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*Review*

# Drugs in Cyclodextrin in Liposomes: How a Suitable Formulation of an Active Substance Can Improve Its Efficiency?

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**Abstract:** The design of new drug delivery systems has been widely sought after. The stability, solubility, and difficulty of targeting active sites for new drugs have always been challenging and remain one of the major drawbacks to the efficiency of certain drugs. Liposomes are phospholipid vesicles enclosing one or more aqueous compartments. Depending on its properties, a drug is embedded in the lipid bilayer or the aqueous medium. Thus, liposomes can act as drug carriers for both lipo- and hydrophilic compounds. New strategies such as "drug-in-cyclodextrin-in liposomes" (DCLs) have been developed as safe and effective carriers for exploiting the inclusion properties of water-soluble cyclodextrins known to form host–guest complexes with lipophilic molecules. Once inclusion complexes are formed, they can be inserted into a liposome aqueous core in order to stabilize it and better control the drug release. Our review will provide an update on the use of DCLs in the field of drug delivery for various kinds of active compounds. While previous reviews focused on the interesting advantages of using this method, such as enhancing the solubility and stability of a drug or controlling and improving drug release, the authors intend to highlight the impact of these nanocarriers on the pharmacokinetic and/or pharmacodynamic properties of drugs.

**Keywords:** drug delivery; cyclodextrins; liposomes; drug-in-cyclodextrin-in-liposome; formulation; cellular uptake

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## 1. Introduction

Research in the field of drug delivery has grown tremendously important throughout the years [1]. In order to facilitate the access of the drugs to their targets and to improve their efficiency, the development of new nanocarriers has been extensively investigated. A wide variety of nanocarriers have been designed, whether for the treatment of immune system-related pathologies [2], cancers and brain-related diseases [3,4], and various diseases or theragnostic agents [5]. Although a lot of nanomaterials are available in the field of drug delivery, this review focused on the supramolecular system known as drug-in-cyclodextrin-in-liposome (DCLs) based on cyclodextrins (CDs) and liposomes. These nanocarriers are used in various applications to have better control over the properties of drugs by modifying their water solubility, their stability, and their pharmacodynamic and/or pharmacokinetic profiles. They are formed from an inclusion complex between cyclodextrins (host) and drugs (guest), which are then incorporated in a liposome (Figure 1). The DCL strategy was first suggested by McCormack and Gregoriadis in 1994 [6]. Considering the properties of liposomes and cyclodextrin, both of which are able to incorporate drugs, they reported a method that combines their properties while avoiding their drawbacks. This drug nanocarrier consists of a drug encapsulated in a cyclodextrin-loaded liposome. This

formulation has been exploited in various areas, such as the pharmacy, cosmetic, and food industries.

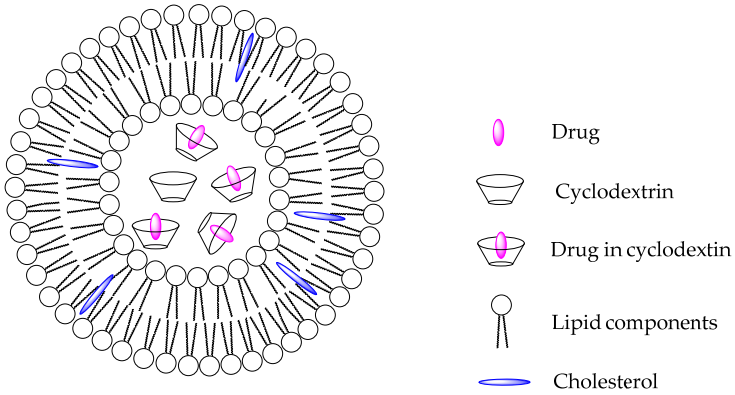



**Figure 1.** Schematic structure of DCL.

In 2014 and 2015, two distinct reviews have been focused on this subject, detailing the different methods used in order to create and characterize such systems. Thus, Chen et al. [7] reported examples of transdermal and antitumor drug carriers, whereas Gharih et al. [8] mainly discussed the difference between the inclusion of cyclodextrin complexes with drugs, drug-loaded liposomes, and DCLs. More recently, Jani et al. [9] (2019) accurately described the advantages of DCL in improving the solubility of anticancer drugs. Hammoud et al. [10] (2019) highlighted the influence of the nature of CDs to stabilize the membrane of the liposome, the effects of the system and its components on the permeations of the cellular membrane, and the consequent modulation of the drug release control. We can finally cite the last reviews of Fernández et al. [11] (2019) and Mura et al. [12] (2020), especially related to cyclodextrins in different carriers, such as liposomes, to promote specific applications and their advantages. To the best of our knowledge, ca. 90 articles deal with DCLs from 1994 to 2023. After a first period from 1994 to 2005 with one article published per year, a constant increase in the number of studies is observed, showing the rise in interest in this promising formulation method (Figure 2). This trend led us to explore the recently described innovations in this field, taking into consideration the combination of cyclodextrins and liposomes in a single formulation as drug carriers and emphasizing their consequences from a pharmacodynamic and/or pharmacokinetic point of view in order to highlight their interest in improving therapeutic protocols.

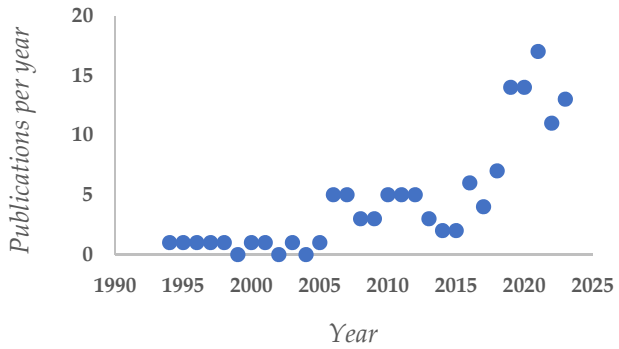



**Figure 2.** Average number of publications related to DCL*. * The evaluation was performed by using the below keywords in SciFinder and Google Scholar: "drug in cyclodextrin in liposomes", "liposome system drug cyclodextrin complex", "hydrophobic drugs liposomal formulation", "cyclodextrin in liposomes", "interaction of cyclodextrins phosphatidyl choline liposomes", "interaction of cyclodextrins DPPC liposomes".

## 2. Drug in Cyclodextrin in Liposome (DCL)

Based on the combined advantages of both cyclodextrins and liposomes, this approach can promote the effectiveness of drugs. Thus, the solubilization and stabilization of the active substance, the control of its release, and its pharmacological activity can be modified by playing with the formulation of DCL.

### 2.1. Inclusion Complexes between a Drug and a Cyclodextrin

Cyclodextrins (CDs) are a family of cyclic oligosaccharides. The most widely used are composed of 6, 7, and 8 α-(1,4)-linked D-glucopyranose units, called α-CD, β-CD, and γ-CD. They have a very particular tridimensional structure, which forms a truncated cone shape with a hydrophobic inner cavity that is open at both ends, named primary and secondary faces according to the nature of the alcoholic moieties present on each face. The cavity sizes are, respectively, 0.57, 0.78, and 0.95 nm for α-CD, β-CD, and γ-CD. CDs are able to accommodate molecules, or some moieties of molecules, forming a host–guest inclusion complex in which trapped molecules (guests) establish non-covalent interactions involving weak electronic interactions, such as Van der Waals, hydrophobic, or dipole-dipole interactions, with the CD cavity. The formation of such complexes consists of a reversible equilibrium between the free guest and the included molecule. This inclusion can be carried out either by the primary face, by the secondary face, or by both faces [13], depending on the affinity for the CD cavity and the stability of inclusion complexes [14]. Several CDs or guests can be involved in the formation of an inclusion complex. Thus, the number of CDs with respect to the number of guests per complex determines the stoichiometry of the complex. The obtained complex can modify the apparent water solubility of the included molecules and prevent their degradation in an aqueous medium. However, some limitations exist with native cyclodextrins due to the size of the cavity, which can hinder the inclusion. Besides, the water solubility of native CDs is relatively low, especially the most commonly used one, β-CD (18 mg/mL) [15]. Therefore, the final CD inclusion complexes sometimes do not reach the desired solubility in water. According to the applications, a change in CD properties is required by modifying their structure in order to affect the cavity size and/or solubility. For this purpose, specific substituents can be introduced by amination, etherification, or esterification of the hydroxyl groups, for instance. The formation of host–guest inclusion complexes with different drugs using native CDs and a wide range of their derivatives is then possible. Among these derivates, hydroxypropyl-β-CD (HP-β-CD) and methylated cyclodextrin derivatives, that is, 3-*O*-dimethyl-β-CD (DIMEB), 2,3,6-*O*-trimethyl-β-CD (TRIMEB), the partially methylated crystallized-β-CD (CrysMEB), and the randomly methylated β-CD (RAMEB), have been widely used in the formation of DCL carrier systems. The abovementioned CD derivatives are commercially available, and their properties afford interesting results. Inclusion complexes are mainly prepared by dissolution in solution and subsequent co-precipitation, spray-drying, or freeze-drying. Other strategies, such as the kneading method, the evaporation of organic solvent, or the sealing method, are used only occasionally [8,14]. The final application of the inclusion complex is then a determining factor in selecting a suitable preparation method. For example, in order to develop an industrialization process, kneading would not be a credible option. Although the freeze-drying method could be industrialized, its high costs and the time of implementation would be hardly compatible with such developments. Finally, inclusion by sealing is only possible in small quantities. Furthermore, the methods used for the preparation of an inclusion complex can affect the characterization results [13–16]. Thus, different values for the association and dissociation constants can be obtained for the same complex. However, the observed orders of magnitude remain similar.

### 2.2. Various Categories of Liposomes

Liposomes are non-toxic spherical vesicles formed by one or more lipid bilayers separating two aqueous media, that is, the inner aqueous core and the outer bulk solution. They are obtained by the self-assembly of lipids, often phospholipids such as phosphatidyl-

choline (PC) and dipalmitoyl-phosphatidylcholine (DPPC), and their properties depend on the number of bilayers, the nature of lipids used, the temperature, the added components, the conditions of preparation, and their environment. In order to modify the rigidity and stability of liposomes, cholesterol molecules, able to establish hydrophobic and polar interactions with the neighboring lipids, can also be introduced into the membrane. It is possible to distinguish various kinds of liposomes (Table 1). 'Classic' liposomes are classified based on their size and number of lipid bilayers (Table 1) [8], namely, the small unilamellar vesicles (SUV), the large unilamellar vesicles (LUV), and the multilamellar vesicles (MLV).

**Table 1.** Main characteristics of 'classic' liposomes.

| Category | Size | Number of Lamellas |
|---|---|---|
| Small unilamellar vesicles (SUV) | 20–100 nm | 1 |
| Large unilamellar vesicles (LUV) | >100 nm | 1 |
| Multi-lamellar vesicles (MLV) | 0.5 µm | 5–20 |

A second category of liposomes corresponds to the so-called 'deformable' liposomes (DefL), whose elasticity properties are higher compared to 'classic' liposomes, allowing better passage through the skin's biological barrier [17]. Their structure is identical to that of classical liposomes, except for the additional introduction of an edge activator that destabilizes the lipid bilayers to increase their deformability. Thus, these liposomes usually provide a better system for dermal and transdermal delivery of drugs. Liposomes can incorporate either hydrophilic molecules within their aqueous core or hydrophobic molecules within their lipid bilayers. Sometimes, liposomes are able to incorporate the same molecule in both compartments by using solubilizing agents, that is, cyclodextrins. This process is called 'double loading' (DLL) as compared to 'single loading' (SLL), where the drug is only located in one part of the liposome.

A third category of liposomes is the ionizable liposome. Anionic and pH-sensitive liposomes, taken up by cells by means of endocytosis and destabilized within endosomes, have emerged as an alternative for non-viral gene carriers thanks to their effective promotion of DNA molecules release into the cytoplasm [18] and as effective anticancer drugs in cancer therapy [19]. Novel cationic liposomes with carboxymethyl-β-cyclodextrin resulted in an improvement in the encapsulation efficiency of pDNA and proved their interest as non-viral gene delivery systems [20].

### *2.3. Cyclodextrins and Liposomes*

Generally, many challenges have to be overcome with liposome-based vectors. Their stability, their encapsulation efficiency, and the rate of release of embedded molecules require accurate control. Indeed, several parameters are sensitive to the incorporation of hydrophobic molecules within liposomes. Hydrophobic molecules are preferentially solubilized in the lipid bilayer, but the volume of the lipid bilayer of liposomes is relatively small. Therefore, to reach the proper concentration of the elected molecules, it may be necessary to reach a high concentration of the molecule in the bilayer. In these conditions, incorporation of the hydrophobic molecule in the bilayer can lead to destabilization of the lipid bilayer and therefore of the liposome, promoting either a too-fast release of the embedded molecules or making the control of the release very tricky. Analogously, the release of hydrophilic molecules embedded in the aqueous core of the liposomes, depending on the stability of the lipid bilayer, could be very difficult to tune. To avoid these problems, the use of cyclodextrins and their derivatives is a very attractive approach [21,22]. CDs can transform a hydrophobic molecule into a hydrophilic CD complex, allowing them to exploit the larger volume of the inner aqueous core of liposomes as compared to the volume of the lipid bilayer [7,8]. CDs can also change the stability and size of the liposome [7,8]. For instance, in the case of a liposome formed from dipalmitoylphosphatidylcholine (DPPC) and cholesterol, TRIMEB causes destabilization of the CD:liposome

system while hydroxypropyl-β-CD (HP-β-CD) does not. Indeed, compared to HP-β-CD, TRIMEB has a greater affinity for lipids and cholesterol, resulting in the extraction of the cholesterol and causing bilayer destabilization [9]. Moreover, the relatively competitive affinity of CD for the guest molecule or for the phospholipids used in liposome formation is also important [23]. When the molecule has a higher affinity than lipids for the CD cavity, the structure of the liposome is generally retained, and the stability of the liposome is kept or even increased. The formation of a strong inclusion complex between the CD and a guest can therefore favor the integrity of the liposomes [24]. The concentration of CDs (complexed or not), the concentrations of lipids, and cholesterol used during the formation of the liposome can also negatively or positively impact the properties of the final DCL system. Consequently, a judicious choice of the type of CD, the guest molecule, as well as the type of liposome prepared and its conditions of formation, is required. Five types of preparation methods were identified to formulate a DCL (Table 2). It is very important to consider all parameters to select the suitable technique because the effect of the system on the pharmacodynamics and pharmacokinetics of the drug may be affected.

**Table 2.** Methodologies for DCL preparation.

| Methodology | Critical Steps of Preparation |
| --- | --- |
| Thin-film hydration (TFH) | Evaporation of an organic solution of lipids, followed by the addition of an aqueous solution of CD/drug inclusion complex |
| Reverse-phase evaporation (REV) | Sonication of a mixture of an organic solution of lipids and an aqueous solution of CD/drug inclusion complex to get a water-in-oil emulsion, followed by evaporation of the organic phase |
| Ethanol injection | Addition of an ethanol solution of lipids to an aqueous solution of CD/drug inclusion complex, followed by evaporation of ethanol |
| Dehydration–hydration on vesicle (DRV) | Evaporation of an organic solution of lipids, followed by addition of an aqueous solution of CD/drug inclusion complex, freeze-drying and hydration of the vesicle with a NaCl solution |
| Freeze and thaw (Fr-Th) | Introduction of phospholipids to a bilayer softening, followed by addition of a buffer solution of CD/drug inclusion complex, freezing and rethawing several times until obtaining vesicles fusion |
| Lyophilization of double emulsions | Preparation of water-in-oil-in-water double emulsion, followed by freeze-drying and hydration |

Several studies have reported the use of DCL for medical and non-medical purposes. Although it is mostly used to improve the pharmaceutical properties of drugs, it has also been studied for the preservation of essential oils [25], agricultural uses [26], or food uses [27]. In most of those cases, DCLs were employed for their protective effect against the external environment of the encapsulated molecules. While it may be a prerequisite for their use as nanocarriers for drugs, the use of DCLs for the development of new medicines is not only based on this characteristic. Indeed, causes of failed marketing authorization of medicines are the low amount of the drug that is able to reach the biological target, as well as, during the pre- and clinical tests, the drug toxicity and the low drug loading. Therefore, DCL may be chosen because they can circumvent some of these drawbacks [7,8,11,28–30]. The present review aims to focus on the DCL nanocarriers used in medicinal chemistry applications and, in particular, on the influence on the pharmacokinetic or pharmacodynamic profile of the vectorized drug.

## 3. Influence of DCL on Compound Pharmacokinetics

The permeability of drugs through biological barriers as well as their solubility in the biological medium is a well-known issue for the development of drugs because the access of the molecules to their target will be either difficult or impossible. The transport of drugs with DCL is a way to modify the pharmacokinetics of the molecules. A wide variety of drugs have been encapsulated in DCL to improve their water solubility as well as their permeability. The absorption of the drug through different biological barriers can be modified, thus helping access to the target when DCL systems are used. Furthermore, DCL may affect the amount of drug loaded inside the system. Almost all the articles related to the formation of DCL made it clear that these factors can be improved using these nanocarriers. A noteworthy fact is therefore that the DCL system may solve various drawbacks of both CDs and liposomes, thus allowing it to be a valid strategy to be used for different kinds of drugs that have various modes of action and different target sites and administration routes.

### *3.1. Improvement in the Bioavailability of a Bioactive Compound*

Thanks to the new methods of drug discovery, a lot of molecules have been developed due to their efficient pharmacological properties. Despite this intensive process, many compounds lack good water solubility, leading to low oral absorption, although this administration route is often highly praised by patients, affording convenience and compliance. In order to avoid this difficulty, which could prevent further stages of development, the formulation of weakly soluble molecules is a great challenge. Among the various investigated nanoformulations, liposomes are undoubtedly the most suitable approach in terms of histocompatibility, cellular affinity, and targeting. DCLs represent an attractive strategy to increase the water solubility of bioactive compounds. Finally, DCL systems are also efficient in improving the pharmacokinetic profile of drugs through other administration routes.

#### 3.1.1. Application to Natural Products

A typical example is the intensive effort made in order to improve the bioavailability of curcumin [31–37]. Curcumin is a natural product extracted from the rhizomes of *Curcuma longa* that could be useful to treat, for instance, osteoarthritis. Due to their polyphenol structure, curcumin molecules form intermolecular hydrogen bonds that reduce their solubility in aqueous media, and their oral bioavailability is consequently poor. Moreover, although curcumin could be incorporated into liposomal membranes, a leakage of lipophilic molecules from liposomal formulations in the blood was observed. Thus, in order to avoid these downsides, the formation of a hybrid system to entrap curcumin in the aqueous compartment of liposomes via a water-soluble curcumin–cyclodextrin inclusion complex was an interesting alternative. Hydroxypropyl-β-cyclodextrin and hydroxypropyl-γ-cyclodextrin were first used to encapsulate curcumin, and the TFH method was implemented to formulate the nanocarrier. The results showed that the main protection of curcumin is provided by the liposome, but the formation of an inclusion complex with cyclodextrin can greatly increase the active substance loading. In addition, as curcumin is sensitive to hydrolysis, the cyclodextrin inclusion complex can modify the chemical stability of the active molecule. The double-loading technique was then used to formulate liposomes in the presence of 2-hydroxypropyl-α/β/γ-cyclodextrins. These vesicles–cyclodextrin systems contained curcumin either in the form of free molecules solubilized in the lipid bilayer or in the inclusion complex curcumin–cyclodextrin embedded in the aqueous core. This approach enhances the curcumin loading into liposomes without affecting the integrity of the bilayers.

Other natural products such as schisandrin, schisantherin, and γ-schizandrin isolated from *Schisandra chinensis* fructus were also used to develop DCL [38]. These lignans have a potential hepatoprotective effect, but their bad taste coupled with their hydrophobic character makes them unsuitable for oral administration. Thus, liposomes encapsulating β-cyclodextrin inclusion complexes loaded with *Schisandra chinensis* fructus extract were

prepared and allowed a significant improvement in the pharmacokinetic parameters by increasing their liver uptake.

Lycopene is a carotenoid mainly found in tomatoes, watermelon, and pink grapefruit. It has many potent biological activities, such as antioxidant, photoprotective, hepatoprotective, and hypolipidemic effects. In order to enhance its low water solubility, double-loaded liposomes encapsulating lycopene/β-cyclodextrin inclusion complex were successfully prepared using the thin-film hydration technique, and these vesicles could prolong its release time [39] and increase its *in vivo* cardioprotective activity.

#### 3.1.2. Application to Commercially Available Drugs

Flurbiprofen is a non-steroidal anti-inflammatory drug widely prescribed as a medication for the treatment of arthritis. In addition to its low aqueous solubility, which limits its oral bioavailability, flurbiprofen leads to severe gastrointestinal side effects, as is usually observed with other non-steroidal anti-inflammatory drugs. To circumvent these difficulties, liposomes formulated using three different techniques and three different cyclodextrin derivatives, that is, β-cyclodextrin, hydroxypropyl-β-cyclodextrin, and sulfobutylether-β-cyclodextrin, were used [40]. The relative bioavailability of the obtained inclusion complex flurbiprofen/β-cyclodextrin-loaded liposomes was significantly improved compared to pure flurbiprofen, inclusion complexes of flurbiprofen/β-cyclodextrin, or flurbiprofen-loaded liposomes (Figure 3).

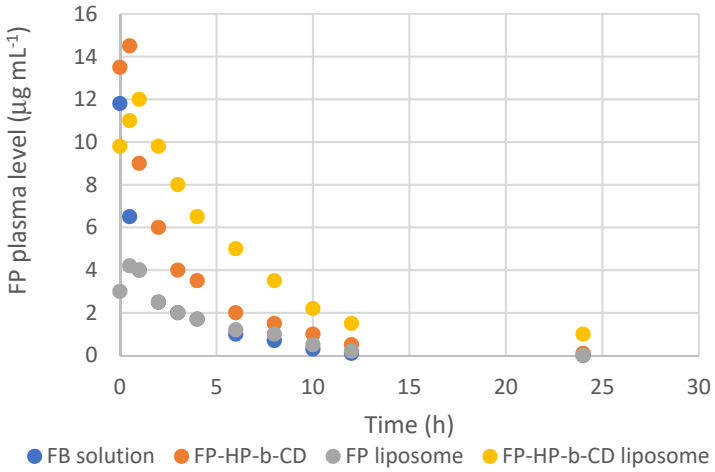


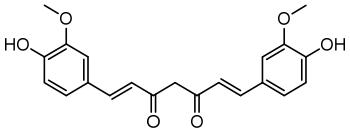
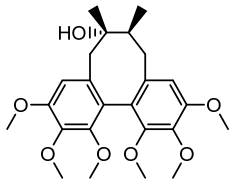
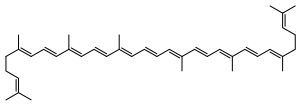
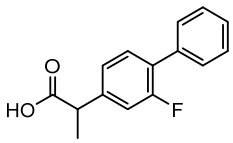
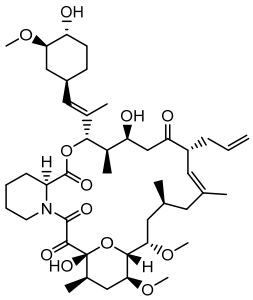

**Figure 3.** Plasma concentration–time curves of flurbiprofen (FP) solution, FP-HP-β-CD, FP liposomes, and FP-HP-β-CD-loaded liposomes. Data were extracted from [40].

Tacrolimus is a macrolide with a very strong immunosuppressant effect. It is combined with other immunosuppressive drugs for the prevention of transplant rejection. The investigation based on a copolymer-modified liposome-containing tacrolimus/β-cyclodextrin inclusion complex showed a greater solubility of tacrolimus, leading to better cellular uptake and intestinal mucous membrane penetration of the lipophilic drug/β-cyclodextrin complexes [41]. This strategy opens the way to the development of new, promising carriers for oral delivery of tacrolimus.

#### 3.1.3. Related Parameters Influenced by the DCL

When a DCL is developed to improve the oral bioavailability of a bioactive compound, the aqueous solubility is generally not the single factor modified by the nanocarrier. All the previous examples showed that side parameters were also changed, most often to promote the own pharmacological activity of the vectorized derivative and/or lessen its drawbacks (Table 3).

**Table 3.** Other parameters improved by DCL.

| Bioactive Compound | | | Related Parameters Affected by DCL | |
|---|---|---|---|---|
| **Origin** | **Name** | **Structure** | **To Promote the Main Activity** | **To Decrease the Side Effects** |
| Natural products | *Curcumin* |  | – | Sensitivity to hydrolysis |
| | *Schisandrin (+analogs)* |  | Liver uptake | Bad taste |
| | *Lycopene* |  | Cardioprotective effect | – |
| Drugs | *Flurbiprofen* |  | – | Gastrointestinal effects |
| | *Tacrolimus* |  | Intestinal mucous membrane penetration | – |

Thus, the use of vesicles as solubility enhancers provides a synergy that could optimize the behavior of a drug from a therapeutic point of view.

### *3.2. Improvement of the Physiological Barriers Crossing*

The nature of the human barriers that a drug has to cross to reach the target site depends on the route of drug administration. For that purpose, the topical use of a bioactive molecule requires efficient permeability of the skin and mucosa, while the parenteral delivery of a drug commonly involves the overcoming of epithelial membranes, tumor stroma, extracellular matrixes, and the blood–brain barrier (BBB). Especially, the crossing of the BBB constitutes a very challenging task to reach the central nervous system without damaging it. The liposome/cyclodextrin/drug supramolecular strategy is undeniably a promising approach to avoid the current limitations encountered when a compound is topically or parenterally administered.

#### 3.2.1. Vesicular Strategies for Effective Transdermal Delivery of Drugs

There is a particular interest in the development of novel vesicular approaches for effective transdermal delivery. The most attractive approach to the design of vesicles for transcutaneous bioactive delivery is the use of elastic or deformable liposomes. The structure of the elastic liposome is based on the use of phospholipids combined with an

edge activator. Due to these features, elastic liposomes provide easier penetration through the *Stratum corneum*, the outermost part of the skin, and an efficient delivery of bioactive compounds into the skin compared to classical liposomes.

Caffeic acid is a well-known phenolic derivative found in fruits, vegetables, coffee beans, and *Glycyrrhiza glabra* root. It has many beneficial effects, such as potential antiviral, anti-inflammatory, antioxidant, and antidepressant activities. A DCL system that combines the advantages of drug–cyclodextrin inclusion complexes and elastic liposomes to enhance skin permeation was implemented for the transdermal delivery of caffeic acid. Hydroxypropyl-β-cyclodextrin was selected as the host for the phenolic compound, and Tego® Care 450 as a PEG-free surfactant constituted the edge activator of elastic liposomes [42]. This DCL involving hydroxypropyl-β-cyclodextrin showed a higher skin permeability compared to both classic and elastic liposomes without cyclodextrin, proving the great interest of DCL systems as drug delivery vehicles for transdermal delivery.

Meloxicam, a specific cyclooxygenase inhibitor (COX-2), was also used to develop a transdermal delivery system based on β-cyclodextrin and elastic liposomes [43]. The combined cyclodextrin complexation and elastic liposome entrapment led both to an enhancement of the water solubility of the drug and a better drug permeability through the skin.

More recently, an effective liposomal formulation of butamben, a local anesthetic agent used in topical, dermal, and mucosal formulations, was developed [44]. To obtain a nanocarrier endowed with enhanced skin delivery, methyl-β-cyclodextrin (RAMEB) was selected as the best carrier for the drug. Thus, the purpose of this study was the development of deformable liposomes bearing butamben as a cyclodextrin complex, aimed at enhancing its therapeutic efficacy. Therefore, two kinds of liposomes were prepared: on the one hand, double-loaded liposomes bearing the lipophilic drug in the bilayer and its hydrophilic cyclodextrin inclusion complex in the aqueous core, and on the other hand, single-loaded liposomes bearing the free butamben in the bilayer. *In vivo* experiments on rabbits proved that double-loaded liposomes were significantly more effective than single-loaded ones in prolonging butamben anesthetic effects because the presence of the drug–RAMEB complex in the vesicle core acted as a reservoir of the active ingredient.

#### 3.2.2. Vesicular Strategies for Increasing the Permeability of the Blood–Brain Barrier

Due to the importance of neurodegenerative diseases, the design of nanocarriers able to transport a drug to the brain is of great importance. Nonetheless, the blood–brain barrier has special filtering properties to potentially prevent xenobiotics from reaching the brain and the spinal cord. Actually, the blood–brain barrier is made up of endothelial cells tightly joined by numerous transmembrane and intracytoplasmic proteins that prevent the brain uptake of most drugs. Thus, DCL systems are a promising alternative to overcome the limits of conventional medicines.

Monflier et al. showed the interesting effectiveness of a supramolecular assembly liposome/CD/adamantoylsaccharide [45]. The system was based on well-defined cyclodextrin-coated liposomes as drug carriers and adamantoylsaccharides as blood–brain barrier-interacting ligands. By this approach, the modified liposome had a 5-fold improved ability to enter the blood–brain barrier-endothelial cells compared to the non-coated liposome in an *in vitro* model of the blood–brain barrier.

Another study was focused on the development of injectable liposomes and DCL formulations encapsulating estetrol, a metabolite of estradiol able to prevent cerebral ischemia in premature babies, in order to enhance its crossing through the blood–brain barrier [46]. These two systems were capable of increasing up to 10-fold the passage of estetrol through an *in vitro* model of the blood–brain barrier. The slight increased passage through the BBB observed for the DCL system as compared to the simple liposomal formulation can be due to the higher stability and lower leakage of encapsulated estetrol in the lipid vesicles of the DCL system.

#### 3.2.3. Vesicular Strategies for Effective Gene Delivery

Genetic therapy is a treatment strategy that relies on the efficient delivery of genetic material within a targeted cell population. Since the cellular uptake of naked DNA is highly inefficient due to its large molecular size and the low stability of plasmids in biological fluids, pH-sensitive anionic liposomes have been proposed to improve the intracellular delivery of nucleic acids [18]. Silva et al. demonstrated that the embedding of functionalized β-cyclodextrin/DNA complexes into pH-sensitive liposomes does not compromise their efficiency [47]. Recently, multicomponent self-assembled supramolecular nanovesicles based on an amphiphilic derivative of β-cyclodextrin and phosphatidylcholine liposomes functionalized with four structurally different adamantyl guanidines have been successfully applied as non-viral gene delivery vectors [48]. The study of Štimac et al. demonstrates that the DCL strategy can be profitably improved by proper design and engineering.

### *3.3. Control of the Release Time of the Drug*

Some pharmaceutical dosage forms are specially formulated to release the active substances slowly or in repeated small amounts. This dosage form is called modified or controlled release. The main objective is to design a device able to release a drug over a prolonged time period, leading to less fluctuation in drug blood levels and reducing the administration frequency.

The DCL strategy could be useful in this case, as exemplified by the study on the antipsychotic substance risperidone [49]. The formulation of this drug based on a long-acting injectable microsphere exhibits a delayed response profile, reaching three weeks until risperidone is released. To avoid this inconvenience, patients have to take oral forms of risperidone to stabilize the plasma level of the drug during this latency. In order to improve this co-administration of medicines, a novel co-administration therapy was investigated by developing conventional liposome and DCL systems obtained with various methods. When the drug is embedded in liposomes, its encapsulation efficiency is higher than in the DCL system, but risperidone in liposome-containing cyclodextrin is released faster compared to the liposome alone, although liposomes have been demonstrated to be more stable in the latter system.

Analogously, it has been demonstrated that the DCL formulation enhances the liposomes potential to deliver siRNA to cell lines *in vitro*. Cruz et al. highlight that the addition of βCD to siRNA embedding liposomes, thanks to the capacity of βCDs to bind to both siRNAs and liposomes, most likely by forming H-bonds to surface phosphate groups, significantly improves the retention of siRNAs inside a liposome, enhancing their transfection efficiency and stability in the cell culture medium [50].

## 4. Influence of DCL on Compound Pharmacodynamics

The composition of drugs can influence the mode of action of the active substance. Liposomes and cyclodextrins have been shown to have their own properties as drug carriers and delivery enhancers of therapeutic agents. Classical liposomes were largely used in the field of oncology and are very interesting tools to administer cytotoxic compounds. They improve the efficiency of the treatment by limiting the side effects with higher concentrations of active substances. Considerable research efforts were also focused on the implementation of strategies for the delivery of local anesthetic agents. Thus, their encapsulation in liposomes or cyclodextrins led to novel formulations able to control the release of the drug. Indeed, prolonged action of these anesthetic agents with a predictable duration of action would be an optimized solution. Analogously, in the case of antibiotics, the drug has to reach its target at a determined concentration and for a prolonged period of time to provide better therapeutic prospects. In all these different medical applications, approaches based on DCL could offer promising ways to develop novel generations of drug delivery systems.

### 4.1. Formulation for Anticancer Drugs

Hypericin is a natural pigment with attractive properties for application in photodynamic therapy. Nevertheless, its hydrophobic character impedes its administration at therapeutic doses because it aggregates in aqueous media, leading to a loss of photodynamic activity. To overcome this limitation, the encapsulation of hypericin can be performed in liposomes with increased membrane rigidity. Bakowsky et al. studied the influence of loading hypericin with the pigment hydroxypropyl-β-cyclodextrin inclusion complex via the dehydration–rehydration vesicle method on the direct phototoxicity of human ovarian carcinoma cells [51]. They showed that these liposomes could deliver the photosensitizer to the tumor site in a more protected manner. This preferential accumulation area in tumor tissue is a key point in establishing a liposomal combination therapy for a safe and selective tumor treatment.

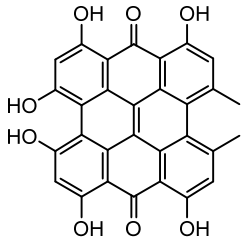
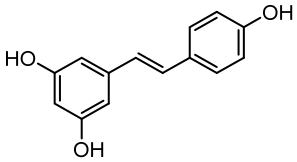
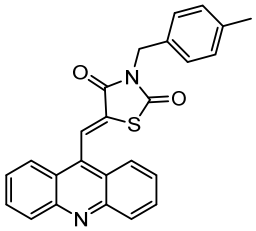
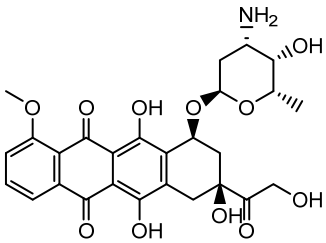
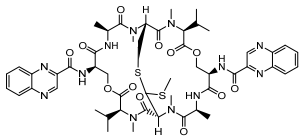
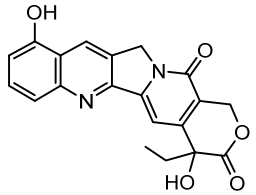
E. Soo et al. have prepared three formulations incorporating resveratrol, a known potential and natural anticancer drug [52]. The first is based on conventional liposomes loaded with the active compound in the bilayer, the second involves liposomes loaded with drug–cyclodextrin inclusion complexes within the aqueous core, and the third corresponds to mixed liposomes loaded with resveratrol and resveratrol–cyclodextrin inclusion complexes. The chemotherapeutic activity of these formulations and free resveratrol was evaluated in HT-29 colon cancer cell lines. The cytotoxicity profile of the liposomes was dose-dependent and enhanced compared to free resveratrol. At lower concentrations of 50 μM, the two liposomes with cyclodextrin inclusion complexes had higher antiproliferative properties, suggesting that more drug was available to inhibit the growth of cancer cells. These results open the way to preclinical research into the treatment of colorectal cancer by using this kind of nanocarrier.

Another example relates to an acridine derivative representative of a series of new anticancer agents developed as more effective and less toxic drugs. Medonça et al. studied the inclusion of this compound in hydroxypropyl-β-cyclodextrin and hydroxypropyl-γ-cyclodextrin, the latter leading to a less stable complex [53]. The authors demonstrated that the CD nanoencapsulation of the drug into hydroxypropyl-β-cyclodextrin improves the penetration of the active substance into the cells, and the nanoencapsulation in the liposomes enhances its antiproliferative activity thanks to a better solubility and therefore an improved loading. Indeed, the obtained findings showed that the nanoencapsulation of cyclodextrin inclusion complexes in liposomes ameliorates the cytotoxic activity and could be used for *in vivo* studies compared to the effectiveness of the free drug.

The DCL approach is especially suitable for enhancing the therapeutic index of drugs. Thus, Arima et al. proved that pegylated liposomes entrapping a doxorubicin complex with γ-cyclodextrin led to the retardation of tumor growth and the improvement of survival rate without suppressing the increase in body weight after intravenous injection in mice [54]. These results revealed that the antitumor effects of doxorubicin were increased by using these CD complexes in the liposome system. The authors suggest that the slow release of doxorubicin from pegylated liposomes and the improved stability of the pegylated liposomes cause high levels of the drug in solid tumors *in vivo*.

Echinomycin is a peptide, including quinoxaline moieties, with potent antitumor and antimicrobial activity due to its DNA bis-intercalator properties. The encapsulation of the echinomycin/γ-cyclodextrin inclusion complex was carried out into PEGylated liposomes, and then the antiproliferative and anti-invasive effects were evaluated against U-87 MG glioblastoma cells [55]. The liposomes showed potent antiproliferative and anti-invasive effects against this cell line. These DCL formulations could, therefore, be a promising alternative for glioblastoma because this disease is the most common and aggressive type of malignant brain tumor. Currently, the survival rate of patients is not significantly increased by available therapies. (See Table 4).

**Table 4.** Example of DCL formulations with anticancer drugs.

| Bioactive Compound | Target | Related Parameters Affected by DCL | Ref. |
| --- | --- | --- | --- |
|  *Hypericin* | Human ovarian carcinoma cells | Delivery of the photosensitizer to the tumor site in a more protected manner | [51] |
|  *Resveratrol* | HT-29 colon cancer cell lines | Increase in availability and higher antiproliferative properties | [52] |
|  *LPSF/AC04* | T47D (breast cancer) cell line | Improvement in the penetration of the active substance into the cells and enhancement in its antiproliferative activity thanks to better solubility | [53] |
|  *Doxorubicin* | Mice-bearing colon-26 tumor cells | Direct introduction of liposomes into cells, enhancing the therapeutic index | [54] |
|  *Echinomycin* | U-87 MG glioblastoma cells | Direct introduction of liposomes into cells, leading to potent antiproliferative and anti-invasive effects against this cell line | [55] |
|  *10-Hydroxycamptothecin* | HepG-2, A549, and SGC-7901 cells | Higher efficiency due to the gradual release of the drug (up to 72 h) | [56] |

Moreover, 10-Hydroxycamptothecin is a natural product and one of the representative drugs in the camptothecin series. It interferes with DNA replication by forming stable Topo I-DNA complexes. The research is currently focused on pharmaceutical forms able to improve their solubility, stability, and targeting so as to reduce their toxicity and improve their bioavailability. An inclusion complex of 10-hydroxycamptothecin with 2-hydroxypropyl-

β-cyclodextrin was first prepared, followed by its encapsulation with liposomes. Chen et al. demonstrated the ability of the 10-hydroxycamptothecin formulation to inhibit three kinds of cancer cell lines—that is, the HepG-2, A549, and SGC-7901 cell models—and found that the drug had a good inhibition after 72 h. Consequently, the formulation has better inhibition activity than commercially available hydroxycamptothecin [56].

### 4.2. Formulation for Anesthetic Drugs

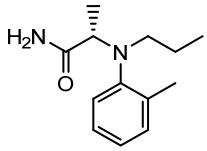
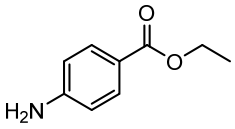
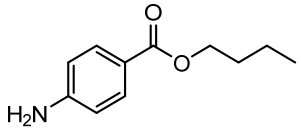
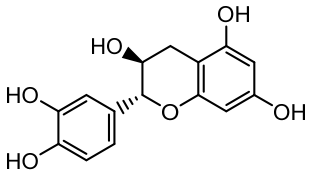
Local anesthetic agents often lack a sufficient delay of action in order to have a true clinical advantage, especially for regional anesthesia in surgery and dental practice as well as for the regional control of acute and chronic pain. Bragagni et al. implemented four different liposomal formulations, that is, liposomes loaded with prilocaine base as complex with hydroxypropyl-β-cyclodextrin in the aqueous phase, liposomes loaded with prilocaine hydrochloride in the aqueous phase, liposomes loaded with prilocaine base in the lipophilic phase, and "double-loaded" liposomes containing free prilocaine base in the membrane bilayer and its hydroxypropyl-β-cyclodextrin complex in the aqueous compartment [57]. They showed that inclusion complex formation with cyclodextrin increased the prilocaine anesthetic effect. For this purpose, the approach based on double loading gives the best results, demonstrating the shortest onset time of anesthetic activity and the longest duration of the anesthetic effect.

The same strategy was developed by Maestrelli et al. in the case of benzocaine and butamben, which are used as local anesthetics in topical, dermal, and mucous formulations [58]. They have a rapid effect, but their duration of action is clearly shorter compared to that of pain. In order to extend the anesthetic effect, the authors studied the interaction of these drugs with native β-cyclodextrin and hydroxypropyl-β-cyclodextrin before preparing double-loaded deformable liposomes. Their efficiency was evaluated *versus* the activity obtained in the case of classic liposomes involving the free drugs in the aqueous phase of the vesicles. Although benzocaine and butamben have different physicochemical properties, similar results were obtained and proved that the approach based on a drug complexation in hydroxypropyl-β-cyclodextrin led to a significant enhancement in both strength and length of the drug therapeutic effect (See Table 5).

### 4.3. Formulation for Improving the Efficiency of Antibiotic Treatments

Cyclodextrins and liposomes can lead to an efficient strategy to avoid antibiotic resistance in bacteria. Liposomes entrapping inclusion complexes of catechin in β-cyclodextrin have been developed as a potential approach to prevent the development of resistance induced by *Staphylococcus aureus* against methicillin [59]. Catechin is a natural product isolated from cashew nuts that exhibits antibacterial properties. This kind of DCL can inhibit the multiple virulence factors of *Staphylococcus aureus*, especially inhibiting biofilm growth, slime production, EPS synthesis, lipase production, bacterial mobility, hemolytic activity, proteolysis, and autolysin, or preventing staphyloxanthin production. It could be used as a novel approach to reduce the risks of pathogenesis caused by methicillin-resistant *Staphylococcus aureus* in human beings.

**Table 5.** Example of DCL formulation with anesthetic and antibacterial drugs.

| | Bioactive Compound | Related Parameters Affected by DCL | Ref. |
|---|---|---|---|
| Anesthetics |  *Prilocaine* | Shorter onset time of anesthetic activity and the longest duration of effect | [57] |
| |  *Benzocaine* | Significant enhancement of both strengths and length of the drug's therapeutic effect | [58] |
| |  *Butamben* | | |
| Antibiotic |  *Catechin* | Effective against *Staphylococcus aureus* resistant to methicillin | [59] |

## 5. Conclusions

In this review, significant impacts of drug-in-cyclodextrin-in-liposome (DCL) complexes on both pharmacokinetic and pharmacodynamic properties were reported. Compared to either cyclodextrins or liposomes individually used, DCL allows substantial improvement in a wide range of parameters for both commercial drugs and biologically active compounds.

Encapsulating drugs in DCL improves water solubility as well as permeability through different barriers, increasing the bioavailability and greatly enhancing the pharmacokinetics of the target molecules. In the case of topical application, DCL significantly increases transdermal permeability. DCL systems are also able to improve the permeability of the blood–brain barrier, specifically against neurodegenerative diseases. The release of the drug over time, either slowly or quickly, can be better controlled using DCL. For antibiotic treatments, DCL can also bypass some antibiotic resistance from bacteria.

DCL was shown to influence the mode of action of the encapsulated drugs. It can deliver active substances in a more protected manner to the target site, improving effectiveness, reducing side effects, and enhancing the therapeutic index of drugs.

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