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Nanoemulsions for Intravenous Drug Delivery

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Introduction

Nanoemulsions are composed of nanoscale droplets of one immiscible liquid dispersed within another. Many drugs are hydrophobic, which leads to limited water solubility, causing the delivery of water-insoluble drugs to be a primary focus of drug delivery research. Emulsions provide a central oil core, stably dispersed in water, that can act as reservoir for hydrophobic drugs. While emulsions have long been used for topical administration, the small size of nanoemulsions makes them attractive for parenteral delivery. In addition to solubilization of hydrophobic drugs, emulsions can reduce pain or irritation upon injection, improve pharmacokinetics, allow for new forms of administration, and can provide for sustained or targeted release.

Emulsion Definitions

Emulsions have been broadly defined as two immiscible phases dispersed within another (Becher, 2001). In principle, this definition could apply to a number of systems including, but not limited to, gas-in-liquid, solid-in-liquid, or gas-in-solid. In conventional usage, however, the term solely refers to a liquid-in-liquid dispersion, The definition supplied by the United States Pharmacopeia is that emulsions are two-phase systems in which one liquid is dispersed throughout another liquid in the form of small droplets (*The United States Pharmacopeia*, 2006). Emulsions are not thermodynamically stable, but the stability can be improved by additives such as surfactants and finely divided solids. Within that definition there is no defined size boundary, either inherent or implied.

The two phases are referred to as the continuous and the dispersed phase, with the dispersed phase typically present as a smaller volume percentage. Highly concentrated emulsions, where the dispersed phase is more than 50 vol%, have been studied, but the high viscosity makes them unsuitable for intravenous drug delivery applications. A dispersion of oil in water, of most interest for pharmaceutical applications, is referred to as

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an oil-in-water (o/w) emulsion. For o/w emulsions the emulsifying agent is typically more soluble in the aqueous phase. The reverse emulsion, water-in-oil, is abbreviated w/o and is stabilized by surfactants that are more stable in the oil phase. Multiple phase emulsions such as w/o/w have been increasingly investigated for drug delivery use in recent years, but will not be addressed further here (Khan, Talegaonkar, Iqbal, Ahmed, & Khar, 2006).

Microemulsions

Microemulsions are isotropic, thermodynamically stable systems composed of oil, water, and surfactant. First reported by Schulman in 1943, the term microemulsion was coined by the same author in 1959 (Hoar & Schulman, 1943; Schulman, Stoeckenius, & Prince, 1959). The choice of nomenclature has been lamented by multiple authors as "confusing an emulsion phase with a thermodynamic phase" (Becher, 2001; Mason, Wilking, Meleson, Chang, & Graves, 2006), but it is still the historic and traditional usage. While thermodynamic stability, and not size, is the defining hallmark of a microemulsion, the droplet sizes are below 100 nm (and many times much smaller) (Jadhav, Shaikh, Ambade, & Kadam, 2006).

Nanoparticles

Nanoparticles are considered submicron colloidal systems, generally composed of polymers (Hillaireau & Couvreur, 2006). This general category can be divided into nanospheres, which consist of a polymeric matrix with drugs dispersed throughout, and nanocapsules, which have a polymeric wall enveloping a liquid core. In principle, the nature of the stabilizer should not alter the type or classification of the aggregate. Therefore, liquid containing nanocapsules should be considered as a subset of emulsions, not a fundamentally different vehicle. For that reason, this review will consider all systems with a dispersed oil core that is liquid at room temperatures (and in vivo), whether stabilized by polymers or not. One type of nanoparticle, which is differentiated from any of the above terms, is a solid lipid nanoparticle (SLN) with a lipid core that is solid at room temperature. During formation of SLNs the solid lipid is first melted, then emulsified as a liquid to form an o/w emulsion, and cooled to allow the lipid to solidify. Due to the similarity in formation and content, these particles have been referred to as "emulsions with solid fat globules" (Siekmann & Westesen, 1998). Though inclusion of these systems would not be inappropriate within this chapter, as stated previously only emulsions that have a liquid core at room temperature will be considered.

Nanoemulsion Definitions

As mentioned previously, the term emulsion carries with it no size connotation. Historically there have been efforts to delineate the smallest end of the size range, generally below 1 μm, with unique nomenclature such as miniemulsions (El-Aasser & Sudol, 2004) ultrafine emulsions (Nakajima,

1997) and submicron emulsions (Amselem & Friedman, 1998). Recently, the term nanoemulsion has come into more widespread usage, but it has not been used with consistent meaning. There have been three ways the term has been used. The first is as a replacement for the term microemulsion, to refer to systems that are defined by thermodynamic stability but have a small size (Sarker, 2005). The second is a more precise incarnation of the terms listed above, such as miniemulsion, to refer to emulsions that are smaller in size than 1 μm, i.e., in the nanometer range (Solans, Izquierdo, Nolla, Azemar, & Garcia-Celma, 2005). The third usage parallels the term nanotechnology in referring to emulsions that have a size near 100 nm (Mason et al., 2006). Though the prefix micro might give a misleading size connotation considering the low nanometer size range of microemulsions, the longstanding historical precedence of the term microemulsion should prevent change to the nomenclature. Furthermore, if the term was changed, it would be best to remove the word emulsion from the name completely so that there is no confusion over the level of thermodynamic stability. Microemulsions are thermodynamically stable, whereas emulsions, regardless of the size, are not. For these reasons, the term nanoemulsion should not be used as a substitute for the term microemulsion to refer to a thermodynamically stable system.

The lack of thermodynamic stability is a trait of all emulsions, so size is the only remaining distinguishing criteria. The US government's National Nanotechnology Initiative has defined nanotechnology as "the understanding and control of matter at dimensions of roughly 1–100 nm," which has become the conventional usage of the word. In correlation with the term nanotechnology, some have sought to define nanoemulsions as emulsions with droplet sizes below 100 nm. Even within that definition, there have been divided opinions as to whether the size should refer to the radius or the diameter (Mason et al., 2006). Though this usage might match current language trends, it establishes an arbitrary size boundary because emulsion properties do not instantly change upon crossing the 100 nm threshold.

Nanomemulsions are often mentioned as being translucent or transparent, rather than the characteristic opaque, milky white of traditional emulsions (Mason et al., 2006; Solans et al., 2005). While emulsion droplets do become translucent or transparent at sizes smaller than the optical wavelengths of visible light, the transition does not happen precisely at 100 nm. Furthermore, optical properties of an emulsion are also dependent on the volume fraction of the dispersed phase. A milky-white emulsion can become translucent upon dilution, though there is no reduction in the particle size. Since the properties of the droplet do not change upon dilution, neither should the nomenclature be changed.

It has also been stated that nanoemulsions are stable to creaming or sedimentation (Solans et al., 2005). The stated justification is that at smaller particle sizes the Brownian motion of the droplets is great enough to overcome the effects of gravity (Mason et al., 2006). However, creaming is dependent not only on size but also on the relative densities of the two phases. An o/w emulsion with an oil density of 0.97 g/mL will be much more resistant to creaming than an o/w emulsion with an oil density of 1.8, even if both emulsions have droplets of the same size. Though it is possible

to have nanoemulsions in which creaming does not occur, as with optical properties there is no specific cutoff at 100 nm.

Others have used the term nanoemulsion to mean emulsions that have a diameter in the nanometer range, i.e., below 1000 nm (Solans et al., 2005). This definition offers the most clarity and precision of meaning, by clearly stating that nanoemulsions are emulsions of a certain size, which is indicated by the prefix. A nanoemulsion should then be defined as a heterogeneous system composed of one immiscible liquid dispersed as droplets within another liquid, where the average droplet diameter is below 1000 nm.

However, size is one of the most important variables that can define an emulsion. The name nanoemulsion alone does not eliminate the necessity of using size as a primary defining characteristic. There will be more difference between a nanoemulsion with diameter of 50 nm and one with a diameter of 500 nm, than between a nanoemulsion with diameter of 900 nm and an emulsion with diameter of 1100 nm, even if the names are the same in the former comparison and different in the latter.

Formation of Nanoemulsions

As non-equilibrium systems, the formation of nanoemulsions requires an input of energy. This energy can be supplied by either mechanical equipment or the chemical potential inherent within the components (Solans et al., 2003). Systems that form spontaneously by self-assembly are thermodynamically stable and hence not emulsions.

Though energy is required for formation, the necessary amount varies. An active area of research involves low-energy emulsion formation, so-called spontaneous emulsification or self-emulsification (Gupta & Cannon, 2000; Miller, 2006). In this situation, an emulsion will form spontaneously upon addition of oil and surfactants to water due to the low interfacial tension from high surfactant levels. These systems are envisioned as a technique for oral drug delivery where gentle agitation provided by the gastrointestinal (GI) tract supplies any necessary energy (Gupta & Cannon, 2000).

Phase-Inversion Temperature

Nonionic surfactants containing a polyoxyethylene polar group become more lipophilic with increasing temperatures as the polyoxyethylene chains become dehydrated (Solans et al., 2003). A surfactant that is more soluble in the aqueous phase will favor the formation of o/w emulsions at low temperatures, but at high temperatures w/o emulsions will be more favored. The temperature at which the phase transition occurs has been called the phase-inversion temperature (PIT) (Shinoda & Saito, 1968). Emulsification at the PIT temperature is favorable and requires a minimal energy input, but the droplets formed are unstable (Salager, Loaiza-Maldonado, Miñana-Pérez, & Silva, 1982). Rapid cooling (or heating) can produce kinetically stable emulsions with small droplet sizes and narrow size distributions (Friberg & Solans, 1978). This method is widely

utilized in industrial emulsion formation (Forster & Von Rybinski, 1998). As might be expected, the PIT can be varied by altering the length of either the hydrophobic chain or the polyoxyethylene chain to make the molecule more hydrophobic or hydrophilic.

High-Energy Emulsification

High-energy emulsification methods involve the introduction of mechanical shear through such equipment as high-shear stirrers, high-pressure homogenizers, microfluidizers, or ultrasound generators. A microfluidizer is the piece of equipment most used in the pharmaceutical industry for the production of emulsions (Jafari, He, & Bhandari, 2006). It works by dividing a stream of liquid into two parts, passing each through a narrow opening and then colliding the streams under high pressure. The high-shear forces created by the collision provide very fine emulsions with generally narrow particle size distributions. In typical usage, a coarse emulsion (diameter > 1 μm) is first formed by some other method, and the size of that larger emulsion is reduced in the microfluidizer. The final droplet size and distribution shape will be dependent on both the emulsion components (surfactant amount, oil volume percent, etc.) and the processing parameters (time, temperature, pressure, etc.). As the desired droplet size decreases, the energy required for formation increases. Ultrasonic emulsification is also effective to reduce the size of emulsion droplets into the nanoscale. However, it is only appropriate for a smaller laboratory scale, and not for production level (Walstra & Smulders, 1998).

Emulsion Physical Properties

Though not thermodynamically stable systems, long-term kinetic stability is of paramount importance for nanoemulsions intended for commercial use, especially for drug delivery. Some of the necessary variables to monitor are particle (droplet) size, viscosity, osmolarity, zeta potential, pH, and conductivity for physical and chemical stabilities of the constituent parts (Gupta & Cannon, 2000). Nanoemulsions often contain unsaturated lipids, which can be susceptible to oxidation or hydrolysis.

Emulsion Destabilization

Emulsions in general, nanoemulsions included, can be destabilized by the following mechanisms: creaming (Becher, 2001) (or sedimentation), flocculation (Petsev, Denkov, & Kralchevsky, 1995; Verwey & Overbeek, 1948), coalescence (Kabalnov & Wennerstrom, 1996), or Ostwald ripening (Taylor, 1998). All of these mechanisms have been extensively reviewed elsewhere and therefore the specific relation to nanoemulsions will be emphasized.

Creaming is the separation of emulsion components based on the density of the droplets. The name is derived from the separation of the cream in unhomogenized milk (Becher, 2001). While creaming is usually

considered to be undesirable, the process does not result in irreversible breaking of the droplets. Oil that is less dense than water will rise while oil that is more dense (such as with perfluorocarbon liquids) will settle to the bottom (sediment). It is routinely stated that the small size of nanoemulsions prevents creaming. While this is true in some instances, a more thorough explanation is necessary.

Creaming is driven by gravitational forces. A theoretical treatment called the colloidal law of atmospheres (Russel, Saville, & Schowalter, 1989) has been developed to relate the gravitational potential energy of a droplet at height h above a surface with thermal energy:

$$mgh = k_B T \tag{1}$$

where m is the buoyant mass of a droplet, g is the acceleration of gravity, h is the height, k_B is Boltzmann's constant, and T is the absolute temperature. The mass of a droplet, m, is defined by

$$\frac{4}{3}\pi r^3 \Delta\rho \tag{2}$$

where r is the droplet radius and $\Delta\rho$ is the difference in density between the two phases. If $\Delta\rho$ is 0.1 g/cm^3, a particle with radius of 500 nm will have a gravitational height $\approx$ 0.01 mm, which means that creaming will occur. At the same density a particle with radius of 10 nm will have a gravitational height $\approx$100 cm, well above the height of most containers, and thus creaming will be prevented. However, with that same density difference, a particle with radius of 50 nm will have a gravitational height $\approx$ 0.8 cm so creaming will occur. Furthermore, this analysis only considers a situation where droplets repulse each other and there is no interaction with the solvent. If there is attraction between droplets, creaming can occur regardless of the size. Favorable electrostatic interactions between the droplets and the solvent, which are unaccounted for in this equation, also affect the rate of creaming. The end result is that the smallest nanoemulsions are stabilized against creaming, a significant advantage over macroemulsions, but only if densities of the dispersed phase and the continuous phase are fairly even.

Flocculation refers to a process in which clusters of two or more droplets behave kinetically as a unit, but individual droplets still maintain their identity (Becher, 2001). It is reversible, but may lead to coalescence, which is irreversible. In systems stabilized by nonionic surfactants, the droplets are attracted by van der Waals forces, but repulsed by steric interactions (Solans et al., 2003). The steric repulsion between emulsion droplets, W_s, can be represented by the following equation:

$$W_s \infty k_B T e^{-\pi D/L} \tag{3}$$

where k_B is the Boltzmann constant, T is the absolute temperature, D is the separation distance between droplet surfaces, and L is the film thickness of the adsorbed polymer. If the total interaction energy is smaller than the

energy imparted from Brownian motion, $\sim k_B T$, the particles will remain unflocculated. An increase in the film thickness, L, will increase W_s and thus lead to more stable emulsions. The van der Waals attractive potential between two spherical droplets (with identical radius) is linearly dependent on the radius, with the following relationship (Israelachvili, 1991):

$$W_{vdw} = \frac{-Ar}{12D} \tag{4}$$

where A is the Hamaker constant, r is the radius of the droplets, and D is the distance of separation between droplets. As the radius of the particles decreases, the attractive potential decreases. Therefore, in nanoemulsions with a small radius and large enough film thickness, flocculation can be prevented, another advantage over macroemulsions.

Coalescence is the collision, and subsequent irreversible fusion, of two droplets. The ultimate end of coalescence is complete phase separation. Flocculation precedes coalescence, so the same methods that are appropriate for prevention of flocculation also prevent coalescence. A thick, surfactant film adsorbed at the interface is often sufficient to prevent coalescence, whether in nano- or macroemulsions. However, with the same polymeric thickness, the stabilization will be greater for a nanoemulsion because the polymer layer will be a greater percentage of the total diameter.

Ostwald ripening is the growth in the size of emulsion droplets as the contents of one drop diffuse into another. The driving force for this growth is the difference in chemical potential between droplets, which is generally not substantial for droplets larger than 1 μm. Therefore, Ostwald ripening primarily affects nanoemulsions and is the most serious instability concern for nanoemulsions (Tadros, Izquierdo, Esquena, & Solans, 2004). This effect is related to the Laplace equation for spheres:

$$\Delta p = 2\gamma / r \tag{5}$$

where p is the pressure across an interface, γ is the interfacial tension, and r is the radius of the sphere.

Kelvin adjusted this equation to describe the difference in vapor pressure between a small droplet of a liquid and the bulk liquid, the situation found in an emulsion:

$$RT \ln \frac{p}{p^0} = \frac{2\gamma[V]}{r} \tag{6}$$

where R is the gas constant, T is the absolute temperature, p is the vapor pressure of the bulk, p^0 is the vapor pressure of the droplet with radius r, γ is the interfacial tension, and $[V]$ is the molar volume of the liquid.

As the radius increases, the pressure difference is reduced and the dispersed droplets become more soluble in the continuous phase. If there is any diffusion of the contents of the dispersed phase, large droplets will grow larger at the expense of smaller droplets and the average size of the particle distribution will continually increase. This effect

has been described by Lifshitz and Slyozov (Lifshitz & Slyozov, 1961) and independently by Wagner (Wagner, 1961). According to Lifshitz–Slyozov–Wagner (LSW) theory, the rate of Ostwald ripening, ω, can be expressed by the following equation:

$$\omega = \frac{\mathrm{d}r^3}{\mathrm{d}t} = \frac{8DC_\infty \gamma M}{9\rho^2 RT} \tag{7}$$

where r is the radius of the droplets, t is the time of storage, D is the diffusion coefficient of the molecules of the dispersed phase in the continuous phase, C_∞ is the bulk solubility of the dispersed phase in the continuous phase, γ is the interfacial tension between phases, M is the molar mass of the dispersed phase, ρ is the density of the dispersed phase, R is the gas constant, and T is the absolute temperature. However, the overall diffusion of the dispersed phase is affected by the diffusion across the interfacial layer in addition to the diffusion in the continuous medium, D. If the diffusion across the interface is slower than diffusion through the medium, then the overall rate of ripening will be slower than predicted. As can be seen, the cube of the particle radius varies linearly with time. For an o/w emulsion, the rate of ripening is directly related to the water solubility of the oil and ripening can even be seen in systems where the solubility is in the low nM range.

It should be noted that in the ideal situation of a perfectly monodispersed distribution there would be no ripening because there would be no differences in solubility of droplets based on size. Thus, narrow distributions will be more resistant to Ostwald ripening than broader distributions.

Higuchi and Misra suggested that the addition of a secondary, less water-soluble, component, could slow ripening (Higuchi & Misra, 1962). The slower diffusion of the secondary component will lead to a heterogeneous distribution with smaller droplets enriched in the less soluble component and larger droplets enriched in the more soluble component. However, this internal segregation will be thermodynamically opposed as osmotic pressure will act to limit differences between droplets and equilibrium will eventually be reached. This principle has been successfully applied with hydrocarbon (Taylor, 1998) and fluorocarbon emulsions (Sharma, Lowe, & Davis, 1988; Weers, Ni, Tarara, Pelura, & Arlauskas, 1994).

The rate of ripening of a two-component disperse phase system is represented by the following equation (Kabalnov & Shchukin, 1992):

$$\omega_{mix} = (\phi_1/\omega_1 + \phi_2/\omega_2)^{-1} \tag{8}$$

where ϕ represents the volume fraction and the subscripts 1 and 2 refer to the more and less water-soluble components, respectively. As ϕ_2 becomes larger, it becomes the dominant term until it solely controls the ripening rate. With a properly chosen additive, Ostwald ripening can be effectively eliminated.

There have been contradictory reports within the literature regarding the effect of excess surfactant upon emulsion stability (Taylor, 1998). In some instances, the rate of ripening has increased as the amount of surfactant has increased, whether the excess surfactant is present in the form of vesicles (Krafft, Rolland, & Riess, 1991) or micelles (Capek, 2004; Izquierdo et al., 2002; Taylor, 2003). One of the justifications cited for such an effect is that the supramolecular aggregate (micelle or vesicle) provides a reservoir to solubilize excess oil, thus increasing the effective solubility of the oil in water. As can be seen in Eq. (7), as the solubility, C_∞, increases, so too does the rate of ripening. However, a decrease in the ripening rate as the amount of surfactant increases has also been reported (De Smet, Deriemaeker, & Finsy, 1999; Liu, Sun, Li, Liu, & Xu, 2006). In these cases, it has been proposed that the oil solubilized in the micelles is not dispersed in the continuous phase, and therefore is not subject to the same mass transfer between droplets. In this argument, C_∞ is lowered as oil is withdrawn from the continuous phase into micelles, thus causing the ripening rate to decrease. An additional study showed that alkane emulsions stabilized by hexaethylene glycol dodecyl ether were unaffected by surfactant concentration (Hoang, La, Deriemaeker, & Finsy, 2003).

Though Ostwald ripening can be present in nanoemulsions, it has some advantages for pharmaceutical development. The ripening rate provides clear criteria for determining the acceptability of formulations. Commercialization of an emulsion mandates stability for at least 18 months (Gupta & Cannon, 2000). The rate of ripening allows the estimation of long-term stability, which in turn suggests guidelines for specifications and expiration dates. If the ripening rate is too rapid, then the nanoemulsion will be unacceptable for pharmaceutical use.

The instability of emulsions can also have some drawbacks. For emulsions intended for parenteral injection the FDA requires sterilization (*The United States Pharmacopeia*, 2006). The most commonly employed form, terminal heat sterilization such as with an autoclave, can often affect the physical stability of the emulsion droplets. Additionally, emulsions are often stored at 5°C for greater stability, which places limits on the product use and storage (Gupta & Cannon, 2000).

Emulsions for Intravenous Drug Delivery

Historical

Though not traditionally labeled as nanoemulsions, phospholipid-stabilized soybean oil emulsions were the first approved intravenous emulsion (Benita, 1998) and have been used clinically as i.v. nutritional supplements for over 40 years (Driscoll, 2006). With an average particle diameter around 300 nm these systems fall in the nano range, which makes them the first therapeutic nanoemulsion. Either egg or soy lecithin is used to emulsify an oil from a natural vegetable source (e.g., soybean). Because of the stabilizer, these emulsions are sometimes called lipid microspheres. Phosphatidylcholine (PC) is the primary component of lecithin, but the amount varies depending

on the degree of purification. Lipoid E80, a commonly used surfactant mixture, contains 80% PC. Phosphatidylethanolamine is the secondary component, followed by phosphatidylserine and phosphatidylinositol present in smaller amounts (*The United States Pharmacopeia*, 2006). As they are not intended for drug delivery per se, the history and development of these emulsions for nutritional purposes will not be discussed further (Figure 15.1).

Intravenous emulsions, like all parenteral products, are required to meet pharmacopeial requirements to be sterile, isotonic, nonpyrogenic, nontoxic, biodegradable, and stable, both physically and chemically (Benita & Levy, 1993). The biocompatibility of emulsions for parenteral nutrition, and concomitant FDA approval, has led them to be a template for future nanoemulsion development. The initial examples of nanoemulsions for drug delivery were all conceptually similar to nutritional emulsions. This similarity could take two forms: either extemporaneous development, where a drug is added to a pre-made commercially available emulsion, or de novo emulsification where an emulsion is made "from scratch" using the same components found in nutritional emulsions (Klang & Benita, 1998). In recent years, development has continued into more innovative emulsion formulations, such as varying the emulsified oil and/or including polymeric emulsifiers.

A number of excellent reviews concerning nanoemulsions for drug delivery appeared in the late 1990 s to 2000 (Buszello & Müller, 2000;

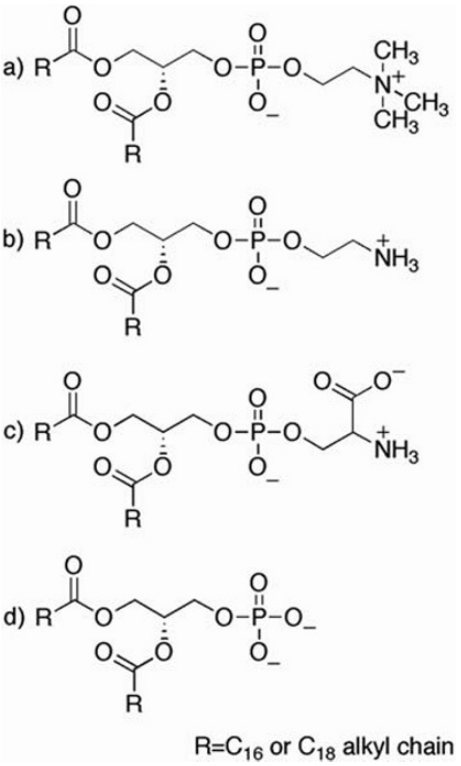

Figure 15.1 Structure of phospholipids present in lecithin, often used as an emulsifier for nanoemulsions: (**a**) phosphatidylcholine (**b**) phosphatidylethanolamine, (**c**) phosphatidylserine, and (**d**) phosphatidylinositol.

Table 15.1 Nanoemulsions that have been marketed for intravenous delivery. Emulsions for parenteral nutrition are not included in the list. Companies listed are those that originally developed the emulsion. In subsequent years, corporate mergers may have led to name changes, e.g., Zeneca has now become AstraZeneca.

Trade name	Drug	Company	Use	Market
Diprivan®	Propofol	Zeneca Pharmaceuticals, UK	General anesthesia	Worldwide
Limethason®	Dexamethasone palmitate	Green Cross, Japan	Chronic rheumatoid arthritis	Japan and Germany
Lipo-NSAID® Ropion®	Flurbiprofen axetil	Kaken Pharmaceutical Co., Japan	Post-operative and cancer pain	Japan
Liple®	Alprostadil (PGE1)	Green Cross, Japan	Peripheral vascular disorders and maintenance and patent ductus arteriosus	Japan
Diazemuls®	Diazepam	Kabi-Pharmacia, Sweden	Treatments for excitation, anxiety, tension, sedation, muscle spasm, convulsions, tetanus, delirium	Europe, Canada, Australia
Diazepam-Lipuro®		Braun, Germany		
Dizac®		Ohmeda Pharmaceuticals		
Etomidat Lipuro®	Etomidate	Braun, Germany	General anesthesia	Germany
Fluosol-DA®	Perfluorodecalin, Perfluoro-tripropylamine	Green Cross, Japan	Artificial blood substitutes	Worldwide

Gupta & Cannon, 2000; Klang & Benita, 1998). For that reason, specific examples in the following section will exclusively focus on the time period since 2000. Furthermore, though nanoemulsions have also been widely studied for oral and topical delivery, intravenous delivery will be the sole focus of this review. A comprehensive list of all marketed nanoemulsions for intravenous delivery (nutritional emulsions excluded) can be found in Table 15.1. Extended discussions for each example can be found elsewhere (Klang & Benita, 1998).

Marketed

[Table 15.1] presents nanoemulsions that have been marketing for intravenous delivery.

Current Research

While it is apparent that nanoemulsions (as with any drug delivery vehicle) will only be investigated if it is believed that an emulsion formulation can improve drug performance in some way, the nature of that enhancement can take many forms. Perhaps the most obvious improvement is for the solubilization of drugs with a low aqueous solubility.

Other areas of study include the reduction of pain/irritation upon injection, reduced toxicity of the drug in vivo, improved pharmacokinetics of the drug, or the possibility of a new method of delivery (e.g., intravenous versus oral administration). Nanoemulsion formulations can also provide drug targeting or multiple functionalities with imaging coupled to therapy. Finally, nanoemulsions can create a new therapeutic area that would not be present without emulsions, such as with fluorocarbon emulsions intended for blood substitutes. The following paragraphs will discuss examples for each of the above categories. Of course, some examples of nanoemulsion formulation will encompass more than one of the above categories, so there will be some unavoidable overlap. Table 15.2 shows a representative list of drugs that have been investigated for intravenous delivery via nanoemulsions since 2000.

Table 15.2 A representative list of drugs investigated for intravenous delivery via nanoemulsions since 2000.

Drug	Reference
All-*trans* retinoic acid	Chansri, Kawakami, Yamashita, and Hashida, 2006
Amphotericin B	Müller et al., 2004
BCH	Shawer, Greenspan, Oie, and Lu, 2002
Carbamazepine	Akkar & Müller, 2003a
Clomethiazole	Nordén, Siekmann, Lundquist, and Malmsten, 2001
Cyclosporin A	Kim, Choi, and Lee, 2002
Dexamethasone palmitate	Seki et al., 2004
Flunarizone	Wang, Wang, Zhang, He, and Tang, 2007
Halofantrine	Mosqueira, Legrand, and Barratt, 2006
Indomethacin	Cruz et al., 2006; Palakurthi, Vyas, and Diwan, 2005; Pohlmann, Weiss, Mertins, da Silveira, and Guterres, 2002
Itraconazole	Akkar and Müller, 2003b
Lorazepam	Medina, Salvadó, and del Pozo, 2001
Menatetrenone	Ueda et al., 2004
mTHPC	Bourdon, Mosqueira, Legrand, and Blais, 2000
Nalbuphine	Wang, Sung, Hu, Yeh, and Fang, 2006
Nimodipine	Yu, He, and Tang, 2006
Norcantharidin	Lixin, Haibing, Xing, Ruiying, and Dawei, 2006
Paclitaxel	Constantinides et al., 2000; Dias, Carvalho, Rodrigues, Graziani, and Maranhão, 2007; Tiwari, Tan, and Amiji, 2006
Pazufloxacin Mesylate	Liu, Huang, Peng, Liu, and Wu, 2007
Probucol	Ishida et al., 2004
Prostaglandin E1	Komori, Aiba, Kushima, Kawasaki, and Kurosaki, 2007
Resveratrol	Hung, Chen, Liao, Lo, and Fang, 2006
Silymarin	Abrol, Trehan, and Katare, 2004
SPK-843	Mozzi, Benelli, Bruzzese, Galmozzi, and Bonabello, 2002
Xanthone	Teixeira, Alonso, Pinto, and Barbosa, 2005
Zinc phthalocyanine	Primo et al., 2007

Solubilization of Poorly Soluble Drugs

As solubilization of poorly soluble drugs is the most apparent application for nanoemulsions, many examples can be found in prior reviews (Gupta & Cannon, 2000; Klang & Benita, 1998). Therefore, only a single representative example will be discussed here. Lorazepam is injected intravenously for premedication and sedation before an operation. It is usually administered as a solution in organic solvents such as propylene glycol. The highest concentration that can be achieved in an aqueous diluent (5% dextrose in water) is 0.05 mg/mL. A phospholipid-stabilized soybean oil emulsion was able to stably emulsify lorazepam at 1 mg/mL, a 20-fold increase, which could significantly reduce the volume needed for injection (Medina et al., 2001).

While emulsion solubilization has been applied to a host of lipophilic drugs, it traditionally cannot be employed if the drug has limited solubility in oils that have regulatory acceptance. One way to counter that deficiency is to position the drug directly in the interfacial lecithin layer. This has previously been achieved by dissolving the drug together with lecithin in an organic solvent, evaporating the solvent, and then using that mixture for de novo emulsification (Lance, Washington, & Davis, 1995). Unfortunately, this method is impractical on an industrial scale. Müller and coworkers devised a method to make the solubilization of drugs at the interfacial layer more feasible on a large scale. With this methodology, termed SolEmuls®, solid nanocrystals of the drug are homogenized with commercially available lipid emulsions, without the need of any organic solvents. In this way, they were able to stably emulsify carbamazepine (Akkar & Müller, 2003a), itraconazole (Akkar & Müller, 2003b), ketoconazole (Akkar, Namsolleck, Blaut, & Müller, 2004), and amphotericin B (Müller et al., 2004).

Reduced Pain/Irritation

At the direct site of intravenous injection, some drugs can cause local irritation. These drugs, as well as certain cosolvents in aqueous solutions, can also cause phlebitis, an inflammation of a vein that can lead to pain or redness. Nanoemulsions eliminate the need for cosolvents, as well as encapsulating drugs that might otherwise be irritants, and in both cases can reduce local irritation upon injection.

Polyene antifungal agents, like amphotericin B, contain a large macrocycle with a series of conjugated double bonds and are typically insoluble in water. Recent efforts have focused on synthesizing new agents that retain the strong antifungal activity while improving the water solubility (Strippoli, D'Auria, Simonetti, Bruzzese, & Simonetti, 2000). One candidate, SPK-843, showed promising antifungal activity but gave mild phlebitis upon repeated intravenous injections. Therefore, it was proposed to study the intravenous injection of SPK-843 in an emulsified form (Mozzi et al., 2002). The drug was extemporaneously added to commercial Intralipid emulsions. After formation, the venous toxicity of the nanoemulsion was tested in the ear vein of rabbits and compared to a 5% glucose solution

of SPK-843. A solution of 5% glucose alone (w/o drug) was tolerated for 17 infusions before vein occlusion due to phlebitis, but when the drug was added only three infusions could be tolerated. With the Intralipid alone, 13 infusions were tolerated, and when the drug was introduced the number remained at 13. Therefore, an emulsified form of the drug reduced the chance of phlebitis compared to an aqueous solution.

In patients with ruptured aneurysms, vasospasm of the cerebral arteries can lead to delayed ischemic deficits, which are responsible for morbidity and mortality. To reduce morbidity and mortality caused by these reasons, nimodipine (NM) is the only available therapy. It is given orally, but its bioavailability is limited. Intravenous injection, as an ethanol solution, has been studied, but leads to irritation. A soybean oil nanoemulsion with lecithin and Tween-80 as the emulsifiers was proposed as a method to reduce irritation (Yu et al., 2006). The irritation was measured both by the rabbit ear vein test, as in the prior example, and by the rat paw lick test. Upon injection of the NM-ethanol solution 100% of the rats licked their paws and the average number of licks was 12, suggesting both a high frequency and a high intensity of pain. With the NM-nanoemulsion formulations, only 60% of the rats licked their paws, and the average number of licks decreased to five, indicating that the both occurrence and intensity of irritation decreased. Furthermore, the nanoemulsions did not alter the pharmacokinetic parameters, as compared to the ethanol solution. Therefore, the in vivo performance was maintained as the pain was reduced.

In another study (Lixin et al., 2006), an anti-cancer drug, norcantharidin, was also formulated in lecithin/Tween-80-stabilized nanoemulsions and the irritation was studied using the rat paw lick test. In this instance, the frequency of rats that licked their paws remained the same at 100% between a solution formulation and a nanoemulsion formulation, but the average number of licks decreased from 18.2 to 8.7, indicating that the intensity of pain was diminished.

Reduced Toxicity of Drug

Beyond the site of injection, drugs or their delivery vehicles can also cause irritation or toxicity once in the body. Paclitaxel is an important chemotherapeutic agent used in the treatment of breast, ovarian, colon, and non-small cell lung carcinomas. The commercially available product Taxol® (Bristol-Myers Squibb) is formulated in a 1:1 v/v mixture of ethanol and polyoxyethylated castor oil (Cremophor EL). Cremophor EL has been associated with bronchospasms, hypotension, and other hypersensitive reactions. To reduce the toxicity associated with Cremophor EL, incorporation of paclitaxel into a wide variety of drug delivery vehicles, including liposomes, micelles, emulsions, and cyclodextrins, has been investigated. A representative nanoemulsion example will be described. Constantinides and coworkers created a nanoemulsion that did not employ any lecithin but used Vitamin E-TPGS (α-tocopherylpolyethyleneglycol-1000 succinate) and Poloxamer 407 to emulsify Vitamin E (DL-α-tocopheryl) as the oil phase (Constantinides et al., 2000). The nanoemulsion droplets had a mean diameter of 67 nm, with 99% below

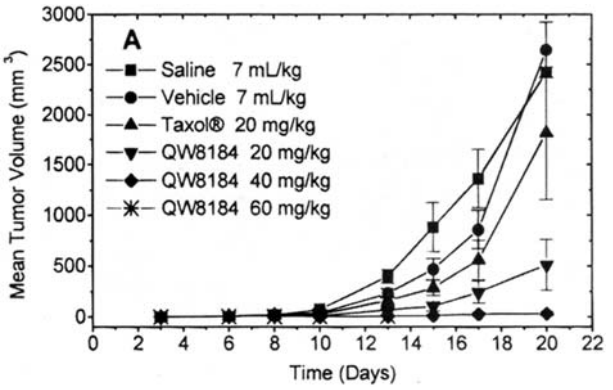

Figure 15.2 Mean tumor regression of B16 melanoma cells in response to QW8184, a paclitaxel nanoemulsion, and Taxol® as a function of time on q3d × 5 schedule. Error bars represent SEM ($n = 8$). Reproduced with permission from Springer.

150 nm, meaning that the emulsions could be filter sterilized with a 0.22 μm filter, an objective of the study. The maximum tolerated dose (MTD) was determined in mice using a tail vein injection. For the commercially available Taxol® formulation the MTD was approximately 20 mg/kg whereas for the nanoemulsion formulation it was approximately 70 mg/kg, over three times greater. The efficacy of the nanoemulsion formulation was assessed with B16 melanoma, a fast-growing solid murine tumor. Nanoemulsions showed increasing efficacy at increasing dosage amounts and were better than the commercial formulation in all cases (Figure 15.2).

Improved Pharmacokinetics

Pharmacokinetics is concerned with the fate of external substances introduced to the body, specifically the extent and rate of absorption, distribution, metabolism, and excretion of compounds. Improving these parameters for more favorable drug performance is a primary objective of drug delivery research in general and for nanoemulsions specifically. One specific parameter that will be mentioned multiple times is the area under the concentration–time curve, abbreviated AUC.

Nalbuphine is a morphine-like drug and one of its advantages over morphine is that it lacks significant withdrawal symptoms. However, due to its short elimination half-life and poor oral bioavailability it needs to be injected every 3–6 h (Lo, Schary, & Whitney, 1987). Prodrugs of nalbuphine have been investigated for parenteral administration, and Fang and coworkers sought to use nanoemulsions for both nalbuphine and its prodrugs (Wang et al., 2006). Egg phospholipid was used as the main emulsifier, along with cosurfactants Brij 30, Brij 98, and stearylamine. Depending on the emulsifier composition the average size ranged from 167 to 314 nm. It was found that the stearylamine-containing emulsions had the highest prodrug encapsulation, while the incorporation of Brij 98 reduced prodrug entrapment, suggesting that the nature of the oil/water interface and the co-emulsifier may affect drug loading. In vivo pharmacokinetic profiles indicate that the plasma concentration of nalbuphine and its prodrugs was enhanced by incorporation into nanoemulsions (Figure 15.3).

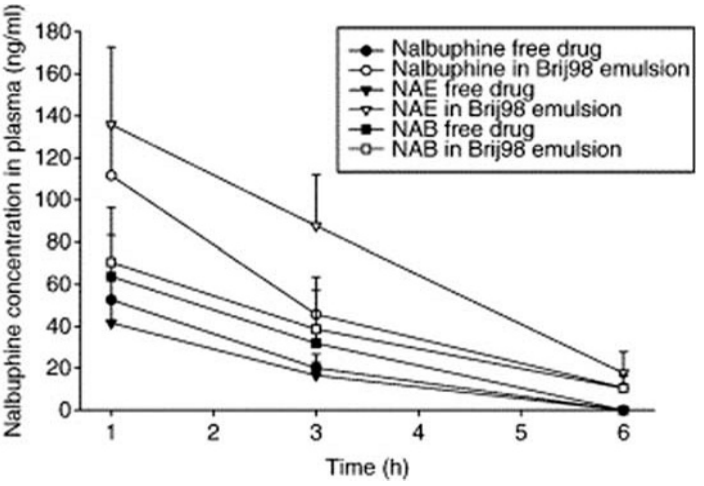

Figure 15.3 In vivo nalbuphine concentration in plasma versus time profiles after intravenous injection of nalbuphine and prodrugs nalbuphine enanthate (NAE) and nalbuphine benzoate (NAB). The free drugs were injected in pH 4 buffer and the emulsions were stabilized by lecithin and Brij 98. Each value represents the mean and SD ($n = 4$). Reproduced with permission from Elsevier.

Tang and coworkers studied the nanoemulsion delivery of flunarizine, a drug used for migraine prophylaxis in which oral administration is marked by low bioavailability and slow absorption (Y. J. Wang et al., 2007). A flunarizine solution (with 5% PEG 400 and 0.2% Tween-80 as stabilizers) and flunarizine-loaded nanoemulsions (both at 1 mg/mL) were compared. While all of the other pharmacokinetic parameters showed no significant difference, the AUC was 1.68 times greater for the nanoemulsion, demonstrating a prolonged circulation time in rats.

Cerebral malaria is a medical emergency that requires treatment that can rapidly reach effective active drug concentrations in vivo (Watkins, Woodrow, & Marsh, 1993). Halofantrine is a well-tolerated and effective antimalarial drug that acts more rapidly than quinine or mefloquine. However, it is given orally and its slow dissolution prevents the rapid therapeutic impact needed to treat cerebral malaria. An intravenous formulation could provide rapid delivery of the drug. A previously investigated parenteral formulation (Krishna et al., 1993) showed local irritation and toxicity. To improve upon those characteristics Barratt and coworkers investigated o/w water nanoemulsions. They demonstrated (Mosqueira et al., 2004) that an emulsion stabilized by poly-D,L-lactide (PLA) and its copolymer methoxy-polyethylene glycol-*co*-poly-D,L-lactide (PLA-PEG) increased the AUC more than sixfold compared to the previous intravenous formulation. In a later work (Mosqueira et al., 2006) poloxamer 188 and lecithin were used in different surfactant combinations with the PLA and PLA-PEG. In all examples, Miglyol® 810 N, a medium chain triglyceride, was used as the oil phase and lecithin as one of the surfactants. Overall, particle diameters of the drug-loaded emulsion ranged from 200 to 350 nm. It was found that the addition of PEG-PLA copolymers increased the emulsion stability compared to those prepared with lecithin alone as well as providing a more consistent, sustained release, which may have application in the treatment of cerebral malaria cases.

Other studies have also shown how PEG coating can affect nanoemulsion circulation and performance in vivo. Palakurthi and coworkers studied the biodisposition of nanoemulsions containing indomethacin, a

non-steroidal anti-inflammatory drug (NSAID) (Palakurthi et al., 2005). Encapsulation in nanoemulsions stabilized by phosphatidylcholine and cholesterol reduced the clearance of the drug by 1.4 compared to the free drug. When the nanoemulsion also contained a DSPE-PEG (1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-polyethylene glycol 2000) conjugate in the stabilizing layer, the drug clearance was reduced by a factor of 3. Because of the reduced uptake into tissues such as the liver and spleen, 7.5 times more of the PEG-coated nanoemulsions ended up at the site of inflammation compared to the uncoated nanoemulsion.

Circulation times were also studied by Ueda and coworkers with nanoemulsions containing menatetrenone and stabilized by polyoxyethylated-hydrogenated castor oils (HCOs) (Ueda et al., 2004). They found that plasma half-lives and liver uptake of nanoemulsions stabilized by HCOs with 10 PEG units were similar to and larger than, respectively, emulsions stabilized by egg yolk phospholipids. However, when the length of the PEG chain was increased to 20 and 60 units there was a marked increase in the circulation time and decrease in the liver uptake, suggesting that there is minimum length of PEG necessary to see improved pharmacokinetic parameters.

In another example, an o/w nanoemulsion of cyclosporin A was compared to commercially available formulations for both oral and intravenous delivery (Kim et al., 2002). When the nanoemulsion was delivered intravenously there was little difference with the commercial i.v. formulation (CIPOL Inj.®) and when delivered orally the AUC was actually less than the commercial oral formulation (Sandimmun Neoral®). However, with both routes of administration the pharmacodynamic efficiency was greater and pharmacodynamic availability was improved twofold versus the commercial formulations.

New Method of Delivery

There are many examples where a drug that is normally delivered orally can be incorporated into a nanoemulsion and injected intravenously, some of which are discussed in the pharmacokinetics section. Another instance where nanoemulsions can open up a new method of delivery is the case of volatile anesthetics for general anesthesia. Volatile anesthetics are low-boiling liquids that are given as gases by inhalation. However, if injected directly into the bloodstream, the time for the anesthetic to equilibrate with the lungs is eliminated, which leads to a more rapid onset of anesthesia. Because direct i.v. delivery of the neat anesthetic causes pulmonary damage and death (Kawamoto, Suzuki, & Takasaki, 1992; Kopriva & Lowenstein, 1969; Sandison, Sivapragasam, Hayes, & Woo-Ming, 1970), fat emulsions have been successfully utilized as a means of delivery for halothane, isoflurane, and sevoflurane (Chiari et al., 2004; Eger & MacLeod, 1995; Musser, Fontana, & Mongan, 1999; Zhou, Luo, Liang, & Liu, 2006). However, the modern volatile anesthetics (except for nitrous oxide) are all highly fluorinated, which reduces their solubility in classic hydrogenated oils and makes them more soluble in fluorinated oils. The solubility of sevoflurane in Intralipid (30%) is limited to a mere 3.5%

(Zhou et al., 2006). Cuignet et al. have demonstrated that the presence of Oxygent (a perfluorocarbon emulsion) greatly increases the blood:gas partition coefficient of isoflurane, sevoflurane, and desflurane compared to Intralipid (Cuignet, Baele, & Van Obbergh, 2002). Building upon this information, it was found that a nanoemulsion stabilized by a fluorinated surfactant, with a fluorinated secondary additive to slow the Ostwald ripening, was capable of stably emulsifying up to 25% sevoflurane, a sevenfold increase over Intralipid. The efficacy and safety of this formulation for intravenous delivery was tested in rats with bolus dosing and found to safely induce anesthesia, from which recovery was smooth and rapid (Fast, Perkins, Pearce, & Mecozzi, 2008).

Drug Targeting

As with other drug delivery vehicles, targeted emulsions are of interest. Targeting can either be active, such as the inclusion of a secondary component in the stabilizing monolayer that has a recognition or functional element for a specific site, or passive, where the final destination is dependent on the size or surface characteristics of the particles.

Active Targeting

Rapidly dividing cells, such as cancer cells, require higher amounts of cholesterol to build cell membranes. Low-density lipoprotein (LDL) is the natural carrier of cholesteryl esters in the body and therefore certain tumors have elevated LDL-receptor activity. Lu and coworkers (Shawer et al., 2002) developed a phospholipid-stabilized nanoemulsion to solubilize a cholesteryl ester of carborane, cholesteryl 1,12-dicarba-closo-dodecaborane-1-carboxylate (BCH), which mimics the natural core of LDL and can be used for boron neutron capture therapy (BNCT). The mean emulsion particle size was 155 nm. If preincubated together, human LDL particles and the nanoemulsions only gave one band in agarose electrophoresis, demonstrating particle interaction and ability of BCH to transfer to the human LDL. Cell culture data showed sufficient uptake of BCH in rat 9L glioma cells for the levels necessary for BNCT.

Cholesterol-rich nanoemulsions that mimic LDL have also been studied over the years by Maranhão and coworkers. In a recent example (Dias et al., 2007), paclitaxel was solubilized in cholesteryl oleate nanoemulsions stabilized by egg phosphatidylcholine. The pharmokinetics of the nanoemulsion and its ability to concentrate the drug in tumors was studied in patients with gynecologic cancers (ovarian, cervix, endometrium). It was shown that paclitaxel in nanoemulsions was stable in the bloodstream and the pharmacokinetic profile was improved compared to the commercial formulation (Figure 15.4). Furthermore, on average 3.5 times more paclitaxel was concentrated in malignant tissues versus normal tissues. Together, these results pave the way for future clinical trials of paclitaxel nanoemulsions.

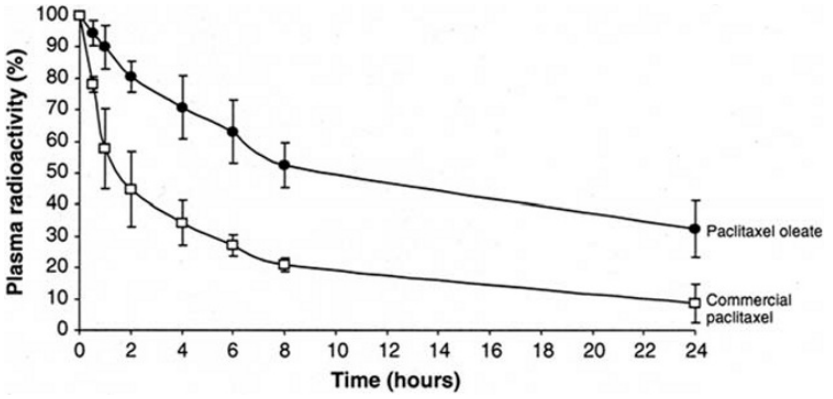

Figure 15.4 Plasma decay curve of nanoemulsion-associated paclitaxel oleate labeled with [^{3}H]-paclitaxel oleate (*filled circle*) and commercial labeled [^{3}H]-paclitaxel (*open square*). Plasma samples were taken over 24 h for radioactive counting in scintillation vials. Results are presented as mean ± SD (*bars*). Reproduced with permission from Springer.

Nanoemulsions have also been used for cell targeting by surface modification with carbohydrates. Hashida and coworkers tested a series of glycosylated emulsions, including galactosylated, mannosylated, and fucosylated. In the first example, a galactose-cholesterol conjugate (Gal-C4-Chol) was inserted into the phosphatidylcholine monolayer of a soybean oil emulsion for hepatocyte-selective targeting in a weight ratio of 70:25:5 soybean oil:PC:Gal-C4-Chol (Ishida et al., 2004). Nanoemulsions containing the Gal-C4-Chol had a higher rate of liver uptake than both emulsions without the Gal-C4-Chol and liposomes with it. Furthermore, a model lipophilic drug, probucol, showed efficient delivery to the liver as compared to Gal-liposomes (Figure 15.5).

A subsequent study showed that the same effect could be achieved with both mannosylated and fucosylated cholesterol conjugates (Yeeprae, Kawakami, Higuchi, Yamashita, & Hashida, 2005). Later work showed

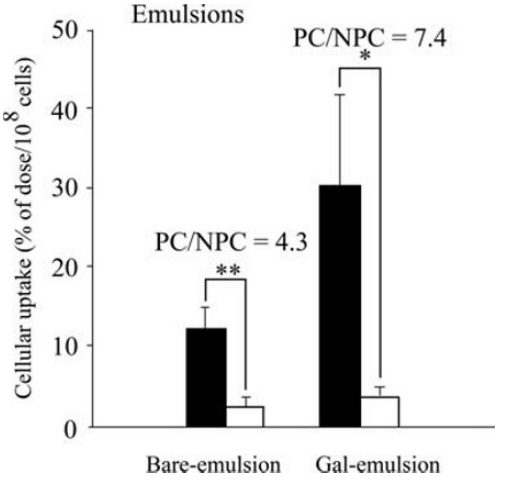

Figure 15.5 Hepatic cellular localization of [^{3}H]-labeled nanoemulsions after intravenous administration into mice. Radioactivity was determined 30 min post-injection in parenchymal cells (PC, filled bar) and non-parenchymal cells (NPC, unfilled bar). Each value represents the mean + SD of three experiments. Statistically significant differences from the control group are shown by *$p<0.05$, **$p<0.01$. Reproduced with permission from Springer.

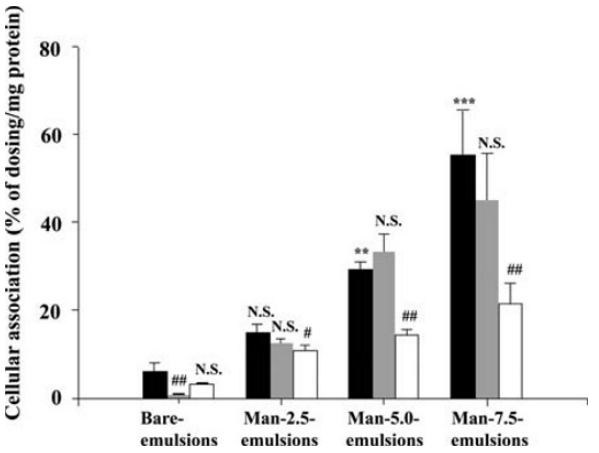

Figure 15.6 Hepatic cellular localization of emulsions after intravenous injection in mice. Radioactivity was determined 30 min post-injection in parenchymal cells (PC, unfilled bar) and non-parenchymal cells (NPC, filled bar). Each value represents the mean ± SD of three experiments. Statistically significant differences between PC and NPC in each group are shown by $*p<0.05$, $***p<0.001$; N.S., not significant. Reproduced with permission from Elsevier.

that the density of mannose on the surface plays a key role in the rate of uptake (Yeeprae, Kawakami, Yamashita, & Hashida, 2006). Nanoemulsions which contained only 2.5% mannose showed no difference in liver accumulation versus the control emulsions, but 5.0 and 7.5% mannose showed an increasingly greater amount of uptake (Figure 15.6).

Some amphiphiles, such as 1-*O*-alkylglycerols, can transiently open the blood brain barrier (BBB) to improve the brain delivery of some anticancer agents (Erdlenbruch, Jendrossek, Eibl, & Lakomek, 2000). Rambhau and coworkers used a variety of 1-*O*-alkylglycerols as cosurfactants along with lecithin to stabilize a soybean oil emulsion for the solubilization of carbamazepine (CBZ), used in the treatment of seizures (Madhusudhan, Rambhau, Apte, & Gopinath, 2007). CBZ is available for oral and chewable tablets, but intravenous delivery could allow for more rapid treatment in acute seizures. The nanoemulsions were around 200 nm in diameter. In emulsions containing 1-*O*-decylglycerol, the brain/serum concentration ratio was 3.0 after 30 min, suggesting the potential for brain targeting.

Passive Targeting

Passive targeting is mainly accomplished by altering the size of injected particles and is a justification for many drug delivery vehicles in place of a free drug. With a particle diameter of 200–300 nm, traditional emulsions for parenteral nutrition rapidly enter the liver and are removed from circulation. Using the same ingredients found in nutritional emulsions (soybean oil and purified egg lecithin), Seki and coworkers created nanoemulsions with a diameter between 25 and 50 nm and larger ones with a 200–300 nm diameter (Seki et al., 2004). It was found that there was a lower liver uptake of the smaller particles, which enabled a longer plasma half-life. The incorporated drug, dexamethasone palmitate (DMP), is an anti-inflammatory agent. It is well known that inflammation sites have leaky capillary walls, which allow smaller particles to passively diffuse across. With a greater plasma half-life, the smaller nanoemulsions passed

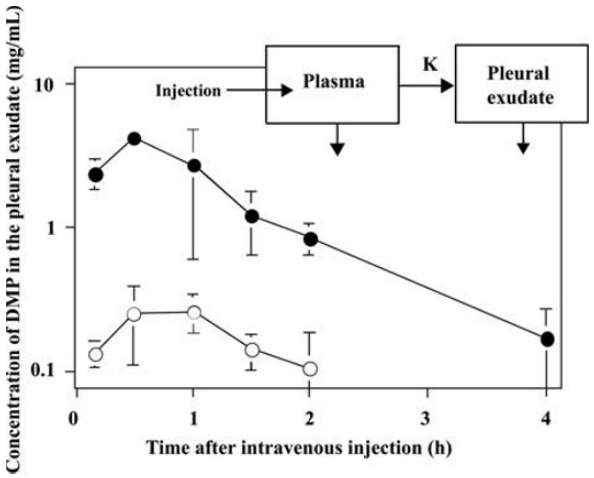

Figure 15.7 Concentration of dexamethasone palmitate (DMP) in the pleural exudate after the intravenous administration of nanoemulsions, incorporating DMP, to rats with experimental pleuritis (dose, 2.5 mg/kg). Each point represents the mean ± SD of five rats. *Closed circles* are nanoemulsions with average diameter 25–50 nm; *open circles* have average diameter 200–300 nm. Reproduced with permission from Elsevier.

into the inflammation sites with greater efficiency, delivering more than three times greater the amount of DMP than with the larger particles (Figure 15.7).

In the prior example, small size was desired so that the nanoemulsion particles could avoid liver uptake. However, in some situations the liver is the target destination of the drug, which makes a larger droplet size more desirable. All-*trans* retinoic acid (ATRA) is an anti-cancer agent that has been studied for the treatment of liver cancer metastasis. Oral administration has been studied, but bioavailability was highly variable, suggesting a possible improvement with intravenous delivery (Ozpolat, Lopez-Berestein, Adamson, Fu, & Williams, 2003). Hashida and coworkers formulated a soybean oil nanoemulsion that was stabilized by either egg phosphatidylcholine (PC)/cholesterol or egg PC/DSPE-PEG and cholesterol to incorporate ATRA (Chansri et al., 2006). The average particle diameter was

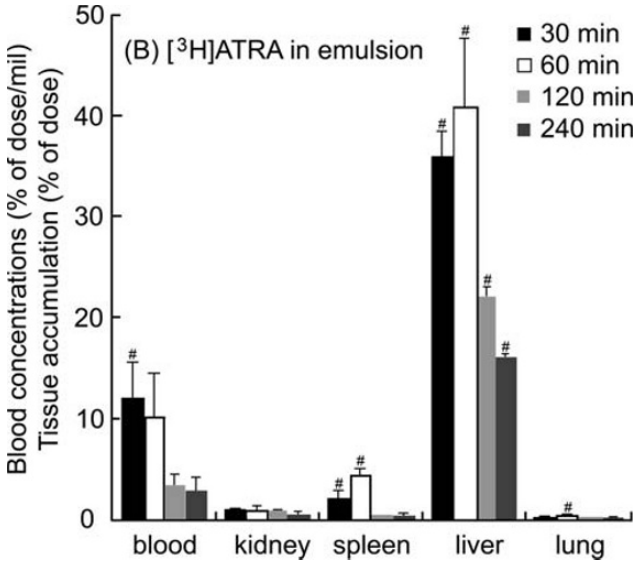

Figure 15.8 Distribution profiles of [^{3}H]all-trans retinoic acid (ATRA) incorporated nanoemulsions after intravenous injection in mice. Results are expressed as the mean + SD of three mice. Statistically significant differences for the emulsified form of [^{3}H]ATRA compared to the non-emulsified form are shown by #$p<0.01$. Reproduced with permission from Elsevier.

133 nm. The nanoemulsions were stable in the presence of albumin in the blood and showed a statistically greater accumulation in the liver, compared to the free drug (Figure 15.8). In CT26 tumor cells, it was shown that emulsified ATRA reduced the number of metastatic nodules and liver weight, compared to a saline solution, as well as ATRA loaded in hydrogenated castor oil (HCO-60) micelles.

Silymarin is a hepatoprotective agent that has a positive effect on metabolism and physiology of liver cells. Abrol and coworkers sought to use nanoemulsions for the delivery of silymarin, which could be passively targeted at the liver (Abrol et al., 2004). Physical characterization studies showed enhanced release of the silymarin from the nanoemulsions, but no in vivo work has been performed yet.

Multiple Functionality

A recent development in nanoemulsions, as with other delivery systems, is the combination of therapeutic and imaging capabilities together in one system. This approach couples drug delivery with tissue imaging to allow simultaneous delivery of the drug and visualization of the physiological effects. Amiji and coworkers devised a 20% pine nut oil emulsion stabilized by egg phosphatidylcholine for the simultaneous solubilization of paclitaxel and gadolinium ions (Gd^{3+}), for enhanced tissue contrast for magnetic resonance imaging (MRI) (Tiwari et al., 2006). The standard nanoemulsions had an average particle diameter of 90.4 nm. In addition to the phosphatidylcholine, a diethylenetriaminepentaacetic acid (DPTA)-phosphatidylethanolamine (PE) complex was added. The PE positions itself at the oil–water interface leaving the DPTA solvent exposed. DPTA is a known chelator for Gd^{3+}, and the high affinity provides for tight binding in an aqueous environment. MRI T_1 relaxation measurements showed similarity between the nanoemulsion and reported literature values for the commercial imaging agent Magnevist®, suggesting the appropriateness of the nanoemulsion as an imaging agent. The nanoemulsions also successfully delivered the paclitaxel to MCF-7 carcinoma cells in vitro, demonstrating the dual functionality, but the drug delivery performance was not superior to an aqueous paclitaxel solution.

New Therapies

Perhaps the most widely studied example of emulsions opening up a new therapeutic area, which would not be available otherwise, is the case of fluorocarbon emulsions for use as artificial blood substitutes. This topic has been extensively reviewed (Krafft, Riess, & Weers, 1998; Riess, 2001, 2005), and so will not be discussed in great detail here. Briefly, though, fully fluorinated molecules (perfluorocarbons, PFCs) have extremely low polarizability, which leads to low van der Waals interactions between molecules. The limited intermolecular forces in PFCs cause them to behave as nearly ideal, gas-like fluids, which allows them to dissolve significant amounts of gas such as O_2. If injected directly, these liquids could form an

oil embolism that could ultimately lead to death, but as an emulsified form, these liquids show no adverse effects in vivo and are able to transport and deliver tremendous amounts of oxygen. Fluosol-DA is an example of a fluorocarbon emulsion that garnered FDA approval, though it was not a commercial success because of practical limitations (the emulsion had to be stored frozen because of stability concerns, thawed and mixed with two annex solutions prior to use, and discarded no more than 8 h after mixing).

Conclusion

The development of new methodologies for the emulsification of o/w biphasic systems has allowed for the formation of nanoemulsions, which possess a stability and particle size that make them particularly suitable for the intravenous delivery of hydrophobic drugs. The combination of different surfactants, additives, and lipids has extended the utility of nanoemulsions to a wide array of drugs. Furthermore, the application of nanoemulsions in drug delivery has made possible the development of new therapies. The safety and efficacy of the use of nanosized emulsions in human patients has been demonstrated with a number of approved products, and current research is continually expanding the usefulness and applicability of nanoemulsion formulations for intravenous delivery. Specifically, the possibility of using nanoemulsions for both imaging and drug delivery has the potential of having a profound impact on the next generation of drug delivery systems.

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