Chapter 6
The Potential of Nanoemulsions in Biomedicine

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Abstract Nanoemulsions are nano-sized oil-in-water or water-in-oil emulsions with a number of applications in biomedicine. Nanoemulsions are highly versatile systems, in terms of composition and physicochemical properties, which can be tailor-made using simple and mild technologies to associate a great variety of drugs and fulfil the requirements for a wide range of pharmaceutical applications. This chapter aims to provide the reader with an overview on compositions and manufacturing methodologies and covers the most recent applications that have been reported in the field of drug delivery.

Abbreviations

BCS	Biopharmaceutical classification system
CFC	Chlorofluorocarbon
CPI	Catastrophic phase inversion

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GRAS	Generally Recognised As Safe by United States' Food and Drug Administration agency
HAMPA 1	Azobenzene substituted poly(sodium acrylate)
HIV	Human immunodeficiency virus
HLB	Hydrophilic–lipophilic balance
LCT	Long chain triglycerides
LSW	Lifshitz-Slezov-Wagner
MCT	Medium chain triglycerides
NIRF	Near-Infrared Fluorescent (Imaging)
O/W nanoemulsion	Oil-in-water nanoemulsion
PARG	Polyarginine
PCL	Poly(ε-caprolactone)
PCL-PEG	Poly(ε-caprolactone)-poly(ethylene oxide)
PEG	Poly(ethylene glycol)
PELC	Poly(ethylene oxide)-block-poly(lactide-co-ε-caprolactone)
PIC	Phase inversion composition
PIT	Phase inversion temperature
PLA	Poly(lactic acid)
PLA-PEG	Poly(lactic acid)-poly(ethylene oxide)
PLGA	Poly(lactic-co-glycolic) acid
PLGA-PEG	Poly(lactic-co-glycolic)-poly(ethylene oxide)
S:O ratio	Surfactant-to-oil ratio
SANS	Small Angle Neutron Scattering
SAXS	Small Angle X-ray Scattering
SEM	Scanning Electron Microscopy
Smix	Mixing ratio of surfactant and co-surfactant
TEM	Transmission Electron Microscopy
W/O nanoemulsion	Water-in-oil nanoemulsion

6.1 Nanoemulsions: Versatile Drug Carriers for Pharmaceutical Applications

Emulsions are mixtures of two immiscible phases, wherein an emulsifier (surfactant) is added in the continuous or external phase to stabilise the dispersed droplets (internal phase). Emulsions are classified as oil-in-water (O/W), when oil droplets are dispersed in aqueous medium, or water-in-oil (W/O), when the internal phase is formed by water.

Emulsions can be further classified depending on droplet size into coarse emulsions and nanoemulsions. The latter are colloidal systems that contain droplets in the nanometer range (typically 10–300 nm). Microemulsions, which also fit in that definition, will not be covered here in detail. In this review, nanoemulsions will be taken to mean kinetically, but not thermodynamically stable, spherical nano-sized emulsions.

Aqueous O/W nanoemulsions constitute the most common type (see Sect. 6.2). Their potential in biomedicine is enormous. They are particularly suited to entrap

hydrophobic drugs in their oily core hence increasing their aqueous levels and absorption. A number of formulations are currently marketed, as for example Novasorb® and Restasis®, or undergoing clinical trials, e.g. CS-1000.

In comparison, research involving W/O nanoemulsions in biomedicine is less extensive (see Sect. 6.3). This is mainly related to the oily nature of the external phase, which restricts administration to non-intravenous routes and limits oral use due to palatability. In spite of this, W/O nanoemulsions remain theoretically suited to the encapsulation and delivery of hydrophilic drugs and macromolecules therefore holding a great potential in drug delivery.

Polymer-coated nanoemulsions, otherwise known as nanocapsules, differ from conventional nanoemulsions in terms of stability, drug loading, and kinetics of drug release (see Sect. 6.4). Nanocapsules are highly versatile systems that can accommodate hydrophilic and hydrophobic agents either dissolved in the oily core or adsorbed onto the shell. Additionally, based on the type of polymeric coating, the surface properties can be conveniently tailored to control their biodistribution and interaction with cells.

This review aims to provide the reader with an overview of the potential of nanoemulsions in biomedicine. It compiles information concerning manufacturing procedures, physicochemical characterisation, and summarises the main biomedical applications for this type of drug delivery systems.

6.2 O/W Nanoemulsions

Aqueous O/W nanoemulsions destined for pharmaceutical applications are typically made from materials listed as GRAS (Generally Recognised As Safe by United States' Food and Drug Administration agency). The selections of the oil, surfactants, and process of manufacturing are critical steps in obtaining nano-sized emulsions and ensuring colloidal stability. The different types of oils that have been reported, summarised in Table 6.1, have been selected from a wide range of materials. Non-ionic or amphoteric surfactants such as poloxamer, lecithin, and Tween® 80 are the most common surfactants used.

6.2.1 Emulsification Methods

Emulsification methods for manufacturing nanoemulsions can be divided into high- and low-energy processes (Tadros et al. 2004; Anton and Vandamme 2009) (for illustration see Fig. 6.1).

High-energy methods (Sect. 6.2.1.1) are based on the application of strong shear forces to break down the oil phase into small droplets (Schultz et al. 2004; Tadros et al. 2004; Delmas et al. 2011). Although highly effective in generating small droplets, the heat generated may be detrimental for labile drugs. In these cases, milder

Table 6.1 Components of O/W nanoemulsions

Oils	Common surfactants
Long chain triglycerides	Span® 85 (sorbitan trioleate)
Soybean oil	Labrafil M 1944 CS® (glycerides and PEG 300 ester mixture)
Carthame oil	Capmul® MCM (mono diglyceride of capric and caprylic acids)
Medium chain mono-, di-, or triglycerides	Vitamin E TPGS (α tocopheryl acid succinate ester/PEG 1000)
Miglyol® 812 N (capric and caprylic acid triglycerides)	Cremophor EL® (polyethoxylated ricin oil)
Captex® 355 (capric and caprylic acid triglycerides)	Myrj® 52 (polyoxyethylene glycol 2000 monostearate)
Labrafac Lipophile WL 1349® (capric and caprylic acid triglycerides)	Tween® 80 (polysorbate 80)
Imwitor® 742 (capric and caprylic mono-, di-, and triglycerides)	Labrasol® (caprylocaproyl polyoxyl-8 glycerides)
Short chain triglycerides	Natural lecithins
Triacetin (triester of glycerol and acetic acid)	Lipoid 80
Fatty acid esters	Phospholipids
Ethyl oleate	Poloxamer® F68
Capmul® PG8 (propylene glycol monocaprylate)	
Capric acid	
Other oils	
Castor oil	
Coconut oil	
Corn oil	
Cottonseed oil	
Bran rice oil	
Evening primrose oil	
Fish oil	
Jojoba oil	
Olive oil	
Linseed oil	
Peanut oil	
Pine nut oil	
Safflower oil	
Squalene	
Sunflower oil	
Sesame oil	
Wheatgerm oil	

low-energy emulsification methods (Sect. 6.2.1.2) may be more appropriate (Izquierdo et al. 2004; Tadros 2005; Anton and Vandamme 2009). Low-energy methods rely on adjusting the composition or environmental conditions to provoke emulsification, (Tadros et al. 2004; Anton and Vandamme 2009; McClements 2011; Gutierrez et al. 2008). Low-energy methods present the added benefit of potentially high yields and are easily scalable.

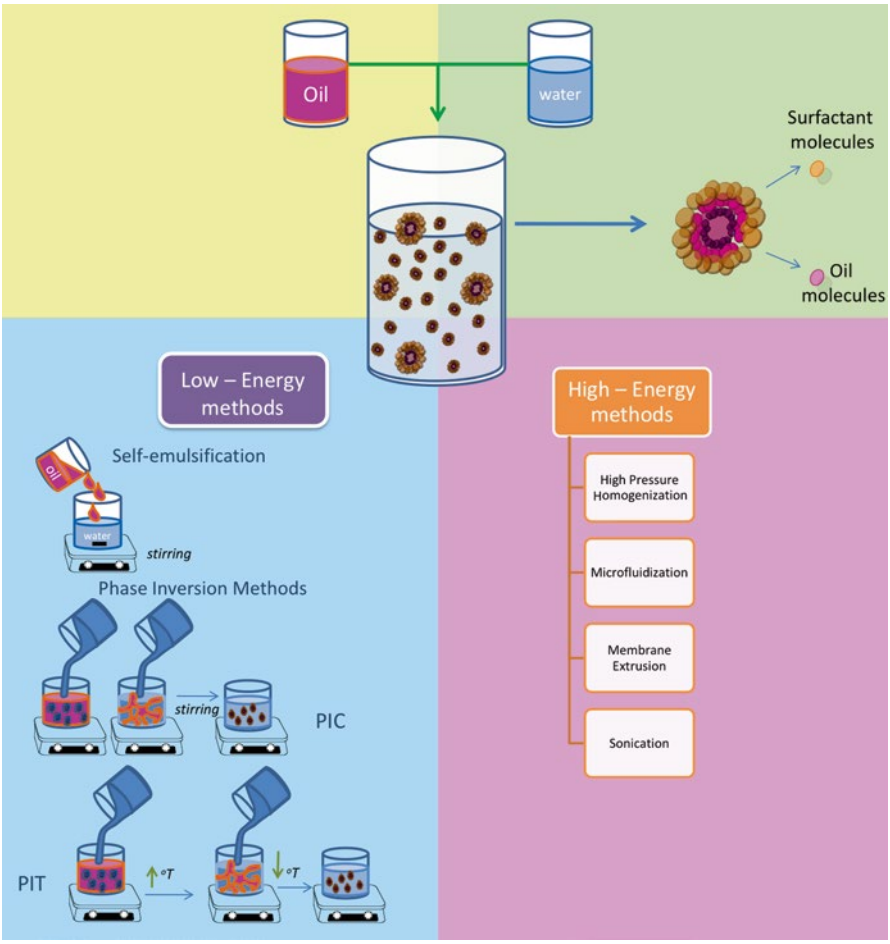

Fig. 6.1 Schematic representation of the most common high- and low-energy manufacturing methodologies applied to the preparation of nanoemulsions

6.2.1.1 High-Energy Emulsification Methods

Homogenization

This method consists of applying intense disruptive forces such as shear, turbulence, and cavitation, into a high-pressure valve homogenizer, to a coarse primary emulsion (Schultz et al. 2004; McClements 2011; Koroleva and Yurtov 2012). Surfactants with a high rate of absorption such as Tween® 20 are required to maintain stability (Taisne et al. 1996; Marie et al. 2002; Koroleva and Yurtov 2012).

Microfluidization

Microfluidization is based on the use of a network of microchannels allowing streams of both the oil and water phases to collide, resulting in the dispersion of the internal phase (Utada et al. 2007a, b). Here, droplet size is controlled not only by the nature and composition of each phase and the emulsifier concentration, but also by the geometry of the microchannels (e.g. co-directional, in opposite directions, T-shaped intersection), and the pressure applied to the liquids (the higher the pressure the smaller the size) (McClements 2010; Koroleva and Yurtov 2012).

Membrane Extrusion

In this method, the dispersion is obtained either by pumping an oil phase through the pores of an extrusion membrane (with the aqueous phase being on the other side of the extrusion phase), or by pumping a coarse emulsion in order to obtain smaller droplet sizes (van der Graaf et al. 2005; Koroleva and Yurtov 2012).

Ultrasonication

Probe sonication produces strong cavitation, a process generating growth, collapse, and oscillation of gas bubbles with release of energy, creating high local temperatures and high shear forces, allowing the preparation of fine nanoemulsions (Abismaïl et al. 1999; Gaikwad and Pandit 2008; Delmas et al. 2011).

6.2.1.2 Low-Energy Emulsification Methods

Self-emulsification

Self-emulsification is the simplest low-energy method. It consists in mixing a lipophilic phase (oil, surfactants, and a water-miscible solvent) with the aqueous phase, with or without additional stabilisers, under vigorous stirring at room temperature. This allows both phases, which were initially thermodynamically stable, to enter a metastable state in which spontaneous emulsification becomes possible. The turbulence created at the interface induces the displacement of the water-miscible solvent and the surfactant from the lipophilic phase to the aqueous phase. This transfer from the oil phase to the water phase results in an increase of the oil/water interfacial area, causing the spontaneous formation of oil droplets (Rahman et al. 2013; Anton and Vandamme 2011).

Emulsification Based on Phase Inversion

Phase inversion methods exploit the ability of an emulsion to switch from one type (W/O) to another (O/W) in response to changes in temperature volume ratio or

composition. Such alterations affect the surfactants' packing parameter (p), a property defined by (6.1):

$$p = v_o / a_e . l_o \tag{6.1}$$

where v_o is the surfactant tail volume, l_o is the tail length, and a_e is the equilibrium area per molecule at the aggregate surface (Nagarajan 2001; Yan et al. 2007).

In brief, surfactants with $p < 1$ are more soluble in water and favour the formation of O/W emulsions, while surfactants with $p > 1$ favour the formation of W/O emulsion as they are more oil-soluble (McClements 2011).

1. Phase inversion based on changes in temperature.

   This method uses temperature to control the affinity of surfactants for the different phases. The temperature at which the affinity of a given surfactant system changes from the dispersed to the external phase, is known as the *phase inversion temperature* (*PIT*) (Anton et al. 2007; McClements 2011).

   As shown in Fig. 6.2, at temperatures below the PIT the surfactant present a $p < 1$ (O/W emulsion). When the temperature increases, the affinity of the surfactant for the aqueous phase decreases, causing the droplets to coalesce leading to the formation of a bicontinuous microemulsion. When the temperature is raised well above the PIT the surfactant becomes more oil-soluble, $p > 1$, favouring the formation of a W/O emulsion. By cooling down to a temperature below the PIT the O/W system will form again, giving rise this way to an O/W nanoemulsion.
2. Methods based on changes in composition

   In the *Phase Inversion Composition* (*PIC*) *method*, the temperature is fixed and the relative affinity of the surfactant for the different phases (i.e. change in p) occurs by altering the composition (Solè et al. 2006; Maestro et al. 2008; McClements 2011).

   The *Catastrophic Phase inversion* (*CPI*) *method* also relies on changes to composition to induce emulsification. For example, phase inversion is achieved by slowly increasing the water volume fraction of a W/O emulsion. Here, however, the phase change is abrupt (catastrophic), without a transitional phase as occurs in the PIC. Droplet size and size distribution will vary with stirring speed and surfactant concentration (Fernandez et al. 2004; Bilbao-Sainz et al. 2010).

6.2.2 Physicochemical Characterization

As with many other colloidal systems, droplet size and surface charge are determined by dynamic light scattering and laser Doppler anemometry, respectively (McClements and Dungan 1995; Heurtault et al. 2003; Bohren and Huffman 2007). Additional information on size and shape can be obtained using Small Angle Neutron Scattering (SANS), Small Angle X-ray Scattering (SAXS), or electron microscopy. Furthermore, electron microscopy techniques, in particular sophisticated cryo-Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM), can deliver high-quality images of nanoemulsions in their

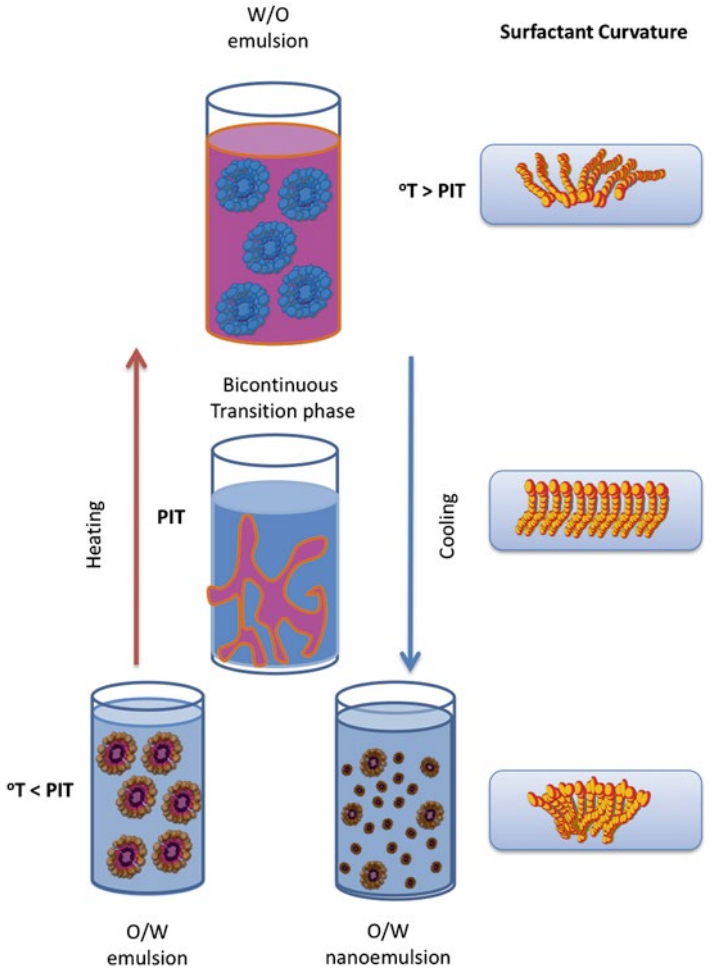

Fig. 6.2 Mechanism of the generation of nanoemulsions using the PIT method

natural state (Klang et al. 2012). In addition to droplet characterisation, the mechanical properties of nanoemulsions are often studied. Nanoemulsions are elastic systems and their elastic storage modulus G' can be measured by means of a rheometer (Howe and Pitt 2008; Pal 2011).

6.2.2.1 Modulating Droplet Size

Droplet size of nanoemulsions can be modulated through selection of proper manufacturing processes and composition. For example, small droplets are more easily obtained by high-energy methods which confer sufficient energy to the system to

breakdown even the smaller droplets (Tadros et al. 2004). Very fine nanoemulsions may also be prepared by low-energy methods, but require a careful selection of the materials, mostly with regard to the surfactants (Maestro et al. 2008). Indeed, surfactants lower the surface tension thus reducing the energy required to break a drop into small droplets. Several authors have reported that hydrophilic surfactants, such as Tween® 80 and Pluronic® 68, contribute towards reducing the oil droplet size compared to more hydrophobic ones (Seijo et al. 1990; Bouchemal et al. 2004). Surfactants with hydrophilic poly(ethylene glycol) (PEG) can also help to control droplet size (Wooster et al. 2008), although their ability to do so may decrease with the length of the PEG chain (Delmas et al. 2011).

The surfactant molecular geometry also influences the size of the dispersed phase and overall stability of the nanoemulsions. The length of the tail and the molecular conformation favour a more efficient packing of the surfactant molecules resulting in a reduction in size of the oil droplets. Most nanoemulsions are stabilised by synthetic surfactants, which tend to have long hydrophilic tails with a molecular size between 2 and 10 nm and consist of non-ionic and/or polymeric surfactants, as for example azo benzene-substituted poly(sodium acrylate) (HMPA-1) (Tadros 2005; Galindo-Alvarez et al. 2011).

6.2.2.2 Modulating Droplet Surface Charge

The surface charge of O/W nanoemulsions can be modulated through the addition of specific components. For example, by adding ionic surfactants, cationic or anionic nanoemulsions can be formed. Cationic nanoemulsions can complex negatively charged hydrophilic macromolecules, such as antisense oligonucleotides (Bruxel et al. 2011; Hagigit et al. 2012), and have increased cell adhesion/penetration properties as a result of interactions with the negatively charged cell membranes (Baspinar and Borchert 2012; Lallemand et al. 2012). Additionally, masking the charge of nanoemulsions by the addition of stealth polymers such as PEG is a strategy to avoid unspecific interactions with blood components after intravenous delivery, thus improving circulation times (Furtado Mosqueira et al. 2001; Huynh et al. 2009; Jokerst et al. 2011).

6.2.3 Kinetic Stability and Ostwald Ripening Effect

Emulsions, like most coarsely dispersed systems, are inherently unstable. As such, these systems will tend to revert to the state with the lowest free energy over time, under specified conditions of temperature, pressure, and composition (Atkins and De Paula 2010). There lies the main difference between microemulsions and nanoemulsions.

As shown in Fig. 6.3 microemulsion formation decreases the free energy of the systems rendering such formulations thermodynamically stable (Anton and Vandamme 2011; McClements 2012). In this respect, microemulsions are closer in

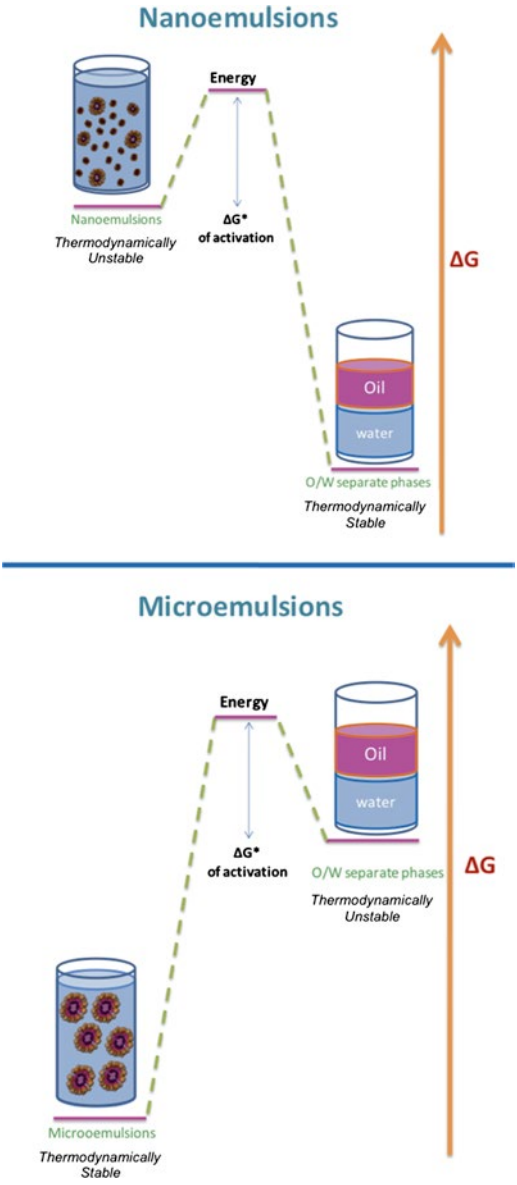

Fig. 6.3 Formation of nanoemulsions and microemulsions: free energy diagram of the two systems compared to the separated phase states

behaviour to micelles (Israelachvili 2011). Oppositely, nanoemulsions obey Gibbs' free energy law. These systems present higher free energy due to the increase in surface area produced by the dispersion of the oil phase. In time, the nanoemulsions will tend to revert back to two distinct phases: the system is said to be thermodynamically unstable. However, the rate of destabilisation is so slow that they are considered kinetically stable. In fact the height of the energy barrier that exists between the emulsified and non-emulsified states (Fig. 6.3) determines the stability

of the nanoemulsion: the higher the energy barrier to overcome, the longer is the stability of the nanoemulsion. In addition to this, the stability will also be influenced by other mechanisms responsible for the interaction between particles, such as Brownian motions, gravitational forces, and shear energy.

Physical instability of coarse emulsions usually manifest itself through flocculation (i.e. droplet aggregation), creaming (i.e. droplet rise through the medium), Ostwald ripening, or coalescence (i.e. when two droplets merge into a single larger droplet), with the latter potentially leading to the irreversible breaking of the formulation (Fredrick et al. 2010). As the very small droplet size of nanoemulsions reduces the incidence of coalescence, the Ostwald ripening effect is the main cause of instability (Anton and Vandamme 2011).

The Ostwald ripening effect is defined as the growth of the largest droplets at the expense of the smallest ones (Tadros et al. 2004). This phenomenon is due to differences in the chemical potential among oil droplets of different sizes. As the chemical potential increases for droplets of smaller size, due to a heightened Laplace pressure, the smallest droplets tend to transfer mass to the largest ones across the continuous phase (Delmas et al. 2011). The Ostwald ripening effect can be avoided by controlling the physical properties of the oil phase and the nature of the surfactant (Webster and Cates 1998). For example, Ostwald ripening was prevented by a large molar volume of long chain triglyceride (LCT) oils, which makes them insoluble in water thus providing a kinetic barrier to Ostwald ripening (Wooster et al. 2008). Another interesting strategy to increase the stability is the incorporation of well-known stabilising polymers, such as PEG (Wooster et al. 2008; Delmas et al. 2011).

As instability is associated with an increase in droplet size, measuring the change in size over time has been the basic method for studying nanoemulsion stability (Porras et al. 2004; Tadros et al. 2004; Wooster et al. 2008). When destabilisation is caused by coalescence, the dependence between size increase and time is given by the Deminière equation (6.2), where r is the average droplet radius after t, r_0 is the value at $t=0$, and ω is the frequency of rupture per unit of surface of the film (Deminière 1998; Peng et al. 2010).

$$\frac{1}{r^2} = \frac{1}{r_0^2} - \left(\frac{8\pi}{3}\right)\omega t \tag{6.2}$$

For Ostwald ripening, the rate is given by the Lifshitz-Slezov and Wagner theory (LSW) according to (6.3), where r_c is the critical radius of the system at any given time; c (∞), the bulk phase solubility; γ, the interfacial tension; V_m, the molar volume; ρ, the density of the water; R, the gas constant; and T, the absolute temperature (Lisfshitz and Slezov 1961; Taylor 1995).

$$\omega = \frac{dr_c^3}{dt} = \frac{8c_{(\infty)}\gamma DV_m}{\rho RT} \tag{6.3}$$

Plotting r^2 or r^3 against time can then identify the actual process driving instability. A linear relationship between the achieved former and time identifies coalescence as the main driving force while the plot between r^3 and time will be linear if Ostwald ripening is the main process.

6.2.4 O/W Nanoemulsions as Drug Carriers

Adequate selection of the manufacturing technique and materials allows tailoring of O/W nanoemulsions with specific physicochemical properties. This could be done to favour high entrapment efficiencies or favourable biodistribution of both lipophilic and hydrophilic drugs (Peltier et al. 2006; Makidon et al. 2008; Hamouda et al. 2010; Zhang et al. 2011b; Hagigit et al. 2012).

Nanoemulsions can host poorly soluble drugs in their hydrophobic core, providing additional protection from acid or enzymatic degradation (Kotta et al. 2012). Encapsulation may also facilitate membrane translocation and increase drug bioavailability (Beg et al. 2011; van Hoogevest et al. 2011). Interestingly, the use of O/W nanoemulsions is not limited to hydrophobic compounds. Although far less common, positively charged nanoemulsions can bind hydrophilic biomolecules through electrostatic interactions, thus enabling the delivery of new therapeutic entities such as oligonucleotides (Teixeira et al. 1999; Bruxel et al. 2011; Hagigit et al. 2012), plasmid DNA (Liu and Yu 2010) or antibodies (i.e. tyroglobulin) (Wang et al. 2012). More detailed information is provided in Sect. 6.5.

6.3 W/O Nanoemulsions

Water-in-oil (W/O) or oily nanoemulsions are systems where small water droplets are dispersed into an organic, water immiscible phase. The aqueous phase can consist of pure water, saline, a buffer, or a drug solution; while alkanes (Porras et al. 2008), carbon dioxide (Psathas et al. 2002), fluorocarbons (Krafft et al. 2003), isopropyl myristate (Sadtler et al. 1999; Uson et al. 2004), and squalene (Huang et al. 2009c) have been explored as the external phase.

6.3.1 Emulsification Methods

In order to achieve small droplet sizes, high-energy methods have been preferred for the preparation of W/O nanoemulsions (Sect. 6.2.1.1) (Sadtler et al. 1999; Landfester et al. 2000; Butz et al. 2002; Courrier et al. 2004b; Taden et al. 2004; Tang et al. 2012). Low-energy emulsifications methods have also been suggested, albeit with mixed results. On one hand, stable water-in-isopropylmyristate nanoemulsions (60–160 nm) were successfully obtained by phase inversion at constant temperature (Uson et al. 2004). Yet, this method failed when applied to water-in-decane systems (Porras et al. 2008). This was reportedly caused by the presence of a multiphase system including solid surfactant and liquid crystals (Porras et al. 2008). Equally, $NaCl_{aq}$-in- CO_2 miniemulsions prepared using the PIT method presented small droplet size (<300 vs >700 nm for formulations prepared using high-pressure

homogenisation), but were reported to be stable for only short periods of time (Psathas et al. 2002). Further evaluation of low-energy methods is therefore required to enable their widespread use for the preparation of oily nanoemulsions.

6.3.2 Pseudo-ternary Phase Diagrams

It has been suggested that phase diagrams alone may not allow for clear discrimination between O/W nanoemulsions and microemulsions (Anton and Vandamme 2011; Fryd and Mason 2012; McClements 2012). Despite these reservations, phase diagrams have repeatedly been reported in the study of W/O nanoemulsions (Porras et al. 2004, 2008; Uson et al. 2004), albeit often combined with light scattering analysis, as an additional tool for the identification of the type of emulsion formed (see below for details).

In order to determine the amount of oil, water, and surfactant required for the formation of nanoemulsions, phase diagrams are constructed at known surfactant: oil (S:O) ratios and varying water content (Porras et al. 2004). Combinations of surfactants are generally preferred to enable the dispersion of maximal amounts of water with one of the surfactants being hydrophobic (low hydrophilic–lipophilic balance, HLB) (Porras et al. 2004, 2008; Uson et al. 2004). The most advantageous mixing ratio (Smix) of each surfactant can be determined by assessing their ability to solubilise water at a constant S:O ratio, typically 15:85 or 5:95 for water in decane systems (Porras et al. 2004, 2008). Differences in the versatility of nanoemulsions stabilised with a single surfactant or a mix of surfactants are highlighted in a phase behaviour study of mixtures of isopropyl myristate, water, and hydrophobic Cremophor® WO7 alone or in combination with Cremophor® EL. In this case, the addition of Cremophor® EL allowed for the formation of more complex systems with a variety of phases that can accommodate larger amounts of water and are more amenable to the formation of W/O nanoemulsions (Uson et al. 2004). Alternatively, phase diagrams can be constructed for different Smix (Uson et al. 2004), which can be cumbersome when screening for a large number of different surfactants.

Once the surfactant combination has been decided, the phase diagram can be constructed (Fig. 6.4). The identification of the type emulsion, microemulsion, nanoemulsion, or coarse is usually performed by light scattering (Mengual et al. 1999). One implement calls for back-scattering measurements to be performed at different cuvette heights and time points (Porras et al. 2008). The formation of coarse emulsions will show changes in size depending on where the measurement is taken due to sedimentation of the dispersed phase. However, it is thought that neither microemulsions nor nanoemulsions will produce such changes and can only be differentiated by following size over time (Porras et al. 2008).

Using this method, the phase behaviour of water–decane mixtures revealed that microemulsions, nanoemulsions, and finally coarse emulsions were formed in sequence when the proportion of water was increased (Porras et al. 2004, 2008). For decane mixtures, stabilised with a combination of Span™ 20:Tween® 80 (62:38),

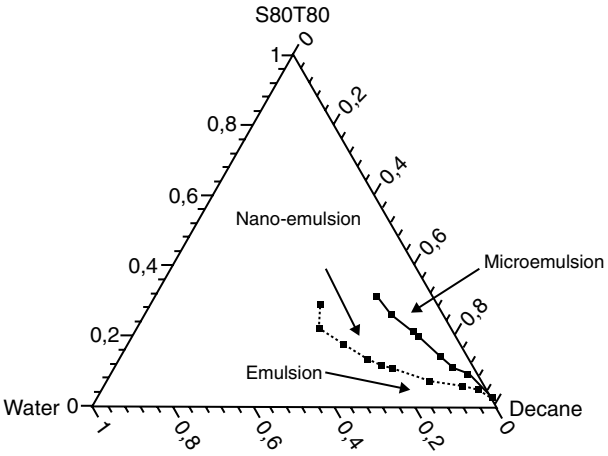

Fig. 6.4 Pseudo-ternary phase diagram showing regions of microemulsion, nanoemulsion, and emulsion for a decane in water nanoemulsions stabilised with Span™ 80 and Tween® 80 (51:49) at 25 °C. Surfactant:decane ratio 5:95. Reproduced from Porras et al. (2008) with permission

the nanoemulsion region was found at water contents between 3 and 20 %, for S:O ratios kept at 15:85 (ca. 68–82 % decane; 12–15 % surfactant) (Chiesa et al. 2008). In this region, emulsions were stable against phase separation, but prone to increases in droplet size, a distinctive feature confirming that nanoemulsions rather than microemulsions were obtained (Chiesa et al. 2008; Porras et al. 2008).

6.3.3 Particle Size, Surface Properties, and Stability

Similarly to aqueous emulsions, droplet size changes with the proportions of the different components and the nature of the surfactants (Uson et al. 2004; Porras et al. 2008). With other components kept at a constant ratio, increases in water and surfactant will produce larger (Porras et al. 2004; Uson et al. 2004; Peng et al. 2010) and smaller (Porras et al. 2004) droplets, respectively. Both occurrences can be explained by changes in interfacial area and interfacial tension (Friberg and Vesable 1985). Increasing the proportion of oil, at a constant water:surfactant ratio, will simply dilute the nanoemulsion and bears little impact on droplet size (Uson et al. 2004). Expectedly, the ratio of the different surfactants can also have an impact on droplet size. For example, in the case of water-in-isopropyl myristate nanoemulsions, droplet size initially decreases when the ratio of Cremophor® WO 7:Cremophor® EL, or hydrophobic to hydrophilic surfactant, was increased from 2:1 to 6:1 (Uson et al. 2004). Size then reached a plateau before increasing again slightly at a ratio of 9:1.

The stability of W/O nanoemulsions will be determined by the amount of surfactant, which allows uniform coating of the droplets and the formation of small and homogeneously dispersed droplets. The discrete size of the dispersed phase droplets

offers some protection from sedimentation (Uson et al. 2004). Yet, rapid phase separation may still occur when the surfactant mixture cannot adequately stabilise the water droplets. This has been observed in water-in-decane emulsions where formulations stabilised with mixtures of Span™ 20:Tween® 20 rapidly separated (Porras et al. 2008).

Ostwald ripening remains the most common cause of instability for W/O nanoemulsions (Courrier et al. 2004b; Porras et al. 2004, 2008; Uson et al. 2004). Yet, for given S:O ratios and surfactant mixtures, coalescence may become the prime destabilisation mechanism at higher water contents, as a result of the thinning of the protective surfactant layer (Porras et al. 2008). This was observed for water-in-decane nanoemulsions. At an S:O ratio of 15:85, emulsions were destabilised through Ostwald ripening for water contents of 11–14 wt% but mainly by coalescence for 16 wt% (Porras et al. 2008). In water-in-fluorocarbon nanoemulsions, destabilisation due to Ostwald ripening could be slowed down by decreasing the solubility of the droplets through the addition of NaCl to the dispersed phase (Sadtler et al. 1996).

6.3.4 W/O Nanoemulsions as Drug Carriers

W/O nanoemulsions have been studied for various applications including as a scaffold for the formation of nanoparticles (Landfester et al. 2000) or as coolants (Chiesa et al. 2008). In comparison to aqueous nanoemulsions, very few examples of pharmaceutical use of W/O formulations have been reported (see Sect. 6.5 for details). Indeed, many of the systems described as W/O nanoemulsions seem to better fit the definition of microemulsions as the formulations (1) were often described as being thermodynamically stable, (2) presented a high surfactant content (>40 %), and (3) could be prepared by simply mixing all the ingredients, although some have reported the use of probe sonication (Wu et al. 2001a, b; Wang et al. 2008a). Nevertheless, oily emulsions have shown potential for the delivery of water-soluble agents (Sadtler et al. 1998; Wu et al. 2001a, b; Courrier et al. 2004a; Wang et al. 2008a; Hwang et al. 2009; Fan et al. 2011; Zhang et al. 2011a; Tang et al. 2012) and to improve the oral bioavailability of Class III BCS (Biopharmaceuticals Classification System) drugs and transport of proteins through the skin (Zhi et al. 2005; Gundogdu et al. 2011; Russell-Jones and Himes 2011).

6.4 Polymer-Coated O/W Nanoemulsions: Nanocapsules

Nanocapsules, formed by an inner oil phase and coated by a hydrophilic or hydrophobic polymer, have been widely explored in the field of drug delivery over the last years (Damgé et al. 1988; Quintanar-Guerrero et al. 1998; Prego et al. 2005, 2006a, b; Peltier et al. 2006; Letchford and Burt 2007; Cattani et al. 2010;

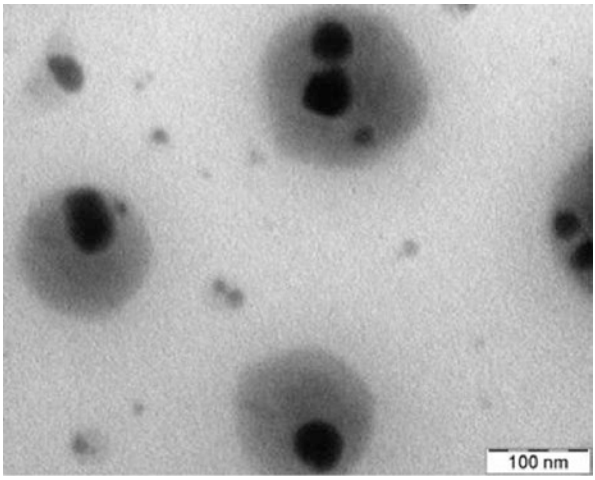

Fig. 6.5 TEM micrograph of poly(lactic acid) poly(ethylene oxide) nanocapsules prepared by interfacial deposition of preformed polymers

Ngwuluka 2010; Anton et al. 2012; Battaglia and Gallarate 2012). For illustration, Fig. 6.5. shows a TEM micrograph of poly(lactic acid)-poly(ethylene oxide) (PLA-PEG) nanocapsules.

6.4.1 Coating Materials

Shell-forming polymers may be selected from a wide range of biomaterials, including hydrophobic and hydrophilic materials, as compiled in Table 6.2. In turn, particle size, polydispersity index, and zeta potential of nanocapsules are clearly influenced by the molecular weight, charge, solubility, and chemical structure and architecture of the coating polymer (Prego et al. 2006a, b; Goldberg et al. 2007). The type of polymer, the nature of the drug, and the specific interactions between them and other components of the nanocapsules have a strong influence on drug loading and drug release properties (Cauchetier et al. 2003). For example, the use of polyarginine as a shell-polymer led to a significant reduction of the particle size, in comparison to the uncoated nanoemulsion, and to an inversion of the superficial charge from negative to positive (Lozano et al. 2013). In the case of chitosan nanocapsules, the amount of chitosan had to be carefully modulated to improve the association of salmon calcitonin as both drug and polymer competed for interaction with the negatively charged acid group of the stabiliser lecithin (Prego et al. 2006a, b).

Selection of the appropriate polymeric shell could also influence the behaviour of the systems in vivo (Damgé et al. 1988, 1990; Oyarzun-Ampuero et al. 2013). In this context, the mucoadhesive and permeation enhancer polysaccharide chitosan has been selected for the preparation of nanocapsules for transmucosal delivery of

Table 6.2 Polymers reported for the preparation of nanocapsules

Shell composition	Properties	Active agents	Pharmaceutical applications	Ref.
Poly(ε-caprolactone) (PCL)	Biodegradable	Indomethacin	Oral drug delivery	Cattani et al. (2010)
	Biocompatible		Ocular drug delivery	Cruz et al. (2006)
	Controlled Release			Poletto et al. (2008)
	Good mechanical properties			Calvo et al. (1997)
Poly(ε-caprolactone)-poly(ethylene oxide) (PCL-PEG)	Biodegradable	Carvacrol	Imaging and diagnostic	Ameller et al. (2003), Furtado Mosqueira et al. (2001)
	Biocompatible		Cancer therapy	
	Controlled release			
	Good mechanical properties			
	Long-circulating systems			
Poly(lactic-co-glycolic) acid (PLGA)	Biocompatible		Delivery of antimicrobial drugs	Abdel-Mottaleb et al. (2011)
	Biodegradable	Ibuprofen	Transdermal drug delivery	Iannitelli et al. (2011)
	Controlled release	Carvacrol		
	FDA approved			
Poly(lactic-co-glycolic)-poly(ethylene oxide) (PLGA-PEG)	Biocompatible	Carvacrol	Imaging and diagnostic	Ameller et al. (2003), Furtado Mosqueira et al. (2001)
	Biodegradable		Cancer therapy	
	Controlled release			
	Long-circulating systems			
Poly(lactic acid) (PLA)	Biocompatible	Fluconazol	Ocular drug delivery	de Assis et al. (2008)
	Biodegradable	Atovaquone	Cancer therapy	Cauchetier et al. (2003)
	Controlled release			
	Low toxicity			
Poly(lactic acid)-poly(ethylene oxide) (PLA-PEG)	Biocompatible	Fluconazol	Cancer therapy	Furtado Mosqueira et al. (2001), de Assis et al. (2008)
	Biodegradable	pDNA	Imaging and diagnostic	
	Controlled release			
	Long-circulating systems			

(continued)

Table 6.2 (continued)

Shell composition	Properties	Active agents	Pharmaceutical applications	Ref.
Chitosan	Mucoadhesive	Indomethacin	Oral drug delivery	Prego et al. (2005)
	Biocompatible	Salmon calcitonin	Ocular drug delivery	Calvo et al. (1997)
	Permeation enhancer			Prego et al. (2006a)
	Biodegradable			
	Transfection agent			
Chitosan-poly(ethylene oxide) (Chitosan-PEG)	Mucoadhesive	Salmon calcitonin	Oral drug delivery	Prego et al. (2006b)
	Biocompatible	Docetaxel	Cancer therapy	Torrecilla et al. (2013)
	Permeation enhancer		Nanovaccines	
	Biodegradable			
	Transfection agent			
	Long-circulating system			
Polyaminoacids	Biocompatible	Docetaxel	Cancer therapy	Lozano et al. (2013)
	Biodegradable	pDNA	Nanovaccines	González-Aramundiz et al. (2012)
	Permeation enhancer			
	Vaccination adjuvant			
	Transfection agent			
Hyaluronic acid	Biocompatible	Docetaxel	Cancer therapy	Oyarzun-Ampuero et al. (2013)
	Biodegradable			
	Transfection agent			
	Mucoadhesive			
	Active targeting			
	Long-circulating systems			

macromolecules such as pDNA, antigens, or peptides, as detailed in Sect. 6.5. Results demonstrated an improved effect of chitosan nanocapsules in drug retention, absorption, and therapeutic efficiency, in comparison with uncoated nanoemulsions, thus highlighting the positive role of the polymeric coating (Prego et al. 2005, 2006a, b, 2010; Shu et al. 2010; Vicente et al. 2010; Gaspar et al. 2011).

Further improvement of the properties of nanocapsules could be achieved through surface modification with PEG. PEGylation can be carried out by incorporation of PEG fatty acids esters, also reported as PEGylated nanoemulsions, or by using preformed PEGylated polymers such as poly(lactic acid)-PEG (PLA-PEG), poly(lactic-co-glycolic) acid-PEG (PLGA-PEG), poly(ε-caprolactone)-PEG (PCL-PEG), or chitosan-PEG to form the shell (Furtado Mosqueira et al. 2001; De Campos et al. 2003; Prego et al. 2006b). Apart from the well-known stealth behaviour that PEG confers on formulations (Gabizon 2001), PEGylation decreases the toxicity of the nanocarriers, improves nanocapsules stability, and has an influence on the physicochemical properties of the resultant nanosystems (de Assis et al. 2008; Vonarbourg et al. 2009; Battaglia and Gallarate 2012). Additional surface modification could lead to the design of stealth nanocapsules decorated with specific ligands, in order to actively target disease sites, improving drug accumulation (Béduneau et al. 2007; Morille et al. 2009; Laine et al. 2012; Torrecilla et al. 2013).

6.4.2 Formulation Technologies

Most of the methods described for the preparation of nanoemulsions can be adapted for nanocapsules manufacturing. High- and low-energy methods including emulsification-coacervation (Lertsutthiwong et al. 2008, 2009), simple or double emulsification (Moinard-Checot et al. 2006, 2008; Grigoriev and Miller 2009), phase-inversion temperature-based technology, (Heurtault et al. 2002), in situ polymerization (Ngwuluka 2010), and interfacial deposition of preformed polymers (Calvo et al. 1997; Prego et al. 2006a, b; Lozano et al. 2008, 2013) have all been described.

6.4.2.1 Simple or Double Emulsification

The selection of one or another for a pharmaceutical formulation will depend on the solubility of the drug to be entrapped into the oily droplets. In all cases hydrophobic polymers form the shell, and high-energy shear forces such as ultrasonication are required.

During *simple emulsification* (O/W), lipophilic drugs are incorporated into the organic inner phase, which is emulsified in an aqueous phase containing a stabilising agent such as cholic acid or poly(vinyl alcohol). The polymer that will form the capsule is typically added to the oil phase (Quintanar et al. 2005; Moinard-Checot, et al. 2008; Poletto et al. 2008).

The *double emulsion* method (W/O/W) combines two emulsification steps. In the first instance a W/O nanoemulsion is formed, which contains the drug, solubilised in the inner phase. This oily emulsion is then emulsified in a secondary aqueous phase (Quintanar-Guerrero et al. 1998; Poletto et al. 2008).

6.4.2.2 In Situ Interfacial Polymerization

This technique involves the formation of nanocapsules through the polymerization of monomers at the interface of two non-miscible liquids. Polymerization conditions differ depending on the selected polymer. For example, acrylic acid and acrylamide derivatives can be polymerized in the presence of persulfate salt (Scarioti et al. 2011; Zhao et al. 2011a, b). In the case of alkyl cyanoacrylate monomers, anionic polymerization can be initiated by the presence of nucleophilic groups (Aboubakar et al. 1999; Nicolas and Covreur 2009). Poly(alkyl cyanoacrylate) nanocapsules showed an exceptional ability to encapsulate insulin into the oily core and to improve its effectiveness in vivo by the oral route (Damgé et al. 1990, 1997). This method does not require the application of high energies. The main disadvantage is the toxicity that may be caused by free monomers. Toxicity of the monomers has been related with their local concentration, the length of the alkyl chain, and thus, cell membrane bioadhesion (Dillingham et al. 1983; Huang and Lee 2006).

6.4.2.3 Interfacial Deposition of Preformed Polymers

This is a mild technique to obtain O/W nanocapsules that involves the deposition of preformed polymers at the oil–water interface (Mora-Huertas et al. 2010). As it was described before for the self-emulsification method (Sect. 6.2.1), this procedure is based on the diffusion of the organic solvent to the aqueous phase in which the surfactant, if needed, is dissolved. Hydrophobic polymers are incorporated in the organic phase. As the organic solvent diffuses the polymer becomes insoluble, just allowing the deposition of the hydrophobic polymer at the oil nanodroplet interface (Cauchetier et al. 2003; Teixeira et al. 2005; Stella et al. 2007; de Assis et al. 2008; Cattani et al. 2010).

Hydrophilic polymers are incorporated in the aqueous external phase. In this case, additional electrostatic interactions are involved in their deposition at the oil nanodroplets interface (Lozano et al. 2008, 2013; Garcia-Fuentes and Alonso 2012). An alternative is to incubate the polymer with preformed anionic of cationic nanoemulsions.

6.4.3 Nanocapsules as Drug Carriers

O/W-polymer-coated nanoemulsions can host drugs both in the oil core and onto the polymeric shell. As a consequence, nanocapsules are seen as highly attractive

delivery systems and hold an enormous potential for a wide range of applications, being particular examples detailed in the next section (Calvo et al. 1997; Damgé et al. 2007; Huynh et al. 2009; Teixeira et al. 2010; Lozano et al. 2013; Torrecilla et al. 2013).

6.5 The Potential of Nanoemulsions in Therapeutics

In this section we aim to provide an overview of the potential of nanoemulsions in therapeutics, supported by specific examples that illustrate the most recent advances in the field.

6.5.1 Transmucosal Drug Delivery

The delivery of drugs and macromolecules by non-invasive transmucosal routes is a promising approach in therapeutics. Nanoemulsions can be specifically designed to overcome mucosal barriers and other limitations of every modality of administration.

6.5.1.1 Oral Delivery

Despite its advantages, i.e. easiest of administration and patient compliance, the oral route remains a challenge for formulation scientists. Drug nanocarriers are expected to (1) confront the gastrointestinal track and its harsh environment, preserving the integrity of the encapsulated drugs, (2) overcome the intestinal mucosa, and (3) promote drug absorption.

As mentioned in Sect. 6.2, O/W nanoemulsions are well-suited for the encapsulation of poorly soluble hydrophobic drugs (Shafiq et al. 2007; Chen et al. 2011; Parveen et al. 2011; van Hoogevest et al. 2011). An improvement in drug solubility is related to an increase in drug bioavailability as dissolution is often a rate-limiting step to absorption (van Hoogevest et al. 2011).

Examples of improved oral bioavailability upon encapsulation in nanoemulsions include the case of Sylimarin, a mixture of flavolignans rich in Silybin with hepatoprotectant properties, which saw its oral bioavailability in rats increased by six and fourfold compared to Sylimarin in suspension and the marketed formulation (SYLBON®) (Parveen et al. 2011), respectively. Similarly, upon oral administration of a nanoemulsion of the pro-drug ramipril, the bioavailability of the active metabolite ramiprilat was increased by 2.94 and 5.4-fold with respect to conventional capsules and a drug suspension (Shafiq et al. 2007).

Enhanced oral bioavailability can also be achieved by preventing the interaction of the encapsulated drug with efflux transporters such as the P-glycoprotein (P-gp). The essential oil eugenol is rich in terpenoids and is an inhibitor of P-gp-mediated transport (Yoshida et al. 2005). Eugenol-based nanoemulsions (isopropyl myristate,

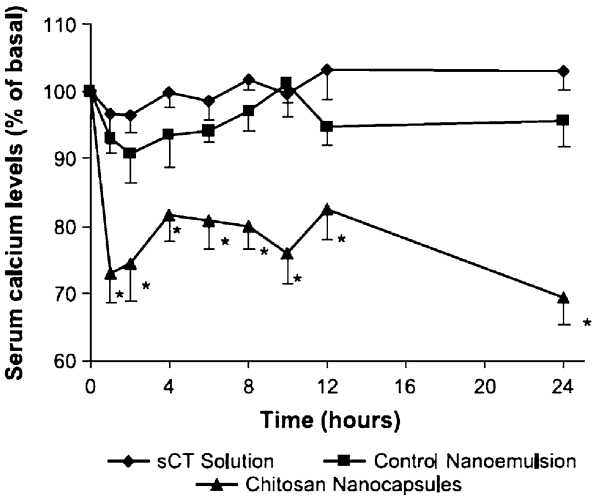

Fig. 6.6 Changes in serum calcium levels after oral administration to rats of salmon calcitonin associated to chitosan nanocapsules (*triangle*) or non-coated O/W nanoemulsions (*square*). As a control, a solution of the peptide in solution (*diamond*) was used. Reproduced from Prego et al. (2006a) with permission

eugenol, and Tween® 80) have then been proposed for the oral delivery of colchicine, a P-gp substrate (Shen et al. 2011). Results show a 2.1-fold increase in oral bioavailability of colchicine compared to the drug in solution (Shen et al. 2011). Nanoemulsions prepared without eugenol (isopropyl myristate and Tween® 80) also enhanced bioavailability but to a lesser extent (1.6-fold).

Finally, the inclusion of mucoadhesive polymers by increasing retention time through prolonged interaction with the intestinal mucosa can also improve the absorption of encapsulated drugs (Prego et al. 2005). Chitosan, a well-known mucoadhesive polymer, was used to coat medium chain triglyceride (Miglyol® 812) and lecithin nanoemulsions (chitosan nanocapsules) destined for oral peptide delivery. As it can be observed in Fig. 6.6, an improved efficacy (threefold) was observed when the peptide salmon calcitonin was associated with chitosan nanocapsules, compared to uncoated nanoemulsions (Prego et al. 2005, 2006a, b). Furthermore, polymer coating plays an important role in improving drug stability under physiological conditions (Fukui and Fujimoto 2009). In fact, insulin encapsulated in chitosan-glucomannan- (Wang et al. 2008b) and poly(isobutyl cyanoacrylate)-coated nanocapsules (Damgé et al. 1988, 1990; Watnasirichaikul et al. 2002) showed improved (1) stability and pH resistance of sensitive drugs in vitro, (2) drug absorption, and (3) a therapeutic effect in both rats and dogs, in comparison to the free insulin.

6.5.1.2 Pulmonary and Intranasal Delivery

Water-in-fluorocarbon nanoemulsions constitute an attractive carrier for the delivery of drugs to the lung (Sadtler et al. 1996; Krafft 2001; Krafft et al. 2003).

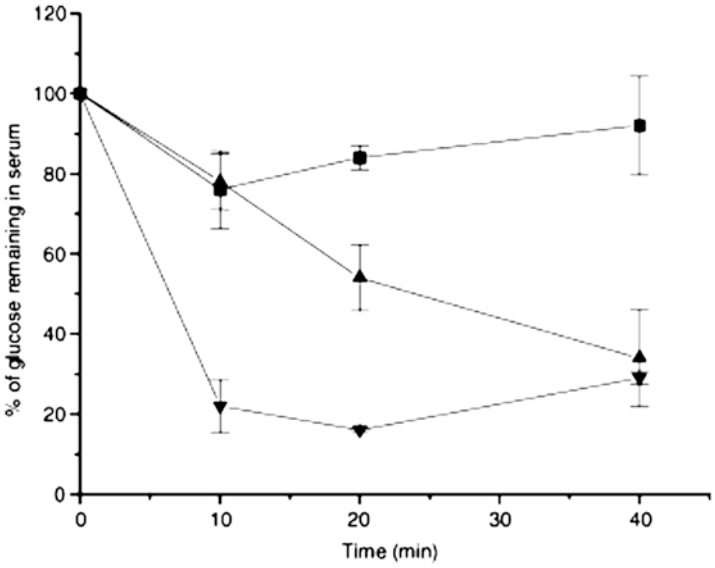

Fig. 6.7 Changes in blood glucose levels in mice following the intranasal instillation of a drug-free (*squares*) and insulin-loaded (*triangles*) water-in-perfluorooctyl bromide nanoemulsion or of an insulin aqueous solution (*inverted triangles*). Data is expressed relative to the blood glucose levels measured in control-untreated mice. Reproduced from Courrier et al. (2004a) with permission

Fluorocarbons are biologically inert materials, miscible with hydrofluoroalkanes (used as propellants) (Butz et al. 2002) and able to solubilise large amounts of oxygen (Riess 2005). One of the main challenges in the formulation of fluorocarbon is their high hydrophobicity and lipophobicity that limits the type of surfactants that can be used as stabilisers. To this end, several (*F*-alkyl)alkyl dimorpholinophosphate C_nF_{2n+1} $C_mH_{2m}OP(O)[N(CH_2CH_2)_2O]_2$ (F_nC_mDMP) surfactants were evaluated (Krafft et al. 1991; Sadtler et al. 1998). Out of the different candidates, F_8C_{11}DMP surfactant was able to produce stable fluorocarbon nanoemulsions with an average size around 100–120 nm (Sadtler et al. 1999; Courrier et al. 2004b), which were shown to be highly efficient in controlling the release of a model hydrophilic fluorescent probe. A later report verified that they could be used to homogenously and reproducibly deliver low-molecular weight caffeine from a CFC-free pressurised metered-dose inhaler, providing preliminary indications that water-in-fluorocarbon nanoemulsions could become successful inhaled drug delivery systems (Butz et al. 2002).

Water-in-fluorocarbon nanoemulsions were also evaluated for the delivery of insulin in a mouse model, following intranasal instillation (Courrier et al. 2004a). As depicted in Fig. 6.7, insulin loaded in the nanoemulsion produced a rapid hypoglycaemic effect (40 % of blood glucose levels after 20 min) though less pronounced than the insulin solution control (80 %). However, while the effect of the insulin solution seemed to cease after 40 min, the last available time point, glucose levels

continued to decrease when insulin was given as a nanoemulsion, suggesting a more sustained effect. Longer experimental times would thus be required in order to confirm the true potential of these formulations for insulin delivery.

6.5.1.3 Ocular Delivery

Eye diseases are commonly treated by topical administration of the selected drug. However, providing and maintaining the drug concentration at the administration site remains challenging. The design of drug carriers aimed for delivery onto the ocular mucosa is seen as an efficient strategy to prolong ocular residence time of drugs, limiting drainage, and increasing the pharmacological effect at the site of action. In addition nano-sized delivery systems have shown an improved interaction with the ocular mucosa and can even penetrate the corneal and conjunctival epithelia (Maincent et al. 1995; de la Fuente et al. 2008a, b, 2010; Contreras-Ruiz et al. 2011).

O/W nanoemulsions are considered an excellent option for topical delivery of lipophilic drugs to the eye. As a matter of fact, some formulations have already been commercialised (Klang et al. 2000; Lallemand et al. 2012). Restasis® (Allergan, Inc.) was the first O/W emulsion formulation to be approved for commercialization. It consists in an anionic emulsion of Cyclosporin A for the treatment of dry eye and is composed of glycerine, castor oil, polysorbate 80, carbomer copolymer type A, purified water, and sodium hydroxide to adjust pH. Nanoemulsion-based artificial tears such as Soothe® (Bausch & Lomb, Inc.) and Refresh Endura® (Allergan, Inc.) have also been marketed in the United States. Currently the pharmaceutical company Novigali Pharma is developing a technology marketed as Novasorb® for the treatment of dry eye disease with cyclosporine A. Novasorb® contains the cationic lipids benzalkonium chloride and cetylalkonium chloride and polyols such as glycerol, mannitol, or sorbitol. This formulation is now in its phase III Clinical Trial for the evaluation of efficacy, tolerance, and safety (Lallemand et al. 2012).

Since the corneal and conjunctival cell membranes are negatively charged at physiological pH, cationic nanoemulsions are currently explored for an improved interaction (Klang et al. 2000; Lallemand et al. 2012). Klang et al. (2000) compared positively and negatively charged nanoemulsions based on medium chain triglycerides (MCT) for ocular delivery of indomethacin. The positively charged nanoemulsions provided significantly higher drug levels than both the control solution and a negatively charged emulsion in the aqueous humour and sclera-retina. Furthermore, the spreading coefficient of the positively charged emulsion on the cornea was shown to be four times higher than that of the negatively charged emulsion (Klang et al. 2000). Promising results have been recently published by Hagigit et al. (2012) on the use of cationic nanoemulsions based on medium chain triglycerides and also a well-reported cationic lipid for transfection, DOTAP ((*N*-[1-(2,3-dioleoyloxy)-propyl]-*N*,*N*,*N*-trimethylammonium methyl sulphate), for the complexation and delivery of antisense oligonucleotides directed at Vascular endothelial growth factor receptor (VEGF-R2) to reduce neovascularization. Results showed that, upon

topical administration to rats and mice (previously treated to develop corneal neovascularization), there was a substantial inhibition of the process.

Similarly, cationic polymer-coated O/W nanoemulsions have been shown to improve the ocular bioavailability of different drugs such as indomethacin, pilocarpine, metipronalol, or piroxicam (Jacob et al. 1990; Losa et al. 1993; Klang et al. 1996; Desai and Blanchard 1998; De Campos et al. 2003). The corneal penetration of indomethacin loaded into chitosan nanocapsules was improved by a factor of 2, according to drug concentrations measured in the aqueous humour, and with respect to uncoated cationic nanoemulsions upon instillation to rabbits (Calvo et al. 1997). A different composition, based on PECL forming the shell, has also shown potential for this particular application, providing a fivefold increase in cyclosporine concentrations in the cornea compared to the levels measured upon instillation of the free drug. Moreover, this particular nanocapsule's composition specifically target the cornea and decrease systemic absorption, which is usually related to undesirable side effects (Calvo et al. 1994, 1996).

6.5.2 Topical/Transdermal Drug Delivery

Transdermal delivery remains a highly desirable route of administration due to the large skin surface available for absorption as well as limited first-pass effect. However, those advantages are offset by the skin's well-organised structure and low permeability (Neubert 2011). Among the different carriers tested as transdermal delivery system, nanoemulsions seem particularly promising and hold several advantages for the treatment of skin conditions (Abdel-Mottaleb et al. 2011).

The efficacy of O/W nanoemulsions for topical/skin delivery depends heavily on the selection of oil, particularly with regard to its molecular weight: high molecular weight oils favour the formation of more stable nanoemulsions than low-molecular weight oils (Koroleva and Yurtov 2012). Yet, low-molecular weight oils increase the penetration rate and do not leave an oily and greasy skin (Sonneville-Aubrun et al. 2004). Therefore, oils should be selected based on the requirements of the final formulation. Oils containing high levels of antioxidants, such as rice brain oil (Oriza Sativa), a widely used component of sunscreen creams and anti-ageing creams, have also been formulated in anti-irritant nanoemulsions properties (Lerma-Garcia et al. 2009). Bernardi et al. have demonstrated that rice bran oil nanoemulsions have high hydration and moisturising properties when applied on the skin of patients affected by psoriasis or dermatitis while maintaining the normal skin pH (Bernardi et al. 2011). A large proportion of the literature on topical use of nanoemulsions comes from the cosmetic industry. Indeed, nanoemulsions have been included in various formulations, from hydrating creams to hair-colouring products. The L'Oreal group alone possesses dozens of proprietary technologies based on nanoemulsions.

Nanocapsules have also been proposed as potential carriers for transdermal delivery (Teixeira et al. 2010; Abdel-Mottaleb et al. 2011). More specifically, it is expected that an increased affinity of the nanocarriers for the stratum corneum due

to the polymeric coating (PCL nanocapsules) may promote a deeper penetration into human skin in comparison with non-coated nanoemulsions (Alves et al. 2007). This was demonstrated by Teixeira and co-workers for retinyl palmitate, a vitamin A derivative, loaded into PLA nanocapsules (Teixeira et al. 2010).

6.5.3 Nanoemulsions for Disease Management

6.5.3.1 Cancer

Anticancer drugs present biopharmaceutical issues that need to be resolved in order to achieve maximal therapeutic efficacy and low toxicity. Most anticancer drugs suffer from poor solubility or compromised stability in physiological fluids. Moreover, the mechanisms of action tend to be broad and non-specific to tumour cells, affecting the surrounding tissues. The design of suitable carriers intended to overcome these limitations is a key point in the development of novel anticancer therapies (Brigger et al. 2002).

Apart from the increasing solubility of poorly soluble or hydrophobic drugs, nanoemulsions can significantly improve anticancer drugs' therapeutic index. Current commercial taxane formulations include a high percentage of surfactants such as Cremophor® L for paclitaxel and Tween® 80 for docetaxel that are known to cause toxicity (Gelderblom et al. 2001; Engels et al. 2007). Docetaxel, re-formulated as a nanoemulsion manufactured by high-pressure homogenization and composed of egg lecithin, soybean oil, and co-surfactants, resulted in decreased toxicity in healthy mice when administered intravenously. The formulation was shown to be effective in a tumour model of glioma (Gaoe et al. 2012). Another example is provided by Zhang et al. (2011b), who reported the preparation of a lipophilic doxorubicin-oleic acid derivative which was subsequently emulsified in the presence of Lipoid® E80 (egg phospholipids), vitamin E, and soybean oil. This doxorubicin nanoemulsion was able to prevent drug accumulation in the heart, lung, and kidneys vs a doxorubicin solution, while still maintaining high blood levels after intravenous administration to healthy mice.

Another case where the drug therapeutic index was improved is that of melphalan, an anticancer molecule for the treatment of ovarian cancer, generally administered as a tablet or as an injection. After oral administration of the tablet the drug is subject to plasmatic metabolism resulting in: (1) its inactivation and (2) the reduction of bioavailability on repeating dosing; both problems have been addressed with a nanoemulsion formulation (Rajpoot et al. 2012). Capmul® MCM, Tween® 80, and Transcutol® P were used respectively as oil, surfactant, and co-surfactant. Oral administration in mice showed an almost fivefold increase in bioavailability compared to a suspension of the free drug.

Improved therapeutic index and drug pharmacokinetics can be achieved by increasing the drug circulation time through the incorporation of PEG (Beduneau et al. 2006; Allard et al. 2008; Bourseau-Guilmain et al. 2012; Laine et al. 2012).

Moreover, it has been suggested that certain PEGylated surfactants, as PEG-hydroxystearate, could have an inhibitory effect on P-glycoprotein (Huynh et al. 2009). An additional advantage of PEGylated nanoemulsions is that ligands for targeting concrete cell populations can be linked to the PEG moieties (Béduneau et al. 2007; Allard et al. 2008; Bourseau-Guilmain et al. 2012; Torrecilla et al. 2013). Ohguchi et al. have shown that the use of folate-PEG-linked nanoemulsions loaded with aclacinomycin inhibit the tumour growth of folate receptor-positive nasopharyngeal tumours after intravenous injection to mice (Ohguchi et al. 2008). Bourseau-Guilmain et al. reported the efficacy of functionalized PEG nanocapsules with a monoclonal antibody against AC133, a cancer stem cell marker (Bourseau-Guilmain et al. 2012). Furthermore, PEGylated chitosan nanocapsules conjugated to a monoclonal antibody anti-TMEFF-2 showed a significant decrease of the IC_{50} in A549 cells after 24 h of incubation, in comparison with free drug or non-conjugated nanocapsules (Torrecilla et al. 2013).

6.5.3.2 Infectious Diseases

Antimicrobial/Antiparasitic Therapies

Due to the intrinsic ability of O/W nanoemulsions to host drugs with poor aqueous solubility, many studies have focussed on finding new applications or extending the spectrum of application for drugs already known. For example, clortrimazole, a topical antimycotic (i.e. ear, skin), has been formulated in nanoemulsions composed of Capryol™ 90, Solutol® HS 15, and Gelucire® 44/14 as surfactants and tested for the treatment of malaria via oral administration. Borhade et al. (2012) demonstrated the ability of such formulations to inhibit the growth of *Plasmodium Berghei* in mice, showing higher suppression compared to the drug formulated as a suspension. Further, nanoemulsions protected the drug against degradation and remained stable over 6 months, which could turn out to be an economically attractive strategy for the development of anti-malaria therapeutics.

Nanocapsules have been proposed to overcome bacterial resistance to conventional treatments. Biofilms are an organised community of bacterial cells embedded within a hydrated matrix. In this ecosystem, microbial properties are altered and have an increased resistance to antibiotics. Nanocarriers have the potential to diffuse into the mucus environment surrounding the biofilm, deliver the active drug locally, and improve its residence time and effectiveness (Iannitelli et al. 2011; Peulen and Wilkinson 2011). Iannitelli et al. described the use of PLGA nanocapsules with carvacrol core (carvacrol acts as the oily core and is known to inhibit the growth of several bacteria strains) for the treatment of microbial biofilm and showed an improvement in the penetration of carvacrol deep into the core of the biofilm. An additional advantage is that other antimicrobial agents could be encapsulated in the oily carvacrol reservoir to increase the potency of this formulation.

Lastly, an interesting O/W nanoemulsion formulation has been commercialised under the name NanoProtect™. This preparation, made of GRAS materials, has

been tested and evaluated to act as decontamination agent of facilities and equipment contaminated with anthrax. Although not for use in humans, this application highlights the potential of O/W nanoemulsions as a preventive measure to limit transmission of anthrax (Bielinska et al. 2007).

Nanovaccines

Despite of the well-known efficacy of classical vaccines, attenuated, or killed pathogens, subunit vaccines have gained much attention over the last years due to their higher safety and purity. The main disadvantage of these subunit vaccines is their dependency on the use of adjuvants to produce an effective immune response (Correia-Pinto et al. 2013). Regarding this, drug delivery systems could improve the recognition of antigens by antigen-presenting cells, producing an effective immune response (Wadhwa et al. 2012).

The efficacy of W/O coarse emulsions (>1 μm) as adjuvants for vaccines is well-known. Freund's (Freund et al. 1937) and Montanide ISA (Aucouturier et al. 2002) adjuvants are both commonly used emulsion-based veterinary adjuvants. The high efficacy of W/O emulsions stem from their ability to control the release of the antigen and to enhance the immune reaction (Herbert 1968; Schijns 2000; Jansen et al. 2005). Consequently, nanoemulsions could be expected to be beneficial for the same purposes. To date, most of the examples on the use of nanoemulsions as adjuvants in vaccination relate to O/W nanoemulsions, which have served to improve the mucosal response to vaccines administered to the nasal mucosa (Myc et al. 2003; Bielinska et al. 2007; Hamouda et al. 2011; Stanberry et al. 2012).

O/W nanoemulsions have been formulated with the recombinant HIV viral protein gp120 and induced both systemic and mucosal antibody responses after intranasal administration to mice and guinea pigs (Bielinska et al. 2008). Makidon et al. have shown that intranasal needle-free immunisation against Hepatitis B can be achieved with an O/W nanoemulsion of cetyl pyridinium chloride, Tween® 80, and soybean oil as a particulate adjuvant for the recombinant Hepatitis B surface antigen (HBsAg) (Makidon et al. 2008). A nanoemulsion with similar composition formulated with outer membrane proteins of *B. multivorans* developed by NanoBio® Corporation was later shown to achieve in vivo immunisation and protection against pulmonary infection in mice after intranasal administration (Makidon et al. 2010). These formulations are currently being brought through Phase I clinical studies (Johnson et al. 2010; Makidon et al. 2010). Chitosan nanocapsules have similarly been assayed for intranasal needle-free immunisation against Hepatitis B (Vicente et al. 2009, 2010). HBsAg was adsorbed onto the chitosan shell, while immunostimulants were used in the core composition. Results have shown an enhanced effect in long-term immunisation in vivo, after both intramuscular and intranasal administration to mice (Vicente et al. 2010).

Still, the potential of W/O nanoemulsions has not gone unrecognised. Huang et al. have described an elegant multiple nanoemulsion, which draws on the

advantages of W/O nanoemulsions while countering syringeability problems by further dispersing the initial emulsion into an aqueous outer phase (Huang et al. 2009a, b, c, 2010). Their formulation, referred to as a PELC nanoemulsion, combines a polymeric surfactant (poly(ethylene oxide)-block-poly(lactide-co-ε-caprolactone) with a low-molecular weight sorbitan trioleate (Span® 85). The oil phase is composed of the GRAS-listed oil squalene. PELC nanoemulsions systematically produced stronger immune responses compared to vaccine administered without adjuvant, generating IgG titers 1.5-fold to sevenfold higher that were observable up until 26 weeks after intramuscular immunisation (Huang et al. 2009c, 2010); encapsulating the antigen in the internal water phase further improved the response to the antigen compared to formulations where it was contained in the external phase (Huang et al. 2010). However, the immunity bestowed by PELC vaccines was not significantly different from influenza vaccine administered with aluminium-salt adjuvants, an adjuvant commonly found in human vaccines (Gupta 1998).

6.5.4 Emerging Therapeutic Approaches

6.5.4.1 Gene Therapy

Gene therapy has been defined as an interesting therapeutic approach and is based on the administration of genetic material to alter protein expression. Vaccination, cancer therapy, and tissue regeneration are some of the fields in which gene therapy has been used as effective therapeutic tool (Schatzlein 2001; Rolland 2005).

Cationic nanoemulsions have successfully been used for the complexation and delivery of nucleic acids, for example to the ocular surface, (Hagigit et al. 2012). Cationic nanocapsules have also been proposed as gene carriers. In this regard, poly(arginine) (PARG) or PEGylated PARG were selected as cationic polymers to constitute the shell and allow the simple adsorption of nucleic acids (Lozano et al. 2013). Saulnier and co-workers have recently proposed a different approach for efficient association of nucleic acids into the oil core, which involves the encapsulation of preformed lipoplexes into PEGylated nanocapsules prepared by PIT (Huynh et al. 2009). The efficacy of this formulation has been demonstrated in NMRI nude mice (David et al. 2012). For that purpose, a gene encoding for an enzyme able to transform the pro-drug ganciclovir into a cytotoxic metabolite was selected as a new experimental anticancer treatment. The successful expression of the gene in the tumoural tissue (orthotopic SK-Mel28 luc and HTB-72 melanoma models in mice) after intravenous administration of DNA-loaded nanocapsules was evidenced by a significant reduction in the growth of the tumoural tissue after 4 days of treatment with the pro-drug Ganciclovir. Moreover, the specific targeting of these nanocapsules could also be achieved by modifying the PEG chains with galactose for hepatocyte targeting (Morille et al. 2009).

6.5.4.2 Tissue Engineering

Over the last decades, tissue engineering has emerged as a promising field in biomedical research. The goal is to improve cell reassembling into structures similar to the original tissue. A defined biomimetic environment is critical for cell differentiation and proliferation leading to functional tissues (Griffith and Naughton 2002; Ikada 2006). Growth factors play an essential function in this process as they interact with selected receptors. However, it is necessary to control their delivery and to protect them from proteolytic degradation (Malafaya et al. 2007; Chen et al. 2010).

Despite increasing interest in drug delivery systems for tissue engineering, the use of nanoemulsions in this field is still at an early stage. Encapsulation of growth factors allows the protection and controlled release critical to achieve an improved effect on both cell proliferation and differentiation (Shi et al. 2010; Dvir et al. 2011). PLGA and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) nanocapsules have been tested for the sequential delivery of bone morphogenic proteins (i.e. BMP-2 and BMP-7) to mesenchymal stem cells and led to a synergistic effect on osteogenic differentiation, due to the adjusted release of both components (Yilgor et al. 2010).

6.6 Imaging and Diagnosis

Nanoemulsions offer an interesting alternative to the use of radioisotopes for imaging purposes. For example, CS-1000 is a commercially available agent (Celsense Inc., USA) specifically formulated as an aqueous colloidal nanoemulsion of perfluorocarbon polymers. Perfluorocarbons are both hydrophobic and lipophobic and do not associate with cell membranes. They also provide a long-lasting intracellular labelling, as they are not subjected to enzymatic degradation. CS-1000 is undergoing studies as a novel Fluorine-19 delivery agent for detection and quantification of human dendritic cells using Magnetic Resonance Imaging (MRI) (Shan 2004; Bonetto et al. 2011).

Nanoemulsions can also act as platforms with the dual capacity to incorporate substances for drug therapy and detection (i.e. theranostics), in order to enable imaging-guided therapy, as illustrated in Fig. 6.8. This theranostic nanoemulsion is composed of soybean oil, 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy (polyethylene glycol)-2000] ammonium salt (PEG-DSPE). The nanoemulsions incorporate the hydrophobic glucocorticoid prednisolone acetate valerate (PAV), as anticancer agent, iron oxide nanocrystals, for MRI imaging, and the fluorescent dye Cy7, for Near Infrared Fluorescence (NIRF) imaging. This nanotheranostic platform tested in a colon cancer murine model showed accumulation in the tumours, with both MRI and NIRF imaging, while the tumour growth was consistently inhibited due to the action of the encapsulated drug (in comparison with the free PAV, saline, or a drug-free control nanoemulsion) (Gianella et al. 2011).

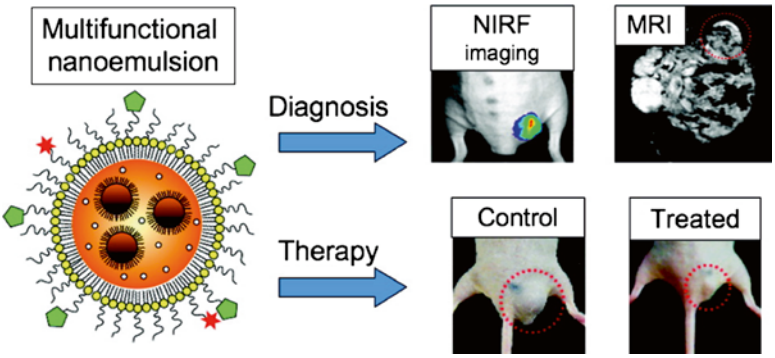

Fig. 6.8 Theranostic nanoemulsion with dual capacity to act as delivery system for (1) anticancer drugs, and (2) contrast agents and fluorescent dyes for MRI and NIRF imaging. Reproduced from Gianella et al. (2011) with permission

6.7 Conclusions

The knowledge accumulated thus far suggests nanoemulsions to be a promising strategy to confront current biomedical needs. Nanoemulsions can be made to contain well-defined, biocompatible, and biodegradable materials, all established and with a good safety record, and can be prepared by mild and scalable technologies. Additionally, nanoemulsion formulations can be tailored to the biopharmaceutical requirements and modality of administration. Importantly, nanoemulsions can be formulated in a case-by-case basis to encapsulate/associate a wide range of molecules, from poor-soluble drugs to highly hydrophilic complex macromolecules. Considering that the quantity of therapeutic molecules emerging from preclinical programmes has grown substantially over the last decade, advances in nanoemulsion development are expected to lead to a burst in the production of new therapeutic/diagnostic entities for biomedical applications.

Problem Box

Question 6.1

Which is the main mechanism related to nanoemulsions instability and how can it be prevented?

Answer 6.1

The Ostwald ripening effect, defined as the growth of the largest droplets at the expense of the smallest ones, is the main cause of instability of nanoemulsions. Controlling the physical properties of the oil phase and the nature of the surfactant can prevent destabilisation of O/W nanoemulsions, as well as coating

(continued)

Problem Box (continued)

the nanoemulsions with a polymeric shell. For W/O nanoemulsions, Ostwald ripening could be slowed down by increasing the ionic strength of the aqueous phase, therefore limiting the solubility of the droplets in the external phase.

Question 6.2

How nanoencapsulation can increase oral bioavailability of drugs/biomolecules? Name some mechaisms.

Answer 6.2

Nanoemulsions can promote drug absorption by (1) improving the solubility of hydrophobic drugs, (2) protecting labile molecules from degradation in the harsh environment of the gastrointestinal track, (3) increasing the interaction with the intestinal mucosa and the retention time, (4) preventing the interaction of the encapsulated drug with efflux transporters such as the P-glycoprotein (P-gp).

Glossary

Capmul® MCM-C8 Glyceryl monocaprylate
Capryol™ 90 Propylene glycol monocaprylate (type II)
Cremophor® WO7 (BASF Corp) Pegylated hydrogenated castor oil manufactured by reacting 1 mol of the oil with 7 mol of ethylene oxide
Cremophor® EL (BASF Corp) Pegylated hydrogenated castor oil manufactured by reacting 1 mol of the oil with 35 mol of ethylene oxide. Now known as Kolliphor EL®
Gelucire® Glycerides and esters of polyethylene glycol
Miglyol® 812 Caprylic/capric triglyceride
NIRF imaging Near-Infrared Fluorescent Imaging
Solutol® HS 15 2-Hydroxyethyl 12-hydroxyoctadecanoate. Now known as Kolliphor® HS 15
Span™ 20 Sorbitan laurate
Span™ 85 Sorbitan trioleate
Sylibin 2-[2R,3R-dihydro-3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-1,4-benzodioxin-6-yl]-2R,3R-dihydro-3,5,7-trihydroxy-4H-1-benzopyran-4-one
Sylimarin A mixture of flavolignans rich in Silybin with hepatoprotectant properties
Tween® 20 Polyoxyethylene(20) sorbitan monolaurate
Tween® 80 Polyoxyethylene(20) sorbitan monooleate

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