fenestrated capillaries, the pores

(fenestrae) are approximately 40–80 nm in diameter and they can be either open (unobstructed) or covered by a thin diaphragm [33]. These capillaries have a continuous subendothelial basement membrane and are situated in the intestinal mucosa, pancreas, glomerulus, peritubular capillaries, endocrine glands, the choroid plexus of the brain, and the ciliary body of the eye [33]. Discontinuous capillaries on the other hand, have large gaps between endothelial cells and are located in the liver, spleen, and bone marrow [33]. The basal membrane is either absent, which is the case for the liver or discontinuous (spleen and bone marrow) [34]. The largest pore size in the capillary endothelium is believed to be about 100 nm [35]. Nanoscopic drug carriers are generally too large to diffuse across the capillaries of continuous endothelium. Their best opportunity for escaping the systemic circulation is through the gaps between the endothelial cells of discontinuous capillaries. Consequently, colloidal drug carriers tend to accumulate in the liver, spleen, and bone marrow.

Control over carrier size can impart some selectivity for the extravascular space of tumoral sites, reducing anticancer drug toxicity toward healthy tissues. This selectivity can be achieved by taking advantage of the difference in capillary structure between tumors and normal tissues. Tumor vasculature is often characterized as porous or ‘leaky’, allowing enhanced permeation of colloidal particles across the endothelium and into the extravascular space. In addition, tumors have poor lymphatic drainage, allowing colloids to be retained in the tissue for longer periods of time [36]. This increased permeation and retention of colloids is called the enhanced permeation and retention (EPR) effect [37]. The optimum size range for colloidal particle accumulation in tumors is generally accepted to be about 50–200 nm [38]. Particles in this size range can be convected from the blood vessel into the extravascular space through the porous vasculature of the tumor. Depending on the porosity of the tumor capillaries, particles above 200 nm may not pass through the pores and will be eliminated more quickly by the MPS. On the other hand, particles less than 50 nm will easily extravasate through the discontinuous endothelium of the liver, spleen, and bone marrow.

As a rule of thumb, for successful accumulation of drug in the tumor by the EPR effect, the concentration of colloidal carriers in the plasma must remain high for more than 6 h [39]. The progressive extravasation of the carrier into the tumor tissue over several hours will result in increasing concentrations of anticancer drug in the vicinity of the cancer cells. Kurihara et al. [40] demonstrated that lipid emulsions under 230 nm in diameter could deliver more RS-1541, a highly lipophilic anti-tumor agent (13-*O*-palmitoyl-rhizoxin), to the tumor site (M5076 sarcoma cells) than larger droplets (Figure 4.4). The low concentrations of RS-1541 detected in the tumor for the larger emulsions is most probably a result of the impermeability of the leaky tumor capillaries to large particles and their faster removal rates from blood. It was also observed that emulsions larger than 220 nm reduced the toxicity of RS-1541 as shown by the higher maximum tolerated dose (MTD) with increasing size (Table 4.3). All emulsions regardless of size (70–380 nm) suppressed tumor growth and improved survival at the MTD. The medium-sized emulsions (220 nm), however, displayed the highest antitumor activity at the MTD as a result of the permeability of the tumor vasculature for the emulsions and reduced toxicity, permitting the injection of a higher dose. Hence, lipid emulsions can augment the delivery

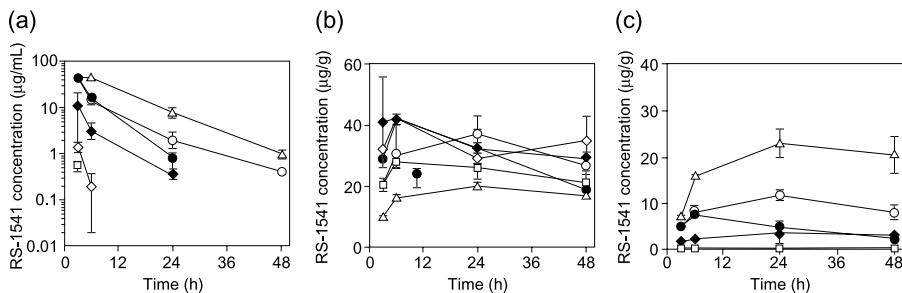


Figure 4.4 Concentrations of 13-*O*-palmitoyl-rhizoxin (RS-1541) in the (a) plasma, (b) liver, and (c) tumor after a single intravenous administration of various sizes of emulsion formulations and a surfactant solution of RS-1541 to mice bearing M5076 sarcoma at a dose of 5 mg/kg. The emulsion droplet sizes were 110 (Δ), 230 (○), 350 (◆), 410 (◇), 630 nm (□), and the surfactant solution (●). Each value represents the mean $\pm$ SE (standard error) of three mice. (Adapted with permission from Springer Science and Business Media Ref. [40] Copyright 1996.)

TABLE 4.3 Antitumor activity of RS-1541 emulsion formulations against M5076 sarcoma at the MTD

Mean diameter (nm)	Dose (MTD) ^a (mg/kg)	Tumor diameter ^b (%)	Tumor growth delay ^c (day)	ILS ^d (%)	Cure (on day 120)
Surfactant solution	6.0	213	17	62	0/6
70	4.5	166	24	66	0/6
100	4.5	113	29	69	0/6
220	15.0	13	>61	>224	4/6
380	40.0	18	56	>195	3/6

^aRS-1541 was given in each formulation to M5076-bearing BDF1 mice via a single intravenous injection at maximum tolerated doses (MTDs) on day 13 after inoculation (six mice were used for each group).

^bTumor diameter on day 44 divided by that on treatment day.

^cDays required for the tumors to reach again the diameter on treatment day following therapy.

^dIncrease in life span: ratio (%) of median survival days in a treatment group of mice to that in the control group (37 days).

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of cytotoxic compounds to tumoral sites and reduce systemic toxicity by suitable selection of the droplet size.

4.4.2 Effect of Lipid Emulsion Composition and Emulsifiers

Composition of the Oil Phase

The composition of the internal phase has also been shown to alter the biodistribution of lipid emulsions. Lutz et al. [41] observed that lipid emulsions composed of medium-chain triglycerides (MCTs) were cleared from plasma more quickly than those prepared with long-chain triglycerides (LCTs). This is probably caused by the faster hydrolysis of MCTs by LPL and hepatic lipases compared with LCTs as a

result of the greater solubility and mobility of shorter-chain triglycerides at the oil/water emulsion interface [42].

Adding free cholesterol has also been shown to alter the metabolism of triglyceride emulsions. Maranhao et al. [22] observed that emulsions with low free cholesterol content (<4% w/w) were metabolized in a manner similar to chylomicrons, as shown by the faster removal rate of triglycerides from the blood than CO as a result of LPL-mediated hydrolysis of the oil and greater uptake of CO than triglycerides by the liver. In contrast, emulsions with high free cholesterol (>6% w/w) displayed similar triglyceride and CO removal rates from blood, and equal uptake by the liver. The group also observed that emulsions containing high free cholesterol bound less Apo-A-I, Apo-A-IV, and Apo-C, and more Apo-E in vitro. Apo-C-II is essential for LPL binding and activation and hinders liver uptake, whereas Apo-E facilitates emulsion uptake by the liver. Hence, the presence of free cholesterol may modify the metabolism of the droplets by altering the binding of apolipoproteins onto the surface.

Phosphatidylcholine Composition

The biodistribution of emulsions can also be altered by the phospholipid emulsifier. Lenzo et al. [43] demonstrated that the nature of the PC affected the metabolism of the emulsion in rats. Five lipid emulsions with different phospholipid emulsifiers were prepared. The phospholipids selected were PC, 1,2-dioleoyl-*sn*-glycero-3-phosphatidylcholine (DOPC), 1,2-dimyristoyl-*sn*-glycero-3-phosphatidylcholine (DMPC), 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC), and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine (POPC). The average composition of each emulsion was similar and size was maintained at about 150 nm. The emulsions were radiolabeled with [^{14}C]triolein (TO) and [^3H]CO or dipalmitoylphosphatidyl-[*N*-methyl- ^3H]choline to monitor the hydrolysis of the triglyceride oil by LPL, the clearance of the entire colloid particle, and the transfer of phospholipids to the HDLs, respectively.

The carriers emulsified with PC or POPC were metabolized in a manner similar to chylomicrons as shown by the rapid removal rate of [^{14}C]TO from plasma, consistent with hydrolysis by LPL, and the efficient uptake of [^3H]CO (remnant emulsions) by the liver. DPPC-based emulsions remained in plasma the longest and the triglycerides associated with this emulsion disappeared very slowly, suggesting that the emulsion was less susceptible to hydrolysis by LPL. Moreover, the phospholipid radiolabel did not transfer to HDLs. A possible explanation for the above observations is the difference in chain unsaturation among the five phospholipid emulsifiers. The authors hypothesized that rapid hydrolysis of the triglyceride oil by LPL and efficient transfer of phospholipids to HDLs requires a chain unsaturation at the glycerol 2-position.

4.4.3 Effect of Surface Charge

Lipid emulsions obtain their surface charge through the use of neutral, anionic, or cationic emulsifiers. Most emulsions used in drug delivery are either neutral or negatively charged because cationic carriers are more prone to aggregate in the pres-

ence of plasma proteins. This susceptibility for aggregation in the bloodstream is a result of the electrostatic interactions with negatively charged plasma proteins. It is generally accepted that surface charge has an effect on the rate of particle uptake by the MPS, although the connection is far from straightforward. Other surface properties, such as the nature of the emulsifier, may take precedence over the effects generated by surface charge. Davis et al. [44] found no clear correlation between ζ potential and the rate of emulsion uptake by mouse peritoneal macrophages, although emulsions with the weakest charge, prepared with the non-ionic surfactant poloxamer 338, had the slowest rate of uptake. Stossel et al. [45] found that emulsions with higher surface charge (positive or negative) were phagocytosed at a faster rate compared with neutral or weakly charged surfaces. Oku et al. [46] observed that uptake by the liver and spleen was greater for positively charged liposomes than for neutral or anionic ones. The higher accumulation of cationic liposomes in the MPS organs may be caused by both particle aggregation in the presence of serum and protein adsorption on to the colloid, which was observed to a lesser extent in neutral or anionic liposomes. Devine et al. [47] found that liposomes bearing a net positive or negative charge activated the complement in a dose-dependent manner, whereas no complement activation was observed for neutral liposomes. Interestingly, long-circulating cationic lipid emulsions have been reported in the literature by careful selection of the emulsifier [48].

Over the past few years, there has been increasing interest in developing cationic lipid–DNA complexes for the improved delivery of genetic material [49, 50]. An advantage of using cationic carriers is the enhanced cellular uptake via endocytosis over neutral or negatively charged carriers [51]. This is a result of the favorable electrostatic interactions of cationic particles with the negatively charged moieties on biological membranes. However, as a result of the tendency of cationic particles to aggregate in the presence of serum, the positive charge will need to be shielded, which will invariably reduce transfection efficiency in the absence of targeting ligands.

4.4.4 Long-circulating Lipid Emulsions

After intravenous administration, colloidal drug carriers are rapidly taken up by circulating monocytes and macrophages in the liver, spleen, and bone marrow. Avoiding the MPS is crucial when the emulsions are to be delivered to non-MPS cells. Prolonged circulation of the drug carrier is also necessary to achieve passive targeting of tumoral tissues via the EPR effect. Modification of the colloidal surface such that the carriers are invisible or ‘stealth’ to opsonins and macrophages is an approach investigated to increase the circulation time of submicrometer emulsions in blood.

Sphingomyelin

The presence of sphingomyelin (SM) at the oil/water interface has been shown to reduce the uptake of the emulsions by the MPS. Takino et al. [27] demonstrated that adding SM to a PC and soybean oil formulation increased the circulation time of the submicrometer emulsions in blood and decreased liver and spleen uptake. The emulsions were composed of PC:soybean oil (1:1) and PC:SM:soybean oil

(0.7:0.3:1) with [¹⁴C]CO incorporated as a radiolabeled tracer. The AUC of the SM emulsion was 1.6 times larger than that of the one emulsified with PC only. Similarly, Redgrave et al. [21] observed that increasing the amount of SM enhanced the circulation time of the carrier in plasma (Figure 4.5) and reduced uptake by the liver

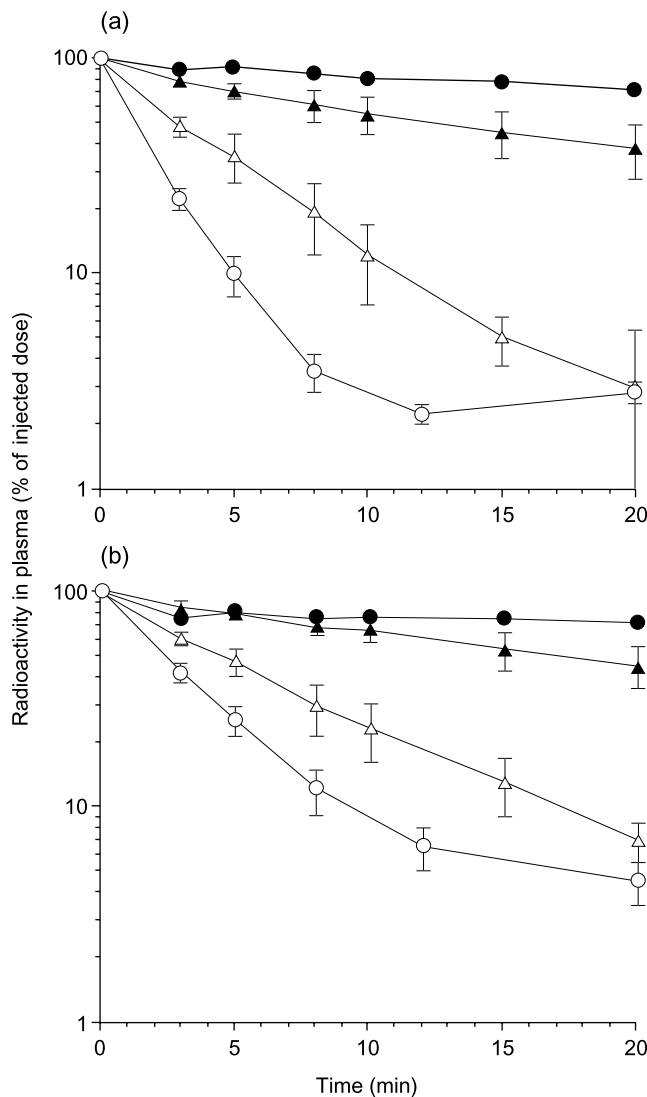


Figure 4.5 Radioactivity in plasma of triolein (TO) and cholesteryl oleate (CO) labels after injection of emulsions stabilized by mixtures of sphingomyelin (SM) with egg phosphatidylcholine (PC). TO-CO-cholesterol emulsions stabilized with mixtures of SM and PC were injected intravenously in conscious rats. Plotted are the data for labeled (a) TO and (b) CO incorporated in the emulsions remaining in the plasma at 3, 5, 8, 12, and 20 min after injection. Results are means $\pm$ SE of at least four experiments for each observation. SM 100% (●), SM/PC 50/50 (▲), SM/PC 25/75 (Δ), PC 100% (○). (Adapted with permission from Elsevier Ref. [21] Copyright 1992.)

(Figure 4.6). Even though SM and PC share a common phosphorylcholine polar head group, there are structural discrepancies between the two molecules that reflect their different physical properties in colloidal systems. SM has a high content of saturated acyl chains relative to naturally occurring PCs and has a stronger hydrogen bonding capacity, which may alter monolayer rigidity and interactions with blood components [52].

Polyethyleneglycol Lipids

A very widely used and effective method to avoid clearance by the MPS is to incorporate polyethyleneglycol (PEG) (also known as polyethylene oxide or PEO) at the colloid surface using a lipid derivative. PEG, a hydrophilic and flexible polymer, creates a zone of steric hindrance around the carrier which decreases the rate and extent of opsonin binding [53]. PEG is widely accepted for intravenous administration because it is a biocompatible, nontoxic, and nonimmunogenic polymer. Moreover, PEG-lipid derivatives are amphiphilic and as a result can be used as a co-emulsifier as well. Liu et al. [29] observed the influence of PEG molecular mass on the biodistribution of lipid emulsions composed of castor oil and PC. The PEG-lipid derivatives investigated were dioleoyl *N*-(monomethoxy-PEG-succinyl)phosphatidylethanolamine (PEG-DOPE) (M_r 1000, 2000, and 5000 Da) and PEO 20 sorbitan monooleate (Polysorbate 80). Emulsion droplet size was maintained at about 200 nm so that the circulation behavior was dependent only on the surface properties of the emulsions. It was observed that emulsion circulation time in blood

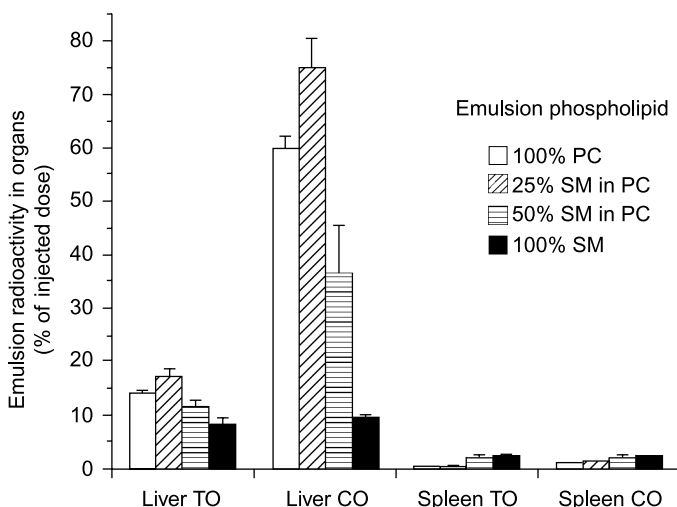


Figure 4.6 Radioactivity in the liver and spleen of triolein (TO) and cholesteryl oleate (CO) labels after injection of emulsions stabilized by mixtures of sphingomyelin (SM) with egg phosphatidylcholine (PC). TO-CO-cholesterol emulsions stabilized with mixtures of SM and PC were injected intravenously in conscious rats. Organ uptakes of radioactive TO and CO labels in the emulsions were measured 20 min after injection. Results are means $\pm$ SE of at least four experiments for each observation. By analysis of variance the differences between groups were statistically significant with $P < 0.01$ for liver TO, <0.001 for liver CO, <0.01 for spleen TO, and < 0.025 for the spleen CO. (Adapted with permission from Elsevier Ref. [21] Copyright 1992.)

depended on the length of the PEG chain. PEG₂₀₀₀-DOPE and PEG₅₀₀₀-DOPE kept the emulsions in the blood the longest. Approximately 60–70% of the injected dose remained in the blood after 30 min. PEG₁₀₀₀-DOPE and Polysorbate 80 emulsions demonstrated comparable behavior in vivo with 47% of the injected dose remaining in the blood after 30 min. The high emulsion concentration observed in the blood for PEG₂₀₀₀-DOPE and PEG₅₀₀₀-DOPE translated into lower accumulation in the liver. Consequently, coating an emulsion surface with PEG of sufficient chain length can confer long circulating properties to submicrometer emulsions.

Hoarau et al. [38] evaluated two different processes to incorporate 1,2-distearoyl-*sn*-glycero-3-phosphatidylethanolamine-*N*-monomethoxy-[PEG] (PEG-DSPE) into lipid nanocapsules. The conventional method was the first investigated and involved the addition of PEG-DSPE with the other surfactants during the emulsification of the oil. The second method evaluated was after insertion, wherein an aqueous micelle solution of PEG-DSPE was added to the preformed lipid nanocapsules and then incubated for 90 min at 60°C. The authors observed that the post-insertion method enhanced the amount of PEG-DSPE that could be incorporated into the nanocapsule compared with the conventional process. For the conventional method, the amount of PEG₂₀₀₀-DSPE and PEG₅₀₀₀-DSPE could not exceed 3.4 and 1.5 mol% of the total surfactants, respectively, because physical instability would occur. In contrast, PEG-DSPE could be incorporated into the lipid nanocapsules at higher quantities (6–10 mol%) using the post-insertion method, regardless of the PEG chain length. Consequently, the pegylated lipid nanocapsules prepared by the post-insertion technique circulated longer in blood as a result of the greater PEG density at the surface (Figure 4.7), e.g. the AUC increased fivefold as the proportion of PEG₂₀₀₀-DSPE increased from 1.7 to 10 mol%.

Other Methods to Enhance Circulation Time

Surfactants containing PEO chains such as, PEO-*b*-poly(propylene oxide)-*b*-PEO (PEO-*b*-PPO-*b*-PEO, poloxamers) and PEO-hydrogenated castor oil (cremophors) have also been investigated to enhance the hydrophilicity of emulsion surfaces to reduce opsonin binding and uptake by the MPS. Lee et al. [54] demonstrated that emulsions coated with poloxamer 338 reduced the amount of ibuprofen octyl ester delivered to the MPS organs. Ueda et al. [55] investigated the influence of ethylene oxide number in PEO-hydrogenated castor oil surfactants on menatetrenone clearance rate from plasma and distribution to MPS organs. They observed that a minimum of 20 ethylene oxide units ($M_r = 880$ Da) is required to prolong menatetrenone circulation time in plasma. The prolonged circulation of emulsions containing more than 20 ethylene oxide units translated into a lower accumulation of menatetrenone in the liver. Menatetrenone incorporated into emulsions with 10 ethylene oxide units were rapidly removed from plasma and taken up to a greater extent by the liver.

4.4.5 Active Targeting of Selected Cells

Drug delivery systems utilizing ligands that specifically recognize determinants on the surface of target cells have been extensively investigated in liposomes [56–58] and macromolecular prodrugs [59]. However, few studies have been done with emul-

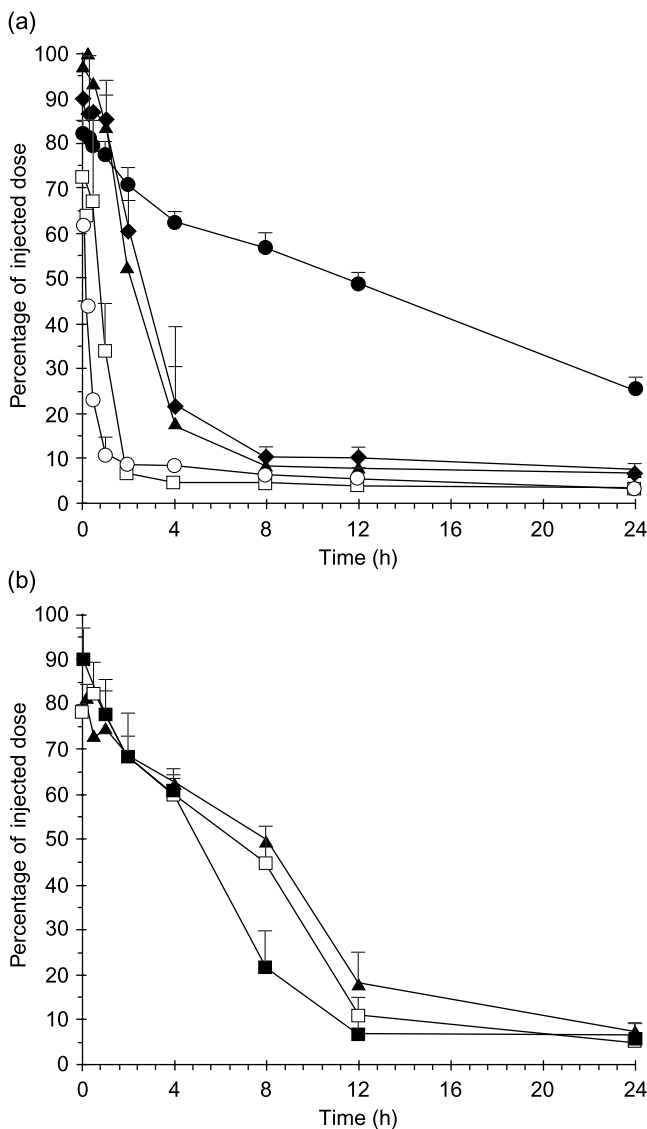


Figure 4.7 Blood concentration–time profile of stealth liposomes and different lipid nanocapsule formulations prepared by (a) the conventional or (b) the post-insertion method. Mean $\pm$ SD (standard deviation) ($n = 3 - 5$). (a) Pegylated liposomes (●), plain lipid nanocapsules (○), pegylated lipid nanocapsules with 1.7 mol% 1,2-distearoyl-*sn*-glycero-3-phosphatidylethanolamine-*N*-monomethoxy-[polyethyleneglycol] (PEG₂₀₀₀-DSPE) (□), pegylated lipid nanocapsules with 1.4 mol% PEG₅₀₀₀-DSPE (▲), and pegylated lipid nanocapsules with 3.4 mol% PEG₂₀₀₀-DSPE (◆). (b) Pegylated lipid nanocapsules with 6 mol% PEG₂₀₀₀-DSPE (■), pegylated lipid nanocapsules with 6 mol% PEG₅₀₀₀-DSPE (□), and pegylated lipid nanocapsules with 10 mol% PEG₂₀₀₀-DSPE (▲). Formulations were injected intravenously at a dose of 2 mg lipids/rat. (Adapted with permission from Springer Science and Business Media Ref. [38] Copyright 2004.)

sions. Incorporating ligands on to the emulsion interface is a promising method to enhance specificity towards the target site(s). For this method to be successful, lipid emulsions must have the appropriate ligand(s) anchored on to the surface, and must be able to reach the target cells, bind to the receptors, and either enter the cell or empty the contents in the vicinity of the cell.

Lipid Emulsions Associated with Apolipoprotein E

Apo-E has an affinity for both the remnant and LDLs on hepatocytes and is an important mediator in the uptake of emulsions and lipoproteins by the liver. Incorporating Apo-E on lipid emulsions provides an opportunity to target hepatocytes. Rensen et al. [60] investigated the possibility of using lipid emulsions associated with Apo-E as drug carriers for a model antiviral prodrug, iododeoxyuridine-oleoyl (IDU-OL₂), to target hepatocytes selectively for improved therapy of hepatitis B viral infection. The emulsions were prepared using natural lipids (PC, lysophosphatidylcholine, TO, and CO) and had a mean size of approximately 80 nm to mimic natural chylomicrons. The lipid emulsions were radiolabeled with [¹⁴C]CO and [³H]IDU-OL₂ to track the in vivo distribution of the whole droplet and prodrug, respectively. After intravenous injection, lipid emulsions pre-loaded with Apo-E were removed faster from serum and were taken up more by the liver than the control emulsions (lipid emulsions not pre-loaded with Apo-E) (Figure 4.8). The uptake of the carrier by the liver reached about 70% of the injected dose for the Apo-E pre-loaded emulsion compared with only 30% for the control. The prodrug exhibited similar removal rates from serum and uptake by the liver as the carrier. The authors also showed that the carrier and prodrug were mainly taken up by parenchymal cells with little accumulation in the endothelial or Kupffer cells. Introducing lactoferrin, a glycoprotein that blocks Apo-E-mediated uptake of lipoproteins by parenchymal cells, before injecting the lipid emulsions, resulted in a considerable reduction in emulsion uptake by the liver.

Sugar-coated Emulsions to Target Hepatocytes

Incorporating Apo-E on to the lipid emulsion is a complex process that may cause reproducibility and stability issues [61]. Another method to enhance selectivity for hepatocytes is to incorporate sugars such as galactose on the surface of the lipid emulsion, to target the carbohydrate receptors on hepatocytes. Ishida et al. [61] investigated the biodistribution of galactosylated (Gal) and nongalactosylated emulsions after intravenous injection in mice. The results demonstrated that the Gal emulsion was more quickly removed from the blood compared with the bare emulsion, whereby the AUC values for the Gal emulsion and bare emulsions were 1.9 and 3.7 (percentage of dose × h/mL), respectively. In addition, the uptake of the Gal emulsion by the liver was 3.2 times greater than that of the bare emulsion. Moreover, Gal emulsions were taken up 7.4-times more by parenchymal cells than by non-parenchymal cells, compared with only 4.3 times for the bare emulsions. These findings suggest that introducing galactose on the surface of lipid emulsions is a promising method for delivering drugs to hepatocytes.

Antibody-peptide Conjugation onto Long-circulating Lipid Emulsions

Cancer cells often overexpress certain antigens or receptors, which provides another possible method to enhance the selectivity of anticancer drugs toward tumor tissues

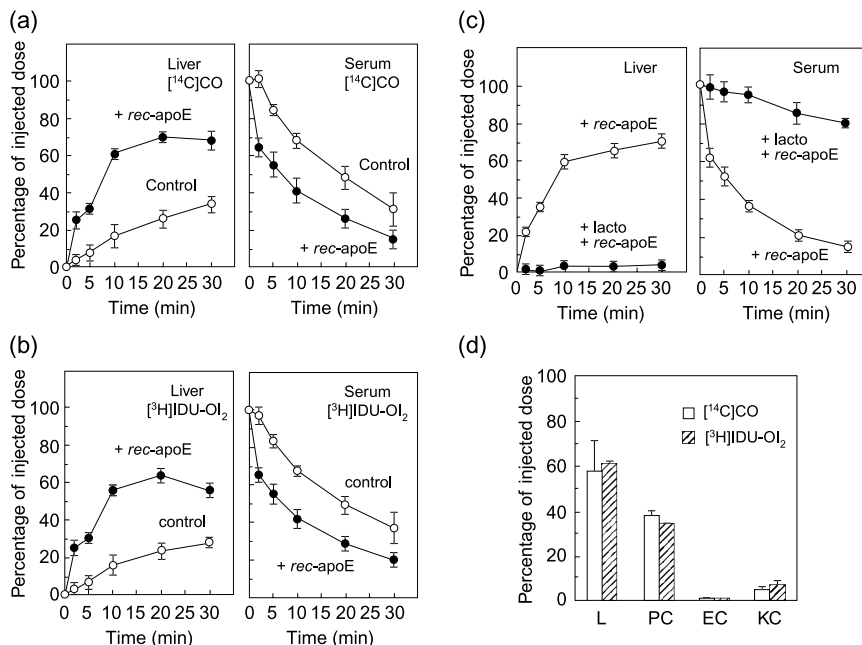


Figure 4.8 Liver uptake and serum decay of the control and human recombinant (*rec*) Apo-E-enriched emulsion iododeoxyuridine-oleoyl ($[^3\text{H}]\text{IDU-OL}_2$) in rats, in the absence or presence of lactoferrin. Control and *rec* Apo-E-enriched emulsions, double-labeled with $[1-^{14}\text{C}]\text{cholesteryl oleate}$ ($[^{14}\text{C}]\text{CO}$) and $[^3\text{H}]\text{IDU-OL}_2$ were injected into fasted anaesthetized rats. (a, b, c) At the indicated times, the liver uptake and serum decay of (a) $[^{14}\text{C}]\text{CO}$ and (b) $[^3\text{H}]\text{IDU-OL}_2$ were determined. (c) The liver uptake and serum decay of *rec* Apo-E-enriched emulsions were also determined after preinjection of lactoferrin. (d) At 30 min after injection of emulsion *rec* Apo-E-IDU-OL₂, the liver was perfused. Total liver (L) association was determined and parenchymal (PC), endothelial (EC), and Kupffer (KC) cells were subsequently isolated. Values are means $\pm$ SD of three experiments. (Adapted with permission from Macmillan Publishers Ltd Ref. [60] Copyright 1995.)

[58]. Antibodies, antibody fragments, or synthetic peptides can be incorporated onto the carrier surface specifically to recognize the antigen/receptors on cancer cells and offers a possible solution to the nonspecific and slow uptake of colloidal carriers by cancer cells. Linking antibodies to liposomes has been widely studied; however, much more progress is required for its successful application [58]. In theory, the techniques applied to liposomes can be carried over to lipid emulsions. Ideally, the tumor-targeting ability of antibodies can be coupled with the long-circulating properties of pegylated lipids. To avoid the interference between the PEG chain of pegylated lipid emulsions and the antibodies incorporated into the emulsion surface, antibodies linked to PEG chains have been developed in recent years. Lundberg et al. [62] successfully conjugated an anti-B-cell lymphoma monoclonal antibody (LL2) on to a lipid emulsion by coupling LL2 to PEG-DSPE. The immunoreactivity of the LL2-conjugated emulsion was tested by determining the binding affinity

to WN, the anti-idiotypic antibody to LL2. The results showed that increasing the density of LL2 at the surface enhanced the binding of the emulsion to WN by up to 40 antibodies per droplet.

4.4.6 Drug Leakage from Emulsions

Drug retention within the droplet after intravenous administration is another important factor to consider when designing lipid emulsion, not only for dissolution purposes but also as carriers for lipophilic drugs. Controlling the biodistribution of the entire droplet will not enhance the therapeutic effect if the drug is released from the carrier before it reaches the target site. Takino et al. [27] suggested that the drug must have adequate lipophilicity ($\log P > 9$) to remain sufficiently incorporated in the emulsion in the blood circulation. The influence of lipophilicity on drug leakage was observed by Kurihara et al. [63]. The group evaluated the pharmacokinetics of two lipophilic anticancer agents, rhizoxin and RS-1541, incorporated into lipid emulsions after intravenous injection in rats. The lipophilicities of rhizoxin and RS-1541 were very different with $\log P$ values of 1.9 and 13.8, respectively. After intravenous injection, rhizoxin was removed much more quickly from plasma than RS-1541 and distributed more to the liver, lung, and intestine. The different pharmacokinetic profiles can be attributed to the lower retention of rhizoxin within the lipid emulsion after injection. Similarly, Sakaeda et al. [64, 65] found that Sudan II, with a $\log P$ of 5.4, was rapidly released from the lipid emulsion in plasma. Consequently, the lipid emulsion did not alter the pharmacokinetics of Sudan II. The lipophilicity of a drug can be increased by chemically modifying it. A drawback of this approach is, however, that chemical modifications may reduce efficiency or completely inactivate the drug.

4.5 PREPARATION OF EMULSIONS FOR INTRAVENOUS ADMINISTRATION

To be used for intravenous administration, emulsions must be biocompatible, biodegradable, nontoxic, sterile, isotonic, physically and chemically stable, and non-immunogenic [1]. Moreover, droplet size must be small enough to avoid forming pulmonary emboli. To achieve these requirements, the excipients, additives, and manufacturing conditions must be carefully selected. In addition, a complete physicochemical characterization of the emulsions is necessary, followed by a long-term stability testing schedule on all promising formulation candidates. This section describes the basic factors that need to be considered when developing emulsions for intravenous injection.

4.5.1 Excipient and Formulation Considerations

Internal Phase (Oils)

To comply with the essential requirement of biocompatibility, research on injectable emulsions has primarily focused on the use of vegetable oils (triglycerides) as the

oil phase [66]. Triglyceride oils can be characterized as long, medium or short chain, depending on the number of carbon atoms per hydrocarbon chain. LCTs contain 14, 16, 18, 20, or 22 carbons in a fatty acid chain, which may or may not be unsaturated [24]. MCTs are derived from coconut oil and contain saturated fatty acids with chains of 6, 8, 10, or 12 carbons [1, 24, 67]. Lastly, short-chain triglycerides (SCTs), such as triacetin and tributyrin, have chain lengths of only two and four carbons, respectively. LCTs and MCTs should be considered in the initial stages of formulation because many of these oils are approved for injection and are found in a number of FDA (Food and Drug Administration) approved products [67] (Table 4.4). The choice of oil usually depends on the solubility and stability of the drug. MCTs have 100-fold greater water solubility than LCTs, and consequently are typically better solubilizers for drugs because most hydrophobic drugs have some polarity [24, 67]. Kan et al. [68] reported that triglycerides with short fatty acid chains were better solubilizers for paclitaxel, a lipophilic anticancer drug. They reported that paclitaxel solubility increased as the number of carbons per hydrocarbon chain decreased, e.g. tributyrin (C4) and tricaproin (C6) provided greater paclitaxel solubility than tri-caprylin (C8) and other plants oils with a mixture of 6–22 carbons per hydrocarbon chain. Triacetin (C2) gave the highest paclitaxel solubility at a value of 75 mg/mL. Triacetin is not, however, approved for injection and might be difficult to emulsify as a result of its relatively high water solubility.

Vitamin E (DL- α -tocopherol) has been investigated as an alternative biocompatible oil to triglycerides to solubilize highly lipophilic drugs [69–71]. Constantinides et al. [70–72] have formulated a submicrometer emulsion of paclitaxel with high drug loading (8–10 mg/mL) using vitamin E as the internal phase and D- α -tocopheryl polyethyleneglycol 1000 succinate (TPGS) and poloxamer 407 as the emulsifiers (TOCOSOL-paclitaxel). This formulation is less toxic and has greater antitumor activity in mice bearing B16-melanoma tumors than the commercial formulation for paclitaxel (Taxol). At the MTD for Taxol (20 mg/kg), TOCOSOL-paclitaxel showed greater tumor regression than Taxol on a q3dx5 dosing schedule.

TABLE 4.4 List of oils used in commercial emulsions for parenteral nutrition

Oils	Commercial product name	Manufacturer
<i>LCTs</i>		
Cottonseed oil	Lipofundin	B. Braun
Safflower oil	Liposyn	Abbott Laboratories
Soybean oil	Intralipid	Kabi-Pharmacia
	Soyacal	Alpha Therapeutics
	Travamulsion	Travenol Laboratories
	Liposyn III	Abbott Laboratories
	Lipofundin S	B. Braun
	Trivé 1000	Egic
Safflower oil : soybean oil <i>LCTs</i> + <i>MCTs</i>	Liposyn II	Abbott Laboratories
Soybean oil : <i>MCTs</i> , (1 : 1)	Lipofundin MCT/LCT	B. Braun

LCT, long-chain triglyceride; MCT, medium-chain triglyceride.

Moreover, tumor growth was suppressed further at higher doses of this formulation (40 and 60 mg/kg). TOCOSOL-paclitaxel is currently in phase III clinical trials [72]. In addition to being a solubilizer for poorly soluble drugs, vitamin E may provide some therapeutic value. Bartels et al. [73] examined the influence of vitamin E, administered intravenously in an emulsion before surgery, on ischemia and reperfusion (I/R) injury in a double-blinded study on 68 patients. I/R injury is usually an outcome of liver surgery, which causes oxidative stress and cell damage. The results of the study indicated that administering vitamin E before surgery may reduce the impact of I/R injury in liver surgery. The antioxidant activity of vitamin E has also been shown to protect against doxorubicin-induced cardiotoxicity in animal studies [74, 75]. Moreover, vitamin E was found to enhance the anticancer activity of doxorubicin on human prostatic carcinoma cells in vitro [76].

Another possible internal phase for intravenous emulsions is perfluorocarbons. Emulsions containing perfluorochemicals have been investigated as contrast agents for diagnostic tissue imaging or as carriers for the transport of oxygen offering an alternative to blood transfusions [77, 78]. Perfluorochemicals are chemically inert, synthetic molecules containing carbon and fluorine atoms, and are capable of dissolving considerable amounts of oxygen [78]. They are hydrophobic and as a result require emulsification for dispersion in aqueous media. Several types of perfluorochemicals have been investigated such as, perfluorooctyl bromide ($C_8F_{17}Br$), perfluorodecyl bromide ($C_{10}F_{21}Br$) and perfluorodichlorooctane ($C_8F_{16}Cl_2$). Imavist (formally known as Imagent) and Oxygent are perfluorocarbon emulsions presently undergoing clinical trials as an ultrasound contrast agent and artificial blood substitute, respectively.

Emulsifiers

The purpose of surfactants is to emulsify the oil phase and provide physical stability against flocculation and coalescence during storage, which may be for extended periods of time. Surfactants provide physical stability by reducing the oil–water interfacial tension and promoting droplet–droplet repulsion. Injectable emulsions are frequently emulsified with natural lecithins obtained from either egg yolk or soybeans. These lipids are biocompatible and biodegradable, and have relatively good emulsifying properties [79]. Lecithins are differentiated by the nature of the head group, and the length and degree of saturation of the acyl chains. The head group can be phosphatidic acid (PA), ethanolamine (PE), serine (PS), or PC, and determines the surface charge of the emulsion. At pH 7, PE and PC head groups are uncharged, whereas PA and PS head groups are anionic. Surface charge can promote long-term emulsion stability by electrostatic repulsion and can influence its biodistribution in vivo.

The length and degree of saturation of the acyl chains greatly influences the gel–liquid phase transition temperature (T_c) and the surface properties of lipid bilayers (liposomes) and monolayers (emulsions). The T_c refers to the temperature at which the lipids shift from a highly ordered gel state to a less ordered fluid. Saturated lipids generally have phase transitions above room temperature (e.g. T_c of 1,2-distearoyl-*sn*-glycero-3-phosphatidylcholine (DSPC; $C_{18:0}$) is $58^\circ C$) [12, 79]. Introducing unsaturations or reducing the length of the acyl chains decreases the T_c .

substantially (e.g. T_c of DOPC (C18:1) is -22°C and of DMPC (C14:0) is 23°C) [12, 79]. Most natural phosphatides have chain lengths of 16–18 carbons; however, chains with as few as four carbons also exist. Nii et al. [79] observed that both PC acyl chain length and degree of chain unsaturation influenced the ability of the lipid to emulsify tricaprylin (glyceryl trioctanoate). PCs with shorter and saturated acyl hydrocarbon chains were more effective emulsifiers because they produced emulsions with smaller mean globule size with less change in appearance and droplet size over time.

Lipid emulsions are often co-emulsified with a biocompatible synthetic surfactant to enhance emulsification properties. An example of an oil requiring co-emulsification is vitamin E. Previous studies have shown that stable tocopherol emulsions cannot be prepared with lecithin as the sole emulsifier [72]. A possible explanation for this observation is the greater polarity of tocopherol compared with vegetable oils as a result of the presence of a hydroxyl group on the aromatic ring. The enhanced polarity of tocopherol may solubilize more lecithin in the emulsion core, resulting in less emulsifier at the interface to stabilize the system. Consequently, a more hydrophilic co-emulsifier is required to emulsify tocopherol. The synthetic surfactants approved for intravenous injection are few and include polysorbates, cremophors, and poloxamers.

To aid in the initial selection of emulsifiers, the hydrophile–lipophile balance (HLB) method is widely used. HLB is a system that classifies surfactants on an arbitrary scale, based on the relative proportions of the hydrophilic and hydrophobic parts on the molecule. Each surfactant is given a number, usually between 0 and 20. If the HLB value is high, the surfactant has a relatively large number of hydrophilic groups and is more soluble in water. In contrast, surfactants with a low HLB are more hydrophobic and will consequently be more easily dispersed in organic phases. In general, stable w/o emulsions are formed from surfactants with a low HLB, whereas those with a high HLB are typically used to make stable o/w emulsions. HLB values for several synthetic emulsifiers that are approved for intravenous injection are listed in Table 4.5. The HLB method also classifies the oil, but in terms of HLB ‘required’ ($\text{HLB}_{\text{required}}$). The $\text{HLB}_{\text{required}}$ specifies the HLB of the emulsifier that will produce the most stable emulsion. Oils are usually given two $\text{HLB}_{\text{required}}$ values, one to produce a stable o/w emulsion and the other for a stable w/o emulsion. This method allows the formulator to match the HLB of the emulsifiers with the $\text{HLB}_{\text{required}}$ of the oil to produce a stable emulsion. The $\text{HLB}_{\text{required}}$ values to produce a stable o/w emulsion for cottonseed oil, safflower oil, and soybean oil are 7.85, 7.72, and 7.66, respectively (Crodamol catalogue).

The HLB concept is advantageous in the initial screening stage of emulsion development because it reduces the number of emulsifiers to consider for a given type of oil. Although the formulator should be aware that the HLB method has serious limitations, arising from the fact that only the molecular structure of the individual surfactant is considered and the emulsion as a whole is ignored [8]. For instance, the HLB method does not take into account pertinent factors such as the conformation of the surfactant, salinity of the aqueous phase, or temperature [80]. Consequently, even if HLB and $\text{HLB}_{\text{required}}$ are correctly matched, the emulsion produced may not be stable.

TABLE 4.5 Several non-phospholipid surfactants approved for intravenous administration in at least one country or under clinical investigation

Chemical name	Common names	Properties	M_r	Reference	HLB	Reference
Poly(ethylene oxide) 35 castor oil	Cremophor EL	Nonionic	2515	[117]	13.5	[118]
Poly(ethylene oxide) 40 castor oil	Cremophor RH 40	Nonionic	N/A	—	14–16	[119]
Poly(ethylene oxide) 60 castor oil	Cremophor RH 60	Nonionic	N/A	—	15–17	[119]
Poly(ethylene oxide) 20 sorbitan monolaurate	Polysorbate 20	Nonionic	1225	[120]	16.9	[120]
Poly(ethylene oxide) 20 sorbitan monopalmitate	Polysorbate 40	Nonionic	1282	[120]	15.6	[120]
Poly(ethylene oxide) 20 sorbitan monooleate	Polysorbate 80	Nonionic	1310	[121]	15.0	[120]
PEO ₈₀ - <i>b</i> -PPO ₂₇ - <i>b</i> -PEO ₈₀ ^a	Poloxamer 188	Nonionic	8400	[122]	29	[118]
PEO ₁₀₁ - <i>b</i> -PPO ₃₆ - <i>b</i> -PEO ₁₀₁ ^{a,b}	Poloxamer 407	Nonionic	12600	[123]	22	[124]
D- α -Tocopheryl polyethyleneglycol 1000 succinate ^b	TPGS	Nonionic	1513	[121]	13	[72]
Poly(ethylene oxide)-15- hydroxystearate	Solutol HS-15	Non-ionic	958	—	14–16	—
Deoxycholic acid	—	Anionic	392	—	24	[72]
Glycocholic acid	—	Anionic	465	—	N/A	—

^aPoly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly(ethylene oxide).^bIn phase III clinical trials.

Aqueous Phase

The isotonicity of an injectable emulsion is important in order to avoid disturbing the state of cells in contact with the formulation. The final osmolarity should be between 200 and 300 mosmol/kg and can be achieved by adding isotonicizing agents such as glycerol, sorbitol, and xylitol to the aqueous phase. Glycerol is more commonly used and can be found in most parenteral emulsions including Intralipid, Lipofundin N, Liposyn, and Soyacal. Ionic agents such as sodium chloride can also adjust osmolarity; however, they should be avoided as the ions can destabilize emulsions (see earlier). The pH of the final emulsion may need to be adjusted and this can be done by adding small amounts of HCl or NaOH. The desired pH is usually between 7 and 8 to maintain physiological compatibility and minimize hydrolysis of the oil and phospholipids [81].

Antioxidants are often added to the emulsion to eliminate or reduce oxidation of the drug, oil, and emulsifier [8, 67]. Common antioxidants used for injectable formulations include α -tocopherol, deferoxamine mesylate, and ascorbic acid [81]. The formulation may also require the use of preservatives to resist microbial growth. Microorganisms may change the physicochemical properties of the emulsion, such

as color, odor, pH, and physical stability, and may present a health hazard [8]. Common preservatives used in injectable preparations include phenol, cresol, and methyl, ethyl, or propyl esters of *p*-hydroxybenzoic acid [82].

4.5.2 Emulsion Preparation

The most common process for manufacturing emulsions is to incorporate the drug during the emulsification of the oil [66]. Another method of incorporating the active ingredient is to add a sterilized solution of the drug dissolved in a solvent to a pre-formed sterilized emulsion. This method is not often done because of stability issues that may be encountered, such as drug precipitation in the aqueous phase and emulsion cracking [1, 4].

The first step in emulsion preparation is usually to dissolve the water-soluble components (isotonizing agent and preservatives) in the aqueous phase and the lipophilic compounds (drug and perhaps the antioxidant) in the oil phase. The emulsifier can be dispersed in either phase. Both phases are typically heated and agitated to facilitate the dispersion of the various components [67]. The oil phase is then added to the aqueous phase. As the oil is added, the mixture is agitated with the aid of a medium-shear mixer and is usually heated. The rate of addition should be optimized because adding the oil phase too quickly can lead to incomplete dispersion of the oil in the aqueous phase [67]. The temperature and duration of heating in the premix stage depend largely on the thermosensitivity of the drug, oil, and emulsifier(s). This premix stage produces a coarse emulsion and can have a substantial impact on the final product [66]. A premix that is uniform with a droplet size under 20 μm generally produces a final emulsion that is more unimodal and physically stable [66].

After the premix stage, droplet size must be decreased to less than 5 μm in diameter and preferably below 1 μm , to avoid blocking the capillaries of the lungs. To produce emulsions with small droplet size, microfluidization or high-pressure homogenization is usually used. Microfluidization is a process whereby a liquid mixture is forced by high pressure through an interaction chamber, which splits the stream into two and then recombines them at ultrahigh velocities [83]. The product can be recycled to reduce droplet size further. The combination of high shear, turbulence, and cavitation generated by this apparatus can produce submicrometer emulsions with a narrow size distribution [84]. In high-pressure homogenization, fluid is forced at high pressure by means of a plunger pump through a very narrow channel. Depending on the type of homogenizer, the fluid may then collide head on with another high velocity stream or hit a hard-impact ring. Droplet size is reduced by cavitation, high shear forces, and high-speed collisions with other droplets [85]. Pressure, temperature, and number of passes are parameters that can be controlled and influence the efficiency of droplet size reduction.

After the desired droplet size is achieved, the formulation is filtered to remove large droplets or debris, and sterilized. Sterilization can be achieved by autoclaving or by filtration through a 0.22 μm cartridge filter. The heat generated by autoclaving can cause the oil and lecithin to hydrolyze, liberating free fatty acids, which will reduce the pH of the formulation. The conditions for sterilization by autoclaving will

need to be selected carefully to minimize the degradation of heat-sensitive products. Filter sterilization, on the other hand, greatly reduces the heat burden on the emulsion, although this process does not provide the same guarantee for sterility as autoclaving [67]. Not all emulsions can be sterilized by filtration, because a mean droplet size of less than 200 nm is an essential requirement. Large droplets may clog the 0.22 μm cartridge filter and prevent sterile filtration [86]. The main manufacturing steps involved in the production of intravenous emulsions are outlined in Figure 4.9.

4.5.3 Emulsion Characterization

Injectable emulsions are often characterized for mean droplet diameter, size distribution, surface charge, and phase inversion temperature. The aforementioned properties are useful in predicting emulsion stability, biocompatibility and in vivo biodistribution. Control over droplet size and size distribution can impart some specificity toward target tissues and are also important predictors of biocompatibility.

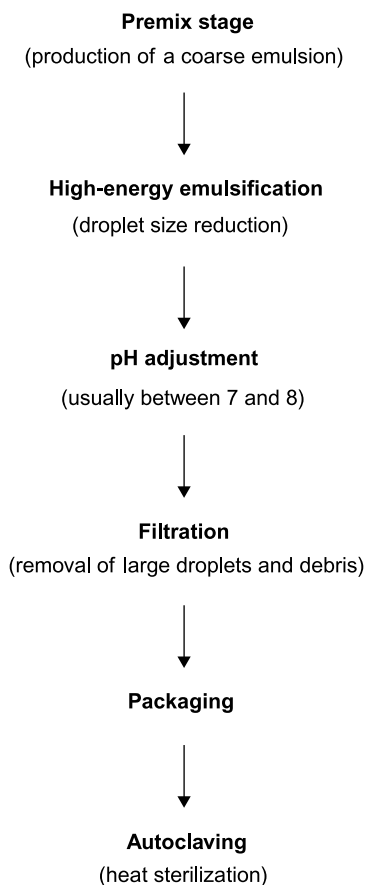


Figure 4.9 The main manufacturing steps involved in the production of intravenous emulsions.

ity because droplets larger than $5\mu\text{m}$ can potentially form pulmonary emboli. The maximum allowable droplet size for intravenous administration is, however, unclear. Emulsions containing few droplets above $5\mu\text{m}$ might not necessarily cause any adverse reaction because capillary blockage may be reversible by droplet degradation and large droplets may pass through small capillaries by deforming [87]. Burnham et al. [88] found that fat droplets larger than $7.5\mu\text{m}$ in diameter could deform and pass through pulmonary vasculature without difficulty. There are a number of techniques available to measure mean droplet size and size distribution of emulsions. Dynamic light scattering (also known as photon correlation spectroscopy or quasielastic light scattering), atomic force microscopy, static light scattering (or intensity light scattering), and electron microscopy are frequently used to determine the size and size distribution for droplets below $1\mu\text{m}$ [38, 89]. However, apart from atomic force microscopy and electron microscopy, the upper limit of detection on these instruments prevents the evaluation of the droplet size distribution above $5\mu\text{m}$. For detection of droplets larger than $5\mu\text{m}$, light obscuration, electrical-sensing zone (Coulter Counter), and optical microscopy are appropriate methods [90–92].

Surface charge measurements are also useful indicators of emulsion biocompatibility. Surfaces with a net positive charge are more likely to aggregate in the bloodstream in the presence of plasma proteins than negatively charged or neutral droplets. Charged surfaces can also impart physical stability to the emulsion by preventing/reducing coalescence on random collisions through electrostatic repulsion (see earlier). The surface charge of an emulsion droplet can be obtained through measurements of ζ potential using laser Doppler anemometry [93]. Lastly, phase inversion temperature, the temperature at which the emulsion changes from o/w to w/o or vice versa, can be a useful predictor of emulsion stability during temperature-altering processes such as heating, emulsification and sterilization [94].

4.5.4 Stability Measurement

The stability of an emulsion formulation is vital for its use in clinical applications. The formulation must display physical, chemical, and microbial stability for at least 1 year, if not more. The difficulty in emulsion formulation is that the system must be stable in aqueous solution, as opposed to polymeric micelles [95] or nanoparticles [96], which can be easily stored as a lyophilized powder whereby they have less opportunity to destabilize. Creaming and a visible layer of oil are classic signs of a physically unstable emulsion, whereas formulation discoloration is a typical indication of chemical instability. A long-term stability testing schedule should be performed on all promising formulation candidates, whereby each emulsion is stored at various temperatures ranging from 4 to 50°C [81]. The emulsions should be monitored for changes in size, pH, drug content, ζ potential, viscosity, electrical conductivity, and chemical composition [81].

Physical Stability

The long-term stability of an emulsion is difficult to estimate and only time can actually tell you whether the formulation is stable. Waiting for extended periods of time

to find out whether a number of formulation efforts are stable is very impractical and there are methods available to accelerate stability testing. Most accelerated tests induce physical instability by increasing the number of collisions between globules. Accelerated tests based on sample heating are not, however, reliable because they do not reflect the environment of samples kept under storage conditions. Heating the sample not only enhances collisions but also diminishes the protective action of adsorbed surfactants, increases the solubility of all components, promotes the degradation of heat-sensitive products, alters the electric double-layer, and reduces surfactant adsorption at the emulsion interface, which can cause a potentially stable emulsion to destabilize, leading to erroneous results [2, 81].

Steam sterilization is considered an acceptable temperature-raising accelerated test because it approximates the environment that an emulsion would experience during autoclaving. Excessive shaking and freezing–thawing cycles are other commonly used accelerated stability testing processes because these techniques predict the conditions that a formulation will be subjected to during transportation and storage [81]. Another good method to estimate emulsion stability is to make size measurements frequently several weeks after the formulations are prepared. Emulsions that increase in size over time during the first few weeks in storage will invariably destabilize [2]. If there is no change in size, the formulator can have some hope that the emulsions will be physically stable long term [2].

Chemical Stability

Injectable emulsions can undergo chemical changes by oxidation and hydrolysis of the oil and/or emulsifier [67]. Chemical instability can be detected by formulation discoloration and changes in pH caused by the increase in free fatty acids. Chemical instability can be reduced by storing the emulsions refrigerated, protected from light, and in sealed containers with a layer of an inert gas, typically argon. These precautionary methods will reduce hydrolysis of the oil and emulsifier(s) [67]. Degradation of the encapsulated drug can also occur during storage. Consequently, the integrity of the encapsulated drug over time must also be determined [67].

Some groups have investigated storing lipid emulsions as a lyophilized powder to overcome some of the stabilized issues encountered when stored in solution [97, 98]. Freeze–drying emulsions is, however, difficult because the droplets may crack during the lyophilization process; it is rarely done. Bensouda et al. [99] evaluated the influence of a number of cryoprotective agents (glucose, mannitol, sorbitol, maltose, lactose, glycine, and dextran) on the success of the freeze–drying process. Glucose, maltose, sorbitol, and lactose provided protection against changes in particle size, whereas mannitol, dextran, and glycine offered no protective action.

4.6 LIPID EMULSIONS FOR THE DELIVERY OF NUCLEIC ACID-BASED DRUGS

Gene therapy is the science in which nonfunctional genes are substituted, altered, or supplemented for the treatment of genetic or acquired diseases. The difficulty in the successful application of gene therapy is the complexity of delivering functional

genetic material such as plasmid DNA, antisense oligonucleotides (ODNs), or small interfering RNA into the cell. This is a result of their rapid degradation in plasma and their inability to cross cell membranes as a result of their hydrophilic and polyanionic nature, and relatively large size. Viral vectors have been extensively investigated for gene delivery [100]. However, concerns about host inflammatory and immune responses have created a demand for nonviral vectors [101–103]. As such, cationic liposomes are commonly investigated because they enhance transfection of DNA into the cell [104, 105]. Nevertheless, a major shortcoming of this technology is the formation of large aggregates with time and the reduced transfection of the liposome–DNA complex in the presence of serum [106].

Cationic lipid emulsions have been considered as alternative nonviral gene delivery vectors to liposomes. Complexation occurs through electrostatic interactions between the nucleic acids and the cationic lipid emulsifiers. The lipid emulsion–DNA complex can be prevented from forming large aggregates in the presence of serum by co-emulsification with an appropriate nonionic surfactant [107]. Yi et al. [108] prepared a cationic lipid emulsion–DNA complex that retained more than 60% transfection efficiency in the presence of 90% (v/v) serum. This formulation was composed of soybean oil, 1,2-dioleoyl-*sn*-glycero-3-trimethylammonium propane (DOTAP) as the cationic surfactant and co-emulsified with 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE) and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine-N-[PEG 2000] (PEG₂₀₀₀-POPE).

The relatively high transfection efficiency of the lipid emulsion–plasmid DNA complex can be attributed to its ability to resist aggregation in the presence of serum, which may be caused by steric stabilization of the PEO chains of PEG₂₀₀₀-POPE. Indeed, Kim et al. [109] observed that adding a co-emulsifier containing a PEO chain such as Polysorbate 80 or the Brij series (PEO 4-, 7-, 10-, or 23-lauryl ether) produced DNA-complexed emulsions that could resist changes in size in the presence of serum. In contrast, surfactants without a PEO group such as sorbitan monooleate (Span 80), mannide oleate (Montanide 80), and oleyl alcohol aggregated in the presence of serum and during DNA complexation. The authors also observed that the presence of PEO in both Polysorbate 80 and Brij interfered with the electrostatic interactions between DNA and the emulsion interface. DNA and cationic lipid interactions were reduced when the Polysorbate 80 content or PEO chain length in the Brij series increased. Despite the progress, much still needs to be done for the successful application of cationic emulsions to deliver genetic material *in vivo*. Indeed, cationic lipid emulsions will most likely face the same problems as other nonviral gene delivery systems.

4.7 CONCLUSION

Lipid emulsions are promising carriers for highly lipophilic drugs as a result of their biocompatibility, reasonable shelf-life, aptness for large-scale manufacture, and ability to solubilize large quantities of drug in their oily core. Lipid emulsions can also alter the biodistribution of incorporated drugs and enhance specificity toward target tissues by passive and active methods. Evading the MPS or natural fat

metabolism is necessary when the encapsulated drug is to be delivered to non-MPS organs or liver parenchymal cells, respectively. Long-circulating lipid emulsions can be obtained by reducing droplet size and grafting long-chain hydrophilic polymers, such as PEO, to the emulsion interface. Moreover, active targeting using ligands that recognize specific determinants on cells can enhance specificity and uptake by target cells. It should be kept in mind that control over droplet biodistribution alone will not enhance the therapeutic effect because the drug may leak out of the carrier before reaching the target site. In general, drugs with higher lipophilicities ($\log P > 9$) are retained better within the emulsion after intravenous administration. In addition to being carriers for lipophilic drugs, lipid emulsions have also been adapted to deliver genetic material and are an alternative nonviral vector to liposomes.

Several therapeutic lipid emulsions are commercially available for clinical use and other formulations are presently undergoing clinical trials. The use of intravenous lipid emulsions in the clinic as drug carrier vehicles will expand as new and less toxic formulations are discovered.

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