CHAPTER

# Lipid-based colloidal carriers for topical application of antiviral drugs 14

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## CHAPTER OUTLINE



## 14.1 INTRODUCTION

The pharmaceutical industry effort today is directed to therapies for human immunodeficiency virus (HIV) and hepatitis C virus (HCV), since the high cost of development has limited the investment in novel antiviral drugs (Littler and Oberg, 2005). Therefore, antiviral research is more likely to be centered on novel therapies for severe diseases, with a long duration and high incidence. The

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existence of vaccines is, of course, also an important factor determining the need for drug development. Even when there is an effective vaccine against a virus, as is the case for hepatitis B virus (HBV), the number of patients who are already infected may afford a large enough market for continued investment in new drugs. These considerations have limited the investment in antiviral drugs to a relatively short list of more severe viral diseases. Therefore, we predict that the development of antiviral drugs over the next 20 years is likely to be focused on HIV, HBV, HCV in view of their medical importance and a continuous need for new drugs to cope with resistance development (Littler and Oberg, 2005).

There are other life-threatening or debilitating viral diseases, such as the ones derived from infections from the *Herpesviridae* family of virus, for which there is a slowdown in the investment of new and more efficient therapies. The reason for this loss of interest is the existence of a drug armamentarium against these viruses that is considered to have enough efficiency to provide treatment for current clinical situations. However, therapeutic treatment of recidivist cases of labial and genital herpes has a rather modest effect in shortening the disease occurrence (Jiang et al., 2016). Furthermore, the majority of antiherpes drugs are administered orally, although some are delivered via intravenous, subcutaneous routes and very few by topical route (Szunerits et al., 2015). Indeed, the topical route is still far from its full potential for antiherpes drugs administration. Despite the general high acceptability of topical formulation by patients, which is also evident from the increasing market for transdermal products, the skin's low permeability limits the number of drugs that can be delivered in this manner (Uchechi et al., 2014). Therefore, current commercial topical formulations for herpes infections treatment have limited efficiency due to low skin penetration of the antiviral agent, requiring the application of about four, up to five times per day for improving the therapeutic effectiveness of the formulation (Durai, 2015; Jain et al., 2011; Lembo and Cavalli, 2010; Sharma et al., 2012; Szunerits et al., 2015).

To sum up, classic therapies against herpesviruses are not totally effective, new drugs are not being synthesized and discovering new solutions for delivery of antivirals already launched in the market is particularly important to improve their topical administration. For all the mentioned reasons, the development of topical formulations containing lipid-based colloidal carriers is a promising approach to promote more effective penetration of antiviral drugs through the skin and thus to reduce the frequency of application and enlarge the number of drugs that can be topically administered.

### 14.1.1 HUMAN HERPESVIRUSES OVERVIEW

The *Herpesviridae* is a large family of DNA-containing, enveloped viruses. This family comprises eight human herpesviruses (HHV), which can be divided, according to biological and genomic similarities, into three subfamilies ($\alpha$, $\beta$, and $\gamma$): (1) the $\alpha$-*herpesvirinae* (*herpes simplex* virus types 1 and 2 (HSV-1 and HSV-2),

*varicella-zoster virus* (VZV or HHV-3)); (2) the *β-herpesvirinae* (*cytomegalovirus* (CMV or HHV-5), HHV-6 and HHV-7); and (3) the *γ-herpesvirinae* (Epstein–Barr virus (EBV or HHV-4) and Kaposi's sarcoma associated with herpesvirus (KSHV or HHV-8)) (Gilbert et al., 2002; Jiang et al., 2016) (Fig. 14.1).

HSV infections are among the most common human diseases, affecting around 21 million people every year and 60%–95% of the population (Jiang et al., 2016). According to a recent survey, approximately 50%–80% of adults suffer from oral and perioral herpes infection that represent one of the most common oral soft tissue diseases encountered in the general population (Jain et al., 2011; Stoopler and Balasubramaniam, 2013). In the immunocompetent host, a primary HSV-1 infection can be either asymptomatic or cause labial and oral lesions. Oral lesions consist of self-limiting gingivostomatitis (5–7 days), often associated with sore throat, due to vesicles on the posterior pharynx (pharyngitis) and on the tonsils (tonsillitis) (Harmenberg et al., 2010; Cunningham et al., 2012). These vesicles, if present, can rupture and form ulcerative lesions with exudates. After the acute gingivostomatitis has passed, the symptoms fade in 2 weeks (Harmenberg et al., 2010; Cunningham et al., 2012). Following primary infection, the virus migrates to the trigeminal nerve ganglion where it can remain latent indefinitely. A variety of stimuli can lead to reactivation of the virus, including environmental triggers such as exposure to ultraviolet light, fever, psychological stress, illness, and menstruation. When reactivated, the virus travels along sensory neurons to the skin and other mucosal sites and causes clinical infections, such as the recurrent episodes of *herpes labialis* (RHL). RHL episodes can be associated with lesions located at the mucocutaneous junction of the lips (known as a fever blister or cold sore) or can be asymptomatic. When symptomatic, episodes typically progress through sequential phases. The first phase is named prodrome and consists of the initial symptoms of reactivation (itching, burning and/or paraesthesia). The prodromal stage occurs in the absence of cutaneous lesions and generally takes 4–5 days. The subsequent phase consists of the appearance of erythema and papule formation. Approximately 25%–50% of RHL episodes do not progress beyond the prodromal or papule stage and are referred to as aborted lesions. Episodes that progress beyond the prodromal stage evolve through postulation (development of vesicles), ulceration and, ultimately, scabbing. Peak viral titers occur in the first 24 h after the vesicular stage, with a subsequent progressive decline as most lesions are converted to ulcers/crust. In the immunocompetent host, RHL are self-limited and generally heal spontaneously within 8–10 days. Regardless, RHL episodes can be frequent, painful, long-lasting and disfiguring. This justifies the administration of antiviral therapy to block viral replication in order to enable shortening the duration of symptoms and accelerate the resolution of lesions. In immunocompromised patients, RHL episodes are usually longer and more severe, potentially involving the oral cavity or extending across the face (Harmenberg et al., 2010; Cunningham et al., 2012; Stoopler and Balasubramaniam, 2013).

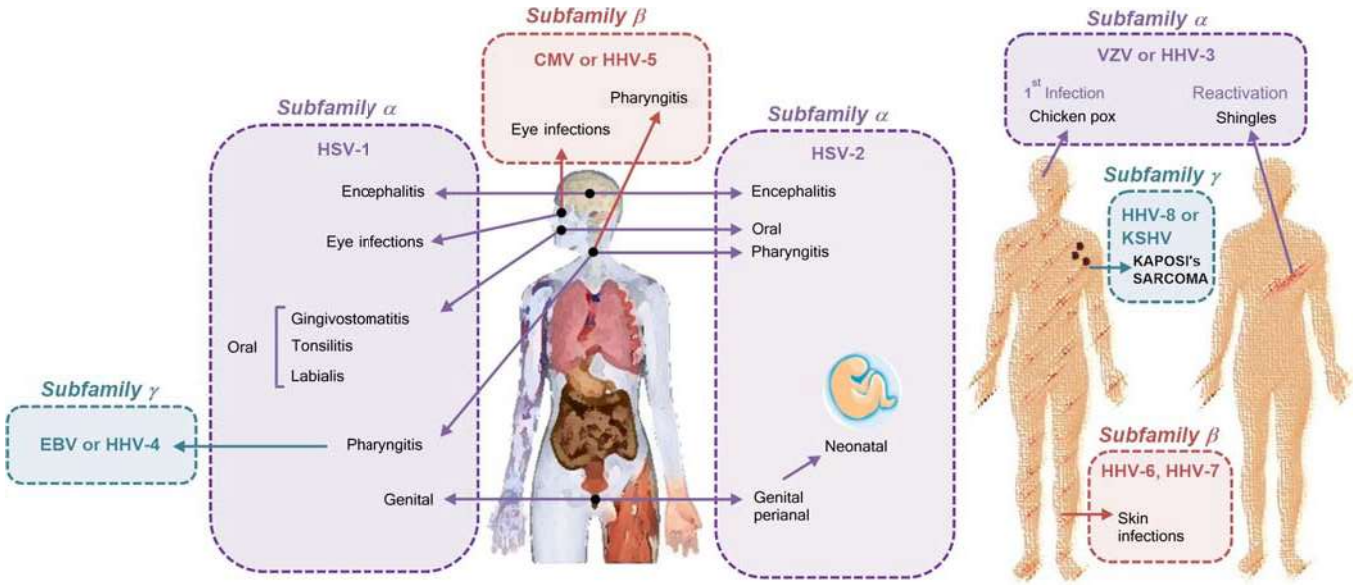



**FIGURE 14.1**

Physiopathology of Herpesviridae family. CMV stands for cytomegalovirus; EBV stands for Epstein–Barr virus; HHV stands for human herpesviruses; HSV stands for herpes simplex virus; KSHV stands for Kaposi's sarcoma virus; VZV stands for varicella-zoster virus.

Although orofacial infections and RHL are commonly associated with HSV-1, the epidemiology has dramatically changed in recent decades. HSV-1 used to be acquired in childhood and adolescence through non-sexual contact. Conversely, HSV-2 was traditionally associated as an etiologic agent of *herpes genitalis*. Due to changes in sexual practices, at least 50% of new cases of *herpes genitalis* in developed countries are now caused by HSV-1. Furthermore, HSV-2 has also been identified as a cause of oropharyngeal infection and *herpes labialis*, although it is still the main cause of recurrent *herpes genitalis* (Harmenberg et al., 2010; Cunningham et al., 2012; Stoopler and Balasubramaniam, 2013). When symptomatic, the pathology of *herpes genitalis* follows a similar progress through sequential phases and episodes, as described for orofacial herpes. The first episode of *herpes genitalis* is characterized by extensive and usually painful genital and perigenital vesicles, pustules, and ulcers, often accompanied by systemic symptoms such as fever, headache, local discomfort, dysuria and inguinal lymphadenopathy. Then the virus enters into latency periods and it is periodically reactivated, causing recurrent episodes of genital lesions (Fife et al., 1997; Jiang et al., 2016).

In addition to the most frequent clinical conditions of orofacial herpes, RHL and *herpes genitalis*, HSV-1 and HSV-2 can also cause a wide range of diseases, from mild conditions to severe infections, such as eye disease (keratitis and corneal blindness) and infections of the central nervous system (CNS), such as, encephalitis (severe infection that involves active viral replication in the brain cortex and its often caused by HSV-1) or meningitis (usually a benign infection of the meninges often caused by HSV-2). HSV infections of the CNS are associated with significant morbidity and mortality, even when appropriate antiviral therapy is administered. Life-threatening HSV infections of the brain can occur from birth throughout life and can be of two types: neonatal HSV infections, which are usually caused by HSV-2, and *herpes simplex* encephalitis (HSE), which occurs in children and adults, and is generally caused by HSV-1. Neonatal HSV infection is usually the consequence of vertical transmission from a pregnant woman with *herpes genitalis* to her fetus. It is acquired either in utero (congenital infection in 5% of cases) or as a consequence of intrapartum contact of the fetus with infected maternal secretions (85% of cases). The remaining cases occur after postpartum exposure to HSV during the first 4 weeks of life. From all babies with neonatal HSV infection, 50% will have CNS disease, and the other 50% will have skin, eye, and mouth disease. In children aged $>6$ months and in adults, HSE remains the most common cause of sporadic fatal encephalitis in the Western world (Whitley and Lakeman, 1995).

Besides HSV, VZV is the other member of subfamily $\alpha$ and is the causative agent of chickenpox (in the first infection) and shingles (after latency and virus reactivation) (Gilbert et al., 2002; Jiang et al., 2016).

The subfamily $\beta$ includes: *cytomegalovirus*, a causative agent of pharyngitis and eye infections, but normally asymptomatic in immunocompetents; HHV-6; and HHV-7, responsible for skin infections (Gilbert et al., 2002; Jiang et al., 2016).

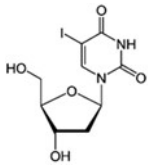
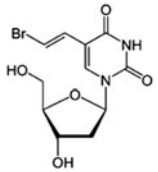
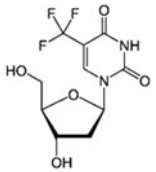
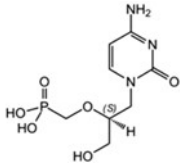
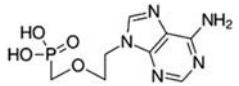
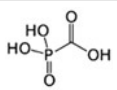
The subfamily γ includes EBV, a causative agent of mononucleosis (also known as kissing disease for its mode of transmission) and HHV-8, the virus responsible for Kaposi's sarcoma (systemic cancer presenting cutaneous lesions that occurs in immunocompromised patients) (Gilbert et al., 2002; Jiang et al., 2016).

Although HHV infections are not usually life-threatening in immunocompetent patients, it can result in severe disease among immunocompromised patients. For instance, acquired immune deficiency syndrome (AIDS) patients may present chronic and/or disseminated cutaneous herpetic lesions and they may experience frequent recurrences of localized or disseminated zoster. Furthermore, CMV is the most common cause of sight-threatening infections among AIDS patients and is associated with significant morbidity and mortality among organ transplant or bone marrow transplant recipients. Furthermore, HSV infections increase the risk for developing HIV infection and contribute to the HIV epidemic (Gilbert et al., 2002).

### 14.1.2 CURRENT ANTIVIRAL AGENTS AGAINST HUMAN HERPESVIRUSES INFECTIONS

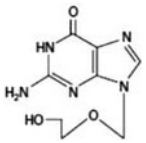
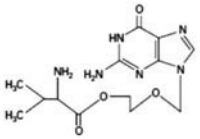
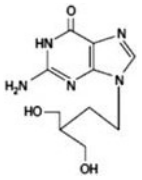
Forty years ago, just three drugs were available for the treatment of viral infections; now, more than 60 compounds have been approved for clinical use. We can say that antiviral drug development is now progressing at the pace antibiotics were 40 years ago. This evolution is mainly due to a greater understanding of viral life cycles, and was driven in particular by the need to fight HIV (De Clercq, 2002, 2008, 2012, 2013a,b; Littler and Oberg, 2005; Razonable, 2011; Stoopler and Balasubramaniam, 2013; Jiang et al., 2016). Therefore, it is not surprising that, from the current antiviral repertoire, at least half of the approved drugs are used for the treatment of HIV. The remaining half are used for the treatment of HBV, HSV, VZV, CMV, influenza, and HCV infections (De Clercq, 2002, 2008, 2012, 2013a,b; Littler and Oberg, 2005; Razonable, 2011; Stoopler and Balasubramaniam, 2013; Jiang et al., 2016). HHV viruses are then relegated to a secondary plan in drug development. However, as previously mentioned, HHV can cause a broad spectrum of diseases in humans that are not usually life-threatening but can be frequent, painful, long-lasting and disfiguring; thus dramatically affecting the quality of life of patients (Razonable, 2011; Cunningham et al., 2012; De Clercq, 2013b; Stoopler and Balasubramaniam, 2013). Furthermore, the established treatments are not always effective or well tolerated, highlighting the need for further development and refinement of formulations that can deliver and improve the available antiviral drugs. For this reason, in this chapter we will stick to a list of antiviral drugs against HHV, to give researchers an overview of the drugs available to be used in new improved formulations (Table 14.1).

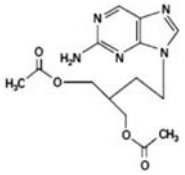
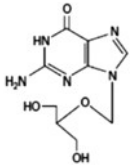
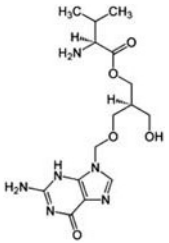
**Table 14.1** Antiviral drugs approved for the treatment of human herpesvirus infections

| Class of Drugs | Antiviral Drug | Chemical Structure | Target Virus | Viral Target and Mechanism of Action | Administration Route | Registered Brand Name | Manufacturer |
|---|---|---|---|---|---|---|---|
| ACYCLIC GUANOSINE ANALOGUES | Acyclovir |  | HSV, VZV, CMV | Inhibition of DNA polymerase after being metabolized to acyclovir triphosphate. | Oral, Topical, Intravenous | Zovirax® | GlaxoSmithKline |
| | Valaciclovir |  | HSV, VZV, CMV | Prodrug of acyclovir. Mechanism of action is the same as acyclovir. | Oral | Zelitrex®, Valtrex® | GlaxoSmithKline |
| | Penciclovir |  | HSV | Inhibition of DNA polymerase after being metabolized to penciclovir triphosphate. | Topical | Denavir®, Vectavir® | Novartis |
| | Famciclovir |  | HSV, VZV | Prodrug of penciclovir. Mechanism of action is the same as penciclovir. | Oral | Famvir® | Novartis |
| | Ganciclovir |  | CMV | Inhibition of DNA polymerase after being metabolized to ganciclovir triphosphate. | Oral, Intravenous, Intraocular implant | Cymevene® Cytovene® | Roche Pharmaceuticals |
| | Valganciclovir |  | CMV | Prodrug of ganciclovir. Mechanism of action is the same as ganciclovir. | Oral | Valcyte® | Roche Pharmaceuticals |

(*Continued*)

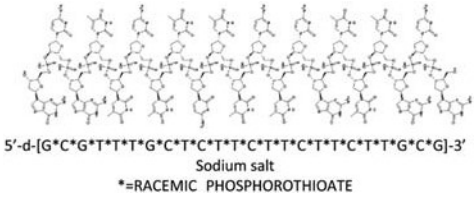
**Table 14.1** Antiviral drugs approved for the treatment of human herpesvirus infections *Continued*

| Class of Drugs | Antiviral Drug | Chemical Structure | Target Virus | Viral Target and Mechanism of Action | Administration Route | Registered Brand Name | Manufacturer |
|---|---|---|---|---|---|---|---|
| | Idoxuridine |  | HSV, VZV | It is phosphorylated to its active form by cellular kinase. It acts as a competitive inhibitor of DNA polymerase and also acts as chain terminator being incorporated in viral DNA. | Topical, Ophtalmic | Herpid®, Stoxil®, Idoxene® Virudox® | Yamanouchi, Astellas |
| **THYMIDINE ANALOGUES** | Brivudin[a] |  | HSV, VZV | It is phosphorylated to its active form by cellular and viral kinase. It acts as a competitive inhibitor of DNA polymerase being incorporated in viral DNA, leading to a reduced integrity and functioning of the viral DNA. | Oral, Topical, Ophtalmic | Zostex®, Brivirac®, Zerpex® | Berlin Chemie, Menarini |
| | Trifluridine |  | HSV, VZV | Inhibits conversion of dUMP to dTMP by thymidylate synthase and inhibits specific DNA polymerases being incorporated in viral DNA, resulting in faulty DNA. | Topical, Ophtalmic | Viroptic® | Glaxo wellcome |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **ACYCLIC CYTIDINE ANALOGUES** | Cidofovir[b] |  | HSV, VZV, CMV | It is phosphorylated to its active form by cellular kinase. It is then targeted at the viral DNA polymerase where acts as chain terminator, being incorporated at the viral DNA chain. | Topical, Intravenous | Vistide® | Pfizer |
| | Adefovir dipivoxil |  | HSV, CMV[c] | It is phosphorylated to its active form by cellular kinase. It is then targeted at the viral DNA polymerase where acts as chain terminator, being incorporated at the viral DNA chain. | Oral | Hepsera® | Gilead sciences |
| **PYROPHOSPHATE ANALOGUES** | Foscarnet |  | HSV, VZV, CMV | Pyrophosphate analogue, interferes with the binding of the pyrophosphate (diphosphate) to its binding site of the viral DNA polymerase, during the DNA polymerization process. | Intravenous | Foscavir® | Astra Zeneca |

(*Continued*)

**Table 14.1** Antiviral drugs approved for the treatment of human herpesvirus infections *Continued*

| Class of Drugs | Antiviral Drug | Chemical Structure | Target Virus | Viral Target and Mechanism of Action | Administration Route | Registered Brand Name | Manufacturer |
|---|---|---|---|---|---|---|---|
| **DETERGENT** | Docosanol |  | HSV, VZV | It blocks the virus from attaching to cells via interference of epithelial cell surface receptors and viral envelope proteins. | | | |
| **ANTISENSE** | Fomivirsen[d] |  5′-d-[G*C*G*T*T*T*G*C*T*C*T*T*C*T*T*C*T*T*C*T*T*G*C*G]-3′ Sodium salt *=RACEMIC PHOSPHOROTHIOATE | CMV | Being complementary in base sequence, it hybridizes with, and thus blocks expression (translation) of, the CMV immediate early 2 (IE2) mRNA. | | | |

*HSV- Herpes simplex virus; VZV-Varicella-zoster virus; CMV-Cytomegalovirus; Chemical structures were created using ACD/ChemSketch 10.0.*
[a]*Brivudin is only approved in some countries.*
[b]*Cidofovir in the commercial form of vistide® was discontinued in 2014. Generics are still available.*
[c]*Adefovir dipivoxil has activity spectrum against HSV, but it is mainly used against Hepatitis B virus as reverse transcriptase inhibitor.*
[d]*Fomivirsen was discontinued in 2002, but is still used in some countries, such Australia, where it has received Orphan Drug Status for CMV-induced retinitis in AIDS patients.*

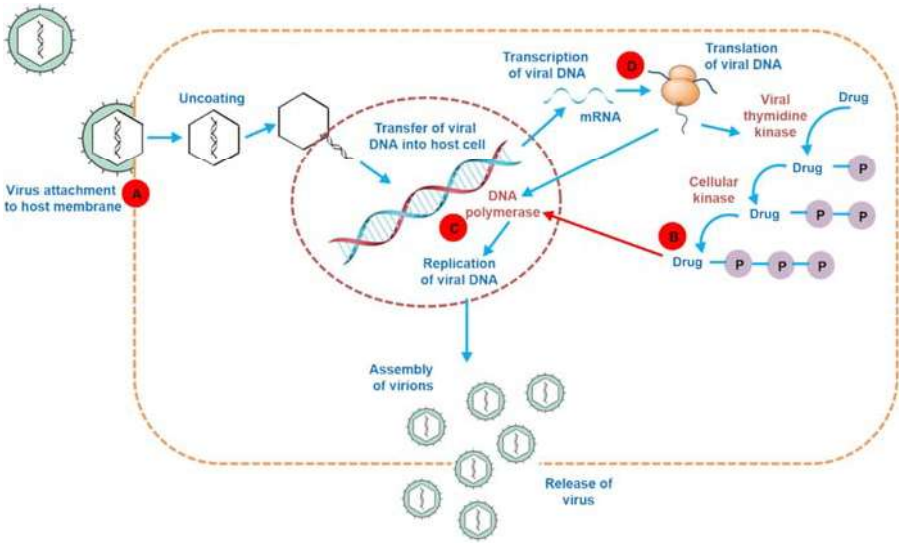



**FIGURE 14.2**

Schematic representation of human herpesviruses cycle and sites of action of antiviral drugs. (A) Drugs that inhibit virus attachment; (B) nucleotide and nucleoside analogues that are activated by phosphorylation and afterwards inhibit DNA polymerase; (C) pyrophosphate analogues that inhibit directly DNA polymerase; (D) antisense drugs that inhibit translation of mRNA.

In contrast to retro-viruses, herpesviruses do not present a reverse transcription step in their replicative cycle, which means that their DNA genome can be replicated by the viral DNA polymerase after the latter has been expressed in the virus-infected cell (Fig. 14.2) (Jiang et al., 2016).

At present, there are six classes of drugs approved for the treatment of HHV infections (Table 14.1); the viral DNA polymerase is the target of four of these classes. The four classes of drugs that target viral DNA polymerase are analogues of cytidine, guanosine, thymidine and pyrophosphate. These analogues need to be phosphorylated intracellularly to the triphosphate form. The first (for brivudin, also the second) phosphorylation step is ensured by the HSV- or VZV-encoded thymidine kinase, or the CMV-encoded protein kinase, and is therefore confined to virus-infected cells. This explains the specific antiviral action of the established antiherpetic compounds. Subsequent phosphorylations are achieved by host cellular kinases. In their triphosphate form, the nucleoside analogues interact with the viral DNA polymerase. They work either as competitive inhibitors of DNA polymerase (blocking its function) or as alternative substrates of DNA polymerase, i.e., for being similar enough to natural substrates, they are incorporated into growing DNA strands preventing their elongation (De Clercq, 2002, 2008, 2012,

2013a,b; Littler and Oberg, 2005; Razonable, 2011; Stoopler and Balasubramaniam, 2013; Jiang et al., 2016).

Besides the drugs that target DNA polymerase (that constitute the majority of anti-HHV drugs), there are two other drugs that present different mechanisms of action. Docosanol is a detergent that hampers the attaching of the virus to the epithelial cell surface, and Fomivirsen is an antisense oligonucleotide sequence that blocks translation of CMV mRNA (Table 14.1 and Fig. 14.2).

## 14.1.3 CHALLENGES IN TOPICAL DELIVERY OF ANTIVIRAL AGENTS

### *14.1.3.1 Pharmacokinetic challenges of antiviral agents*

While the global market for antiviral drugs is constantly witnessing progression due to the existence of unmet needs and population growth, the development of safe and effective antiviral drugs is a complex and difficult task (Szunerits et al., 2015). Currently available antiviral drugs are far from being the perfect solutions to available HHV pathologies. The marketed drugs still have several pharmacokinetic parameters to be addressed when evaluating their therapeutic potential, including:

1. *Bioavailability* upon either topical, oral or parenteral administration. Adequate bioavailability (that is the quantity of drug that reaches the systemic circulation to be distributed in the body) is fundamental for the success of an antiviral and depends both on the *solubility* of the drug in the aqueous media and on the lipid membranes' *permeability* to drugs. Good solubility and permeability are thus considered as markers of adequate oral bioavailability and are essential prerequisites for antiviral drugs (Lembo and Cavalli, 2010). A parameter that allows the evaluation of the balance between permeability in lipid membranes and solubility in aqueous media is the drug partition coefficient (LogP). Besides solubility and permeability, other factors also affect oral bioavailability of an antiviral, including the action of intestinal metabolizing enzymes, efflux transporters, and food (Lembo and Cavalli, 2010).
2. *Plasma half-life ($t_{1/2}$)* is the time required to reach half of the plasma concentration. A short $t_{1/2}$ indicates that the drug is rapidly eliminated and it might not have time to exert a therapeutic effect. A very long $t_{1/2}$ is also unwanted, as it indicates that the drug stays in circulation for long periods and can induce toxicity through bioaccumulation (Szunerits et al., 2015).
3. *Plasma protein binding affinity*. Drugs that demonstrate strong binding affinity to plasma protein have a long circulation time and can cause toxicity through bioaccumulation. Furthermore, a drug highly bound to plasma proteins is not available for distribution in the target tissues and hence has reduced therapeutic efficacy (De Clercq, 2001).

4. *Distribution* through the organism. Permeability of lipid membranes to antivirals is also a required factor to assure drug distribution through the target tissues (e.g., to ensure penetration into the CNS, or through skin or mucosae) (De Clercq, 2001).
5. *Metabolism* through the liver (i.e., cytochrome P-450 drug metabolizing) enzymes and *elimination* through the kidney. These are essential to assure the elimination of drug of the body and to avoid bioaccumulation and subsequent toxicity (De Clercq, 2001).
6. *Toxic side effects* must be considered when the drugs must be administered for a prolonged period, as in the treatment of infections in immunocompromised patients. These side effects may seriously compromise compliance (adherence of the patient to the therapeutic regime) (De Clercq, 2001).
7. *Resistance*. Prolonged antiviral therapy increases the likelihood that drug-resistant strains of the virus will emerge (De Clercq, 2001; Field, 2001, Gilbert et al., 2002; Bacon et al., 2003; Morfin and Thouvenot, 2003; Lembo and Cavalli, 2010; Szunerits et al., 2015; Jiang et al., 2016).

Analyzing these listed pharmacokinetic parameters, it is possible to conclude that all currently available antiviral drugs present problems that reduce their efficacy, such as limited solubility; low lipophilicity, indicating low membrane permeability; short half-life; and a poor bioavailability (Table 14.2) (Balfour, 1999; Sharma et al., 2012). Consequently, high doses and multiple dosing regimens are required which, in turn, can negatively affect patient compliance and cause severe side effects (Lembo and Cavalli, 2010; Jain et al., 2011; Szunerits et al., 2015). Taking the example of acyclovir (ACV), several drawbacks were observed in its physicochemical and pharmacokinetic properties, such as: slight water solubility (1.3 mg/mL at 25°C), poor permeability ($0.12 \times 10^{-6}$–$2.0 \times 10^{-6}$ cm/s), short $t_{1/2}$ (2.5–3.5 h), and poor oral bioavailability (10%–20%) because of its slow and incomplete absorption in the gastrointestinal tract. This indicates that approximately 80% of the administered dose of ACV is never absorbed and for that reason high doses (up to 1200 mg/day) are required for the oral administration of this antiviral agent (Lembo and Cavalli, 2010; Jain et al., 2011; Durai, 2015; Szunerits et al., 2015). This demands a high level of patient compliance, with many patients taking multiple oral doses daily at set times to obtain a relatively constant and high drug level (Szunerits et al., 2015). Indeed, the oral administration of an antiviral is far from being an adequate administration route, as antivirals with a low or variable bioavailability require the use of higher doses and prolonged treatment durations in order to eradicate the virus (Lembo and Cavalli, 2010). However, other administration routes are not the solution for a better antiviral therapy, as they also present several pharmacokinetic problems. For instance, as ACV has limited solubility in water (1.3 mg/mL at 25°C), intramuscular injection or ocular administration is of limited use. Parenteral administration of ACV

**Table 14.2** Pharmacokinetic Parameters of Antiviral Drugs Approved for the Treatment of Human Herpesvirus Infections

| Antiviral Drug | Solubility (mg/mL) | Log *P* | $t_{1/2}$ (h) | Protein Binding (%) | Oral Bioavailability (%) | Toxic Effects |
|---|---|---|---|---|---|---|
| Acyclovir | 1.3 | −1.56 | 2.5–3.5 | 9–33 | 10–20 | Reversible nephropathy; gastrointestinal disturbances, irritation at infusion site; phlebitis; headache; rash; encephalopathy |
| Valaciclovir | 70 | −0.3 | 2.5–5.3 | 13–18 | 54 | Same as acyclovir and thrombotic microangiopathy |
| Penciclovir | 1.7 | −1.1 | 2 | <20 | 0[a] | Mild erythema |
| Famciclovir | 25 | 0.6 | 10 | 20–25 | 77 | Headache; nausea; diarrhea; interaction with drugs |
| Ganciclovir | 2.6 | −1.7 | 2–4 | 1–2 | 8–9 | Bone marrow suppression (granulocytopenia); renal insufficiency; fever, headache, irritation at infusion site; phlebitis; rash; encephalopathy |
| Foscarnet | 1.68 | −2.1 | 3.3–6.8 | 14–17 | 0[b] | Renal insufficiency; electrolyte imbalance (hypocalcemia); nausea; vomiting; anemia; penile ulcers; seizures |
| Idoxuridine | 1.6 | −0.53 | 0.5 | N/A | 0[a] | Irritation; blurred vision and photophobia; corneal clouding and damage of the corneal epithelium may also occur |

*N/A: not available.*
[a]*Only available topically with no appreciable systemic absorption.*
[b]*Oral bioavailability is 0%, therefore only intravenous administration is available.*

is presently available as bolus intravenous injections in the form of strong alkaline (pH 10−11) solution, causing, however, pervascular inflammation (Szunerits et al., 2015). Compared with oral administration, topical administration of ACV leads to tenfold higher concentration over the entire epidermis (Jain et al., 2011). However, this concentration fails to produce the desired therapeutic effect at the site of infection because of the low penetration of ACV in the basal epidermis (Lembo and Cavalli, 2010; Jain et al., 2011). As a consequence, topical formulations of the drug need to be applied 5−6 times per day.

To overcome ACV pharmacokinetic problems, derivates and prodrugs have been synthesized, such as valaciclovir, the L-valine ester of acyclovir, and famciclovir, a prodrug of penciclovir, which show improved oral absorptions in comparison with the parent drug (Lembo and Cavalli, 2010). However, the effectiveness of these antiviral drugs is still constrained by tedious dosing regimens and limited accumulation at the site of infections (basal epidermis and dermis) (Jain et al., 2011).

The anti-CMV drug, ganciclovir, represents another example of an antiviral with very poor bioavailability (6%−9%), requiring the daily administration of a high dose ($>1000$ mg) (Lembo and Cavalli, 2010). Moreover, the oral administration of other antivirals is not viable. For example, foscarnet and cidofovir require intravenous administration because of their extremely low oral absorption and their gastrointestinal toxicity (De Clercq, 2001). For the local treatment of ocular pathologies, including retinitis, the intravitreal administration of ganciclovir and formisen were demonstrated to be more effective than intravenous administration, but multiple administration and high doses are required; intraocular injections are poorly tolerated by patients and run the associated risks (Lembo and Cavalli, 2010).

Finally, another problem of antiviral agents is that the chronic treatment with such drugs can produce moderate levels of drug toxicity, which might lead to serious complications in the patient (Lembo and Cavalli, 2010).

Besides the mentioned physical-chemical and consequent pharmacokinetic limitations of the drugs, prolonged antiviral therapy also increases the likelihood that drug-resistant strains of the virus emerge (Szunerits et al., 2015). Long-term use of ACV treatment has, in particular, resulted in ACV-resistant virus strains in immunocompromised patients. HSV infection in the immunocompetent patients generally requires short-term anti-HSV therapy, and drug resistance does not easily occur. As a result, a low prevalence (range from 0.1% to 0.6%) of HSV resistance to ACV has been reported in normal immunocompetent patients. In contrast, the immunocompromised patients generally require long-term anti-HSV therapy, and they are likely to develop drug resistance that ranges from 3.5% to 10%. Some clinical surveys have reported a rate of ACV-resistant HSV isolates of up to 36% (Jiang et al., 2016). Resistance has also been reported for immunocompromised patients treated with ganciclovir for CMV infections (De Clercq, 2001).

#### 14.1.3.2 Skin and mucosa as challenging sites for drug delivery

Given the challenging pharmacokinetic problems of antiviral drugs, transdermal drug delivery or topical delivery (either on skin or mucosae) could be an alternative and convenient route of administration for a variety of clinical HHV manifestations. These routes possess a number of significant advantages over traditional dosage forms. These include: reducing direct effects on the stomach and intestine, avoidance of first-pass metabolism, increase in patient compliance (administration is usually simple and painless, thus allowing for self-administration and patient comfort), and the fact that the drug concentrations at the dermal layer of the skin may better correlate with the antiviral activity (Sharma et al., 2012). Nevertheless, skin and mucosae constitute barriers and present difficulties for drug delivery that will be discussed in this section.

Human skin is the largest organ of the body, accounting for more than 10% of body mass. Skin has a multifunctional role, but one of the most important functions is its ability to interact with the environment, acting as a protective barrier against foreign aggressions (chemicals, microbes) and also avoiding the loss of excessive endogenous material, such as water (Brown et al., 2006; Uchechi et al., 2014; Hamblin et al., 2016). The skin essentially consists of four layers (Fig. 14.3). The top or uppermost layer of the skin is known as the *stratum corneum* (SC). The SC is comprised of dead cells (corneocytes) composed mainly of lipids (20%) and insoluble bundled keratins (70%), which confers support and mechanical stability to the SC. Around the corneocytes there is a lipid matrix made of lipid bilayers rich in ceramides (40%–50%), fatty acids (15%–25%), cholesterol (20%–25%) and cholesterol sulfate (5%–10%). The SC is actually the main barrier of the skin and its barrier properties may be partly related to its very high density and its low hydration of 15%–20%, compared with the usual 70% for the body. The other skin layers are: the remaining layers of the epidermis (viable epidermis), the dermis, and the subcutaneous adipose tissue. There are also several associated appendages: hair follicles, sweat ducts, glands, and nails (Brown et al., 2006; Uchechi et al., 2014; Hamblin et al., 2016).

Most topically administered drugs do not have the ability to penetrate the SC. Once the SC is a corneocyte-rich layer, transport across it is primarily by passive diffusion in accordance with Fick's Law and no active transport processes have been identified. There are three options for drugs to permeate the SC (Brown et al., 2006; Trommer and Neubert, 2006; Hamblin et al., 2016).

##### 14.1.3.2.1 The intercellular lipid route

The intercellular lipid route refers to the passage of substances between corneocytes through interlamellar regions of the SC that are rich in fluid lipids. The capacity of a drug to permeate the SC through the intercellular lipid route depends on its lipophilicity and molecular weight. Normally, permeation is favored for lipidic and amphiphilic molecules with Log $P$ values of 1–3 and

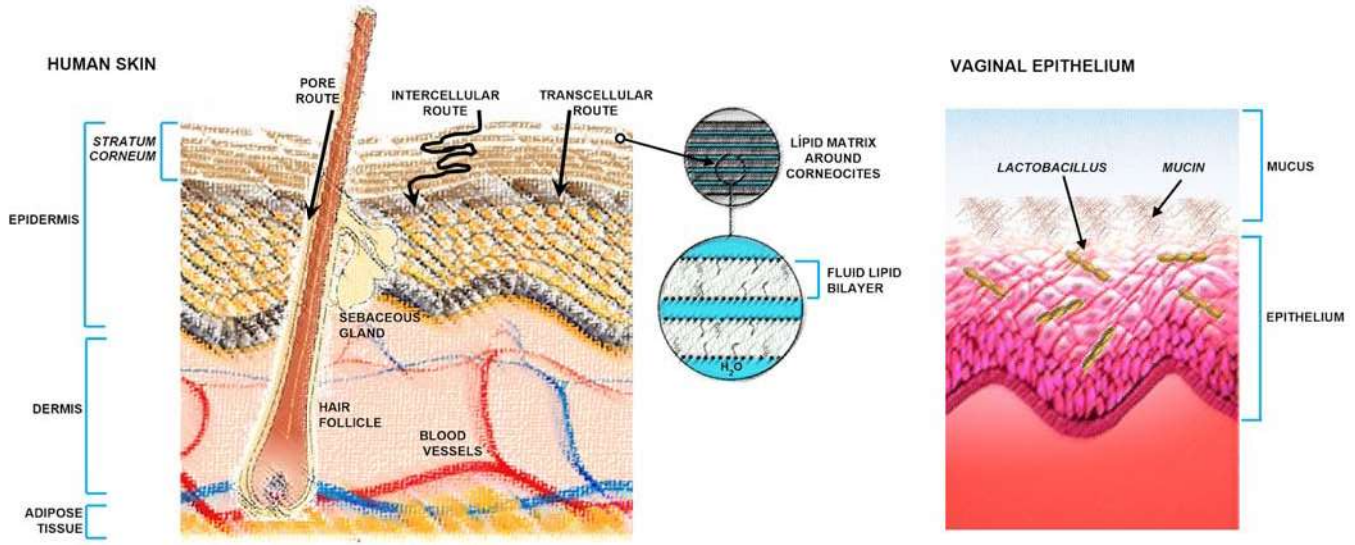



**FIGURE 14.3**

Left: schematic representation of human skin and penetration routes of drugs throughout the skin. Inset shows lipid matrix around corneocites. Right: schematic representation of vaginal epithelium.

molecular weight smaller than 400–500 Da. Hydrophilic molecules diffuse predominantly laterally along surfaces of less abundant and narrow (≤10 nm) water-filled interlamellar spaces, or through free spaces between lamella and corneocyte outer membranes (Hadgraft, 2004; Uchechi et al., 2014).

#### 14.1.3.2.2 The transcellular route

The transcellular route involves crossing the skin by directly passing through both the lipid structures of the interlamellar region and moving across corneocytes with their keratin-enriched intracellular macromolecular matrix. Although this route is considered a shorter path compared with intercellular routes, transcellular diffusion involves higher resistance to the passage of substances because they have to cross both lipophilic and hydrophilic structures, and therefore this is not a usual route for drug permeation (Trommer and Neubert, 2006).

Overall, at least for polar drugs, it is likely, that the transcellular route provides the main pathway during percutaneous absorption. As penetrants become more nonpolar, the intercellular route likely becomes more significant, although whether it entirely dominates, even for nonpolar drugs, is unclear (Barry, 1987).

#### 14.1.3.2.3 Pore route

Since the skin appendages (glands and hair follicles) occupy only 0.1% of the total human skin surface, the contribution to the pore route was primarily neglected. Recent studies have, however, reconsidered the efficiency of follicular routes. Due to the morphological architecture of hair follicles, follicular penetration is a complex process that may have great potential for the absorption of substances below the skin surface. Hydrophilic conduits of pore openings are ≥5 μm (sweat ducts (≥50 μm), pilosebaceous units (5–70 μm), and sebaceous glands (5–15 μm), representing the largest width/lowest resistance of the skin barrier (Uchechi et al., 2014; Hamblin et al., 2016).

To just include the SC layer in skin barrier properties is oversimplified, but it is certain that SC is the main barrier responsible for the protection exerted by the skin against the environment. Moreover, the SC is more lipophilic than both the epidermis and the dermis, which resemble an aqueous hydrogel. In skin lesions, such as those caused by HHV, the SC and even the epidermis (in ulcers) have been removed. Although the papules or nodules show a thickened epidermis, the SC removal and epidermal alterations tend to increase the permeability of drugs and, most significantly, hydrophilic compounds that have low penetration rates into the intact skin (Hamblin et al., 2016).

Mucosal membranes are another important point for topical administration of anti-HVV drugs. Mucosal membranes are moist surfaces lining the walls of various body cavities, such as respiratory, gastrointestinal, and reproductive tracts as well as the nostrils, eyes, and mouth. In mucosal membranes, mucus is the main challenging barrier that drug delivery must overcome (Fig. 14.3) (Sarmento and das Neves, 2014). Mucus is a viscoelastic, adhesive gel that lines the epithelium and

protects it by lubricating, trapping, and removing possible infectious agents. Mucus is composed of mucins, usually negatively charged due to the presence of carboxylate groups (sialic acid) and ester sulfates at the terminus of some sugar units. The pH of the mucus can vary dramatically, depending on the local environment. Typical pH values for the nasal mucus are neutral or slightly acidic (pH = 5.5–6.5), for the eye mucus is ~7.8 and the mouth 6.2–7.4 (Sarmento and das Neves, 2014). In the particular case of vaginal mucosa, it is covered by a thin layer of acidic mucus which provides lubrication and an additional barrier to drug permeation. Lactic acid resulting from the fermentation of host glycogen by lactobacilli is the main factor responsible for its acidic pH around 4–5 (Fig. 14.3). Depletion of normal microbiota results in increased pH (to around neutrality) and facilitates infection (Sarmento and das Neves, 2014). The mucous pH can be seen as another barrier for drug delivery, as it may impact the stability of drugs, and so, their influence should be assessed during therapeutic development. Among other components, vaginal fluid possesses relatively low enzymatic activity; however, the presence of different enzymes, particularly peptidases, may impact on the possible delivery of biopharmaceuticals by this route. Vaginal mucosa is able to be permeated by active molecules, particularly those of low molecular weight (<300 Da) and presenting some degree of hydrophobicity (Sarmento and das Neves, 2014). In addition, small molecules that partition into oil, or other nonpolar solvents, diffuse more slowly through mucus than through water and their diffusion constants in mucus decrease in proportion to their nonpolar/polar partition coefficient (Sarmento and das Neves, 2014). Different low molecular weight compounds have been shown to permeate vaginal tissue at comparable or even higher rates than across buccal or intestinal mucosae. This can also be the case of some higher molecular weight molecules (Sarmento and das Neves, 2014). The surface charge of compounds is also relevant to evaluate mucus permeation. Small cationic molecules can bind tightly, to the negatively charged glycan domains of mucin, decreasing their diffusion across mucus (Sarmento and das Neves, 2014).

### 14.1.4 ADVANTAGES OF LIPID-BASED COLLOIDAL CARRIERS AS TOPICAL DELIVERY SYSTEMS

To improve the therapeutic activity of antivirals present on the market, it is necessary to change the conventional dosage forms. Radically modified formulations of drug dosage forms, such as the design of novel nanodelivery systems for antiviral administration, can improve bioavailability, modify the $t_{1/2}$, and reduce the administered dose to overcome the problems of non-compliance brought about by difficult dosing regimens (Lembo and Cavalli, 2010).

Indeed, an important feature of nanodelivery systems is their ability to encapsulate therapeutic agents that may not otherwise be bioavailable due to poor solubility, short half-life, or inability to maintain contact with their biological target.

These nanoformulations are reported to be superior to traditional formulations with respect to controlled release; the nanosystem can serve as a depot of encapsulated drugs by facilitating their release at a sustained rate over time and allowing continuous interface with their biological target. Sustained release avoids rapid saturation and elimination, thereby allowing the delivery of potent drugs while minimizing side effects. Release rate can also be conveniently controlled by altering the nanosystem composition and physical characteristics. Of these characteristics, nanocarrier size distribution is an important factor in determining the interaction with the cell membrane and the penetration across physiological barriers (Uchechi et al., 2014; Hamblin et al., 2016).

Nanocarriers as drug delivery systems were first intended for use in parenteral or oral routes of administration and, as such, still continue to be the focus of many studies. However, skin application of these nanocarriers is also useful when considering surface effects (film formation and occlusive effects), local effects in the skin (drug delivery in the epidermis and dermis), and systemic effects (deeper drug permeation and transdermal delivery) (Uchechi et al., 2014). In potential uses, apart from those concerned with surface effects, the nanocarrier must overcome the SC barrier in order to deliver the drug more or less deeply into skin layers (Uchechi et al., 2014). Physicochemical properties of nanocarrier systems determine the interaction with biological systems and thus determine how deeply the nanocarrier may penetrate into skin. The main physicochemical properties that affect skin penetration are size, shape, composition, rigidity, and charge in the surface of the nanocarriers (Uchechi et al., 2014).

Regarding the ideal size of nanocarriers for topical administration, there is some controversy; while the small size of nanocarriers may enable diffusion through the pores, size alone does not confer penetration of the SC (Hamblin et al., 2016). Indeed, it was expected that nanocarriers with sizes bigger than 10 nm did not penetrate human skin to any great extent. However, through hydration of the SC (namely by the occlusive effect provoked by the nanocarriers) and using massage, larger channels between the lipids may be created. Furthermore, hair follicles and sebaceous glands offer alternative routes and are potential sites for nanocarrier deposition. In this context, the pilosebaceous unit can serve as a reservoir for nanocarriers, protecting them from the continuous desquamation of the SC. From this reservoir, drugs encapsulated by nanocarriers may enter the epidermis or dermis (Hamblin et al., 2016).

Skin permeability also varies according to different types of shapes of the nanocarriers: spherical nanocarriers seem to be more capable of penetrating skin, compared with ellipsoidal nanocarriers. Furthermore, it has been reported that spherical nanocarriers can reach deeper tissues being located in the epidermis and the dermis, whereas ellipsoidal nanocarriers are mainly located in the layers of the SC and the epidermis (Hamblin et al., 2016).

Composition of the nanocarriers is also an essential parameter conditioning skin penetration. As stated earlier, the intercellular lipid route is one of the most

important ways to penetrate SC. In this context, lipid colloidal nanosystems have the advantage of a lipid composition compatible with the lipid matrix of the intercellular route. The lipids present in the nanosystem composition can interact with lipids present in the SC, changing the structure of the upper skin (Uchechi et al., 2014). This change is beneficial for the penetration of drugs into the SC. Furthermore, if allied to the lipid composition, there is low rigidity (e.g., flexible lipid vesicles) and the nanocarriers may assume a deformable structure and pass through the SC (Uchechi et al., 2014).

Finally, the surface charge of the nanocarriers is also a determinant regarding their ability to permeate skin. Indeed, according to the surface charge, extracellular proteins may adsorb on the surface of applied nanocarriers, including products of skin bacterial flora (Hamblin et al., 2016). This protein corona will influence all further interactions established between the nanocarriers and the skin, and consequently will influence their skin penetration (Hamblin et al., 2016).

Besides skin penetration abilities of nanocarriers, which are important to develop antiviral formulations for topical and transdermal application in skin, it is also important to take advantage of the nanocarriers' ability to permeate the mucus barrier, as a prospect of developing antiviral nanoformulations for topical application in mucosae. In order to reach their targets, drug nanodelivery systems must cross at least the outermost layers of the mucus barrier rapidly to avoid a fast mucus clearance (Sarmento and das Neves, 2014). It is well recognized that, in order to penetrate the mucus, the nanocarriers must avoid adhesion to mucin fibers and be small enough to avoid significant steric inhibition by the dense fiber mesh (Sarmento and das Neves, 2014). Accordingly, it has been shown that nanocarriers of 500 nm coated with a muco-inert polymer cross the physiological human mucus with diffusivities of only fourfold less, compared to their rates in pure water (Sarmento and das Neves, 2014). Positively charged nanocarriers are to be avoided, as they can bind tightly to the negatively charged glycan domains of mucin being trapped in the mucus gels. However, at very high concentrations, positively charged nanocarriers can lead to the collapse of the mucus gel, forming large channels that may provide access to epithelial surfaces for the drugs they carry (Sarmento and das Neves, 2014).

Fig. 14.4 summarizes, in a schematic representation, the nanocarriers' properties required to achieve a better penetration into and across the skin and mucus.

## 14.2 TYPES OF LIPID COLLOIDAL CARRIERS

Despite intensive research in the area of colloidal carriers, the use of nanocarriers in pharmaceutical products is limited. The technology to produce certain types of nanosystems is expensive, and stability and nontoxic effects must also be guaranteed (Días-Torres, 2010). Therefore, the selection of excipients in a formulation is

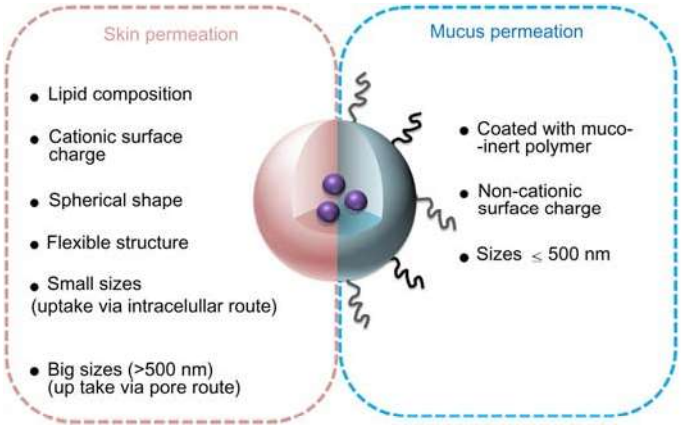



**FIGURE 14.4**

Schematic representation of the nanocarriers' properties required to achieve a better penetration into and across the human skin (left figure) and mucus layer (right figure). Drawn with basis on literature reports (Sarmento and das Neves, 2014; Uchechi et al., 2014; Szunerits et al., 2015; Hamblin et al., 2016).

essential to the overall safety of pharmaceutical products (Lim et al., 2012). In this context, lipids represent a promising option, especially to use as water-insoluble drug carriers, since they are frequently extracted from, or derived based on, natural sources and exhibit physiological biocompatibility and biodegradability. Several lipids used as excipients in pharmaceutical formulations possess well established safety profiles and present FDA approval status (Koo et al., 2005; Lim et al., 2012).

The most investigated lipid colloidal carriers for topical applications in the pharmaceutical field include liposomes, lipid nanoparticles, and micelles. This section offers a general review on the background and development of these lipid-based nanocarriers, with a focus on their composition and main preparation methods. Furthermore, characterization techniques including mean size, zeta potential (ZP), degree of lipid crystallinity and polymorphic changes, encapsulation parameters, drug release, and stability are reported.

### 14.2.1 LIPOSOMES

Liposomes were invented by Dr. Alec D. Bangham and collaborators in the 1960s at the Babraham Institute, University of Cambridge (Bangham and Horne, 1964); the first report on the liposomal encapsulation of a therapeutic agent was published in 1971 (Gregoriadis et al., 1971). These lipid colloidal carriers are defined as nearly spherical shape vesicles, self-assembled lipid structures in which an

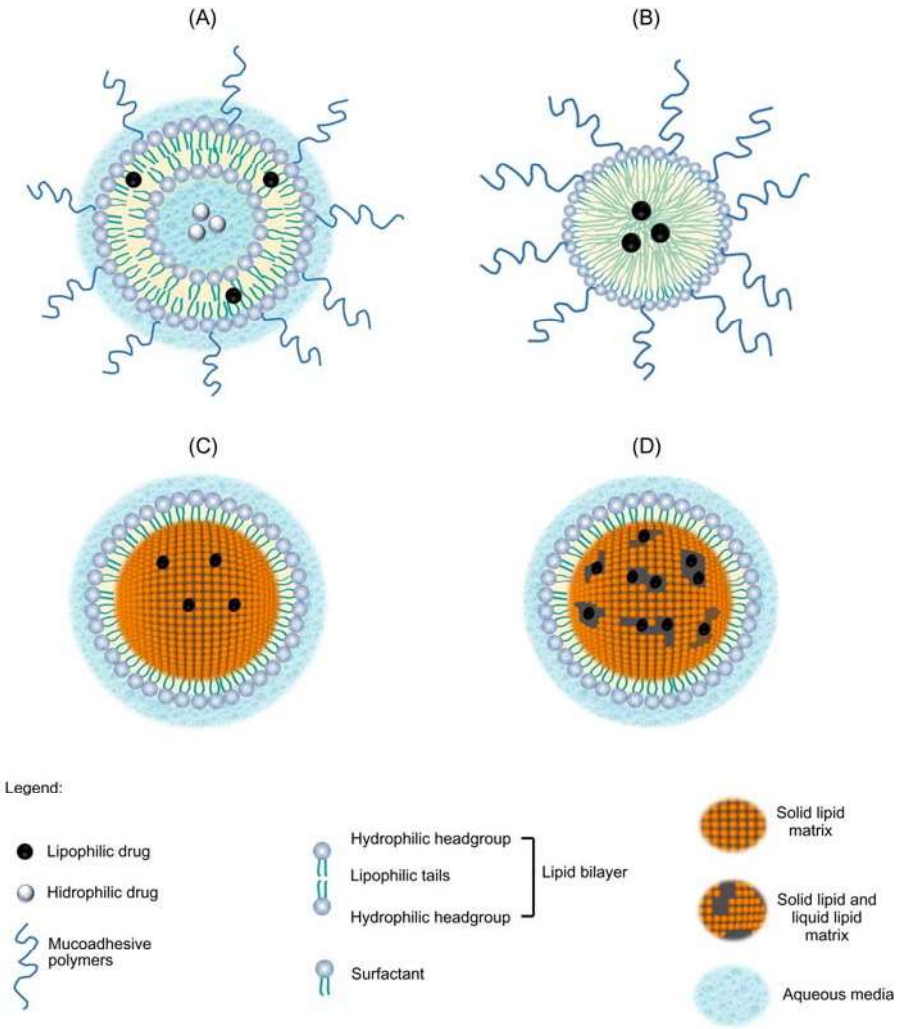



**FIGURE 14.5**

Schematic representation of different lipid colloidal carriers for topical applications: (A) liposomes; (B) micelles; (C) solid lipid nanoparticles (SLN) and (D) nanostructured lipid carriers (NLC).

aqueous core is completely enclosed by one or more lipid bilayers (Fig. 14.5A). Hydrophobic interactions, combined with some geometrical packing factors, are responsible for the formation of lipid bilayers, and van der Waals forces maintain the lipophilic chain packing. Hydrogen bonds and electrostatic interactions

between the water molecules of the aqueous phase and the hydrophilic lipid head groups stabilize the liposomes' structure (Bozzuto and Molinari, 2015).

Despite the first liposomal formulations being composed exclusively of natural lipids, they can contain different lipids that present generally recognized as safe (GRAS) status, such as natural and cell-like membrane lipids (e.g., cholesterol, egg lecithin, and soy lecithin), and/or semi synthetic phospholipids (e.g., dioleoyl phosphoethanolamine, dimyristoyl phosphatidylethanolamine, distearoyl phosphoethanolamine (DSPE), dipalmitoyl phosphatidylcholine (DPPC), dimyristoyl phosphatidylcholine (DMPC), distearoyl phosphatidylcholine (DSPC), etc.) (Silva et al., 2012d). Although most of phospholipids employed to prepare liposomes have neutral charge (e.g., DMPC, DPPC), anionic phospholipids (e.g., dipalmitoyl phosphatidylglycerol, dimyristoyl phosphatidylglycerol) and cationic lipids (e.g., stearylamine, 2-dioleoyl-3-trimethylammonium-propane, dioleyloxypropyltrimethylammonium bromide, dioctadecyldimethylammonium bromide or chloride), can also be used. The latter ones are especially indicated for gene delivery.

According to their size and number of bilayers, liposomes can be generally classified into: small unilamellar vesicles (single lipid bilayer in a vesicle with 25–100 nm in diameter), large unilamellar vesicles (single lipid bilayer in a vesicle with sizes 100–500 nm), giant unilamellar vesicles (single lipid bilayer in a vesicle with sizes $>1$ μm) and multilamellar vesicles (MLVs; several lipid layers separated from one another by a layer of aqueous solution that have a mean diameter between 1 and 2 μm) (Lucio et al., 2010; Pinheiro et al., 2011; Lúcio et al., 2013). The thickness of the liposome membrane measures around 5–6 nm (Días-Torres, 2010).

There are numerous liposomal preparation methods, including: (1) lipid film hydration method flowed by mechanical approaches, such as homogenization techniques (e.g., sonication, microfluidization, extrusion); (2) approaches based on the replacement of organic solvents by aqueous solutions (e.g., ethanol injection); (3) reverse phase evaporation techniques; and (4) techniques based in detergent removal. The selection of the applied method is intrinsically connected with the following parameters: properties of the constituent lipids and of the encapsulated compound; nature of the medium in which lipid vesicles will be dispersed; and therapeutic application. Along with these parameters, it is perceptible that most of the liposomal preparation methods are inadequate for large scale production and that the ethanol injection and the lipid film hydration method, followed by extrusion, are the most frequently used (Allen and Cullis, 2013; Lúcio et al., 2013).

Liposomes present attractive properties that make them distinctive drug delivery carriers. Considering their lipid composition variability and structural properties, liposomes are considered versatile to carry compounds with different solubility, i.e., liposomes may carry (1) water-soluble compounds (located inside the aqueous cavity or at the hydrophilic head-group) increasing their penetration through lipophilic physiologic membranes; (2) poorly water-soluble compounds (located into the lipid bilayer at the fat acid chains) increasing their solubility in

aqueous body fluids (Fig. 14.5A); and (3) compounds with intermediate Log*P* (that partition between the lipid bilayer and the aqueous compartment) (Immordino et al., 2006). Encapsulation efficiency (EE) depends on both the water solubility and Log *P* of the drug, which also determines its location in the liposomal formulation. Furthermore, since liposomes present an identical morphology of cellular membranes and considering the natural materials or their synthetic derivatives used to prepare them, liposomes are considered biocompatible, biodegradable, non-immunogenic, and without or low toxicity. Other advantages of liposomes are: accommodation of drugs in its original form, i.e., not requiring covalent bonds with the carrier, and an ability to protect encapsulated compounds from the external environment (Mozafari, 2005; Diebold et al., 2007; Sanchez-Rodriguez et al., 2015).

Despite the exhaustive research in liposome technology-as-drug delivery carrier since their discovery, it is surprising that not many more liposomal-based products are available in the pharmaceutical market. However, this fact could be explained by some drawbacks associated with liposomes that limit their therapeutic outcomes, such as: in vivo failures (e.g., lack of specificity); some physical and chemical instability phenomena (e.g., aggregation, sedimentation, fusion, phospholipids hydrolysis and/or oxidation), which can occur during storage period and/or under physiological conditions after administration; batch-to-batch variation in manufacturing; low drug-loading efficiency; and the complex regulatory issues that must be accomplished before their passage for clinical use (Bozzuto and Molinari, 2015; Mishra et al., 2010; Chang and Yeh, 2012). Despite liposomes being considered as safe colloidal carriers due to their nature, the addition of non-physiological compounds can induce chemical modifications that are useful to improve their efficacy in drug delivery but can also incite some toxicological effects (Bozzuto and Molinari, 2015). Some of these drawbacks can, however, be reduced by applying different strategies to improve the performance of liposome formulations. In this chapter, we will focus specifically on strategies used to improve topical administration of drug-loaded liposomes.

The first academic work related to the cutaneous application of liposome-based drug formulation was presented at the International Pharmaceutical Federation (FIP) 1979 congress (Mezei and Gulasekharam, 1979) and the research paper was published 1 year later (Mezei and Gulasekharam, 1980). This liposomal formulation contained triamcinolone acetonide, a steroid, and the authors reported a higher drug concentration in the epidermis and dermis, and a lower systemic drug concentration, compared to conventional semisolid formulations (Mezei and Gulasekharam, 1980, 1982).

Liposomes seem to be effective nanocarriers for the topical application of drugs, since their bilayer structure has similarity to the structure of cellular membranes. Therefore, liposomes can function as permeation enhancers and are able to interact/penetrate with the cellular membranes by means of different mechanisms: endocytosis, changes in the ultrastructures of the intercellular lipids on

topical application; fusion, the lipid bilayer of liposomes can fuse and mix with other bilayers such as the cell membrane which promotes the release of its contents; or exchange of lipids that can modify the physical properties of the membrane in the local application (e.g., elasticity) (Murthy and Shivakumar, 2010; Budai et al., 2013; Raj et al., 2012).

Several factors seem to affect the performance of liposome formulations as topical delivery systems, e.g., size, lamellarity, lipid composition, surface charge and/or surface decoration, and elasticity. Despite that their size can vary from a few nanometers to several micrometers, typically the liposomes applied to clinical use range between 50 and 450 nm (Etheridge et al., 2013). In the case of topical cutaneous applications, smaller sizes seem to be required, as the intercellular pores of the SC restrict larger size particles (Hadgraft, 2004; Hamblin et al., 2016). Bigger size particles would only be able to penetrate skin through follicular pores. However, Betz et al. (2005) reported that MLVs were able to deliver a drug within 30 min to the SC, epidermis, and dermis in significantly higher concentrations, compared to conventional topical preparations such as creams, lotions, gels, or emulsions. Regarding the "bigger" size of these colloidal carriers, compared to the skin intercellular pores available for penetration, Cevc and Gebauer (2003) suggested a transport mechanism based on a hydration gradient (i.e., the water activity gradient similar to the occlusive effect found in classical dermoformulations) to explain both dermal penetration and the liposomal movement along the SC pores.

Along with size, the number of phospholipid bilayers (lamellae) is an important parameter, as it influences the encapsulation efficacy of drugs in the liposomes and also controls the drug release (Joo et al., 2013).

The lipid composition of liposomes is also an important factor affecting their performance, as it influences their physicochemical properties such as particle size distribution, surface charge, lamellarity, elasticity (i.e., "fluidity") and, ultimately, stability and drug encapsulation/release. For example, unsaturated phosphatidylcholines from natural sources, such as egg or soybean, provide more fluid and thus more permeable and less stable bilayer structures, while the saturated phospholipids with long acyl chains (e.g., DPPC) result in less fluid and relatively impermeable bilayers (Akbarzadeh et al., 2013). Besides carefully choosing the main phospholipidic components, the presence of some additives (e.g., cholesterol) can influence the membrane permeability of lipid vesicles and their stability (Bozzuto and Molinari, 2015, Gill et al., 2013). For example, the reduction of cholesterol content in liposomal formulation can increase the fluidity of the bilayers, resulting in an increase of bioactive transport through the SC (Murthy and Shivakumar, 2010). Alternatively to phospholipids, vesicular bilayers (termed as niosomes) can also be prepared using different compositions, consisting of a mixture of cholesterol and non-ionic surfactants, such as alkyl ethers, alkyl esters, or alkyl amides (Abdelkader et al., 2014). These surfactants do not suffer from the same drawbacks of phospholipids used to prepare liposomes, which have

variable purity grades. Therefore, from a technological perspective, niosomes are hopeful drug carriers that have additional advantages in comparison to liposomes, such as easier large scale production and lower costs (Gan et al., 2013; Mujoriya et al., 2011).

Apart from composition, surface charge is another important factor affecting physical stability and physiological performance of liposomes (e.g., distribution and interaction with membranes) (Días-Torres, 2010). Liposomes can be surface-charged as neutral, negative or positive, in accordance with the functional groups attached, and medium pH. Charged liposomes have superior stability and less aggregation problems because of the repulsion forces that maintain particles of the same charge apart (Días-Torres, 2010). The surface charge of the liposomes also determines penetration properties (i.e., percutaneous absorption and distribution) of lipid vesicles (Gonzalez-Rodriguez and Rabasco, 2011). Ogiso and collaborators performed histological studies and demonstrated that negatively charged liposomes diffused much faster to the dermis and lower portion of hair follicles across SC compared to the positively charged vesicles (Ogiso et al., 2001). Along with surface charge tuning, liposomes' surface coating with mucoadhesive polymers (i.e., Carbopol, chitosan and pectin) can prolong their residence time in the local of application, enhancing their permeability (Vanic et al., 2013; Joraholmen et al., 2014).

Among other drug carriers, liposomes have seen a great deal of development when considering the vaginal delivery of biopharmaceuticals. However, regarding the penetration in mucosae, the mucoadhesive properties can hinder the penetration of particles in the mucus. In this case, a muco-inert coating and a negative surface charge is preferable to ensure that the liposomes are not retained in the mucus (Sarmento and das Neves, 2014).

Another approach to improve the topical application of liposomal formulations is elasticity enhancement. The aim is to use flexible liposomes, i.e., transformable liposomes or transfersomes, for their ability to penetrate through the skin/mucosa. Transfersomes are liposomes that possess in their composition an edge activator, i.e., a component that destabilizes the liposomal bilayers and increases their deformability (Murthy and Shivakumar, 2010). In this way, transfersomes are able to deform and flow between corneocytes through the intercellular spaces, which enhances skin permeation. The edge activator can be a single-chain surfactant (e.g., sodium cholate, sodium deoxycholate, span 60, span 65, span 80, Tween 20, Tween 60, Tween 80, and dipotassium glycyrrhizinate) or alcohol (in this case the transfersomes are called ethosomes).

### 14.2.2 LIPID NANOPARTICLES

Lipid nanoparticles were invented in the early 1990s and patented by two independent groups: J.S. Lucks and R.H. Muller (Germany) (Lucks and Muller, 1993) and M.R. Gasco (Italy) (Gasco, 1993). These patents are differentiated by the

method of production, using the high pressure homogenization (HPH) and microemulsion techniques, respectively. At this time, beyond these two research groups, Prof. Westesen was also working in this topic (Siekmann and Westesen, 1992). Ever since, lipid nanoparticles have been intensively explored by different academics. They are formulated with lipids that are physiologically acceptable and biodegradable and most of excipients possess GRAS status, approved by the regulatory authorities for human use, which lead to low or non-toxicity (Muller et al., 2011, 2002b; Silva et al., 2012d).

Lipid nanoparticle formulations contain solid lipophilic nanoparticles dispersed in an aqueous medium and physically stabilized with an emulsifying layer using suitable surfactant agents. The original concept of lipid nanoparticle dispersions derived from the oil-in-water nanoemulsions, where the inner liquid lipid(s) of the oil droplets (e.g., fatty vegetable oils or middle chain triacylglycerols) are replaced by lipid(s) that present a solid state at both room and body temperatures. Due to this, lipid nanoparticles continue in a solid state after administration to the body. The drug is dissolved or dispersed in the high-melting fat solid matrix. The lipid phase corresponds to between 0.1% and 30% of the total formulation. Therefore, some of the failures of nanoemulsions as drug delivery systems, such as burst and immediate drug release due to the liquid nature of the oil droplets, physical instability, and lack of protection of labile molecules, could be overcome by using lipid nanoparticles due to the solid nature of the matrix (Silva et al., 2012d). Identical to polymeric nanoparticles, lipid nanoparticles have therefore been effective in modulating the release of encapsulated drugs (Vaghasiya et al., 2013; Mendes et al., 2013) and protecting the labile active compounds against chemical degradations (Niculae et al., 2014; Ravanfar et al., 2016). Although the polymeric nanoparticles have a solid matrix identical to lipid nanoparticles that can control drug release, the production process of lipid nanoparticles can be carried out as an organic solvent–free process, contrary to what generally happens in the preparation of polymeric nanoparticles and avoiding the potential toxicity derived from residues that remain in the final formulation. In comparison to liposomes and polymeric nanoparticles, lipid nanoparticles also have lower cost of excipients and of line production equipment; chemical-stability without the use of lyophilization or spray-drying processes; and the production methods can be easily transferred to industrial-scale production with good reproducibility, a prerequisite for system to be introduced to the market (Muller et al., 2002b; Mehnert and Mader, 2001).

The literature describes various methods, or combinations thereof, to manufacture lipid nanoparticles. Generally, techniques use to achieve the nanometer range particle size are based on two different approaches (Mader, 2014): (1) dispersion techniques (top-down processes) such as HPH, ultrasound, high shear rotor-stator in which high shear force are used. In most cases, the dispersion steps are processed with molten lipids at higher temperatures; and (2) bottom-up techniques such as microemulsion or supercritical fluids methods. In most cases, these

techniques require none or only moderate shear stress and the lipid nanoparticle dispersion is produced from a solution. By changing the environment (e.g., by addition of the aqueous phase, decrease temperature, or pressure change) a decrease of the solubility capacity occurs and lipid nanoparticles are formed. In general, the last approach provides lower lipid nanoparticles concentrations, needs additional purification steps, and requires an increase of processing time.

Lipid nanoparticles have colloidal size, generally ranging from 150 to 300 nm, even though smaller sizes (e.g., $<$100 nm) or larger sizes to (up to 1000 nm) can be achieved (Muller et al., 2011). As in other lipid colloidal carriers, they present a high surface area and a superior ability to penetrate biological membranes, which increases drug bioavailability.

In accordance with composition and inner structure of the lipid matrix, lipid nanoparticles can be divided into two types: solid lipid nanoparticles (SLN) and the nanostructured lipid carriers (NLC). In order to explore the differences between the lipid nanoparticles, Muller and collaborators usually compare their inner structures to a perfectly organized "symmetric brick wall" (in the case of SLN) and a disordered "Welsh natural stone wall" (in the case of NLC) (Fig. 14.5C and D, respectively) (Muller et al., 2011).

SLN correspond to the first generation of lipid nanoparticles and the solid matrix is formed by a solid lipid at room temperature as well as body temperature (Muller et al., 2011; Lucks and Muller, 1993). They are generally composed of 0.1%−30% (w/w) solid lipid phase dispersed in an aqueous solution of 0.5%−5% (w/w) surfactant used as physical stabilizing agent (Pardeike et al., 2009). Due to the crystalline structure of solid lipid, polymorphic lipid transitions may take place (Muller et al., 2002,a,b). Soon after SLN production, the solid lipid recrystallizes in high-energetic lipid modifications, i.e., $\alpha$ and $\beta'$ forms. These are unstable forms, which correspond to low-ordered lipid modification of the particle matrix. Over time, thermodynamically less stable forms tend to transit to more stable polymorphic forms, i.e., $\beta$i and $\beta$ modifications, and acquire a perfect crystalline structure with few imperfections that allow little space to host drug molecules (Das and Chaudhury, 2011; Das et al., 2012). As a consequence, the drug can be expelled from the SLN matrix, especially when highly purified solid lipids are used (Muller et al., 2011). Therefore, the complexity of the physical state of the lipid components contributes to the limited drug-loading capacities of SLNs that may not be able to reach therapeutic drug levels. Moreover, this limited drug-loading capacity (LC) is responsible for a change in drug release profile during storage time, which, in turn, contributes to the poor long-term physical stability of SLN systems (Muller et al., 2002b, 2011).

The aforementioned SLN limitations boosted the development of the second generation of lipid nanoparticles, termed as NLCs, in 1999 (Müller et al., 1999). The lipid phase of NLCs is composed of a mixture of a spatially different lipid molecules, corresponding to a blend of a solid lipid and a liquid lipid (e.g., oil), in a proportion that generally varies between 70:30 and 90:10. The addition of a

liquid lipid leads to a crystal structure with more imperfections in the NLC matrix, which is still solid at both room and body temperatures. The presence of oil prevents the perfect recrystallization of the solid lipid (Muller et al., 2011). The less-ordered inner structure of the NLC matrix allows for: (1) high drug-LC; (2) reduced drug expulsion during storage because the solid lipid in an NLC matrix remains in unstable polymorphic form during longer time periods; (3) modulation of the drug release profile by varying the lipid matrix composition (Das et al., 2012; Silva et al., 2015; Muller et al., 2002a; Mehnert and Mader, 2001). Despite the reported advantages, the superiority of NLCs over SLNs has not been fully established and SLN dispersions have been demonstrated to be also promising drug delivery carriers, presenting good results in terms of long-term stability and high drug-loading efficiency (Kovacevic et al., 2011; Das et al., 2012; Tiwari and Pathak, 2011; Silva et al., 2012b).

Regarding the composition of lipid nanoparticles, the most common solid lipids used are categorized into different classes (Table 14.3). As referred previously, most of these lipids, with the notable exception of cetylpalmitate, are approved as GRAS and are physiologically well tolerated (Shah et al., 2015). In terms of liquid lipids, Miglyol 812 (medium chain triglycerides of caprylic and capric acids) is the most used, even though others (e.g., oleic acid, squalene, Cetiol V) have also been employed.

The selection of an adequate lipid phase is based on its ability to solubilize the drug (Bummer, 2004), which influences the drug EE and loading capacities. As previously mentioned, a combination of lipids with different structures enables a greater load capacity, due to the imperfections that arise in the solid lipid matrix. Nevertheless, a mixture of chemically very different lipids can result in the formation of the so-called supercooled melts, i.e., during lipid nanoparticle production, the emulsion lipid droplets remain in the liquid state and a nanoemulsion is obtained instead of lipid nanoparticles dispersion (Souto et al., 2007). This phenomenon occurs if the critical crystallization temperature is not reached during the cooled step of lipid nanoparticles preparation method (see Section 2.2). The critical crystallization point is much lower than the melting point of the lipid. The critical crystallization temperature depends on the nature of the solid lipid (Heike, 2011). Some lipids, such as trilaurin, tricaprine, and some Witepsol bases, tend to form supercooled melts and for that reason it is not advisable their use in the preparation of SLNs and NLCs if controlled drug release is desired.

The composition of lipids can also influence the physicochemical properties of lipid nanoparticles, such as the size, ZP, and stability of the formulation during storage (Shah et al., 2014). For example, Rai et al. (2008) reported that lipids with superior melting points may lead to an increase in SLN particle size, lipids with shorter fatty acid chains, and considerable amounts of mono and diglyceride-formed SLNs with smaller particle size.

As mentioned earlier, for physical stabilization of the water-lipid interface of the dispersions 0.5%–5% surfactant (i.e., emulsifier agent) is added, depending

**Table 14.3** Typical Lipids Used to Prepare Lipid Nanoparticles

| Solid Lipids | Examples | Melting Point/ Temperature Range (°C) |
|---|---|---|
| Pure triglycerides fats (synthetic monoacid triglycerides) | Triestearin (Dynasan 118) | 70–74 |
| | Tripalmitin (Dynasan 116) | 61–65 |
| | Trimyristin (Dynasan 114) | 55–58 |
| | Trilaurin (Dynasan 112) | ≈45 |
| | Tricaprin | ≈33 |
| Hard fats | Glyceryl monostearate (Imwitor 900) | 54–64 |
| | Glyceryl palmitostearate (Precirol ATO 5) | 52–55 |
| | Glyceryl behenate (Compritol 888 ATO) | 65–77 |
| Fatty acids | Stearic acid | 67–72 |
| | Palmitic acid | 61–64 |
| | Behenic acid | 74–78 |
| Waxes | Celtyl palmitate | 43–53 |
| | Carnauba wax | 78–85 |
| | Beeswax | 62–65 |
| | Emulsifying wax (cetylstearylalcohol and polysorbate 60 4:1, w/w) | 50–55 |
| Mixtures of glycerides | Witepsol E 85 | 42–44 |
| | Witepsol W35 | 33–35 |
| | Witepsol H35 | 33–35 |
| | Witepsol H42 | 41–43 |
| | Softisan 154 | 55–60 |
| | Softisan 100 | ≈35 |
| | Softisan 142 | ≈43 |
| | Soya bean oil | – |
| | Oleic acid | – |
| | Medium chain triglycerides (MCT)/caprylic- and capric triglycerides | – |
| | α-Tocopherol/vitamin E | – |
| | Squalene | – |
| | Hydroxyoctacosanylhydroxystea rate | – |
| | Isopropyl myristate | – |

*Adapted from Mehnert and Mader, (2001); Silva et al., (2012a,b,c,d, 2015); Shah et al., (2015); Mader, (2014).*

on the type and concentration of the lipid phase and the administration route (Mehnert and Mader, 2001). Surfactants can be broadly categorized into three classes, based on their charge: ionic (e.g., sodium cholate, sodium glycocholate, sodium taurocholate), non-ionic (e.g., polysorbate 80, poloxamer 188 and 407, tyloxapol, Span 20, 80 and 85, Brij 78, Tego care 450), and amphoteric (e.g., egg lecithin, soybean lecithin, and phosphatidylcholine) (Mehnert and Mader, 2001). Non-ionic surfactants are less toxic and exhibit less irritation than ionic surfactants (McClements and Rao, 2011). Among the ionic surfactants, cationic surfactants are more toxic than anionic or amphoteric surfactants. The presence of surfactant agents promotes a physical stabilization of the colloidal system, preventing the aggregation of nanoparticles by electrostatic or steric stabilization. Ionic surfactants interfere in the electrostatic stability, whereas non-ionic surfactants produce steric repulsion stability (Shah et al., 2015). However, in reality several non-ionic surfactants used are too small to infer authentic steric stability, and they likely stabilize the colloidal dispersion by the Gibbs-Marangoni effect (Walstra, 1993). In general, low concentrations of surfactants increase the particle size during storage. Despite high concentrations of surfactant reducing surface tension and facilitating particle partitioning during the homogenization step (promoting the reduction of size) (Sinha et al., 2011), they may attribute some toxicity to the lipid nanoparticles. In many cases, the combination of surfactants in the formulation more efficiently prevents the aggregation of the nanoparticles, giving them greater stability and dispersed product (Shah et al., 2014; Mader, 2014).

Some parameters affect the models of drug incorporation and drug release from lipid nanoparticle dispersion, such as the drug partition coefficient (Log *P*) of the drug between the lipid and the aqueous phases, the type of lipid(s) and surfactant(s), and the production methods (Silva et al., 2015). In both lipid nanoparticles, SLNs and NLCs, there are three structural models that describe the drug incorporation into these colloidal carriers (Muller et al., 2002a, 2000). In various situations, and, depending on the partition coefficient, a combination of the three types of systems is obtained. In the case of SLNs, these models are (Fig. 14.6A) (Muller et al., 2000, 2002b): (1) homogenous matrix model, in which drug is molecularly dispersed homogeneously or forms amorphous clusters within the lipid matrix that prolong the drug release; (2) drug-enriched shell model, in which the drug is concentrated on the outer shell of the lipid nanoparticles and is indicated to encapsulate drug that required fast initial drug release (i.e., burst effect); (3) drug-enriched core model, in which drug core is surrounded by a lipid layer or reservoir-type system that forms a membrane-controlled release, governed by Fick's law of diffusion, that prolongs the drug release.

NLCs also possess three different morphologies, based on the location of incorporated drug (Fig. 14.6B): (1) imperfect crystal type, which is reached by mixing solid lipids with small amounts of chemically very different liquid lipids. An imperfectly/disordered structured solid matrix is obtained that accommodates high drug molecules either in molecular form or as amorphous clusters; (2) multiple

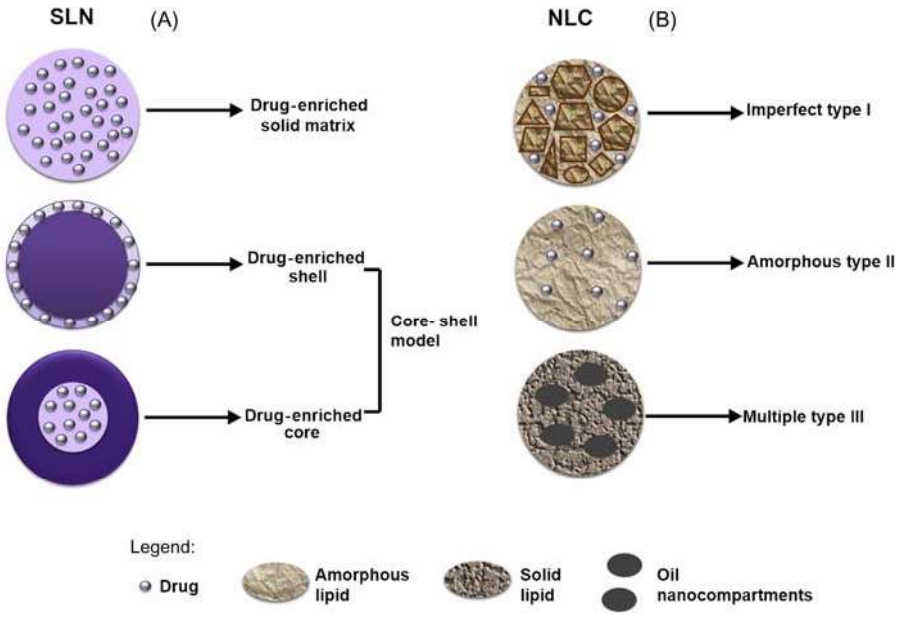



**FIGURE 14.6**

Schematic representation of: (A) Models of drug incorporation into SLN: solid homogeneous matrix enriched with drug (upper), drug-free core with drug-enriched shell (middle), and drug-enriched core with lipid shell (lower); (B) types of NLC: (I) imperfect type, (II) amorphous type, and (III) multiple type.

*Adapted from Uner and Yener (2007).*

type, also called oil-in-lipid-in-water type, in which the solubility of liquid lipid in the solid lipid is exceeded; phase separation and formation of tiny oily nanocompartments within the solid liquid matrix occurs. Drug can be distributed in these oily nanocompartments, being protected from degradation by the surrounding solid lipids and determining a controlled drug release; and (3) amorphous type, which is achieved by mixing solid lipids with special lipids (such as hydroxyoctacosanylhydroxystea rate, isopropyl palmitate, or medium chain triglycerides) to prevent the drug being expelled during the phenomenon of crystallization by maintaining the polymorphism of the lipid matrix, maintaining the lipid core in an amorphous nature.

The unmeasured number of research papers published proved that the topical application of lipid nanoparticles to skin and mucosa has received increasing attention. This fact could be explained by the properties of lipid nanoparticles, such as adhesiveness and stickiness to the lipid film of the SC, or to lipid of the mucosa surface (Muller et al., 2011) as result of hydrophobic interactions, which prolong the residence time at the application site. After topical application and due to the

interactions between the lipids, lipid nanoparticles tend to lose their structure and melt, favoring the skin penetration process (Junyaprasert et al., 2009).

In dermal topical application, the absorption of lipid nanoparticles in the lipid film of the skin allows reinforcing of the protective film and increasing skin hydration through occlusive properties, which are the function of nanoparticle size (Wissing and Muller, 2003). These colloidal systems may also influence skin's permeability, once they cause skin structure disruption (Souto et al., 2007). In the case of mucosal application, the lipid composition of the lipid nanoparticles influences their adhesiveness and residence time to the mucosa. There is some controversy about the utility of muchoadhesiveness. The muchoadhesiveness and residence time in mucosa can indeed be enhanced by using positively charged lipid nanoparticles that adhere stronger to the negatively charged mucosa (Silva et al., 2012d). However, if the goal is that lipid nanoparticles do not get trapped in the mucus but are able to cross it to the epithelium, then, as already explained for liposomes, their charge should be negative and their surface should be coated by a muco-inhert polymer (Sarmento and das Neves, 2014)

#### 14.2.2.1 Lipid-core micelles

Micelles are aggregates of amphiphilic molecules or surfactants, which contain a hydrophilic head-group and a hydrophobic tail, are spontaneously self-assembled when immersed in an aqueous media, and their content is above their critical micelle concentration (CMC). Despite concentration, aggregation occurs under certain temperatures, called the critical micellization temperature (Mittal, 1991), resulting from the orientation of hydrophobic fragments towards themselves into a state of minimum energy that minimizes the exposure of hydrophobic domains to the aqueous environments. Van der Waals forces are established among hydrophobic fragments to form the micelle core (Torchilin, 2005; Martin, 1993). The micelles are thermodynamically stable and typically have a shape close to spherical; the usual size for pharmaceutical purposes is within a range of 10–80 nm (Torchilin, 2007). The size of micelles could depend on the solubilization of drugs, i.e., extensive solubilization may augment the micelle size. Their hydrophobic core facilitates the encapsulation/solubilization of hydrophobic drugs through hydrogen bonding or hydrophobic interactions. A common protocol to prepare drug-loaded lipid-core micelles is the solvent evaporation technique, in which drugs and hydrophilic copolymers-lipid (e.g., polyethylenoglycol (PEG)-DSPE) are first dissolved in a miscible volatile organic solvent. Subsequently, the solvent is evaporated, resulting in the formation of a dry transparent polymer-lipid/drug film, which is then hydrated with an aqueous buffer solution and forms micelles spontaneously upon intensive shaking. The amount of drug that exceeds the solubilization capacity of the micelles precipitates in a crystalline form, which can be removed by filtration (Torchilin, 2005).

Micelles reveal attractive properties that can be applied when used as drug carriers for topical applications. Those include: solubilization of poorly water-soluble drugs consequently improving their bioavailability; loading with as much

as 5–25 wt% of a drug; reduction of the toxicity of many drugs; drug permeation enhancement across the physiological barriers; triggering drug release (by stimuli-responsive micelles made with amphiphilic block-copolymers micelles); and protection of labile drug from possible inactivation (for example, the hydrophilic hydrated layer created by PEG molecules in the PEG 750-dipalmitoyl phosphatidylethanolamine micelles notably slowed the degradation of all-trans retinoic acid by atmospheric oxygen (Wichit et al., 2012)) (Torchilin, 2005; Lim et al., 2012).

Lipid micelles are nanostructures composed by lipid monolayers. The hydrophobic micelle core is formed by phospholipid residue that is very hydrophobic, due to the presence of two long-chain fatty acyls that afford considerable stability to the micelle (Torchilin, 2005) and can solubilize hydrophobic compounds. The polar head groups make up the outer hydrophilic corona (Lim et al., 2012). Water-soluble copolymers (e.g., PEG and polyvinylpyrrolidone (PVP)) can be conjugated with phospholipids or lysolipids (e.g., PEG-phosphatidyl ethanolamine (DSPE)) (Fig. 14.5B). The attached hydrophilic copolymers (e.g., PEG chains) stabilize the colloidal carrier by a steric effect phenomenon, preventing opsonization process and their uptake by the reticular endothelial system in biological media (Webster et al., 2007). Despite other hydrophilic polymers being used, PEG is the most popular for composing the corona of therapeutic micelles, due to advantages such as: low price, low toxicity, efficient steric protection, and being approved for internal applications by regulatory agencies (Torchilin, 2005). The composition and sizes of both micelle core- and corona-forming groups can affect the size of micelle, LC, drug release profile, and biodistribution (Gill et al., 2014). For example, PEG-DSPE conjugates form micelles with a spherical shape, uniform size distribution, and very low CMC values as a consequence of the strong hydrophobic interactions between the double acyl chains of the phospholipids residues. Additionally, PEG-DSPE-based micelles prepared with an increase in PEG chain length resulted in an increase in size of micelles (Ashok et al., 2004).

Micelles can use pore route penetration pathways and consequently improve drug bioavailability in cutaneous application (Bachhav et al., 2011). Additionally, in PEG-DSPE micelles, both PEG and DSPE are chemical promoter agents that can enhance percutaneous permeation/absorption of the drug (Asbill and Michniak, 2000). Taking into consideration their phospholipid nature, the DSPE molecules disturb the lipid bilayer structure of the SC, resulting in the increase partitioning of drug into the skin's layers (Kirjavainen et al., 1999). The PEG molecules can decrease the surface tension of the SC, which results in enhancement of drug diffusion after cutaneous application.

### 14.2.3 CHARACTERIZATION METHODS

Several parameters should be considered for good characterization of lipid colloidal carriers, such as organoleptic characteristics, mean size, ZP, EE, degree of lipid crystalline, and lipid modification.

The mean particle size and size distribution, known as polydispersity index (PI), i.e., variation of particle size around the mean diameter, of colloidal systems are crucial parameters for both physical stability and biopharmaceutical behavior. Although different techniques can be applied for measuring the mean particles size in colloidal dispersions, photon correlation spectroscopy (PCS), also called dynamic light scattering, and laser diffraction (LD) are the most widely used (Mehnert and Mader, 2001). These techniques measure the mean value of particle size regarding the population and not individual size.

The PCS technique provides absolute and reproducible results, measuring the fluctuations in intensity of scattering as a result of the Brownian motion, i.e., the movement of the particles due to the random collision with the molecules of the dispersion medium (Malvern, 2012a). This technique is based on the fact that the particles suspended in a liquid medium are in constant motion. The intensity of the scattered light varies with the particle size as well as the speed of the Brownian motions, whereas the smaller particles disperse less light and diffuse relatively faster than the larger particles. Therefore, the intensity of light scattering is a function of the particle size and the velocity of Brownian motion. The equation of Stokes–Einstein (Eq. (14.1)) describes the relation between hydrodynamic diameter of a spherical particle and the speed with which it moves (Finsy and De Jaeger, 1991):

$$d(H) = KT3\pi\eta D \tag{14.1}$$

where $d(H)$ is the hydrodynamic diameter, $D$ is translational diffusion coefficient which measures the velocity of the Brownian motion, $k$ is the Boltzmann's constant, $T$ is the absolute temperature and $\eta$ is the viscosity of the solution.

PCS is a sensitive, simple, and non-destructive technique. This technique allows measuring in a mean particle size range that varies between a few nanometers and approximately 3 μm, i.e., it is suitable to characterize lipid colloidal carriers. Larger sizes can be measured by LD, since this technique covers a range of sizes from a few nanometers ($\approx$20 nm) to millimeters (Malvern, 2017). The measurement using LD is based on the proportionality between the diffraction angle and the particle radius, according to the Fraunhofer diffraction theory. Smaller particles create more intense scattering at high angles, compared to the larger ones (Mehnert and Mader, 2001). Some authors recommend the use of PCS and LD combined, since the LD technique can detect non-measurable microparticles with the PCS technique (Müller-Goymann, 2004).

The PCS technique can also be used to assess the PI which provides information on the homogeneity of the size particle distribution in a dispersion medium. The monodisperse formulations present lower PI values, i.e., dispersion is more monodisperse if the PI value is closer to zero. Values of PI around 0.1–0.3 represent a relatively narrow size distribution and values equal or higher than 0.5 indicate broad distributions (Liu and Wu, 2010).

The ZP is an indirect measurement of the surface charge of the particles in a dispersion medium. The particles dispersed in an aqueous system acquire surface

charge, mainly due to the presence of ionizable groups or the adsorption of ionic species, affecting the distribution of ions in the surrounding interfacial region and increasing the concentration of the counter ions close to the surface. The magnitude of the ZP is related to the degree of repulsion/attraction between the adjacent particles and is thus a useful indicator of the long-term physical stability indicators of colloidal dispersion (Lage et al., 2012). Generally, it is accepted that ZP values, in absolute terms, higher than 30 mV are ideal for full electrostatic stabilization and have lower tendency for aggregation during storage due to the electric repulsion (Malvern, 2012b). If, however, the particles have low ZP values, the attractive forces exceed the repulsive forces and the particles tend to coagulation or flocculation resulting in poor physical stability of the dispersion (Das and Chaudhury, 2011). Nevertheless, this conclusion is not true for colloidal carriers that contain steric stabilizers, as the adsorption of these compounds will decrease the ZP value, due to the shift in the shear plane of the particle (Muller, 1996).

The degree of lipid crystallinity and lipid polymorphic changes are important parameters to be attended to, since these parameters influence the drug encapsulation capability, the drug release profile, and the long-term stability of the colloidal system (Mehnert and Mader, 2001). Despite various analytical techniques being available, the lipid crystallinity and polymorphic forms are mainly assessed using differential scanning calorimetry (DSC) and X-ray scattering techniques. DSC is a thermal analytical technique that provides information about the melting and crystallization behavior of the solid and liquid constituents of the particles, based on the fact that the structural changes of the lipids have different melting range temperatures and enthalpies. Variations of enthalpy create endothermic or exothermic signals depending on the transition consume energy (e.g., recrystallization) (Müller-Goymann, 2004). The DSC technique consists of submitting the sample to controlled temperature programs, during which thermal transitions can be measured. However, the explanation of experimental results is not always simple. For example, contrary to what is often reported in the literature, an absence of drug melting peak does not confirm molecular dispersion or drug incorporation in the colloidal carriers (Mader, 2014). This technique does not also allow for the identification of the cause of thermal transitions and should be complemented with other methods, such as X-ray diffraction, that allows for the study of molecular structure and discriminates between fusion, polymorphic transitions, water loss, or decomposition of the substance (Bunjes and Unruh, 2007).

Nuclear magnetic resonance and electron spin resonance are sensitive methods which can be employed to distinguish different colloidal species, such as micelles, lipid nanoparticles, liposomes, supercooled melts, and non-encapsulated drug nanometer size ranges that can be formed during the production process of aqueous dispersions. These techniques are useful to investigate the dynamic phenomena and the characteristics of lipid nanocompartments (Mehnert and Mader, 2001).

Microscopic techniques, such as scanning electron microscopy (SEM) and transmission electron microscopy (TEM), atomic force microscopy (AFM),

CryoSEM, and CryoTEM produce images with high resolution and allow measurement of particles smaller than 1 μm (Mehnert and Mader, 2001). These techniques are useful for morphology characterization of colloidal systems.

Electron microscopy allows for visualizing the microstructure. This technique is based on the interaction between electrons and the components of the sample generating several measurable signals, i.e., the electrons can be transmitted, backscattered or diffracted. Both SEM and TEM are useful, since they allow the observation of details, such as shape and surface characteristics.

AFM generates a three-dimensional surface profile and provides information about the surface, including structural, mechanical, functional, and topographic (Das and Chaudhury, 2011) information. It uses the force between a surface and a probing tip, resulting in a spatial resolution of up to 0.01 nm for imaging. The atomic force microscope obtains images quickly enough (about 20 s per image) to allow the observation of in situ processes occurring at interfaces (Mehnert and Mader, 2001). The size of the visualized particles is of the same magnitude compared with the results of PCS measurements.

The determination of drug encapsulation parameters in lipid colloidal carrier, namely EE and LC, is often assessed indirectly, i.e., measuring the amount of drug not incorporated in the aqueous phase using a pre-validated analytical method. Methods such as ultrafiltration and ultracentrifugation are used to separate the lipid colloidal carriers from the aqueous phase. These parameters are determined using the following equations:

$$\text{EE}\ (\%) = 100 \times \frac{\text{total amount of drug} - \text{amount of free drug}}{\text{total amount of drug}} \tag{14.2}$$

$$\text{LC}\ (\%) = 100 \times \frac{\text{total amount of drug} - \text{amount of free drug}}{\text{total amount of lipid}} \tag{14.3}$$

EE defines the ratio between the weight of drug entrapped and the total weight of drug added to the dispersion. LC expresses the ratio between the drug entrapped and the total weight of the lipids. Such parameters are indicative of the production effectiveness. High EE and LC values reduce the amount of formulation necessary to achieve therapeutic levels, which leads to a reduction of the drug used in each administration. Alternatively, EE can be determined using a direct method, i.e., measuring the drug encapsulated into the colloidal carriers, using a solvent that can dissolve both nanocarrier and drug.

Regarding the lipid matrix, a drug-controlled release profile is expected from lipid colloidal dispersions. Generally, the drug release profile is evaluated by placing lipid colloidal dispersions into a dialysis bag, which is then immersed into the dissolution medium and incubated in a shaker. Sink conditions should be maintained throughout the experiment. The drug release data interpretation could be harmonized using mathematical equations, which fit the results under kinetic models that predict the drug release mechanism (Silva et al., 2012c).

Stability studies should be performed in accordance with the International Conference on Harmonization (ICH) Guidelines (ICH, 2003). The sample is stored at various temperature and humidity conditions from a specific period. Drug content and variation in the average particles should be periodically monitored.

## 14.3 INCORPORATION OF LIPID COLLOIDAL CARRIERS IN SEMISOLID FORMULATIONS FOR TOPICAL APPLICATION

The typical low viscosity of lipid colloidal dispersions is a restrictive factor for topical application, since it affects the residence time and consequently compromises the desired therapeutic effect. Therefore, in most cases, their incorporation into commonly used semisolid formulations (e.g., creams, ointments, and gels) represents a requirement to obtain an appropriate consistency product for topical application. This procedure can also improve long-term stability once the colloidal carriers are entrapped in the three-dimensional systems (Pardeike et al., 2009). Therefore, the association of a well established topical formulation with the special characteristics of lipid colloidal carriers in the finished product seems to be a good option for therapeutic efficacy.

The preparation of semisolid formulations containing lipid nanocarriers dispersion can be performed using different approaches (Souto et al., 2007; Wissing and Muller, 2003; Muller et al., 2002b): (1) incorporating colloidal carriers into conventional semisolid formulations (e.g., hydrogels, o/w cream); (2) adding viscosity promoters (i.e., gelling agents) to the aqueous phase of the lipid dispersion; or (3) developing in one step a final product with a high concentration of lipid colloidal dispersion with a desired viscosity.

For preparation of o/w creams containing lipid colloidal dispersions, it is recommended to add highly lipid-concentrated dispersions (e.g., 50% solid content in the case of lipid nanoparticles) in order to overcome eventually problems related with the LC (Souto et al., 2007). This incorporation can be performed either in the course of production or after the cream is produced. In the first case, part of the water in the formulation cream is substituted by the aqueous dispersion of lipid colloidal carriers, followed by the usual production process. In the second situation, the inclusion of lipid colloidal dispersion is carried out in a previously prepared cream that presents reduced water content by stirring at room temperature (Souto et al., 2007). The temperature control is very important to avoid phenomena that disrupt the internal structure of the nanocarriers, which plays a crucial role in the drug release control as well as in the protection of the encapsulated compounds. For example, in the case of lipid nanoparticles, the temperature should avoid the melting of solid nanoparticles.

The incorporation of lipid colloidal carriers into hydrogels represents the most frequently used approach by various research groups (Gupta et al., 2010; Mendes et al., 2013; Tichota et al., 2014; Gong et al., 2013). The inclusion can be made into a pre-prepared hydrogel by a mechanical stirrer and, subsequently, a dilution of the lipid nanocarriers occurs. Alternatively, a gelling agent (e.g., Carbopol, xanthan gum, chitosan) may be added directly into the water-phase of the lipid colloidal dispersion. This last procedure has the advantage of preparing a more concentrated formulation of lipid colloidal carriers. The addition of certain electrolytes, such as sodium or potassium hydroxide, reduces the surface electric charge of the particles (i.e., the ZP), leading to their destabilization and aggregation. Therefore, in pH-dependent gelation processes, it is advisable to use other types of neutralizing agents, such as triethanolamine, Tristan (tromethamine), or Neutrol TE (*N*,*N*,*N*,*N*,-tetra(2-hydroxypropyl)ethylenediamine) (Souto et al., 2007). After preparation of the hydrogel, the aggregation of nano-sized particles is not a problem, since the lipid colloidal carriers are entrapped in the three-dimensional network structure of the hydrogel and become physically stable (Souto et al., 2004).

The drug release from lipid colloidal carriers incorporated into semisolid formulations can be controlled either by the lipid matrix and semisolid tridimensional structure (Wissing and Muller, 2003; Mendes et al., 2013).

Additional characterization methods should be performed in semisolid formulations containing the lipid colloidal dispersion, such as: organoleptic characterization, pH determination, texture analysis, rheological behavior, permeation studies, drug product release, and stability specifications (Tichota et al., 2014; Chang et al., 2013).

The pH determination of topical formulations is extremely important, since it must be compatible with the natural pH of the application site, which is different in skin and mucosa (e.g., normal vaginal pH is 4−5; natural skin surface pH has an average of below 5). In hydrophilic formulations, the analysis of pH can be performed directly in formulation using a pH electrode.

The mechanical properties of a topical formulation include a variety of parameters, such as viscoelastic properties, adhesiveness, firmness, and consistency. These parameters influence the manufacturing processes and might also affect the drug release and subsequent the percutaneous absorption (i.e., drug bioavailability) (Barry, 1983). For characterization of mechanical properties, rheological behavior and texture analyses can be assessed.

Texture analysis is based on the penetration of a probe into the sample at predefined conditions, i.e., depth, force, and velocity; and provides information related to the adhesiveness (bioadhesion), and the firmness of semisolid formulation for topical application. Both parameters depend on the viscosity of the formulation (Jones et al., 1997; Silva et al., 2012a). Adhesiveness measures the force required to overcome the attractive forces between the surfaces of the sample and the probe, when both come into contact. Generally, the topical semisolid

formulation should have high bioadhesiveness, which is desirable to keep the formulation in the locality of application and prolong the contact residence. The firmness of the formulation determines the facility of applying the product. Suitable values of these parameters were achieved in hydrogels containing lipid nanoparticles (Gonzalez-Mira et al., 2012; Silva et al., 2012a).

Generally, the incorporation of aqueous SLNs and lipid colloidal dispersions into semisolid formulations affects their rheological behavior, i.e., flow and deformation properties. The characterization of viscous and elastic behavior, as well as microstructure of the topical formulation, can be performed using flow viscometry, oscillatory rheometry, and transient measurements (Uchechi et al., 2014). The plots of shear stress versus shear rate (rheograms) and some mathematical models, such as power law (Ostwald-de Waele) and power law with a yield stress (Herschel-Bulkley), enable an inferring about the flow behavior (Moreno, 2001; Marcotte et al., 2001). In terms of flow properties, semisolid dosage forms usually display non-Newtonian flow behavior, where the shear stress and shear rate are independent, so the viscosity is not constant. The non-Newtonian systems can be classified into plastic (the formulation starts flowing after achieving a yield value), pseudoplastic (viscosity decreases with increasing cutting speed) or dilatants (an increase in viscosity occurs with increased cutting speed) (Lee et al., 2009; Alves et al., 2011). In addition, non-Newtonian systems may exhibit thixotropy, i.e., the reversible variation of viscosity with time. The thixotropy phenomenon result from the breaking of the three-dimensional structure of the system, resulted from the increase of the shear rate, which is slowly restored with the decrease of shear rate, due to the movement of particles by Brownian motion (Alves et al., 2011; Lee et al., 2009). The thixotropy is a desired property for topical formulations as it contributes to increasing the retention time of the formulation in the locality of application (Souto et al., 2004; Lippacher et al., 2004). Some research studies demonstrated that hydrogels containing lipid nanoparticles showed a plastic behavior with thixotropy both with SLNs (Silva et al., 2012a) as with NLCs (Mendes et al., 2013).

## 14.4 CURRENT FORMULATIONS OF LIPID COLLOIDAL CARRIERS AND PATENTS FOR TOPICAL APPLICATION OF ANTIVIRAL DRUGS

This section presents the current status in the emerging area of nanocarriers as delivery systems for antiviral therapy, providing a summary of reported studies and highlighting some achievements (Tables 14.4 and 14.5).

Besides the nanocarriers containing antiviral drugs, other strategies are being developed as therapeutic alternatives. In a study carried out by Palliser et al. (2006) seven siRNAs targeting three essential HSV-2 genes (UL5 (a component

**Table 14.4** Liposomes Containing Antihuman Herpes Virus Drugs

| Drug | Composition/Preparation Method | Characterization of the Nanocarriers | | | Experimental Model (Route of Administration) | Main Achievements | Reference |
|---|---|---|---|---|---|---|---|
| | | Size (nm) | Zeta Potential (mV) | EE (%) | | | |
| **ACV** | (A) PC:CHOL: (SA:DCP) (1.6:1:0.15) | | | | In vitro: excised cornea of rabbits | • Formulation (A): slow penetration of ACV across the cornea and an increase in the extent of absorption | Law et al. (2000) |
| | (B) PC:CHOL: (DCP:SA) (1.6:1:0.15) | n.i. | − (A) | ≈25 | In vivo: rabbits | | |
| | ACV (1.24 mg/mL) | | + (B) | | Topical administration (instillation on the cornea surface) | • Potential use in the treatment of herpes keratitis | |
| | Lipid film hydration | | | | | | |
| **ACV** | (A) CA:CHOL:CS:PA (4:2.5:2.5:1) | | | | | • All formulations had higher flux and permeability of ACV-palmitate than a common ointment form | Liu et al. (2004) |
| | (B) PC:CHOL:PS (1:0.5:0.1) | (A) 620 | + 85 (A) | | | | |
| | (C) PC:CHOL:SA (1:0.5:0.1) | (B) 640 | + 82 (B) | | | | |
| | (D) PC:CHOL (1:0.5) | (C) 540 | + 90 (C) | n.i. | In vitro: hairless rat skin | • (A) was the most effective in transdermal delivery of ACV-palmitate | |
| | (E) DPPC:CHOL (1:0.5) | (D) 510 | + 58 (D) | | Topical administration | • Presence of CHOL did not reveal difference in transdermal delivery of ACV-palmitate | |
| | (F) PC | (E) 500 | + 50 (E) | | | | |
| | ACV-palmitate (0.5% (w/v)) | (F) 570 | + 49 (F) | | | • (C) had the highest intradermal retention of ACV-palmitate | |
| | Reverse phase evaporation + sonication | | | | | | |
| **ACV** | PC:CHOL:SA (1.6:1.0:0.39) | | | | In vivo: rabbits | • Superior bioavailability of ACV formulation, when compared with the ointment | Chetoni et al. (2004) |
| | ACV (1.24 mg/mL) | 370.9 | + | 22.8 | Topical administration (instillation on the cornea surface) | • Formulation produced a significantly higher $C_{max}$ and $T_{max}$ = 90 min in the aqueous humor | |
| | Lipid film hydration + extrusion | | | | | • Positively charged bind to the corneal surface, leading to an increase residence time favoring the ACV absorption | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **ACV** | (A) PC: CHOL:TB:PG (5:7:1:1) | | | | | | |
| | (B) PC: CHOL:TB:PS (5:7:1:1) | | | | | | |
| | (C) PC: CHOL:TB:PhA (5:7:1:1) | (A) 8200 | | (A) 44.3 | | • Overall, formulations containing PG as negatively charged lipid showed better results | |
| | (D) DMPC: CHOL:TB:PG (5:7:1:1) | (B) 7500 | | (B) 43.2 | | | |
| | (E) DMPC: CHOL:TB:PS (5:7:1:1) | (C) 7200 | | (C) 30.7 | | | |
| | (F) DMPC: CHOL:TB:PhA (5:7:1:1) | (D) 9200 | | (D) 72.2 | | | |
| | (G) DPPC: CHOL:TB:PG (5:7:1:1) | (E) 8100 | | (E) 58.1 | | • The multivesicular delivery system (formulations A to M) as an intradermal depot offers the advantage of a very high loading and controlled release of ACV for an extended period of time | Jain et al. (2005) |
| | (H) DPPC: CHOL:TB:PS (5:7:1:1) | (F) 7600 | | (F) 53.8 | In vivo: rats | | |
| | (I) DPPC: CHOL:TB:PhA (5:7:1:1) | (G) 12,400 | n.i. | (G) 76.6 | Intradermal administration | | |
| | (J) DSPC: CHOL:TB:PG (5:7:1:1) | (H) 12,300 | | (H) 64.2 | | | |
| | (K) DSPC: CHOL:TB:PS (5:7:1:1) | (I) 12,600 | | (I) 62.3 | | • The increase in AUC and decrease in $C_{max}$ reflects that the multivesicular formulations could reduce the toxic complications and limitations of conventional IV and oral therapies | |
| | (L) DSPC: CHOL:TB:PhA (5:7:1:1) | (J) 16,000 | | (J) 82.0 | | | |
| | | (K) 16,800 | | (K) 74.2 | | | |
| | | (L) 14,000 | | (L) 76.3 | | | |
| | (M) PC:CHOL (1:1.5) | (M) 1800 | | (M) 12.8 | | | |
| | ACV (10 mg/mL) | | | | | | |
| | Reverse phase evaporation | | | | | | |
| **ACV** | (A) PC:PG (9:1) | (A) 320 | − 56.2 (A) | 26.84 | In vitro release studies in vaginal fluid simulant | • Negatively and positively charged liposomes yielded similar encapsulation efficiencies and were stable in conditions used to mimic vaginal fluid | Pavelic et al. (2005) |
| | (B) PC | (B) 564 | − 20.4 (B) | 8.09 | | • Positively charged liposomes were the most stable in media with mucin | |
| | (C) PC:SA (9:3) | (C) 385 | + 8.3 (C) | 23.78 | Topical administration to vaginal mucosa | | |
| | ACV (40% (w/v)) | | | | | | |
| | Polyol dilution method + extrusion | | | | | • Even after 24 h of incubation in vaginal fluid simulant more than 35% of the encapsulated ACV was retained in the hydrogel | |

(*Continued*)

**Table 14.4** Liposomes Containing Antihuman Herpes Virus Drugs *Continued*

| Drug | Composition/Preparation Method | Characterization of the Nanocarriers: Size (nm) | Zeta Potential (mV) | EE (%) | Experimental Model (Route of Administration) | Main Achievements | Reference |
|---|---|---|---|---|---|---|---|
| **ACV** | (A) PC + SMP + SA (8.03:1:4.46) | (A) 288 | + 32 (A) | (A) 55 | | • Positively charged liposomes (A,B,C) had a higher ACV entrapment efficiency than negatively charged liposomes | |
| | (B) PC + SMP + ALAB (6.25:1:6.25) | (B) 207 | + 15 (B) | (B) 43 | | | |
| | (C) PC + CHOL + SA (12.5:1:5) | (C) 430 | + 35 (C) | (C) 46 | | | |
| | (D) PC:CHOL (12.5:1) | (D) 339 | − 6 (D) | (D) 30 | In vitro: pig-ear skin | • Elastic positively charged liposomes (A and B) determined a ACV skin flux similar to that of non-elastic positively charged liposomes (C), but higher than that of non-elastic negatively charged liposomes (D) | Peira et al. (2007) |
| | (E) PC + SMP (12.5:1) | (E) 295 | − 13 (E) | (E) 37 | Topical administration | | |
| | ACV-palmitate (4 mg/mL) | | | | | | |
| | Lipid film hydration + sonication | | | | | • Elastic positively charged liposomes (formulation B), was the most efficient improving *in vitro* ACV skin deposition | |
| **ACV** | (A) SPC:SPAN* (95:5) | | | | | • The elastic liposomal formulation for transdermal delivery of ACV provides better transdermal flux, higher entrapment efficiency, ability as a self-penetration enhancer and effectiveness for transdermal delivery as compared with conventional liposomes | |
| | (B) SPC:SPAN* (90:10) | | | | | | |
| | (C) SPC:SPAN* (85:15) | | | | | | |
| | (D) SPC:SPAN* (80:20) | 96–155 | n.i. | 43.1–66.4 | In vitro: hairless rat skin | • In vivo studies showed that on transdermal application of elastic liposomes, the concentration of ACV in plasma was found to be 4.2 times compared with conventional liposomes. Thus it is concluded that the elastic liposomes may be promising vehicles for the transdermal delivery of ACV | Jain et al. (2008) |
| | (E) SPC:SPAN* (75:25) | | | | In vivo: rats | | |
| | *SPAN 40/60/80 | | | | Topical administration | | |
| | ACV (1 mg/mL) | | | | | | |
| | Lipid film hydration + sonication | | | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **ACV** | (A) PC:ACV (10:1) | (A) 1000 | −6.05 (A) | (A) 15.24 | | | |
| | (B) PC:PG:ACV (9:1:1) | (B) 681.5 | −30.00 (B) | (B) 22.12 | | | |
| | (C) PC:ACV (10:1) sonicated | (C) 308.5 | −1.71 (C) | (C) 7.30 | | | |
| | (D) PC:PG:ACV (9:1:1) sonicated | (D) 147.8 | −23.20 (D) | (D) 11.48 | In vitro: phospholipid vesicle-based permeation assay | • The incorporation of ACV into liposomes was found to significantly increase its in vitro permeability and coating with carbopol further enhanced the permeability from the PC:PG liposomes, as well as sonicated PC liposomes | Naderkhani et al. (2014) |
| | (E) PC:ACV (10:1) + COATING | (E) 1000 | −10.34 (E) | (E) 20.34 | | | |
| | (F) PC:PG:ACV (9:1:1) + COATING | (F) 455.3 | −35.80 (F) | (F) 17.92 | Pig mucin | | |
| | (G) PC:ACV (10:1) + COATING sonic | (G) 236.1 | −7.65 (G) | (G) 9.63 | | | |
| | (H) PC:PG:ACV (9:1:1) + COATING sonic | | | | Topical administration | | |
| | COATINGS: CHIT (0.6%) + CARBOPOL (0.1%%) | (H) 64.4 | −31.00 (H) | (H) 5.33 | | | |
| | Lipid film hydration + sonication | | | | | | |
| **IDU** | (A) IDU:PC:CHOL (1:1:1) | | | | | • The skin retention of IDU was enhanced due to its entrapment in the liposomal vesicles | |
| | (B) IDU:PC:CHOL (1:2:1) | | | | | | |
| | (C) IDU:PC:CHOL (1:4:1) | | | (A) 48.6 | In vitro: human cadaver skin | | Seth et al. (2004) |
| | α-TOC (1% (w/w)) | 3470 | n.i. | (B) 68.4 | In vivo: human patients | • The clinical study suggested the improvement of therapeutic efficacy of IDU entrapped in liposomes in treatment of HSV-1 and HSV-2 patients | |
| | | | | (C) 83.5 | Topical administration | | |
| | Reverse phase evaporation | | | | | | |
| **GCV** | PC:CHOL:NaDC (12:1.7:1) | | | | In vitro: corneas of rabbits<br>In vivo: rabbits | • The *in vitro* transcorneal permeability of GCV liposomes was found to be 3.9-fold higher than that of the solution<br>• In vivo ocular tissue distribution of GCV from liposomes was 2–10 times higher in the sclera, cornea, iris, lens, and vitreous humor when compared with those observed after solution dosing | Shen and Tu (2007) |
| | GCV (1.2 mg/mL) | 210 | −52.4 | 51.2 | Topical administration instillation on the cornea surface | | |
| | Reverse phase evaporation + sonication | | | | | • These results suggested that liposomes may hold some promise in ocular GCV delivery | |

ACV, *acyclovir;* ALAB, *l-alanine benzyl ester;* α-TOC, *α-tocopherol;* CA, *bovine brain ceramide;* CHOL, *cholesterol;* DCP, *dicetylphosphate;* DMPC, *1,2-dimyristoyl-*sn*-glycero-3-phosphocholine;* DPPC, *1,2-dipalmitoyl-sn-glycero-3-phosphocholine;* DSPC, *1,2-distearoyl-*sn*-glycero-3-phosphocholine;* GCV, *ganciclovir;* IDU, *idoxuridine;* NaDC, *sodium deoxycholate;* PA, *palmitic acid;* PC, *1,2-diacyl-*sn*-glycero-3-phosphocholine or phosphatidylcholine;* PG, *phosphatidylglycerol;* PhA, *phosphatidic acid;* PS, *phosphatidylserine;* SA, *stearylamine;* SMP, *sucrose monopalmitate;* SPAN 40/60/80, *sorbitan laurate 40/60/80;* SPC, *soy lecithin;* TB, *tributyrine.*

**Table 14.5** Other Lipid-based Colloidal Carriers for Antihuman Herpes Virus Drugs
SLN and NLC Containing Antihuman Herpes Virus Drugs

| | | Characterization of the Nanocarriers | | | | | |
|---|---|---|---|---|---|---|---|
| **Drug** | **Composition/Preparation Method** | **Size (nm)** | **Zeta Potential (mV)** | **EE (%)** | **Experimental Model (Route of Administration)** | **Main Achievements** | **Reference** |
| **PCV** | Lipids: PC, GMS<br>Surfactants: P188; TW80; TW20; Brij 78<br><br>(A) PC:GMS:P188 (1:3:1)<br>(B) PC:GMS:P188 (1:3:1.5)<br>(C) PC:GMS:P188 (1:3:2)<br>(D) PC:GMS:P188 (1:3:2.5)<br>(E) PC:GMS:TW80 (1:3:2)<br>(F) PC:GMS:TW20 (1:3:2)<br>(G) PC:GMS:Brij 78 (1:3:2)<br>PCV (30 mg/mL)<br>Double (W/O/W) emulsion technique | 254.9 | −25 | (A) 48.0<br>(B) 60.3<br>(C) 88.4<br>(D) 92.4<br>(E) 55.9<br>(F) 48.0<br>(G) 51.9 | In vitro: rat skin<br>In vivo: rabbits<br>Topical administration (instillation on the cornea surface) | • The interaction between SLNs and the skin surface changed the apparent morphology of stratum corneum and broke the close conjugation of corneocyte layers, which resulted in the increased permeation of PCV into skin dermis (2-fold more than commercial cream) | Lv et al. (2009) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **ACV** | Lipids: COMPRITOL 888, SPC<br>Surfactants: TW80; (2% (w/v))<br>(A) COMPRITOL 888:SPC (90:10)<br>(B) COMPRITOL 888:SPC (85:15)<br>(C) COMPRITOL 888:SPC (80:20)<br>(D) COMPRITOL 888:SPC (75:25)<br>ACV (2%)<br>Double (W/O/W) emulsion technique | 262 | + 85 (A)<br>+ 82 (B)<br>+ 90 (C)<br>+ 58 (D)<br>+ 50 (E)<br>+ 49 (F) | (A) 45.9<br>(B) 50.4<br>(C) 64.2<br>(D) 46.3 | In vitro: Human cadaver skin and rat skin<br>Topical administration | • In vitro skin permeation studies on human cadaver and rat skin revealed 17.65 and 15.17 times higher accumulation of ACV-SLNs in the dermal tissues in comparison to commercially available ACV cream after 24 h | Jain et al. (2011) |
| **ACV** | Lipids:TS (5% (w/v))<br>Surfactants: P188 (2.5% (w/v))<br>ACV (3 mg/mL)<br>Homogenization and ultrasonication | 236.2 | − 3 | 53.2 | In vitro: synthetic membranes mimetic of skin<br>Antiviral activity against HSV-1 tested by plaque reduction assay on Vero cells<br>Topical administration | • SLN are able to encapsulate high amount of ACV and to increase the diffusion of the drug<br>◦ Thus, it should be supposed that the transport of the ACV by colloidal Systems into the stratum corneum bypasses the main barrier to drug permeation, improving skin delivery<br>• However antiviral activity of SLN was not significantly different from free ACV | Cortesi et al. (2011) |
| **ACV** | Solid lipids: COMPRITOL 888; stearic acid; Cithrol GMS<br>Liquid lipids: CAPRYOL-90; LAUROGLYCOL-90<br>Surfactants: TW40; TW80; Brij 78; P188<br>Hot oil-in-water (o/w) microemulsion technique | 400–777 | − 30 to − 34<br>11.14 (SLN) | 25–92 (NLC) | In vitro: cornea from bovines<br>Topical administration | • The prepared nanoparticles were spherical and within the size range suitable for ocular drug delivery<br>• In comparison to free drug solution, NLCs were capable of having faster permeation through the excised cornea indicating their potential enhanced corneal properties | Seyfoddin and Al-Kassas (2013) |

(*Continued*)

**Table 14.5** Other Lipid-based Colloidal Carriers for Antihuman Herpes Virus Drugs
SLN and NLC Containing Antihuman Herpes Virus Drugs *Continued*

| Drug | Composition/Preparation Method | Characterization of the Nanocarriers: Size (nm) | Zeta Potential (mV) | EE (%) | Experimental Model (Route of Administration) | Main Achievements | Reference |
|---|---|---|---|---|---|---|---|
| **ACV** | *SLN*: CP; D-limonene; Transcutol P; BHT | | | | | | |
| | TW20; TW80 | 137.8 | | 6.2–6.5 mg/g lipid | In vitro: human epidermal membrane | • Iontophoresis enhanced the delivery of ACV when it was loaded in SLN. These results suggest that lipid nanoparticles are a promising drug delivery method that can be combined with iontophoresis to improve skin delivery of hydrophilic drugs | Charo Enput Takun et al. (2015) |
| | *NLC*: CP; OA; D-limonene; Transcutol P | (SLN) | | | | | |
| | BHT | 160.8 (NLC) | −53.1 to 56.4 | | Topical administration | | |
| | TW20; TW80 | | | | | | |
| | ACV (3 mg) | | | | | | |
| | Emulsification and sonication | | | | | | |
| ***Micelles Containing Antihuman Herpes Virus Drugs*** | | | | | | | |
| | Acylcarnitines Sodium glycocholate | | | | | | |
| | ACV (0.1 mM) | | | | In vivo: rat *in situ* nasal model | | |
| | | n.i. | n.i. | n.i. | Topical administration | • Byle salt-acylcarnitine promoted the transport of ACV | Park et al. (1992) |
| | Dissolution at concentrations higher the CMC. Sonication | | | | | | |
| | Acylcarnitines Sodium glycocholate | | | | In vivo: rat in situ nasal model | • Byle salt-acylcarnitine promoted the transport of ACV | Park et al. (1992) |
| | ACV (0.1 mM) | n.i. | n.i. | n.i. | Topical administration | | |
| | Dissolution at concentrations higher the CMC. Sonication | | | | | | |

ACV, *acyclovir;* BHT, *butylated hydroxytoluene;* CP, *cetylpalmitate;* COMPRITOL 888, *blend of different esters of behenic acid with glycerol or glyceryl behenate;* GMS, *glyceryl monostearate ridine;* OA, *oleic acid;* P188, *poloxamer 188;* SPC, *soy lecithin;* Transcutol P, *diethylene glycol monoethyl ether;* TS, *tristearine;* TW20, *TWEEN 20;* TW80, *TWEEN 80.*

of the helicase-primase complex), UL27 (envelope glycoprotein B), and UL29 (a DNA-binding protein)) were prepared and assayed for viral protection. siRNA lipoplexes were efficiently taken up by epithelial and lamina propria cells and silenced gene expression in the mouse vagina and ectocervix for at least 9 days. Intravaginal application of siRNAs targeting the HSV-2 UL27 and UL29 genes was well tolerated, did not induce IFN-responsive genes or cause inflammation, and protected mice when administered before and/or after lethal HSV-2 challenge. In a later study (Wu et al., 2009), one of the viral siRNAs was combined with an siRNA targeting the HSV-2 receptor, nectin-1. Cholesterol-conjugated-siRNAs silenced gene expression in the vagina without causing inflammation or inducing IFNs. The viral siRNA prevented transmission within a day of the challenge, whereas the siRNA targeting the HSV-2 receptor nectin-1 protected for a week, but protection was delayed for a few days until the receptor was down-modulated. Combining siRNAs targeting both a viral and host gene protected mice from HSV-2 for a week, irrespective of the time of the challenge.

Moreover, the encapsulation of peptides in nanocarriers can also be used as a vaccine strategy. Cortesi et al. demonstrated that cationic liposomes, containing the secretory form of HSV-1 glycoprotein B (gB1s) or two related polylysine-rich peptides (DTK1 and DTK2) allowed the inhibition of HSV-1 infection (Cortesi et al., 2006). This approach permitted overcoming the rapid drug loss, the long-time release of DTK1 and DTK2, and the poor corneal permeability of the active agents for ophthalmic delivery. Even though both vaccines did not show total protection, rabbits were sheltered against HSV lethal infection and viral reactivation.

Because of nanotechnology's continuous development and its pivotal role in future scientific and economic development, it is also worthwhile to mention some patents on antiviral delivery and formulation. One invention relates to a pharmaceutical composition comprising an antiviral compound selected from the group consisting of foscarnet, acyclovir, valaciclovir, penciclovir, and famciclovir, in admixture with galactolipids and a polar solvent. The pharmaceutical composition aims to be used in a prophylactic and/or curative treatment of herpesvirus infections in mammals, including man, by topical or parenteral administration (Carlsson et al., 2000). Another invention concerns the use of PVP-iodine liposomes for the treatment of herpes. It also concerns a method for producing a pharmaceutical preparation for the treatment of herpes virus-induced skin lesions, the preparation comprising at least one antiseptic compound in a pharmaceutically effective amount, combined with a particulate, pharmaceutically acceptable carrier (Reimer and Fleischer, 2012).

## 14.5 CONCLUSIONS AND FUTURE PERSPECTIVES

Lipid-based colloidal carriers are promising strategies for therapy purposes. Much research effort in developing novel drug delivery systems has been focused on

controlled release and sustained release dosage forms. Now, considerable efforts should be made to face the challenges and barriers that are hindering nanotherapy to achieve its full potential. In fact, there are still several aspects that need to be considered and optimized for a successful translation of nanocarriers from the laboratory to the clinical setting. For example, most of the studies using antiviral nanostructures are limited to either a few *in vitro* or *in vivo* models. However, accurate and extensive *in vitro* and *in vivo* preclinical studies should be performed before reaching the clinic. Compared to antiviral agents on the market (Table 14.1), the use of nanocarriers as antiviral delivery systems is still in its infancy and the dendrimeric microbicide VivaGel (for prevention of HSV and HIV) is the only commercial pharmaceutical nanocarrier on the market. The analysis of physicochemical characteristics, toxicity, hematocompatibility, delivery, and therapeutic efficiency is mandatory. Dialogue between scientists, clinicians, and industry is therefore indispensable for the future design of novel lipid-based colloidal formulations.

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