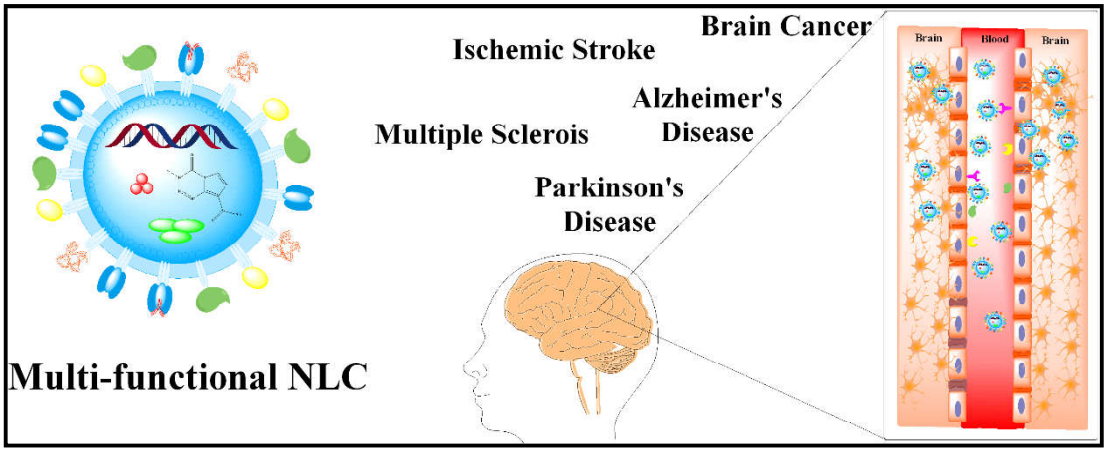
Advances in the design of solid lipid nanoparticles and nanostructured lipid carriers for targeting brain diseases

*Graphical Abstract



A multi-functional nanostructured lipid carrier (M-NLC) can penetrate the blood brain barrier (BBB) after proper surface functionalization and deliver its therapeutic cargo in the diseased tissue.

Advances in the design of solid lipid nanoparticles and nanostructured lipid carriers for targeting brain diseases

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Abstract

Solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) comprise a category of versatile drug delivery systems that have been used in the biomedical field for more than 25 years. SLNs and NLCs have been used for the treatment of various diseases including cardiovascular and cerebrovascular, and are considered a standard treatment for the latter, due to their inherent ability to cross the blood brain barrier (BBB). In this review, a presentation of the most important brain diseases (brain cancer, ischemic stroke, Alzheimer's disease, Parkinson's disease and multiple sclerosis) is approached, followed by the basic fabrication techniques of SLNs and NLCs. A detailed description of the reported studies of the last seven years, of active and passive targeting SLNs and NLCs for the treatment of glioblastoma multiforme and of other brain cancers, as well as for the treatment of neurodegenerative diseases is also carried out. Finally, a brief description of the advantages, the disadvantages, and the future perspectives in the use of these nanocarriers is reported, aiming at giving an insight of the limitations that have to be overcome in order to result in a delivery system with high therapeutic efficacy and without the limitations of the existing nano-systems.

Keywords: SLNs, NLCs, Neurodegenerative diseases, Glioblastoma multiforme, BBB.

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1. Introduction

Over the years, numerous nanostructures of various sizes and shapes have been reported in the literature for the treatment of different diseases. These nanostructures are comprised by various materials either synthetic or natural, and their properties vary depending on the materials used and the functionalization they undergo. It has been more than 50 years since the first appearance of spherical nano/microstructures [1], and during these years these structures have been evolved and found use in many industrial and biomedical applications. In the biomedical field, the evolution of various nanostructures, organic and inorganic, has been rapid due to the imminent need to replace conventional strategies of treating untreatable diseases. In fact, the unmet clinical needs of many diseases were the leading cause for the development of nanostructures with tailored properties, aiming at fabricating a superior drug delivery system, which will specifically target diseased tissues without affecting its healthy niece. Nano-medicinal carriers have been used for the treatment of various diseases including cancer [2,3], atherosclerosis [4–6], intervertebral disc degeneration [7,8], cardiovascular diseases [9,10], and cerebrovascular diseases [11,12].

Due to the complexity of each pathology, each nanostructure has to be properly studied and designed in order to achieve the maximum therapeutic effect with the lowest possible side effects. Most of these nanostructures can be modified in a way that makes them responsive to various internal or external stimuli, a property which is useful for the controlled release of encapsulated therapeutic substances. A significant number of these nanostructures, such as inorganic nanoparticles or other polymeric/lipid nanoparticles, have also been used as diagnostic tools. The combination of therapy and diagnosis led to the fabrication of "theranostic" nanoparticles, but unfortunately, to date, most of these theranostic devices make use only of synthetic polymers and not of lipid-based nanostructures such as solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs).

Among the diseases that these nanostructures target, we find conditions that affect the central nervous system (CNS), the most important of which are brain cancer, ischemic stroke (IS), Parkinson's disease (PD), Alzheimer's disease (AD) and multiple sclerosis (MS). The cause of appearance is different for each disease, and it is affected by genetic and/or environmental factors. This diversity is one of the reasons that the design of drug delivery systems has to be very specific in order to successfully target the diseased area without affecting the surrounding tissues, and to result in its regression or even its complete cure. Even though all the CNS diseases have a different cause, they all share a common characteristic in terms of targeted delivery, and this characteristic is the blood-brain barrier (BBB). BBB is one of the main reasons why the delivery of therapeutic molecules inside the brain is so complex. The tight junctions of the endothelial cells make almost impossible each attempt of delivering drugs into the brain if they are not functionalized with specific targeting segments or if they are not encapsulated inside other nanostructures. In order to overcome this limitation, current therapeutics like drugs, antioxidants, enzymes, genes, DNA/RNA and/or inorganic nanoparticles are properly cloaked inside polymeric or lipid nanostructures that have the ability to penetrate the BBB (**Figure 1**). In the literature, most of the nanoparticulate delivery systems that have been used for treating brain diseases are comprised by synthetic polymers like polyethylene glycol (PEG) or poly (lactide-*co*-glycolide) (PLGA) [11–14]. These polymeric nanostructures present versatility in their size, shape, physicochemical properties, and surface functionalization, that allows them to penetrate the BBB and to deliver their

encapsulated cargo in a controlled and sustained way. Nevertheless, most of these nanoparticulate systems present toxicity issues due to the acidic by-products that are formed during their degradation, rendering them inappropriate for extended use in the brain. An alternative approach with respect to these polymeric nanoparticles is represented by either inorganic nanoparticles (that indeed also present an inherent toxicity) and lipid-based structures. Liposomes, solid lipid nanoparticles, and nanostructured lipid carriers are the most important representatives of the lipid-based nanosystems, and they have been used for the treatment of brain diseases in the last 30 years. These lipid nanostructures are more biocompatible if compared to the polymeric or inorganic nanoparticles, and they have an inherent ability, due to their small size and to their lipid nature, to penetrate the BBB even without any functionalization. One more advantage of these nanostructures compared to the synthetic polymers is the possibility of their production at large scale, and this is one of the reasons why these nanostructures are becoming more and more attractive as a DDS. Unfortunately, to date, very few of these systems have been in clinical trials or commercially available, and one of the possible reasons is their major drawback which lies to their low loading capacity.

The reported literature on polymeric and lipid-based nanoparticulate systems for the treatment of CNS diseases is extensive, and in this review, we are going to briefly describe the advances in the synthesis, characterization, and in *vitro/in vivo* studies for SLNs and NLCs that were carried out during the last seven years. Briefly herein, we are describing the most important brain diseases and some of their main characteristics, followed by a description of the type of lipid-based nanostructures and their fabrication techniques. Finally, an extensive report to the literature on active and passive targeted SLNs and NLCs for CNS, followed by the conclusions and the future perspectives for this type of delivery systems, is carried out.

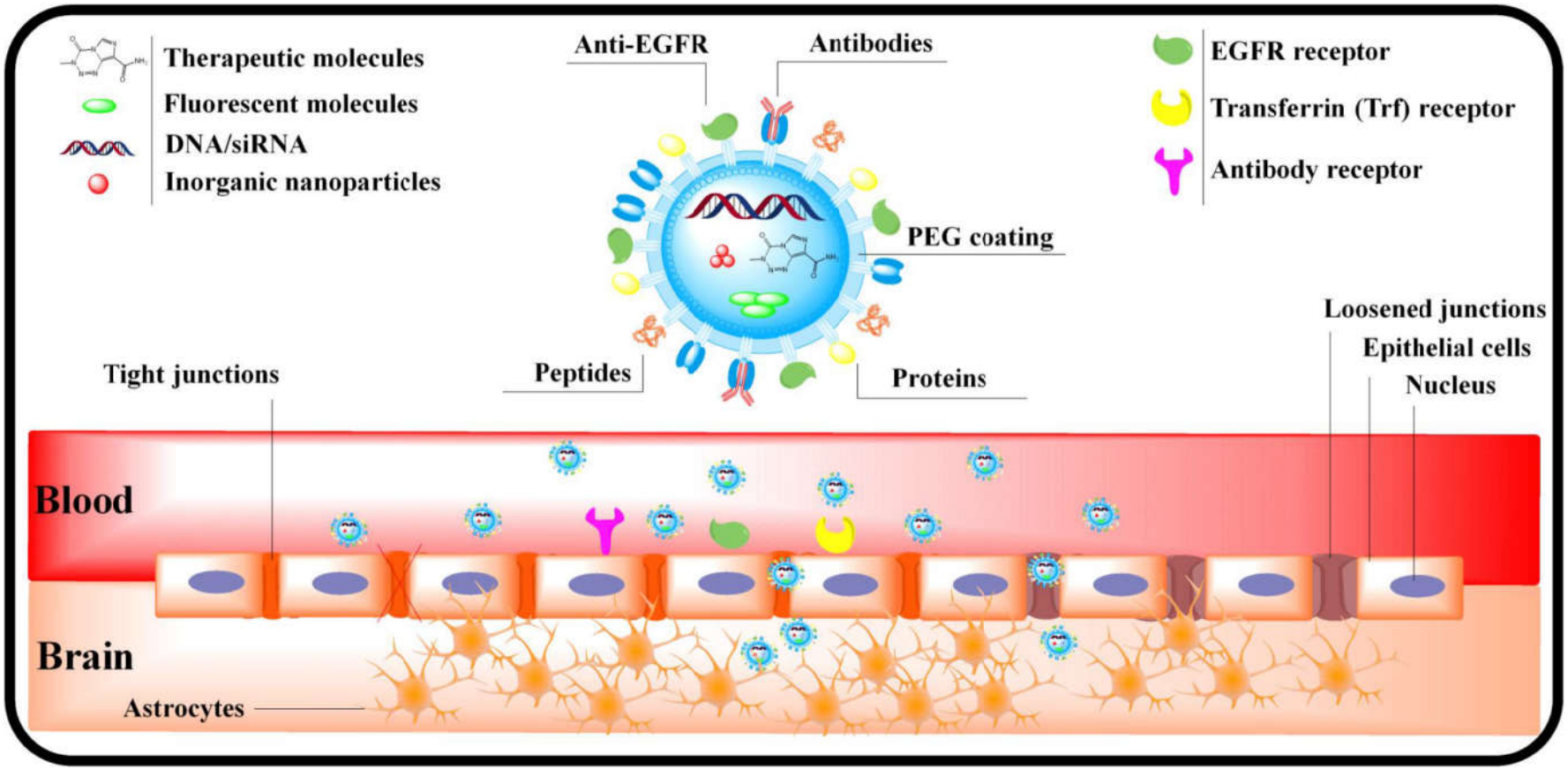


Figure 1. Schematic representation of a multifunctional solid lipid nanoparticle and the targeted delivery of therapeutics. **Upper part:** Potential therapeutics (chemotherapeutic and/or antioxidant substances, fluorescent dyes, genes and inorganic nanoparticles) that can be encapsulated inside the lipid matrix and potential targeting moieties on the outer surface (proteins, peptides, and antibodies), are depicted. **Bottom part:** The representation of the BBB and the penetration of SLNs that are traveling through blood is depicted. The tight junctions of the BBB make the penetration of nanoparticles in the CNS almost impossible (left bottom part). Functionalization of the SLNs with targeting moieties enhances the BBB penetration. In certain diseases, like ischemic stroke, the tight junctions of the BBB are loosened (right bottom part) allowing an easier penetration of the nanoparticles.

2. Solid lipid nanoparticles and nanostructured lipid carriers: synthesis, characterization, and comparison with other DDSs

2.1. Type of lipid carriers

Lipid-based carriers can be divided into various categories depending on their physicochemical properties and the method that is used for their fabrication. The main lipid-based carriers include **1)** niosomes, which are lamellar self-assembled structures that comprise of non-ionic surfactants and cholesterol [15,16], **2)** transferosomes, which are similar to niosomes and to liposomes and they consist of a lipid bilayer created by a lipid matrix that is stabilized by a variety of surfactants [17], **3)** liposomes, which are spherical vesicles created by a lipid bilayer of phospholipids [18,19], **4)** solid lipid nanoparticles which consist of a solid lipid core at room and body temperature [20], and **5)** the nanostructured lipid carriers, the core of which comprises a liquid lipid phase inside the solid lipid phase [21].
Although all of the above-mentioned nanostructures have been used as drug delivery systems for treating brain diseases [20–26], this review will focus only on two types of lipid-based nanocarriers, the SLNs and the NLCs.

2.1.1. Solid lipid nanoparticles

Solid lipid nanoparticles are one of the newest members of the lipid-based nanocarriers family and they made their first appearance almost twenty-five years ago [27–29]. The need of overcoming the limitations of other nanostructured systems (niosomes, transfersomes, micelles, liposomes, emulsions, polymeric nanoparticles) like toxicity, stability and low loading capacities led to their fast development, and since then many studies demonstrating their usefulness in numerous diseases have been published [20,30,31].
SLNs are fabricated using a variety of lipids that share common characteristics including low melting point and solidness at ambient and body temperature, and a variety of surfactants and/or co-surfactants. Some of the main lipids that have been used to date are, monostearin, stearyl alcohol, stearic acid, glycerol monostearate, Precirol® ATO5, Compritol® 888 ATO, cetyl palmitate, while some of the main surfactants that act also as stabilizers are, poloxamer 188, Tween® 80 and dimethyl dioctadecyl ammonium bromide (DDAB). The proper selection of lipids and surfactants as well as the composition of SLNs, approximately 0.1 – 30% w/w for the solid core and 0.5 – 5% w/v for the surfactants, affects their physicochemical properties such as size, polydispersity, surface charge, short and long-term stability, drug loading and release profile.
One of the main reasons for the fast development of SLNs was their ability to effectively deliver in numerous diseased tissues both lipophilic and hydrophilic drugs, as well as other therapeutic molecules including oligonucleotides, peptides, genes and even smaller nanoparticles such as superparamagnetic iron oxide nanoparticles. One more advantage of the SLNs is that they reduce the toxicity of the therapeutic molecule that they transfer protecting them, at the same time, from reticuloendothelial system (RES) clearance. Their inherent ability of poor solubility in water acts also in favor of the encapsulated substance since it results into controlled and sustained release profiles; moreover, their long-term stability allows them to be used for long

periods of time. SLNs are also biocompatible, can be easily sterilized and the used fabrication methods do not require the use of organic solvents which may affect the toxicity of the final product. Moreover, the fabrication of these lipid nanocarriers can be easily scaled up allowing them to be used at industrial scale. Finally, functionalization of the SLNs with specifically modified targeting lipids allows them to actively target desired tissues. Although SLNs have many benefits, they also have a few disadvantages, some of which are the expulsion of the encapsulated therapeutic, the tendency to gelate, and the low encapsulation efficiency (EE) [30][20]. It has to be noted, that the low encapsulation efficiency results from the internal structure of the lipid core, which during crystallization does not allow the creation of empty spaces, making difficult for a potential encapsulated substance to remain trapped inside the solid phase.

2.1.2. Nanostructured lipid carriers

Aiming at improving the inherent disability of SLNs for high loading efficiency, Müller *et al*. [32] proposed a modified version of SLNs where the structure of the solid lipid core contains imperfections that increase the internal free space of the solid, resulting in higher payloads. To achieve this imperfect crystal structure, mixing of liquid lipids with the solid lipid as well as the use of lipids like mono-, di- and triglycerides with different chain lengths, can lead to the desired result [30]. This next generation of SLNs not only presented improved loading efficiencies, yet also demonstrated improved stability as well as prevention in drug expulsion during storage [30,33]. In the case of hydrophobic drugs, increased loading efficiency is achieved due to the inherent property of some drugs to better dissolve in the liquid than solid lipids, and it has already been demonstrated that increased solubility of the drug leads to higher loading efficiencies [34,35].
On the other hand, in the case of hydrophilic drugs, a lipid conjugation approach is followed, where the functional group of the drug (*e.g.*, amine group) can be conjugated with the functional group (*e.g.*, carboxylic acid group) of lipids like oleic acid, through carbodiimide or another type of chemistry. NLCs make use of the same lipids that mentioned in the previous section for SLNs as well as liquid lipids, some of which are almond oil, cetiol, corn oil, Mygliol® 812 N, oleic acid, olive oil, peanut oil, sesame oil[36], soybean oil, Speziol® EOL NF, Tegosoft® M, Tegosoft® P, Suppocire® NC, L-phosphatidyl choline (PC), soy lecithin, and Capmul® MCM C8. Similar are also the surfactants that are used for the NLCs fabrication, some of which are Cremophor® EL, Cremophor®RH, Eumulgin SML, Lutrol F68, Span® 85, Tego Care 450, Speziol® TPGS Pharma, Myrj™ 59, Tween® 20,Tween® 80, Pluronic® F68, N-[1-(2,3-dioleyloxy) propyl]-N,N,N-trimethyl-ammonium chloride (DOTMA). The ratio between the solid lipid and the liquid lipid can range from 4:1 to 1:4, the surfactant concentration from 0.25 to 6 % (w/v), and the total lipid concentration from 1 to 30 % (w/v).

2.2. Fabrication methods

SLNs and NLCs can be fabricated using a variety of methods each one of which has its own advantages and disadvantages. The most common technique that is robustly

used is the high-pressure homogenization technique (HPH). Due to its easiness in handling, its relatively low cost, and its ability of large-scale production, HPH became the most popular technique for the fabrication of lipid nanoparticles. The small size and the low polydispersity of the nanoparticles are two more characteristics that make this technique so attractive. HPH can be categorized into hot and cold HPH method.

In the hot method, the lipid and the desired therapeutic, drug or nanoparticles, are mixed together above the melting temperature of the lipid and after the preparation of a pre-emulsion using an ultrasonic probe or a homogenizer or just stirring/vortexing, the lipid pre-emulsion is homogenized under high pressure. The high energies that are applied to the previously formed nano-emulsion results in sizes that span from the sub-micron range to as low as 40 nm [37].

On the other hand, in the cold homogenization method, after the lipid with or without the therapeutic molecule is melted, it is instantaneously cooled down using dry ice or liquid nitrogen, and milled creating sub-micrometer sized particles which are subsequently homogenized under high pressure, resulting in nanometer scale particles. Cold HPH was invented aiming at overcoming the limitations of hot HPH, one of which was the sensitivity of some therapeutic molecules above a certain temperature [38,39]. In both methods, the use of an organic solvent can be avoided making this technique also environmental friendly.

It has to be noted, that HPH can be used to decrease the size of lipid nanoparticles already formed by other methods, but in this case, a high percentage of smaller nanoparticles or drugs that have already been encapsulated in the lipid core will be released.

Two other methods that make use of high energies to reduce the particle size is the high-speed/shear homogenization technique (HSHT) and the emulsification-ultrasonication technique (EUT) [30,31]. These approaches are more versatile than HPH and the size range, as well as the polydispersity of the particles, depends on the amount of energy that is given to the system. Low energies result not only in high polydispersity and sub-micron sized particles, but also to lower short and long-term stability. Increasing the amount of energy, the size and the polydispersity are reduced, and the stability is increased. In certain cases, a combination of these techniques is applied in order to obtain nano-sized particles. It is noteworthy, that the size and the polydispersity do not only depend on the energy applied to the synthesis of these nanoparticles, but also on the type of the solid lipids, the liquid lipids and the surfactants that are used, since steric hindrances due to functional groups or the length of the chain and crystallization parameters are different among materials. An advantage of this method is that lower amount of lipids, surfactants, and solvent volumes can be used in contrast to HPH, that requires a minimum volume of 1-2 ml.

Another technique used for the synthesis of lipid nanostructures is the solvent emulsification/evaporation method (SEEM). This strategy is based on the oil-in-water (O/W) emulsion approach that is robustly used for the synthesis of polymeric nanoparticles and microparticles like poly(lactide-*co*-glycolide) particles [40–42]. In this technique, the solid and/or the liquid lipid are dissolved into a small amount of organic solvent, immiscible in water, and then they are added to a solution containing the surfactant at a ratio of maximum 10%. The mixed solutions are stirred or homogenized in high speeds, or they are ultrasonicated for a few minutes, aiming at

creating an emulsion. Depending on conditions used in each method, various sizes of nano- and/or microparticles can be obtained. After the emulsification process, the emulsion is left under mild stirring to evaporate the organic solvent, which leads to the hardening/stabilization of the particles [37,43–45]. Compared to the previously mentioned techniques, SEEM does not make use of high temperature, yet it owns the disadvantage of using toxic organic solvents.

In the case of hydrophilic drugs, a double emulsification method can be used. In this case, the hydrophilic molecules are entrapped in dynamic nano- and/or microstructures made by a water-in-oil emulsion (W/O), and, subsequently, this emulsion is mixed with an excess of a water-based surfactant solution and emulsified again creating a water-in-oil-in-water (W/O/W) emulsion. Again, the double emulsion is mildly stirred for a few hours to evaporate the solvent.

The same rationale for the fabrication of nanoparticles is also followed by the solvent displacement method (SDM), but this time the organic solvent is slowly added in a water solution under agitation without the creation of the emulsion. The solution is agitated until the complete evaporation of the solvent. Using the SDM method nanocapsules are fabricated. In contrast to the NLCs fabricated with the emulsion method, these nanocapsules are less stable and a leak of the therapeutic molecule is very common [24,31,42–44]. In order to increase the stability and to avoid drug leakage, the capsules are sprayed-dried or freeze-dried. In both cases, the stability increases but aggregations cannot be avoided. Spray-drying is an alternative method to freeze-drying but it is not used in extent due to the high temperatures and energies that are needed for the fabrication of particles [30,51,52].

The use of supercritical fluids for the fabrication of SLNs & NLCs is another method that is used for the preparation of these nanostructures. An emulsion is once more created; however, in this case, the organic solvent is removed with the help of a supercritical fluid, usually CO_2. The supercritical fluid technology (SFT) is more energy efficient compared to HPH since it does not make use of the high pressures of HPH. In addition, by using the supercritical fluid technology the use of solvents is avoided and the nanostructures are obtained in dry powder, rendering unnecessary a further step of freeze-drying for the stabilization of the nanostructures. Although SFT presents many advantages, until today it has been used mostly for the synthesis of SLNs and not for NLCs [53,54]. A method that has been extensively used for the synthesis of polymeric nanoparticles but not so much for lipid nanoparticles is the fatty acid coacervation technique. In this approach, the alkaline salts of fatty acids coacervate due to proton exchange between the acid solution and the emulsifier, as the pH is lowering. The advantage of this technique is its easiness but a great disadvantage is the size of the formulated particles [55].

2.3. Nanostructures for the treatment of brain diseases

Up to date, the synthesis of nanoparticles for the treatment of brain diseases has been very challenging due to the particularity of delivering therapeutics to the CNS. Numerous delivery strategies have been applied aiming at the fabrication of a delivery system that will combine specific characteristics like high loading efficiency, biocompatibility, and stealth features, but the most important characteristic would be the crossing through the BBB. Thus, inorganic and organic nanoparticles,

functionalized with targeting groups and/or coated with external layers of various biomimetic materials have been studied for this purpose. The most important nanostructures that have been studied for the delivery of therapeutics in the CNS are lipid-based structures like liposomes, SLNs and NLCs, lipoplexes, lipoproteins, polymeric nanoparticles, polymeric micelles, dendrimers, and inorganic nanoparticles including iron oxide, ceria, gold and quantum dots. Each of these systems owns its advantages and disadvantages, and in the next paragraph, we will try to briefly describe them. An overall description of the nanostructures that are used for the delivery of therapeutics to the CNS and their main advantages and disadvantages are given in **Table 1**.

2.3.1. Polymer-based nanostructures

Polymer-based nanostructures are used robustly in the biomedical field due to their versatility in synthesis and functionalization. The synthetic procedures allow their controlled size and shape as well as their loading ability. These polymeric nanostructures can be functionalized with desired ligands in order to target specific disease areas and depending on the monomers that are used for their fabrication, these nanomaterials have the ability to respond to physical/external (alternating magnetic field, light, electric current, ultrasound) [56–59] or biological/internal (enzymes, pH, reactive oxygen species - ROS -) stimuli [42,60–65]. The polymeric nanostructures are the first choice of researchers for the delivery of therapeutic molecules (*e.g.*, drugs, enzymes, genes, DNA, inorganic nanoparticles), and their therapeutic, as well as their diagnostic nature, has been studied extensively. From all the synthesized nanostructures those ones that are mostly used for the treatment of brain diseases are polymeric micelles, polymeric nanoparticles, and dendrimers.

Polymeric micelles

Polymeric micelles are created by the self-assembly of amphiphilic molecules in water above a critical concentration which is called critical micelle concentration. The hydrophobic head creates the core where hydrophobic drugs can be encapsulated while the hydrophilic tale creates the shell which is responsible for the stability of these structures and their prolonged circulation times *in vivo*. The micelle size is comprised within the range 10-100 nm and their dynamic loading ranges from 20 to 30%. Micelles are stable structures that provide controlled and sustained release and can be modified in a way that renders them responsive to external or internal stimuli. Micelles are preferred as a therapeutic vehicle for the treatment of CNS diseases and especially brain cancer because they have the inherent ability to penetrate the BBB and penetrate at the tumor site [66–69].

Polymeric nanoparticles

Polymeric nanoparticles have similar characteristics to micelles concerning their loading efficiency, functionalization versatility, and responsiveness to biological or physical stimuli. The size of the nanoparticles ranges usually from a few nanometers to a few hundreds of nanometers, but for brain applications the preferable size is usually below 200 nm. Polymeric nanoparticles for medical applications are designed in order to be biodegradable and the usual polymers that are used in this kind of applications are poly-ε-caprolactone, polylactides, polyglycolides and their combination poly(lactide-*co*-glycolide). Although these materials are FDA-approved, their low solubility and their degradation in acidic by-products are considered serious

limitations for their use in brain diseases. Moreover, the use of organic solvents for the preparation of the majority of these nanoparticles is one more drawback that may cause increased toxicity issues [66,68,70–75].

Dendrimers

Dendrimers comprise a class of polymeric nanostructures with well-defined architecture, high versatility in functionalization and internal cavities that allow the high encapsulation of drugs, genes, nucleic acids and other therapeutic agents. Dendrimers have been used as drug delivery systems due to their inherent ability to cross the BBB as well as the membranes of cells. Loading of dendrimers can be achieved either by encapsulation due to electrostatic interactions or by conjugation. In addition, the development of pH-sensitive dendrimers made possible the controlled release of encapsulated substances in diseased areas where the pH is acidic. Dendrimers like polyamidoamine (PAMAM) have been mostly used for cancer treatment, but applications for Alzheimer's disease, Parkinson's disease, multiple sclerosis and ischemic stroke have also been developed. Dendrimers, as polymeric structures, share the advantages and disadvantages of polymers but the main drawbacks that prohibit their robust use are the difficulties in scale-up and their inherent toxicity [76–80].

2.3.2. Inorganic nanoparticles

The main advantage in the use of inorganic nanoparticles except for their small size it is also their multi-functionality. Nanoparticles like ceria, iron oxide, gold, and inorganic quantum dots have been used robustly not only due to their therapeutic characteristics, yet also due to their imaging capabilities. Many of these nanoparticles combine both therapeutic and diagnostic ability, falling under the category of "theranostic" nanostructures. An obvious limitation of these nanoparticles is their low biocompatibility compared to the above-mentioned systems [81–88].

2.3.3. Lipid-based nanostructures

Liposomes

As we mentioned before, liposomes are spherical vesicles that consist of one or more phospholipid bilayers. These unilamellar structures of a size range that span from 10 to1000 nm are the predecessors of SLNs & NLCs and comprise the first generation of lipid nanostructures that were used for parenteral drug delivery. Some of the main advantages of liposomes are their high loading efficiency, their low toxicity, and their low antigenicity, while some of their disadvantages include low stability, difficulties in scale-up, fast clearance from the RES and a complex method of fabrication. Although the surface modification of liposomes (PEG coating), and the functionalization of their surface (antibodies, peptides, and aptamers) increases their circulation time, structural stability, and therapeutic ability, this is not enough to make these nanostructures attractive for industrial scale fabrication [23,67,68].

Lipoplexes

The self-assembly of liposomes with nucleic acids through electrostatic interactions results into multi lamellar lipoplexes with positively charged lipid bilayers, separated by the negatively charged nucleic acids. Since lipoplexes are created from liposomes, they share the same advantages and disadvantages that were described above, but also

they present one more disadvantage which is the high affinity of the polycation towards the bound nucleic acid that reduces transfection once inside the cell. Lipoplexes have been used for various brain diseases and more information can be found in the cited literature [89–95].

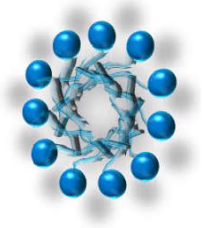
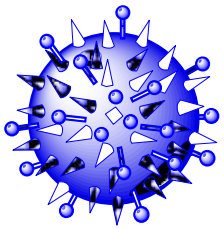
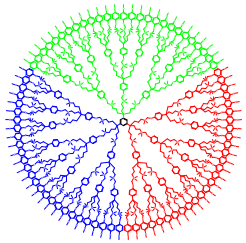
Lipoproteins

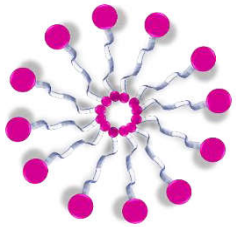
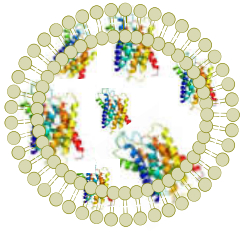
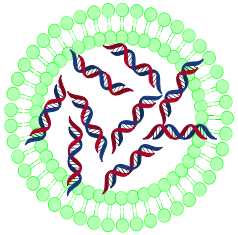
Lipoproteins are natural nanoparticles that are found inside the human body and their main role is the transportation of lipids (*e.g.*, cholesterol), proteins, enzymes, and microRNAs. Lipoproteins have been used as an alternative approach, either alone or in combination with other nanoparticles (*e.g.*, albumin, PEG-PLGA), for the treatment of various diseases including diseases of the CNS, and in principle, their advantages and disadvantages fall under the same category as the ones of liposomes [68,96–100].

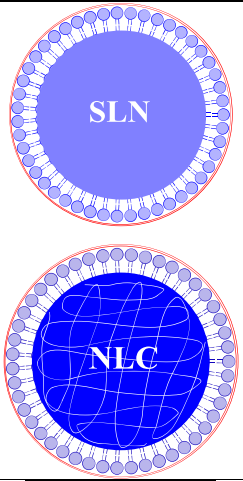
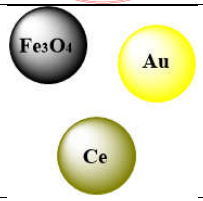
SLNs/NLCs

Solid lipid nanoparticles and nanostructured lipid carriers combine most of the advantages of the above-mentioned carriers, without being restricted by their limitations. One of their advantages that share with the polymeric nanoparticles is their small size, that allows them to easily flow in the blood stream without being uptaken by macrophages. One more characteristic that enhances the stealth ability of these nanoparticles against macrophages is the fact that they are made from natural materials and/or a combination of natural lipids, rendering them more biocompatible compared to their 'polymeric competitors'. Both polymeric nanoparticles and SLNs/NLCs can be functionalized with numerous targeting agents like peptides and antibodies, but in certain cases complex chemical reactions are needed for the functionalization of the polymer-based nanostructures compared to the lipid-ones. One great disadvantage of the polymeric nanoparticles is the burst release of encapsulated therapeutic molecules and their disability of long-term release. In order to achieve a long-term release an extra functionalization/modification is sometimes applied. These disadvantages are not found in the SLNs/NLCs due to their inherent ability of controlled release of various molecules (antioxidants, enzymes, therapeutic agents, *etc.*) for long periods of time. Release of encapsulated molecules is often realized due to the biodegradation of the therapeutic nanocapsule. This biodegradation often leads to increased toxicity when polymeric nanoparticles are used, a phenomenon that can be avoided when using SLNs/NLCs due to the fact that these nanoparticles are made from natural-based lipids that do not affect the extracellular/intracellular environment after their degradation. The fabrication procedure, where no need of organic solvents is necessary if compared to the fabrication of the polymeric structures and which allows reproducibility and high-scale production, the high biocompatibility as mentioned previously, and finally the low immunogenicity that the lipid-based nanostructures present, allow them to be rendered as the best candidates for targeting diseases of the central nervous system. Although this type of nanoparticles demonstrates all of these advantages, it is also characterized by low encapsulation efficiencies which probably is one of the basic limitations that to date has not allowed its mass production and exploitation from the numerous companies within the biomedical field [31,66–68].

Table 1. Advantages and disadvantages of nanostructures designed for targeted delivery of therapeutics to the CNS.

Nanostructure	Material	Type	Advantages	Disadvantages
	**Polymer-based**	Micelles	• Versatility in synthesis and functionalization • Controllable size and shape • High encapsulation efficiency and loading capacity • Responsive to physical (magnetic fields, light, electric current, ultrasounds) and/or biological stimuli (ROS, pH, enzymes) • Delivery of therapeutic molecules (drugs) and/or nanoparticles (iron oxide, cerium oxide, zinc oxide, copper oxide etc.) • Controlled and sustained release • Long term stability • Inherent ability to penetrate the BBB	• Inherent toxicity • Low encapsulation efficiency of hydrophilic therapeutics • Some polymers have low solubility in water and their degradation leads to acidic byproducts (e.g. PLGA) • Difficulties in scaling up • Use of organic solvents during fabrication
		Nanoparticles		
		Dendrimers		

Nanostructure	Material	Type	Advantages	Disadvantages
	**Lipid-based**	Liposomes	• Low toxicity • Low antigenicity • High loading efficiency • Controlled and sustained release	• Low stability • Fast RES clearance • Fabrication methods • Scalability • Reduced transfection due to high affinity of the nucleic acid with the lipid (only for lipoplexes)
		Lipoproteins		
		Lipoplexes		

Nanostructure	Material	Type	Advantages	Disadvantages
	**Lipid-based**	SLNs/NLCs	• Small and controllable size • Stability • Easy surface functionalization • No use of organic solvents during fabrication • High-scale production • Controlled and sustained release • Delivery of hydrophobic & hydrophilic drugs • No toxicity • Low immunogenicity • Biodegradation	• Low loading capacity • Drug expulsion during storage (only for SLNs)
	**Inorganic**	Gold NPs Iron oxide NPs Ceria NPs Quantum dots	• Small size • Multifunctionality • Theranostic use	• Low biocompatibility • Fast RES clearance if they are not functionalized

3. Brain diseases and the role of BBB

3.1. Blood Brain Barrier

BBB is a highly selective barrier that plays a pivotal role to the homeostasis of the brain by regulating the passage of various substances between blood flow and CNS. BBB is part of a bigger structure called neurovascular unit (NVU), that exists along all brain capillaries, and is composed of endothelial cells (EC), pericytes, vascular smooth muscle cells (vSMC), neurons, astrocytes and perivascular macrophages [101]. Except the brain capillaries, there are also peripheral capillaries, the main difference of which lies to the lack of fenestration and intercellular pores, due to the presence of inter-endothelial tight and *adherens* junctions, limiting the transport *via* paracellular pathways. The main molecular components of tight junctions are *zonula occludens* proteins (ZO-1,2,3) claudins, occludin and cingulin, while the main components of *adherens* junctions are cadherins (**Figure 2**) [101]. Molecules like proteins, enzymes and nutrients can travel in and out of the CNS through different pathways. In the next paragraphs, the most important neurological diseases will be briefly analyzed, pointing out their molecular basis, their current limitations and their future perspectives concerning their treatment. The brief description that will follow is intended to give the reader a general view of the major CNS pathologies and does not have the claim of analyzing in detail each disease. For further details, the readers are advised to check the works cited at the end of the review.

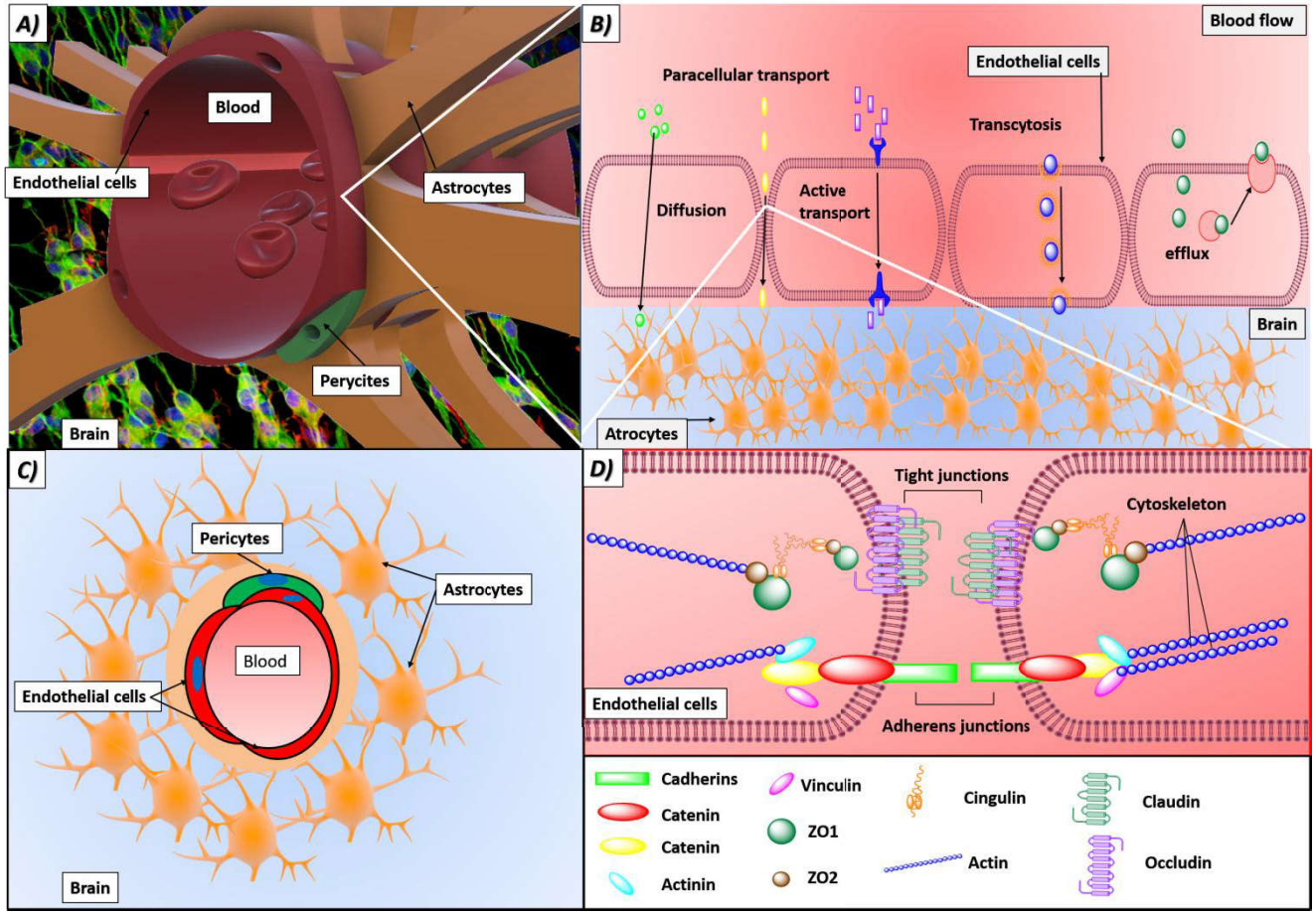


Figure 2. A) and C) Representation of 3D and 2D structures of the BBB with the main components of NVU. B) Representation of the principal transport systems involved in the molecule passage through the brain endothelium. D) Depiction of the molecular constituents of junctions among BBB endothelial cells.

3.2. Brain cancer

Brain tumors can be divided in two macro groups: primary brain tumors which originate from brain tissues, and secondary brain tumors that are the result of the metastatic spreading of cancer cells, originated in other regions. Primary brain tumors are in turn classified into gliomas, central nervous system lymphomas (both of which originates from brain parenchyma and are the most frequent type of brain tumors), meningiomas and pituitary adenomas, that are extraparenchymatous tumors [102]. Gliomas are primary tumor that can originate from neural stem cells, progenitor cells, or from de-diff erentiated mature neural cells transformed into cancer stem cells. They are classified on the basis of both their origins in astrocytomas, oligodendroglioma and mixed oligoastrocytoma, and on the basis of their aggressiveness in grades ranging from 2 to 4. Currently, the most used treatments known for brain tumors involve the use of surgical approaches and/or radiotherapy, that not only present a large amount of risk for the patient, but are also unable to efficiently treat more aggressive form of gliomas [103]. Finally, pharmacological treatments should be able not only to target cancer cells, yet also to destroy brain tumor stem cells in order to prevent a repopulation effect and to prevent all the processes involved in tumor metastatic spreading, including angiogenesis and cell extravasation. In **Figure 3** the cellular mechanisms involved in brain cancer and other CNS diseases are presented.

3.3. Ischemic Stroke

The term stroke is usually referred to an "acute neurologic dysfunction of vascular origin with sudden (within seconds) or at least rapid (within hours) occurrence of symptoms and signs corresponding to the involvement of focal areas in the brain" [104]. Stroke is divided in ischemic, in which blood supply to the brain is interrupted, and hemorrhagic. Ischemic stroke is again divided on the basis of its cause into thrombotic stroke when the cause is an artery thrombus, and in embolic stroke when the cause is the presence of emboli [105]. In both cases the interruption of blood flow to the brain cells causes imbalances of pH, glucose, oxygen, and other nutrient levels, leading to cell death. Currently, the only pharmacological treatment available and approved by FDA for ischemic stroke is the recombinant tissue plasminogen activator (r-tPA), which elicits a thrombolytic action by breaking blood clotting and restoring interrupted blood flow.

3.4. Parkinson's disease

PD is mainly caused by the neuro-degeneration of the dopaminergic neurons in the *substantia nigra pars compacta*, which is a basal ganglia structure involved in reward and movement control. The main symptoms of the disease are tremor, bradykinesia, rigidity, and postural instability. The disease evolves with a progressive loss of movement control that leads to severe respiratory and gastro-intestinal complications, causing in the end the patient's death [106]. To date, the molecular basis of PD is still largely unknown. Currently there are no treatments for PD able to fully cure the disease. All the existent approaches are symptomatic treatments that try either to

restore dopamine levels or to ameliorate movement impairments. The most used pharmacological treatment for PD is still levodopa.

3.5.Alzheimer's disease

AD is one of the most common forms of dementia, with more than 24 million cases worldwide [107]. This disease is characterized by decreased cognitive and physical functions and loss of memory, and it eventually leads to the patient's death due to neuronal cell loss. The disease can manifest itself in the form of familiar AD, usually due to genetically causes, or in the form of sporadic AD caused by environmental factors. The principal areas of the brain affected by AD are neocortex, primarily involved in the processing of sensory information, and limbic system that plays an important role in the control of emotion, instinctive behavior, learning, and short-memory. The basis of the disease is still largely unknown, but its main hallmark is the presence of accumulation of amyloid beta (Aβ peptide) in the form of a plaque. Several forms of familiar AD have been related to the presence of genetic mutations in the sequences of Aβ peptides leading to a higher ratio of accumulation of oligomers, while disruption of clearance system due to genetic causes or environmental factors has been observed in sporadic form of AD. Currently, all the treatments for AD are only able to ameliorate symptoms but cannot lead to the regression of the disease or to its complete cure. Although many therapeutic molecules for the treatment of AD have been proposed, the development of specific drugs able not only to cross the BBB, yet also to inhibit the neurodegeneration and to heal damaged neurons, is still an open challenge.

3.6. Multiple sclerosis

The term Multiple sclerosis (MS) refers to a progressive and chronic disease involving the immune-mediated demyelination of the CNS white matter, involving visual neuron, basal ganglia, brain stem, and spinal cord [108]. Current treatments of the MS can be classified into symptomatic, that try to ameliorate symptoms of the disease, and effective treatment targeted to alter disease progress. A recent work by Dolati *et al*. [108] summarizes the current disease-modifying therapies for MS, most of which act by interfering with B and T cells activity, and reducing BBB disruption.Therefore, the development of carriers able to cross BBB and to specifically target immune cells is a priority in order to obtain more specific treatments with minimal side effects.

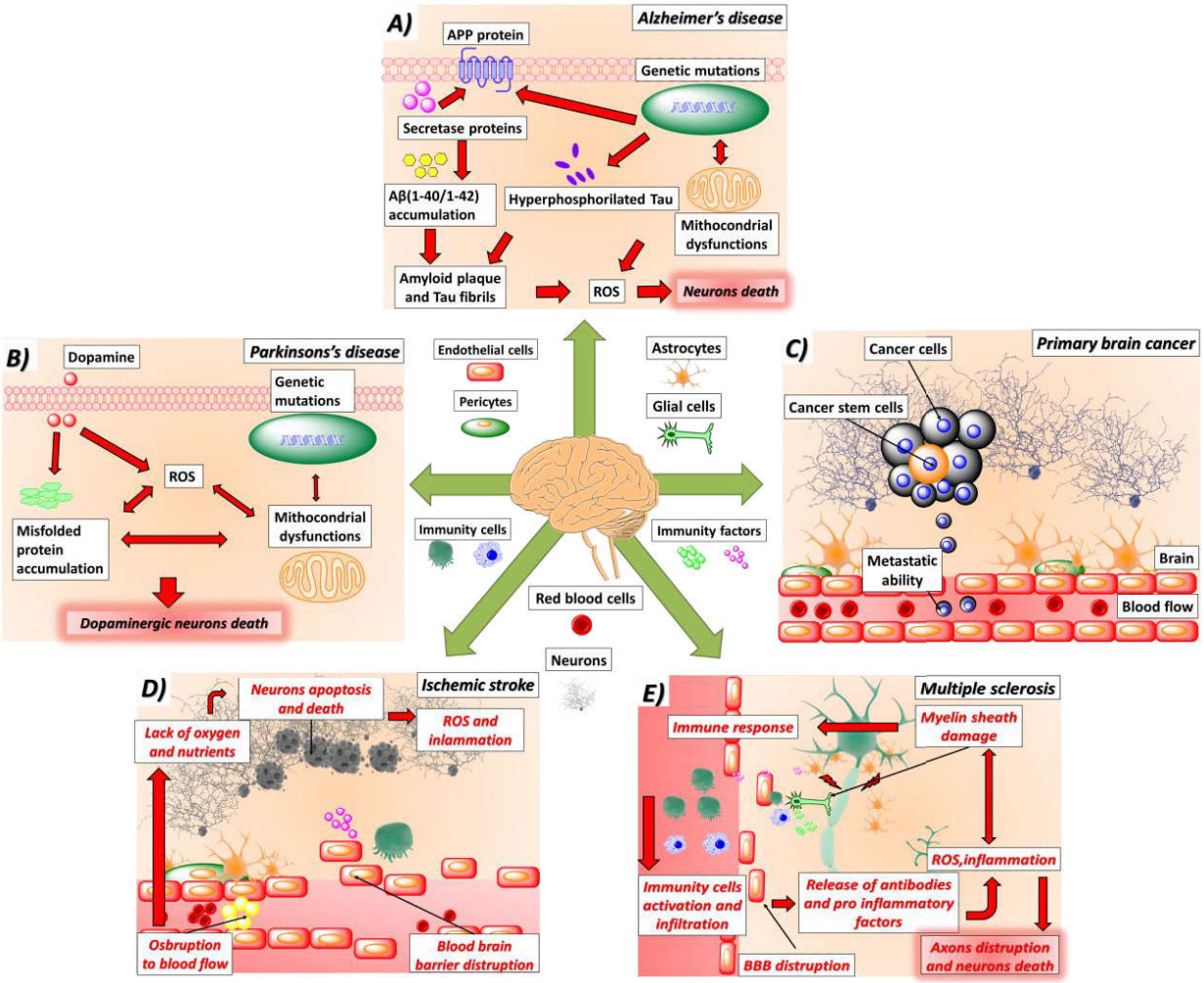


Figure 3. Representation of the main diseases affecting the central nervous system and of the involved cellular mechanisms. A) Alzheimer's disease; B) Parkinson's disease; C) primary brain cancer; D) ischemic stroke; E) Multiple sclerosis.

4. Brain-targeting SLNs and NLCs for CNS diseases

4.1. SLNs and NLCs in brain cancer

Solid lipid nanoparticles and nanostructured lipid carriers have been used for the treatment of various brain diseases and mostly for brain cancer, due to their attractive properties. One of the studies presented in the last seven years was related to the synthesis of cationic solid lipid nanoparticles (CASLNs) which were functionalized with an anti-EGFR receptor aiming at targeting malignant glioblastoma cells [109]. The authors used the microemulsion method (MEM) to prepare cationic SLNs, and they demonstrated that the entrapment efficiency of carmustine (CRN) was related to the concentration of cationic surfactants that they were using, and that the release profile was dependent on the concentration of the cacao butter that it was used. Studies on the U87 cell line demonstrated an effective delivery and antiproliferative properties while studies in human brain microvascular endothelial cells demonstrated the enhanced viability and a decrease in the expression of tumor necrosis factor (TNF)-α.

The same authors, in a follow-up work, have studied a variety of lipids including palmitic acid (PA), cacao butter (CB), stearic acid (SA), Dynasan® 114, Compritol® 888 ATO (CA), cardiolipin, tripalmitin and behenic acid (BA), anticancer drugs including doxorubicin (DOX) and etoposide (ETP) and various targeting groups including aprotinin (Apr), anti-melanotransferrin (Anti-Mtf), folic acid (FA), p-aminophenyl-α-D-manno-pyranoside, serotonergic 1B receptor subtype antagonist (S1BRSA), 83-14 monoclonal antibody (8314Mab), anti-endothelial growth factor receptor (AEGFR), tamoxifen and lactoferrin. Depending on the lipids and on the surface functionalization the size ranged from 80 to 280 nm and the surface charge was either positive or negative and ranged from 14 to 40 mV and to -13 to -38 mV, respectively. The encapsulation efficiency also varied from 17 to 95%. All the *in vitro* studies were carried out using the same cell lines (HBMEC, human U87 malignant glioma, human astrocytes) and the results demonstrated that the SLNs were non-toxic, had antiproliferative effects, and could infiltrate the BBB suggesting their potential therapeutic use in the treatment of glioblastoma multiforme (GBM) [109–117]. A detailed description of the basic characteristics of each study can be found in **Table 2**.

In another study, solid lipid nanoparticles comprised either by Compritol® or Precirol® were prepared using a combination of hot melt homogenization technique (HMHT) and ultrasonic homogenization technique (UHT) and they were loaded with a prototype anticancer drug named edelfosine (EDF). These nanoparticles, the size of which was a little higher than 100 nm, were able to easily accumulate in the brain tissue, something that was attributed to the interaction of the surfactant Tween® 80 with P-glycoprotein (P-gp). These nanoparticles were tested *in vitro* in a C6 glioma cell line as well as *in vivo* in a C6 glioma xenograft tumor. The results showed an antiproliferative effect and a significant reduction in the tumor growth [118].

To overcome the stability problems and the low loading capacity of the SLNs, another group presented the fabrication of NLCs that were loaded with cytarabine (CRB), aiming at targeting meningeal leukemia. The NLCs had an average size of approximately 90 nm and they were coated using Tween® 80 aiming at overcoming the P-gp inhibition. The entrapment efficiency of these nanoparticles was found to be

approximately 50%, which was very high considering the hydrophilic nature of the encapsulated drug. The blank NLCs and the loaded NLCs (Cyt-NLCs) were characterized using differential scanning calorimetry (DSC) and X-ray diffraction analysis (XRD). The *in vitro* release profile of the Cyt-NLCs presented a fast release (ca. 16%) in the first hour but a slower and sustained release for the following 72 h (ca. 90%). Treatment of the EL-4 cell line with blank NLCs did not present any cytotoxic effects, while treatment with the loaded NLCs presented a dose-dependent cytotoxicity which was also higher compared to the treatment with the free drug [119].

The use of small interfering RNAs (siRNAs) is a promising strategy for the treatment of various diseases but due to their instability and their poor delivery into target tissues siRNAs are not robustly used. The use of encapsulated siRNAs in solid lipid nanoparticles was reported in the literature [120], as a way to overcome the limitations that siRNA presents and to enhance its therapeutic efficacy. In this study, low-density lipoprotein (LDL) was conjugated with a PEGylated c-Met (tyrosine-protein kinase Met or hepatocyte growth factor receptor) siRNA aiming at forming spherical nanoparticles. The *in vitro* studies in U87MG demonstrated a decrease in cell proliferation and a down-regulation of the expression levels of c-Met. The *in vivo* experiments in a U87MG xenograft tumor demonstrated an enhanced accumulation of the SLNs to the brain tumor, a down-regulation again in c-Met levels, and also a tumor suppression rendering this nanoparticle a potential candidate for the treatment of glioblastoma multiforme.

SLNs of cetyl palmitate (CP), stabilized with Tween® 60 or Tween® 80 were used for internalization studies, and the results demonstrated that the synthesized nanoparticles of a mean diameter of 200 nm and a surface charge of -20 mV were internalized by gliomas (A172, U251, U373 and U87 cell lines) in a higher amount than macrophages (THP1 cell line). The assessment of the cellular uptake mechanism was carried out using nanoparticles loaded with rhodamine 123 (R123), and it was found that the nanoparticles were internalized by a clathrin-dependent endocytic pathway [121]. A few months later, the same nanoparticles, prepared with a different technique and with a small modification in the used materials, were loaded with the drug camptothecin (CPT) aiming at increasing its therapeutic activity. *In vitro* studies demonstrated an increased uptake of the nanoparticles from porcine brain capillary endothelial cells (BCEC) compared to macrophages (RAW 264.7). In addition, cell viability of BCEC was decreased in CPT-loaded SLNs compared to free CPT. The *in vivo* studies in this work revealed the successful delivery of R123 to the brain [122]. A follow-up study using the same nanoparticles, but modified with 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), revealed that both loaded and unloaded nanoparticles have an orthorhombic sub-cell packing that is preserved in contact with the DMPC membrane that helps in the efficient release of CPT. In addition, biodistribution studies demonstrated that the concentration of the encapsulated CPT was higher in the serum and the brain compared to the free drug and that the loaded CPT could be detected in the brain even after 24 h, where the free drug could be detected only up to 8 h [123].

A targeted approach of NLCs based on CP and three different fatty amines was also presented at the same time, and in this work, the NLCs were loaded with etoposide

and functionalized with an anti-transferrin receptor aiming to create a targeted delivery system for the treatment of myelogenous leukemia. The nanostructures were prepared by combining the emulsion-solvent evaporation method and the ultrasonication method, and the functionalization of the particles with transferrin (Trf) was carried out in two ways: using covalent conjugation and physical coating. The size of the particles ranged from 125 to 250 nm and their surface charge from +25 to +45 mV. The K562 cell line was used for the *in vitro* cytotoxicity studies using MTT, and flow cytometry was used for cell uptake investigation. The functionalization of the nanostructures with Trf decreased their surface charge and their loading efficiency, and also increased their size. Nevertheless, the loaded NLCs conjugated with Trf showed higher cellular uptake and enhanced antiproliferative effect with respect to the unconjugated ones [124].

A different approach was used from another group to fabricate lipid nanocapsules (LNC). These nanocapsules had a size of approximately 90 nm and a surface charge of +11 mV. The nanocapsules were functionalized with the L1 peptide and, in contrast to other published studies, they were not loaded with drugs, yet instead with a locked nucleic acid (LNA) aiming at silencing the oncogenic miR-21. The *in vitro* studies in the U87 malignant glioma (U87MG) cell line demonstrated a reduction of miR-21 expression, which resulted in an increased cell sensitivity after radiotherapy [125].

Curcumin is a well-known antioxidant, the therapeutic properties of which have been suggested for the treatment of brain cancer. Unfortunately, due to its low hydrophilicity, its delivery across the blood brain barrier is minimal. A study in which curcumin was loaded into lipid nanocapsules prepared by interfacial deposition of polymers was presented a few year back. Here, lipid nanostructures of approximately 200 nm and a negative zeta potential have been fabricated and it has been demonstrated that curcumin had a sustain release profile for over 48 h. The *in vitro* studies in the U251MG cell line showed that the curcumin-loaded LNCs (C-LNC) were more cytotoxic compared to the free curcumin. Moreover, *in vivo* studies on rats bearing C6 gliomas presented a decrease in tumor size and malignancy, and prolonged the animal survival when they were treated with C-LNCs, in comparison to the animals that were treated with the same dose of the free drug [126].

Lipid nanocapsules were also used from another group, but this time the encapsulated therapeutic molecule was indomethacin. The results from these studies carried out in C6 and U138-MG glioma cell lines showed that the indomethacin-loaded lipid nanocapsules (IndOH-LNCs) reduced cell viability by inducing apoptotic cell death. Furthermore, an interesting finding of this study was that the IndOH-LNC did not affect the viability of human astrocytes suggesting the selectivity of these LNCs towards glioma cells. Moreover, it was shown that IndOH-LNC modulated the cell cycle dynamics and promoted the cell differentiation of glioblastoma cells [127].

The next published work presented the fabrication of solid lipid nanoparticles loaded with resveratrol (RVR). These nanostructures presented a size ca. 250 nm and a ζ-potential of about -25 mV. The authors in this work showed that RVR-loaded nanoparticles had the same cytotoxic effect as the non-encapsulated drug and that the RVR concentration in the brain was higher (17.2800 ± 0.6344 mg/g) when the drug was encapsulated inside the SLNs, compared to the free drug (3.4500 ± 0.3961 mg/g).

It has to be noted that the nanoparticles were not functionalized with a targeting moiety, proving their inherent ability of passive targeting into the brain [128].
Another system based on lipid nanoparticles was presented almost at the same time, where SLNs were fabricated using the fatty acid coacervation technique (FACT). Doxorubicin was encapsulated inside these SLNs aiming at enhancing its permeation through an *in vitro* BBB model. The disadvantage of this technique is the large size of the fabricated nanoparticles wherein the specific case ranged from 278 to 1600 nm. Nevertheless, it was demonstrated that the permeation of doxorubicin through an hCMEC/D3 cell monolayer (BBB model) was higher when DOX was encapsulated inside the SLNs compared to the free DOX, and also that DOX-loaded SLNs did not affect the cytotoxicity in three types of glioblastoma cells (hCMEC/D3, primary human glioblastoma cells (CV17, 01010627), U87MG)[129].
The fatty acid coacervation technique was used once more to fabricate spherical nanoparticles, of diameter 300-600 nm, loaded with paclitaxel (PTX). These nanoparticles had a positive surface charge (8-20 mV) and their loading efficiency spanned from 25 to 90%. The SLNs were able to penetrate an hCMEC/D3 cell monolayer which was used as a BBB model. From this study, it was concluded that even though stearyl amine positively charged nanoparticles gave the best results, the positive charge was not affecting so much the permeation ability. In addition, an increased toxicity towards glioblastoma cells was presented from the PTX-loaded SLNs [130].
Curcumin was used once more as an anticancer drug in another study, where the authors encapsulated the drug in NLCs (CRM-NLCs). The fabricated system had a size of 150 nm, a polydispersity index (PDI) of 0.18, a ζ-potential of -20 mV and a loading efficiency ca. 90%. The loaded NLCs not only presented enhanced cytotoxicity towards the U373MG cell line, but they also presented higher accumulation to the brain (86201.0 ± 8182.1 ng/g after 120 min) compared to the free curcumin (54321.0 ± 2098.8 ng/g after 180 min) suggesting this drug delivery system as a potential candidate for the treatment of GBM [131].
Hyaluronic acid (HA) was used as another targeting approach for the treatment of GBM since it has the ability to bind to the CD44 receptor which is overexpressed in this type of cancer. The authors of this work fabricated lipid-based nanoparticles (LNP) loaded with polo-like kinase 1 (PLK1) siRNAs (siPLK1), and demonstrated an enhanced reduction in the expression of PLK1 mRNA that resulted in increased cell death. The fabricated HA-LNPs had a size of 100 nm, a slightly negative charge of -8 mV and an entrapment efficiency of 80%. HA coating increased the permeability of the nanoparticles even under shear flow, and the *in vivo* studies in an orthotopic xenograft model showed reduced mRNA levels by more than 80% and a significantly prolonged survival of the treated mice. It is noteworthy that these results were beyond any published report in this model [132].
SLNs loaded with bevacizumab (BVZ) were reported elsewhere [133]. The nanoparticles were prepared using the coacervation technique, and even though their minimum size was 500nm, the *in* vitro results demonstrated that the BVZ-loaded SLNs had the ability to penetrate the hCMEC/D3 monolayer of cells and to increase the activity of BVZ by 100- to 200-fold compared to the free BVZ.

Temozolomide (TMZ) is one of the most robust drugs that are currently used for the treatment of brain cancers, and a published report demonstrated its therapeutic advantage when it is loaded inside NLCs [134]. In this work, TMZ was encapsulated along with DNA and their therapeutic efficacy was evaluated against U87MG cells. The fabricated NLCs had a size of 180 nm, a ζ-potential of +23 mV, an encapsulation efficiency of 83% and a gene loading of 91%. In addition, *in vivo* studies demonstrated a high gene transfection and anti-tumor activity. In another report, the authors fabricated NLCs loaded with TMZ and functionalized with an arginine-glycine-aspartic acid (RGD) peptide [135]. The nanostructures had a size of 120 nm, a positive surface charge of +28 mV and an encapsulation efficiency of 85%, and presented an inhibitory effect against U87MG glioma cell that renders them a promising strategy for the treatment of *gliomatosis cerebri*.

An interesting study from the same group was published one year later, and reported the advantages and disadvantages among SLNs, NLCs and polymeric nanoparticles (PNPs) prepared by the solvent diffusion technique (SDT) for the delivery of TMZ to the brain [49]. All the nanoparticles had a size around 100 nm and an entrapment efficiency of 80%. The difference in these nanoparticles was in the surface charge: while the PNPs exhibited a high positive charge, the SLNs and NLCs instead a highly negative one. From all the above formulations the TMZ-loaded NLCs (TMZ-NLCs) displayed the best antitumor activity both *in vitro* against U87MG cell line and *in vivo* in a mice-bearing malignant glioma model. The same group after a while presented the fabrication of NLCs loaded with both TMZ and DNA (TMZ/DNA-NLCs).

In another study, the therapeutic efficacy of SLNs and NLCs loaded with TMZ and vincristine (VCR) was evaluated [136]. The SLNs & NLCs were compared with SLNs and NLCs loaded only with TMZ (T-SLNs & T-NLCs). The *in vitro* and *in vivo* studies presented a better anti-tumor activity using the NLCs and also the co-delivery of the two encapsulated drugs (VT-SLNs & VT-NLCs) was better than the use of only TMZ. The inhibition rates for the T-SLNs, T-NLCs, VT-SLNs, and VT-NLCs were 42.94%, 70.26%, 55.70% and 83.17%, respectively, while the inhibition rate for the free TMZ solution was 26.34%.

NLCs loaded with curcumin (Cur-NLCs) were reported in a recent study [137]. The fabricated NLCs had a size of 210 nm and an encapsulation efficiency of 88%. The results showed that the IC50 value of Cur-NLCs was 75% lower than that of the free curcumin, proving the enhanced activity of the Cur-NLCs. Moreover, the encapsulation of curcumin prolonged its half-life and increased its levels in the blood by 6.4-folds. Further studies showed an enhanced efficiency of Cur-NLCs in mice bearing A172 xenografts with an inhibition ratio of 82.3% on tumor growth, and also showed that the inhibition effect was mostly due to apoptosis and not necrosis.

The most recent study for the treatment of GBM that was reported [138] made use of PTX-loaded NLCs that were functionalized with Trf. In this report, different formulations of NLCs were fabricated and tested against U87MG cells. The NLCs had a size ca. 200 nm, a surface charge of +25.7 mV, an EE of 92% and a loading capacity of 5.38%. The above values were slightly decreased after the conjugation of the NLCs with Trf (Tf-PTX-NLCs), but its cytotoxic effectiveness was increased compared to unconjugated NLCs. Finally, *in vitro* release profiles showed a slow and sustained release over a period of time.

The last reported study for the treatment of glioblastoma multiforme was presented a few months ago [139,140]. The authors of this study fabricated lipo-polymeric nanoparticles (LPNPs) by using a combination of C15 lipids, DSPE-epoxy-PEG 2000 and polyethyleneimine (PEI) of molecular weight 600. The size of the nanoparticles spanned from 40 to 135 nm, depending on the ratio between the lipid segment and the PEI segment. The LPNPs were conjugated with an RNAi and targeted the brain tumor-initiating cells (BTICs), which have been reported to be responsible for therapy resistance, recurrence, and progression of diffuse gliomas. The delivery of the LPNPs in an *in vivo* established brain model was achieved using a convection-enhanced delivery method (CED) which it has to be noted that at this stage is under clinical trials. The results showed a significant extension of median survival in two patient-derived BTIC xenograft mouse models of GBM. Another important finding of this study was that limited dosing is insufficient to overcome tumor growth despite a clear attenuation of malignant tumor growth.

Table 2. Basic properties and characteristics of SLNs & NLCs used for the treatment of brain cancer

Type of carrier	Solid lipid	Liquid lipid	Emulsifiers/ stabilizers/ surfactants	Preparation method	T (°C)	Size (nm)	Zeta (mV)	EE (%)	DL (%)	Therapeutic molecule	Targeting moiety	*In Vitro*	*In vivo*	Ref
PNP	PLGA	N/A	PVA	SDT	25	109.1 ± 4.3	-28.2 ± 3.1	83.6 ± 3.2	10.8 ± 1.1	TMZ	N/A	U87MG	BALB/c nude mice (5–6 week-old, 18–22 g)	[49]
SLN	StA	Soya Lecithin	DDAB	SDT	25	94.6 ± 3.1	+41.2 ± 4.1	82.3 ± 2.8	9.6 ± 0.9					
NLC	Compritol® 888 ATO, SPC	Soya Lecithin	Cremophor® ELP, Tween® 80 & DDAB	SDM	80-85	121.4 ± 5.6	+29.1 ± 2.4	81.4 ± 3.7	5.2 ± 0.6					
SLN	StA, CB, DSPE-PEG-COOH 5000	-	HTMAB, SDS	MEM	75	180 -560	-2 to -10	50 to 81	N/A	CRN	Anti-EGFR	HBMECs, U87MG	N/A	[109]
SLN	StA, CB, DSPE-PEG-COOH 5000	-	HTMAB, SDS	MEM	75	N/A	N/A	38 to 60	N/A	DOX	Anti-EGFR	HBMECs, U87MG	N/A	[110]
SLN	PAc, CB, DPPC, DSPE-PEG2000-COOH	-	cholesteryl hemisuccinate, taurocholate, Tween® 80 1-butanol	HSHT	25	110-170	-20 to -30	35-85	N/A	DOX	Apr, Anti-Mtf	HBMECs, Human astrocytoma, U87MG	N/A	[111]
SLN	Dynasan® 114 PAc, StA, DSPE-PEG2000-CA, DSPE-PEG2000-AM	-	cholesteryl hemisuccinate, taurocholate L-A-phosphatidylcholine type II-S	O/W emulsion	75	80-240	-14 to -38	N/A	N/A	ETP	APMP, FA	HBMECs, Human astrocytoma, U87MG	N/A	[112]
SLN	Compritol® 888 ATO, CB, DSPE-PEG2000-CA	-	TTMAB, SDS	MEM	75	130-210	N/A	55-95	N/A	CRN	S1BRSA	HBMECs, U87MG, Human astrocytes	N/A	[113]

Table 2 (Continue). Basic properties and characteristics of SLNs & NLCs used for the treatment of brain cancer

Type of carrier	Solid lipid	Liquid lipid	Emulsifiers/ stabilizers/ surfactants	Preparation method	T (°C)	Size (nm)	Zeta (mV)	EE (%)	DL (%)	Therapeutic molecule	Targeting moiety	*In Vitro*	*In Vivo*	Ref
SLN	Compritol® 888 ATO, StA, CA, DSPE-PEG2000-CA	-	cholesteryl hemisuccinate, taurocholate, Tween® 80 1-butanol	MEM		125-210	-13 to -32	71.7 ±4.1	N/A	ETP	8314Mab, AEGFR	HBMECs, U87MG	N/A	[114]
SLN	Tripalmitin CB, CA DSPE-PEG2000-COOH	-	cholesteryl hemi-succinate taurocholate, n-butanol, L-A-phospatidylcholine type II-S	MEM, HSHT	25	80-150	+20 to +40	30-85	N/A	ETP	Anti-Mtf	HBMECs, U87MG, human astrocytes	N/A	[115]
SLN	Behenic acid, Tripalmitin, CB, DSPE-PEG2000-COOH	-	cholesteryl hemisuccinate taurocholate, L-butanol, Tween® 80, L-A-phosphatidylcholine type II-S	MEM, HSHT	25	100-180	+17.5 to +32.5	12.5-85	N/A	CRN	TMX, Lf	HBMECs, U87MG, human astrocytes	N/A	[116]
SLN	Compritol® ATO 888. Tripalmitin, StA, Cholesterol, DSPE-PEG2000-COOH		cholesteryl hemi-succinate taurocholate, L-butanol, L-A-phospatidylcholine type II-S	MEM	75	180-280	+14 to +26	17-75	N/A	ETP	Anti-Mtf, TMX	HBMECs, Human U87MG, Human astrocytes	N/A	[117]
SLN	Compritol® 888 ATO	-	Tween® 80	HMHT & UHT	60	111.2 ±3.1	−20.6 ± 2.1	85.53 ±6.92	18.49 ±2.77 (µg/mg)	EDF	N/A	C6 rat glioma cells	BALB/c mice	[118]
NLC	Precirol® ATO5	Oleic Acid	Tween® 80	EUT	75	105.4 ± 2.5	−21.6 ± 3.1	84.62 ± 4.98	15.31± 3.29 (µg/mg)	CRB	N/A	Leukemic EL-4	N/A	[119]
	StA				80	90.7 ±4.28	-24.2 ±1.89	49.5 ±2.24	N/A					
SLN	Chol, DOPE, DC-chol, Cholesteryl oleate, glyceryl triolate	-	-	SEvaM	60	117.4 ± 11.7	37.3 ± 2.3	N/A	N/A	siRNA	c-MET	U87MG	Male Balb/c-nu mice (6-week-old)	[120]

Table 2 (Continue). Basic properties and characteristics of SLNs & NLCs used for the treatment of brain cancer

Type of carrier	Solid lipid	Liquid lipid	Emulsifiers/ stabilizers/ surfactants	Preparation method	T (°C)	Size (nm)	Zeta (mV)	EE (%)	DL (%)	Therapeutic molecule	Targeting moiety	*In Vitro*	*In Vivo*	Ref
SLN	CP	-	Tween® 60, 80	EUT	55-65	130-165	-18 to -23	N/A	N/A	R123	N/A	A172, U251, U373, U87, THP1	N/A	[121]
SLN	CP or Dynasan® 114 or Witepsol® E85	-	Tween® 20, 40, 60, 80	HPH	55-65	<200	-8 to -14	74-97	0.5-2	CPT, R123	N/A	Porcine BCEC, RAW 264.7	Wistar rats (230–250 g)	[122]
SLN	CP, DMPC	-	Tween® 60, 80	HMHT & UHT	55-65	131-160	-18 to -24	91-93	N/A	CPT	N/A	A172, U251, U373, U87, THP1	male Wistar rats (250–300 g)	[123]
NLC	CP, Stearylamine or Dodecylamine or Spermine	Octyl dodecalnol, Soy lecithin	Poloxamer 188	SDM & EUT	60	125 to 247	+25 to +45	19 to 90	0.39 to 1.86	ETP	Trf	K562	N/A	[124]
LNC	Labrafac™ WL 1349	-	Solutol® HS15, Lipoïd® S75-3	Phase inversion	85	88.21 ± 2.14	11.09 ± 1.09	N/A	N/A	LNA	L1 peptide	U87MG	N/A	[125]
LNC	Sorbitan monostearate & PCL	Grape seed oil	Tween® 80	IDP	25	196 ± 1.40	-9.56 ± 0.66	100	0.50 ±0.02 mg/ml	CUR	N/A	C6 glioma, U251MG	male Wistar rats (8 weeks old, 220–260 g)	[126]
LNC	Sorbitan monostearate, &PCL	Labrafac CC	Tween® 80	IDP	40	231.0 ± 4.0	−7.0±1.3	100	0.998 ± 0.010 mg/mL	IndOH	N/A	C6 glioma, U138MG, astrocytes	N/A	[127]
SLN	Compritol® 888 ATO	-	Tween® 80, PVA	SEEM, EUT, HSHT	25	248.3± 3.80	-25.49 ± 0.49	33.93 ± 1.21	3.08 ±0.10 µg/mg	RVR	N/A	C6 glioma	Female adult Wistar rats (150–200 g)	[128]
SLN	PAc, StA, Arachidic acid, Behenic acid	-	PVA 9000, Fatty acid sodium salt	FACT	75	278-1600	N/A	40-79	N/A	DOX	N/A	hCMEC/D3, primary human glioblastoma cells (CV17, 01010627), U87MG	N/A	[129]
SLN	Stearylamine, Cholesterol	-	PVA 9000, Sodium behenate	FACT	75	299-985	-3 to +24	25-98	16 to 108 (PTX µg/BA mg)	PTX, Coumarin 6	N/A	hCMEC/D3, U87MG, primary human GBM cells: NO3 & CV17	N/A	[130]

Table 2 (Continue). Basic properties and characteristics of SLNs & NLCs used for the treatment of brain cancer

Type of carrier	Solid lipid	Liquid lipid	Emulsifiers/ stabilizers/ surfactants	Preparation method	T (°C)	Size (nm)	Zeta (mV)	EE (%)	DL (%)	Therapeutic molecule	Targeting moiety	*In Vitro*	*In Vivo*	Ref
NLC	Precirol® ATO5	Capmul®, Soya lecithin	Tween® 80	HPH	85	146.8	-21.4±1.87	90.86	N/A	CUR	N/A	U373MG	Male Wistar rats (250–270 g)	[131]
HA-LNP	Dlin-MC3-DMA, DSPC, Chol, DMG-PEG, DSPE-PEG-NH_2	-	-	Microfluidic micro-mixture	25	100.7±3.00	-8.2±0.7	80.0 ±11.0	N/A	siRNA	Hyaluronic Acid	T98G, U251, U87MG	athymic BALB/c nu/nu mice	[132]
SLN	Sodium Stearate, Sodium Palmitate	-	AOT, HPMC	FACT	75-85	515-1213	N/A	30-31	~30µg/mg	BVZ	N/A	HUVEC, hCMEC/D3	N/A	[133]
NLC	Compritol® 888 ATO, and SPC RGD-PEG-DSPE	Soya lecithin	Cremophor® ELP, Tween 80 & DDAB	SDM	80-85	179	+23	83	GL: 91%	TMZ & DNA	RGD	U87MG	BALB/c nude mice (5–6 week-old, 18–22 g)	[134]
NLC	Compritol® 888 ATO, SPC	Soya Lecithin	Cremophor® ELP & DDAB	SDM	70-75	With RGD 118.3 ± 2.6 w/o RGD 93.6 ± 2.1	With RGD +28.9 ± 2.9 w/o RGD +23.3 ± 2.7	With RGD 84.7 ± 3.2 w/o RGD 85.3 ± 2.3	With RGD 5.6 ± 0.5 w/o RGD 7.3 ± 0.9	TMZ	RGD	U87 MG	BALB/c nude mice (5–6 week-old, 18–22 g)	[135]
SLN	StA	Soya lecithin	DDAB	SDT	25	179.1 ± 5.2	+35.7 ± 3.8	VCR: 84.5 ± 4.6 TMZ: 80.8 ± 3.2	VCR: 7.6 ± 0.9 TMZ: 10.3 ± 2.3	TMZ & VCR	N/A	U87 MG	BALB/c nude mice (5–6 week-old, 18–22 g)	[136]
NLC	Compritol® 888 ATO & SPC	Soya lecithin	Cremophor® ELP, DDAB	SDM	70-75	117.4 ± 2.8	+ 29.8 ± 3.2	VCR: 85.4 ± 2.8 TMZ: 88.9 ± 3.6	VCR: 5.7 ± 0.5 TMZ: 6.8 ± 0.7	TMZ & VCR	N/A	U87MG	BALB/c nude mice (5–6 week-old, 18–22 g)	
NLC	Tripalmitin	Oleic Acid	Tween® 80	HSHT, HPH	70	214	N/A	88.6	27.4	CUR	N/A	A172	Female nude mice, 5-6 weeks old, A172 xenograft	[137]

Table 2 (Continue). Basic properties and characteristics of SLNs & NLCs used for the treatment of brain cancer

Type of carrier	Solid lipid	Liquid lipid	Emulsifiers/ stabilizers/ surfactants	Preparation method	T (°C)	Size (nm)	Zeta (mV)	EE (%)	DL (%)	Therapeutic molecule	Targeting moiety	*In Vitro*	*In Vivo*	Ref
NLC	Cholesterol, Triolein, Stearylamine	Soya Lecithin	Poloxamer 188	SEEM	45	205.4 ±11	25.7 ±6.22	91.8 ±0.5	5.38 ±0.03	PTX	Trf	U87MG	N/A	[138]
LPNP	C15 lipid DPSE-epoxy-PEG2000	-	PEI	Microfluidics	90	40-135	-	-	-	RNAi	N/A	GBM6, GBM12, GBM26, GBM43, MGG8, MES83, bEnd3, C8D1A	Athymic nude male or female mice (6-8 week old)	[139], [140]

4.2. SLNs and NLCs in ischemic stroke

Even though ischemic stroke is one of the diseases with highest percentages of morbidity and mortality worldwide, the therapeutic solutions that are presented in the literature using SLNs and NLCs during the last seven years are very limited. One of the reasons may be the use of similar polymeric systems which give the advantage of more versatile surface functionalization compared to the lipid nanostructures.

One of the studies that were presented seven years ago made use of the lipophilic drug vinpocetine (VIN), which is used for chronic cerebral vascular ischemia [141]. Its lipophilic character is responsible for its low bioavailability as well as its short half-life. Aiming at overcoming these limitations, VIN loaded NLCs (VIN-NLCs) with a size from 100 to 200 nm, a slightly negative charge and an encapsulation efficiency of approximately 95% were fabricated. The results of this study demonstrated a sustained release profile with no burst release, as well as an increase of 322% in the bioavailability compared to the free drug.

A few years after this study, other two scientific publications presented the enhanced therapeutic efficacy of VIN after its encapsulation. In the first study, different formulations of SLNs were prepared with the high shear/speed homogenization technique (HSHT) aiming at finding the optimum formulation for the loading and the release of VIN. The results of the best formulation showed an encapsulation efficiency of more than 83% and a sustained release profile for over 96 h indicating its potential use as a controlled delivery system [142]. In the second study, a different approach for the fabrication of VIN-NLCs was used. The authors of this work fabricated complexes of vinpocetine, cyclodextrin (CD), and tartaric acid (TA), that were encapsulated inside Compritol® ATO 888-based NLCs. The *in vivo* studies that were carried out in New Zealand rabbits demonstrated an enhanced oral bioavailability of 522% compared to the free VIN, and of 92% compared to VIN-NLCs [143].

The antioxidant properties of curcumin were also used for the treatment of ischemic stroke according to one study that was published in 2013 [144]. Here, the authors compared the therapeutic effect of free curcumin and SLN-encapsulated curcumin, and the results showed an improvement of 90% in cognition and 52% inhibition of acetylcholinesterase levels. In addition, the levels of numerous enzymes including glutathione (GSH), superoxide dismutase (SOD), catalase, and others were significantly increased suggesting the therapeutic efficacy of the delivery system.

One of the few targeted strategies for the treatment of ischemic strokes made use of PEGylated lipid nanoparticles (PLNs) that were loaded with 3-n-butylphthalide (NBP) and were conjugated with the FAS ligand antibody [145]. From the *in vivo* studies, it was shown that the PLNs accumulate in OX42 positive microglia cells in the ischemic region of a mouse model, and they improve the brain injury after ischemia in much lower dosages than the free NBP.

The neuroprotective properties of encapsulated baicalin against ischemic stroke were used by two different groups. In the first study, where baicalin was encapsulated in SLNs of approximate size 100 nm and a negative ζ-potential of -50 mV, the results showed a higher accumulation of the loaded baicalin in the cerebral cortex and in brain stem, compared to the unloaded drug, and an improved bioavailability and

stability [146]. A targeted approach using baicalin encapsulated in NLCs was presented three years later [147,148]. In this work, PEGylated cationic SLNs conjugated with an OX26 antibody, presenting similar encapsulation efficiency and smaller size compared to the previously reported work, were used. The authors, after fabricating and characterizing in detail their formulations, proceeded with the *in vivo* work. Their pharmacodynamic studies showed a reduction in the content of aspartic and glutamic acid and an increase in the concentrations of glycine, taurine, and γ-aminobutyric acid during ischemia-reperfusion. Furthermore, it was also shown that the bioavailability of baicalin in the cerebral spinal fluid of rats under the cerebral ischemia-reperfusion injury was higher, and that also its uptake into the brain was improved.

Table 3 presents in detail the basic characteristics and properties of the lipid nanostructures that were used for the treatment of ischemic stroke.

Table 3. Basic properties and characteristics of SLNs & NLCs used for the treatment of ischemic stroke.

Type of carrier	Solid lipid	Liquid Lipid	Emulsifiers/ stabilizers/ surfactants	Preparation method	T (°C)	Size (nm)	Zeta (mV)	EE (%)	DL (%)	Therapeutic molecule	Targeting moiety	*In Vitro*	*In vivo*	Ref	Year
NLC	Compritol® ATO 888, GMS	Miglyol®812N, Lecithin	Solutol® HS15, Poloxamer 188	HPH	85	107-177	-13 to -25	94.9 ±0.4	1.80± 0.12	VIN	N/A	N/A	Male Wistar rats (200–250g)	[141]	2010
SLN	StA, Precirol® ATO5, GMS, GTP	-	Tween® 80 Pluronic® F68	HSHT-EUT	N/A	123-464	-10 to -23	83-95	N/A	VIN	N/A	N/A	N/A	[142]	2013
NLC	Compritol® ATO 888	Miglyol®812N	Solutol® HS15	EUT	85	89-148	-12 to +13	59-86	N/A	VIN	N/A	N/A	New Zealand white male rabbits, (2.5±0.2 kg)	[143]	2014
SLN	Compritol® ATO 888 Soy lecithin	-	Tween® 80	MEM	82-85	134.6 ± 15.4	N/A	81.92 ± 2.91	92.33 ± 1.63	CUR	N/A	N/A	Male Wistar rats (250–300 g)	[144]	2013
PEG-SLN	GMS, MCT, PEG- monostearate	-	Tween® 80	MEM	70	38-60	N/A	~94	~15,5	NBP	Fas ligand antibody	N/A	Adult male C57BL/6J wild-type mice were	[145]	2014
NLC	Tripalmitin, Gelucire®, SPC, Vitamin-E	-	Poloxamer 188	EUT	80	93-102	-50 to -57	91-94	N/A	Baicalin	N/A	N/A	Male Wistar albino rats (200–250 g)	[146]	2012
SLN	SL-100, Compritol® ATO 888, DC-Chol	-	Myrj™ 52	SEvaM	70	27-47	+11 to 0	83-98	2.9 – 4.0	Baicalin	OX26 antibody	N/A	Male Sprague-Dawley rats, 260 ± 10 g	[147,148]	2015

4.3. SLNs and NLCs in Parkinson's disease

Parkinson's disease is another type of neurodegenerative disease that leads to a progressive movement disorder. PD is not directly life threatening, but its symptoms significantly reduce the quality of life of people that live with this disease. To date, there is no real cure for PD but the therapeutic treatments reduce some of the patient symptoms.

A few studies have been presented over the years for the treatment of PD, a number of which included the use various drugs encapsulated in SLNs and NLCs, including apomorphine (APO). In one of the studies [149], APO was encapsulated in three different formulations: SLNs, NLCs and lipid emulsions (LEs), and a comparative study of these formulations were carried out. Depending on the type of the nanostructure, the size, the surface charge, the encapsulation efficiency and the release profile were different, with the LEs to give the best results concerning the sustained release. On the other hand, *in vivo* real-time bioluminescence experiments on the brain of male Sprague-Dawley rats, using sulforhodamine B (SRB), demonstrated that NLCs could accumulate to selected brain regions compared to the other two formulations.

Another study using the same drug was carried out aiming at assessing the feasibility of oral apomorphine delivery by using SLNs [150]. This study showed that emulsifier variations affect the physicochemical properties of the SLNs, that may influence their *in vivo* performance. *In vivo* experiments demonstrated that SLNs improved APO bioavailability and were able to deliver the drug to the brain striatum suggesting the potential use of the SLNs as a delivery system for APO through oral administration.

A different strategy using APO as the main drug was published from the same group two years later [151]. In this work, the authors encapsulated two APO-based prodrugs, diacetyl apomorphine (DAA) and diisobutyryl apomorphine (DIA) inside NLCs and they assessed their physicochemical properties. The release studies revealed a slower release profile for the DIA, and the hydrolysis study indicated that the prodrugs underwent bioconversion in plasma and brain extract. The hemolysis and the LDH release revealed a good tolerance by erythrocytes and neutrophils. Finally, the authors used PEGylated and non-PEGylated NLCs, P-NLCs, and N-NLCs respectively, the bioimaging results of which showed a greater extent of accumulation of P-NLCs compared to N-NLCs in the brain.

The encapsulation of ropinirole (RPN), a commercial drug that is used for the treatment of PD, in a hybrid polymer-lipid nanoparticles (PLNs) is another strategy that has been reported in the literature [152]. The nanoparticles in this study were fabricated aiming at delivering RPN through the intranasal route, and it was found that the hybrid nanoparticles presented good mucoadhesive properties without significantly damaging the nasal mucosa. A comparative study between the PLNs and a commercial formulation demonstrated similar experimental results, suggesting the superiority of the PLNs since less amount of drug was needed in order to have the same therapeutic effect as the commercial drug.

RPN was once more used as the encapsulated substance inside NLCs in a study the main goal of which was to study the effect of surface charge on the brain *via* the nasal route [153]. Anionic and cationic NLCs with an absolute surface charge of 34 mV were fabricated, the former of which caused mild inflammation to the nasal

epithelium, while the latter caused the destruction of the lining mucosal nasal epithelium. In addition, even though a higher accumulation in the brain was observed for the cationic NLCs, the anionic presented the highest drug targeting efficiency.

Gelatin nanostructured lipid carriers (GNLs) are another type of hybrid lipid nanoparticles that have been reported for the treatment of PD [154]. The authors of this work fabricated hybrid phospholipid-based gelatin nanoparticles and loaded them with a basic fibroblast growth factor (bFGF), aiming at the delivery of this growth factor into the brain, through the intranasal route. The size of the nanoparticles was approximately 140 nm with a surface charge of -38 mV. The *in vivo* studies in a rat model of hemiparkinsonism revealed that the GNLs could successfully deliver the bFGF in olfactory bulb and striatum, yet not in prefrontal cortex or hippocampus.

A detailed description of the basic characteristics and properties of the SLNs and NLCs that are used for the treatment of PD is given in **Table 4**.

Table 4. Basic properties and characteristics of SLNs & NLCs used for the treatment of Parkinson's disease.

Type of carrier	Solid lipid	Liquid lipid	Emulsifiers/ stabilizers/ surfactants	Preparation method	T (°C)	Size (nm)	Zeta (mV)	EE (%)	DL (%)	Therapeutic molecule	Targeting moiety	*In Vitro*	*In vivo*	Ref
NLC	CP, DSPE-PEG5000	Squalene	Pluronic® F68, Myverol Forestall, Tween® 80	HSHT-EUT	85	373-430	+42 to +51	59-61.5	N/A	APO, SRB	N/A	N/A	Male Sprague-Dawley rats (230–270 g)	[149]
SLN	CP	-				333.1 ±13.8	+36.5 ±0.5	50.3 ±1.7						
LE	-	Squalene				435.9 ±35.2	+54.1 ±1.3	67.2 ±0.8						
SLN	Tripalmitin, HSPC, GMS	-	Pluronic® F68, L-ascorbic acid	EUT	80	154.97 ± 2.83	23.27 ± 0.70	90.38 ± 0.04	N/A	APO	N/A	N/A	Wistar albino rats (200–250 g)	[150]
	Tripalmitin, HSPC, PMS					63.20 ± 0.98	7.23 ± 0.25	91.03 ± 0.14						
NLC	CP	Sesame oil	Pluronic® F68, Myverol	HSHT-EUT	85	213.5 ±1.5	−20.8 ±2.8	N/A	N/A	DAA, R800	N/A	Erythrocytes	Female nude mice (ICR-Foxn1nu strain, eight weeks old)	[151]
	CP, DSPE-PEG5000		Pluronic® F68, Myverol, Forestall			250.1 ±3.3	48.4 ±0.6							
PLN	Stearylamine, Dynasan® 114	Soya lecithin	Pluronic F68, HPMC	SDM	65	98 to 287	+21 to +45	66 to 76	10 to 12.5	RPN	N/A	N/A	Male albino mice (25 ± 2 g)	[152]
A-NLC	Compritol® 888 ATO, Labrafac™ Lipophile WL1349	-	Pluronic® F127, Pluronic® 68, Tween® 80, Epikuron 200	HSHT	80	82 to 314	-39 to +34	32 to 79	N/A	RPN	N/A	N/A	Male Wister albino rats (200–250 g)	[153]
C-NLC	Compritol® 888 ATO, Labrafac™ Lipophile WL1349, Stearylamine													
GNL	Gelatin, HSPC, Chol	-	Poloxamer 188, D, L-glyceraldehyde	Water-in-water emulsion, Freeze-drying		143 ± 1.14	−38.2 ± 1.2	86.7 ± 1.1	4.60 ± 0.01	bFGF, APO	N/A	N/A	Sprague–Dawley rats	[154]

4.4. SLNs and NLCs in Alzheimer's disease

Alzheimer's disease is characterized by memory loss and other cognitive inabilities, that render the patients incapable of dealing with daily tasks. The progression of the disease varies depending on the patient, but ultimately leads to death with a mean life expectancy from three to nine years. Numerous studies have been reported for treating AD, and in this section we are going to focus on the studies that make use of the various lipid nanoparticles.

One of the drugs that have been used for the treatment of AD is Huperzine A (HupA). HupA is cholinergic that acts as a cognitive transmitter and can potentially help AD patients. Two studies that made use of encapsulated HupA in SLNs and NLCs have been presented during the last seven years. In the first study [155], the authors prepared CP-based NLCs loaded with HupA, and they studied their morphological characteristics as well as their physicochemical properties. The size of the NLCs was 120 nm and their surface charge was -22.93 mV. The NLCs presented a good loading efficiency (89.18%), although their loading capacity was not so high (1.46%). The *in vitro* release profiles showed a controlled drug release for up to 96 h. In the second reported study [156], the authors encapsulated HupA in three different formulations aiming at finding the optimum strategy for its transdermal delivery. The three formulations were microemulsions (MEMs), SLNs and NLCs. The sizes of these formulations were close to the ones of the NLCs in the previously reported study, but their surface charge was different depending on the method. The *in vivo* studies, carried out on male Swiss Albino mice, demonstrated that the MEMs exhibited the superior (147.68 ± 9.42 μg/cm^2) cumulative amount of drug permeation followed by the NLCs and SLNs. In addition, skin irritation experiments for 48 h using gels demonstrated that the latter is safe for skin use.

Quercetin (QRT) is a polyphenol that belongs to the group of flavonoids. As a polyphenol, QRT exhibits antioxidant properties and has been used for the treatment of various diseases like cancer and atherosclerosis. The therapeutic effect of encapsulated QRT inside SLNs for the treatment of AD was reported a few years back [157]. The authors in this study fabricated QRT-loaded SLNs based on compritol, aiming at the fabrication of a system that could penetrate the BBB after intravenous administration. The size of the fabricated SLNs was less than 200 nm, they exhibited a positive surface charge of 22 mV and a good encapsulation efficiency of 85.73%. The *in vivo* data showed that the rats treated with QRT-loaded SLNs exhibited better memory-retention *vis-à-vis* than the rats that were treated only with pure QRT.

One of the characteristics of AD are the irregular amounts of the neurotoxic beta-amyloid (Aβ) peptide which undergoes aggregation and forms oligomers, fibrils and plaques; a possible therapeutic approach is the treatment of these oligomers. A research reporting the targeting of these Aβ amyloid aggregates was one of the studies that made use of SLNs in order to fabricate a targeted delivery system for AD [158]. The authors fabricated two types of nanoparticles, liposomes, and SLNs, and made a comparative study of their physicochemical properties. The immunostaining studies that were carried out demonstrated that anionic phospholipids are suitable for targeting the Aβ peptide. Based on this, SLNs comprised of stearic acid, Phospholipon 90G, sodium taurocholate and phosphatidic acid (PA) or cardiolipin (CL) were able to target the $A\beta_{1\text{-}42}$ aggregates, while the plain SLNs could not. The

authors also observed that the PA/CL modified nanoparticles did not bind to bovine serum albumin (BSA). Unfortunately, this work was not supported from *in vivo* studies.

Partial targeting of the brain due to the Tween® 80 emulsifier, that was used for the fabrication of SLNs loaded with piperine (PPR), was reported by another group [159]. In this work, the authors assessed the behavioral and the biochemical effect of their nanoparticles in an experimentally induced AD model. The SLNs reduced the values of SOD and the values in immobility while increasing the acetylcholinesterase values. The synthesized pharmaceutical formulation was compared with the commercial drug donepezil and exhibited better results in the treatment of AD. In this study, the minimum therapeutic efficacy of PPR was found at a concentration of 2 mg/kg body weight of rats.

Low-density lipoprotein (LDL) mimicking SLNs were reported as another form of brain targeting nanoparticles [160]. In this work, SLNs made of the same lipids as LDL were loaded with curcumin and their surface was functionalized with the protein lactoferrin (Lf) (Lf-mNLCs). The *in vitro* studies in brain capillary endothelial cells (BCECs) showed an increased uptake (about 1.5 folds) of the Lf-mNLCs compared to the plain NLCs, with the result being better in the *ex vivo* studies, where the accumulation at the BBB of penetrating Lf-mNLCs was about 3 times higher than the plain ones. Finally, it was proved from the pharmacodynamic studies that AD progression could be controlled.

SLNs aiming at the treatment of AD were also reported by other groups. Each of these groups made use of various lipid matrices and used different drugs. The most important studies were those ones that made use of lipoyl-memantine (LA-MEM) [161], sesamol, and chrysin. In the study where LA-MEM was used, the authors synthesized and fully characterized LA-MEM loaded SLNs. The results demonstrated that the particles were stable in both simulated gastric fluid (SGF) and simulated intestinal fluid (SIF), and that presented a controlled release profile.

A more detailed study was presented by the group that synthesized sesamol-loaded SLNs [162]. The *in vivo* studies were performed on an experimental model of AD, where rats were injected intracerebroventricularly with the glucosamine-nitrosourea compound streptozotocin (ICV-STZ rats), that produces reduced cognition and increased cerebral aggregated Aβ fragments, total tau protein, and Aβ deposits. The results demonstrated that the chronic treatment with free sesamol and with sesamol-loaded SLNs in a dose-dependent manner could effectively restore cognitive deficits and help with the mitigation of nitro-dative stress and cytokine release.

The last reported publication for the treatment of AD presented the use of chrysin as a model drug inside SLNs [163]. The loaded nanoparticles were able to protect against neuronal damage that was induced by the administration of $A\beta_{25–35}$. Furthermore, the therapeutic effect of the nanoparticles was demonstrated by the reduction of all the antioxidant and non-antioxidant enzymes in the hippocampus, as well as from the increase in the lipid peroxidation and of the acetylcholine esterase.

A detailed description of the basic characteristics and properties of the SLNs and NLCs that are proposed for the treatment of AD is given in **Table 4**.

Table 5. Basic properties and characteristics of SLNs & NLCs used for the treatment of Alzheimer's disease.

Type of carrier	Solid lipid	Liquid lipid	Emulsifiers/ stabilizers/ surfactants	Preparation method	T (°C)	Size (nm)	Zeta (mV)	EE (%)	DL (%)	Therapeutic molecule	Targeting moiety	*In Vitro*	*In vivo*	Ref
NLC	CP	Miglyol® 812	Soybean phosphatidylcholine, Solutol® HS15	EUT-HPH	52	120	-22.93± 0.91	89.18± 0.28	1.46± 0.05	HupA	N/A	N/A	N/A	[155]
MEM	-	Capmul® MCM	Transcutol® P, Tween® 80	MEM	25	148.3 ± 5.1	−4 ± 7.83	98.82 ± 1.12	N/A	HupA	N/A	N/A	Male Swiss Albino mice (25–30 g, 4–6 weeks old)	[156]
SLN	GMS	-			60-63	119.1 ± 6.9	−20 ± 5.81	98.66 ± 1.67						
NLC	GMS	Capmul® MCM			60-63	137.1 ± 10.4	−17.5 ± 7.16	99.38 ± 1.08						
SLN	Compritol® ATO 888	-	Tween® 80	MEM	70-75	152 to 4620	-20 to -5	85.73	N/A	QRT	N/A	N/A	Male Wistar rats (180–200 g)	[157]
Liposomes	Cholesterol, Sphingomyelin, & gangliosides, Sm, PC, PE, Chol, PG, PA, CL	-	-	Extrusion	40	141-178	-11 to -49	N/A	N/A	N/A	PA	N/A	N/A	[158]
SLN	StA, PA		Phospholipon 90G, sodium taurocholate	MEM	70	46-76	-39 to -43							
SLN	GMS	-	Epikuron 200, Tween® 80, 20	SDT	53±3	277.6 ± 2.4	−45.0 ± 1.7	68.2	N/A	PPR	N/A	N/A	Albino Wistar rats	[159]
NLC	PC, Cholesterol oleate	Glycerol triolate	-	SEvaM	40	75-163	-3.5 to -17	87-97	2.60 ± 0.17	CUR	Lf	BCEC	Sprague-Dawley rats (180–220g) and ICR mice (18–22g)	[160]
SLN	StA	-	Brij 78	SEvaM	75	160-195	-27 to -35	88-92	12.5± 1.30	LA-MEM	N/A	N2a NB, PHWB	N/A	[161]
SLN	Compritol® ATO 888, Soy lecithin	-	Tween® 80	MEM	82-85	40-70	N/A	75.97± 2.91	97.57 ±1.63	Sesamol	N/A	N/A	Male Wistar rats (200–230 g, 3 months old, ICV-STZ)	[162]
SLN	StA, Lecithin	-	Taurocholate	HSHT	75	240.0 ± 4.79	−40.4± 2.54	86.29± 3.42	71.10 ±3.12	Chrysin	N/A	N/A	Adult male Sprague–Dawley rats (250–300 g)	[163]

4.5. SLNs and NLCs in multiple sclerosis

Even though many reports in the synthesis and characterization of SLNs and NLCs have been published the last seven years for the treatment of various CNS diseases, including brain cancer, ischemic stroke, Parkinson's and Alzheimer's disease, only two of them have been reported for the treatment of multiple sclerosis.

In the first study, the synthesis of SLNs loaded with the drug riluzole (RLZ) is reported [164]. The synthesized particles had a size less than 90 nm and a negative surface charge of approximately -46 mV which was responsible for the high stability of the dispersion. The *in vivo* studies in adult Sprague-Dawley rats demonstrated that the concentration of the encapsulated RLZ was higher in the brain and lower in other body organs compared to the free RLZ, suggesting that the SLNs were able to penetrate the BBB and to enhance the delivery of RLZ in the brain.

In the second reported study, SLNs were used once again but in this case loaded with methylprednisolone (MEP) and functionalized with two glycoprotein antigens, anti-contactin 2 (anti-Cntn2) and anti-neurofascin (anti-Nfasc). Even though the SLNs presented good release profiles and no toxic effects when they were tested against U87MG cells, it was interesting the fact that the plain SLNs exhibited better penetration ability to the BBB compared to the PEGylated and functionalized anti-Cntn2/anti-Nfasc SLNs.

Table 6. Basic properties and characteristics of SLNs & NLCs used for the treatment of multiple sclerosis.

Type of carrier	Solid lipid	Liquid lipid	Emulsifiers/ stabilizers/ surfactants	Preparation method	T (°C)	Size (nm)	Zeta (mV)	EE (%)	DL (%)	Therapeutic molecule	Targeting moiety	*In Vitro*	*In vivo*	Ref	Year
SLN	Compritol® 888 ATO	-	Epikuron 200, taurocholate sodium salt	MEM	75	88 ± 4	-45.8 ± 1.0	-	14.5	RLZ	N/A	N/A	Adult male Sprague–Dawley rats (250-300 g)	[164]	2010
SLN	Cholesterol, Sphingosine, Pphosphatidyl-ethanolamine, phosphatidylcholine, sphingomyelin, phosphatidylserine	-	Tween® 80	SEvaM	25	101-210	-8.7 to + 1.37	34 -50	5-14	MEP, coumarin 6	anti-Nfasc, anti-Cntn2	U87MG	Mice induced with multiple sclerosis	[165]	2017

4.6. SLNs and NLCs in for treating other diseases of the CNS

In the previous paragraphs, a detailed description of the reported SLNs and NLCs formulations that were used during the last seven years for the treatment of the main CNS diseases has been performed. Although a great number of publications that covered many of the CNS diseases were presented, there is still a significant amount of scientific studies that deal with other CNS diseases. Since the focus of this review is on the use of SLNs and NLCs for the treatment of the main CNS diseases, we are not going to describe in detail these studies. Nevertheless, due to the significance of these scientific studies, we briefly describe in **Table 7** the basic properties, the basic characteristics and the therapeutic targets of the fabricated systems that were used in the last seven years, hoping to help the reader to understand the extent of the capabilities of the described lipid nano-formulations.

Table 7. Basic properties and characteristics of SLNs & NLCs used for the treatment of various diseases of the CNS.

Type of carrier	Solid lipid	Liquid lipid	Emulsifiers/ stabilizers/ surfactants	Preparation method	T (°C)	Size (nm)	Zeta (mV)	EE (%)	DL (%)	Therapeutic molecule	Target	Targeting moiety	*In Vitro*	*In vivo*	Ref
SLN	GMS	-	Poloxamer 407	SIT	58	180 to 271	-39.8 to -22.34	75.81	N/A	SMV	Ischemia	N/A	N/A	Balb/c mice (25–30g)	[166]
	Compritol® 888 ATO				70			68.16							
NLC	GMS	Oleic Acid			58			>90							
NLC	CP	Soy lecithin, octyl-dodecanol	Poloxamer 188	SDT-EUT	50	154 ±16	−10±0 .5	N/A	47± 0.8	VPA	Bipolar disorder, Migraines	N/A	N/A	Male Wistar rats (180–210 g)	[167]
NLC	GMS, SA, MCT	Soya lecithin	Vitamin-E	SEvaM	70	215.2	−20.1 ±1.22	89.95± 0.16	3.05± 0.01	QRT	Antioxidant	N/A	N/A	Male Kunming mice (20 ± 2 g)	[168]
SLN	Compritol® 888 ATO, Precirol® ATO5	-	Ploxamer 188, Tween® 20, 80	EUT	85	311-424	-33to -36	N/A	N/A	QRT	Antioxidant	N/A	N/A	N/A	[169]
NLC	GMS, MCT	Soy lecithin	DDAB	HSHT	70	126.6 ±8.48	40.5± 0.8	89.3±0. 56	3.95± 0.18	QRT	Antioxidant	N/A	N/A	Healthy male C57BL/6J mice (18–20 g)	[170]
NLC	GMS, MCT	Soy lecithin	Poloxamer 188	HSHT	75	129±1 5.5	-27.8 ±5.9	95.98 ± 0.63	4.21 ± 0.07	CUR	Antioxidant	N/A	N/A	Healthy Sprague-Dawley rats (190–220 g)	[171]
SLN	Compritol® 888 ATO, Soy lecithin	-	Tween® 80	MEM	82-85	N/A	N/A	N/A	N/A	CUR	Antioxidant	N/A	N/A	Wistar rats, Balb/c mice, (20 and 30 g), New Zealand rabbit (2.0–2.5 kg)	[172]
NLC	Tridodecanoin, Lipoid MCT, Labrafac™ Lipophile WL1349, Glyceryl behenate, Precirol® ATO5, Labrafac™ CC, PC	Labrafac CC	Tween® 80, Poloxamer F68, Cremorphor® EL, Cremorphor® RH 40	SEvaM-EUT	4	90.50± 0.2	-20.39 ±0.89	94.39± 2.00	3.29 ±0.32	CUR	Antioxidant	N/A	RAW 264.7, bEnd3	Sprague-Dawley rats (180–220 g), ICR mice (18–22 g)	[173]

Table 7 (Continue). Basic properties and characteristics of SLNs & NLCs used for the treatment of various diseases of the CNS.

Type of carrier	Solid lipid	Liquid lipid	Emulsifiers/ stabilizers/ surfactants	Preparation method	T (°C)	Size (nm)	Zeta (mV)	EE (%)	DL (%)	Therapeutic molecule	Target	Targeting moiety	*In Vitro*	*In vivo*	Ref
SLN	Glyceryl oleate, CP	-	Ceteth-20, Isoceteth-20	Phase Inversion	90	30-95	N/A	N/A	N/A	IDB	Neurodegenerative diseases	N/A	Astrocytes	N/A	[174]
SLN	Glyceryl oleate, CP	-	Ceteth-20, Isoceteth-20, Oleth-20	Phase Inversion	90	35-40	-3 to +3	N/A	0.7-1.1	IDB	Neurodegenerative diseases	N/A	N/A	N/A	[175]
NLC	Witepsol® H 175	Capmul® MCM	Tego Care 450	EUT	~45	162-324	-25.94 to -49.2	65-89	N/A	IDB, ILO	Schizophrenia	N/A	N/A	Wistar rats (200–250 g)	[176]
SLN	Softisan® 100, SA, PA	-	DDAB, Tween® 80	QESD, HSHT, EUT	0	175-680	-8 to +61	50-90	N/A	IDB	Neurodegenerative diseases	N/A	N/A	N/A	[177]
SLN	StA	-	Epikuron 200, propionic acid, butyric acid, sodium taurocholate	MEM	70	157-161	-35 to -37	N/A	N/A	BCF	Spasticity	N/A	N/A	healthy male Wistar rats (age 9 weeks; 285 ± 4 g)	[178]
SLN	Compritol® 888 ATO	-	Pluronic® F127	SEvaM	70	148.0 ±0.85	−25.35 ±0.45	N/A	59.65 ± 1.18	RSP	Schizophrenia, bipolar disorder, and irritability caused by autism	N/A	N/A	Balb/C mice of either sex (~25 g)	[179]
SLN	Compritol® 888 ATO, Phospholipon® 80H	-	Tyloxampol®	HSHT, HPH	80-85	155-207	-27 to -38	>90	N/A	RSP	Schizophrenia, bipolar disorder, and irritability caused by autism	N/A	Caco-2	N/A	[180]
NLC	Compritol® 888 ATO, CP, Precirol® ATO5, PEG-8 beeswax	Labrafac CC, almond oil, castor oil, olive oil, oleic acid	Tween® 80, Poloxamer 188	EUT	70	151.6 ±7.6	11.75 ± 2.96	96.64 ±4.27	N/A	LMT	Epilepsy & bipolar disorder	N/A	N/A	*Ex vivo*- Goat nasal mucosa *In vivo*-Male Wistar rats (150 -- 200 g)	[35]
NLC	GMS	Oleic acid	Tween® 80	HSHT, EUT	70	167.3 ±7.52	-4.33 ±1.27	83.50 ±2.48	N/A	ASN	Schizophrenia	N/A	N/A	Male Charles Foster rats (200–240 g)	[181]
NLC	GMS	capryol PGMC	Pluronic® F68, (sodium taurocholate	HSHT, EUT	N/A	137.2 ±2.88	-31.53 ±11.21	79.15 ±4.17	9.73 ±3.22	DLX	Behavioral disorders	N/A	N/A	Swiss albino Wistar rats of either sex (150-250 g)	[182]

Table 7 (Continue). Basic properties and characteristics of SLNs & NLCs used for the treatment of various diseases of the CNS.

Type of carrier	Solid lipid	Liquid lipid	Emulsifiers/ stabilizers/ surfactants	Preparation method	T (°C)	Size (nm)	Zeta (mV)	EE (%)	DL (%)	Therapeutic Molecule	Target	Targeting moiety	*In Vitro*	*In vivo*	Ref
NLC	Crodamol SS®, Crodamol GTCC®, SPC, DSPE-PEG2000-MAL	Soya lecithin	Solutol® HS15	EUT	70	96.46 ±0.15	-0.78±0.35	93.11 ±2.67	N/A	TXL	Brain cancer	pCPP	HT-1080	N/A	[183]
NLC	Stearylamine, Precirol® ATO5, GB	Kolliphor® P188, MCT	Vitamin-E, Soybean lecithin	HSHT	5 °C above melting point	190 ±10	-43.5 ±1.2	98.5	14	DCB	Brain cancer & Hodgkin's disease	N/A	N/A	N/A	[184]

5. Conclusion & Future Perspectives

Medicinal formulations fabricated owing to nanotechnological development have been extensively used in the biomedical field in the last decade. These formulations can be in the form of inorganic or organic nanoparticles, hydrogels, spherical or worm-like micelles, dendrimers, nanorods, nanotubes and others, and can comprise several different materials. The combination of various materials is what gives these nanostructures their versatile properties, and what makes them so attractive in nanomedicine.

To date, the majority of the nanostructures that have been used for the treatment of various diseases are made of synthetic polymers like PLGA. Although many studies that make use of polymeric nanoparticles have been presented, and although many of the products of these studies have been commercialized, there is still a great need of finding therapies for untreatable diseases like cancer, HIV, ischemic stroke, Alzheimer's disease, Parkinson's disease, multiple sclerosis, myocardial infarction, atherosclerosis, and others. The polymeric nanostructures demonstrate great advantages including versatility in functionalization, controllable sizes and shapes, high concentrations of encapsulated substance and ability to be used in the theranostic field, but they also present disadvantages, the most important of which are difficulties in scaling up, use of organic solvents during their fabrication, biocompatibility, cytotoxicity and immunogenicity. Numerous fabrication and functionalization techniques have been developed in order to overcome these disadvantages, and to a certain extend these techniques have been successful, but without resolving completely these problems. Thus, new nanostructures had to be developed aiming at overcoming the limitations of these systems.

Lipid-based formulations can be characterized as the next generation of drug delivery systems, since they share most of the advantages of the polymeric nanostructures without their harmful disadvantages. These lipid-based formulations, the most studied of which are solid lipid nanoparticles and nanostructured lipid carriers, are gaining more and more space in the biomedical field for the treatment of numerous diseases, and especially brain cancer and neurodegenerative diseases. Their small size and their inherent ability to cross the BBB, even without any surface functionalization, make them excellent candidates for the treatment of various CNS diseases.

In this review, we tried to give an insight of the most important brain diseases and the reported studies that make use of SLNs and NLCs for their treatment, during the last seven years. We have described a variety of fabrication and characterization techniques for the synthesis of these nanostructures and we gave a detailed description of their use in the CNS. It is obvious from all the reported studies that SLNs and NLCs have been advancing over the years. New fabrication and functionalization techniques have been applied, allowing the SLNs and NLCs to successfully deliver their therapeutic cargo to specific tissues in a controlled and sustained manner. In addition, their cloaking, using stealth technologies like PEGylation, allows them to circulate through blood for long periods of time without being cleared by the RES. When this cloaking technology is combined with the targeting ability, the therapeutic efficacy of the nanostructure is enhanced. Aiming at this enhancement, a great number of papers have presented the therapeutic effects of targeted SLNs and NLCs to the brain, but what it has to be noted here is that many of the results demonstrated

an accumulation of these brain-targeted nanoparticles to other body organs (*e.g.*, liver, heart, lungs, *etc.*), that raises concerns whether their role is more beneficial than harmful. In our opinion, more specific targets, like overexpressed proteins or enzymes that lead to the activation or inactivation of pathways, have to be found, aiming at increasing the accumulation of these nanoparticles to the brain and at enhancing their therapeutic efficacy. Another point that has to be considered is the loading of these nanostructures. To date, most of the studies that have been published mostly demonstrate the encapsulation of drugs and no other therapeutics, like genes, enzymes, DNA/RNA, inorganic nanoparticles and others. In addition, these studies present the encapsulation of only one, or maybe two therapeutic substances. A potential reason for this is the inability of SLNs and NLCs for high loading of therapeutics in their lipid matrix, and these are among the main challenges that need to be resolved in order to increase their therapeutic efficacy in a targeted tissue. The loading of more than two different substances in the nanostructures or the combination of inorganic nanoparticles with other therapeutic molecules will transform the SLNs and the NLCs into multifunctional nanocarriers, rendering them able for a multi-approach treatment. Furthermore, the encapsulation of fluorescent dyes or fluorescent nanoparticles along with other therapeutic segments will transform these therapeutic nanostructures into theranostic nanostructures, that will be able to demonstrate in real time their therapeutic efficacy.

Finally, it has to be noted that albeit SLNs and NLCs have greatly advanced during the last few years, to date none of these nanostructures have been able to successfully reach the clinical trials stage. Thus, advances in molecular biology that will offer novel targets, a better in-depth analysis on the causes of the CNS diseases, and a proper design for SLNs and NLCs taking into consideration the above-described limitations have to be carried out before being able to achieve the perfect lipid-based nano-delivery system.

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Abbreviations

General abbreviations

* AD=Alzheimer`s Disease, Aβ= β amyloid, Aiso = After Isotonisation, A-NLCs = Anionic Nanostructured Lipid Carriers, AOT = Sodium dioctyl sulfosuccinate, Aster = After Sterilization, APMP = p-aminophenyl-?-D-manno-pyranoside, APP= amyloid precursor protein, Apr = Aprotinin, ASP = Aspirin, BBB=blood brain barrier, BHR =benzoic acid-2-hydroxy-2-D-ribofuranosylhydrazide, Biso = Before Isotonisation, C15 lipid = Pentadecylic acid, CB = Cacao Butter, Chol = Cholesterol, C-NLCs = Cationic Nanostructured Lipid Carriers, CNS= Central nervous system, Cntn2 = Contactin 2, c-MET = tyrosine-protein kinase Met or hepatocyte growth factor receptor, CPPs = Cell Penetrateing Peptides, CSF=Cerebrospinal Fluid, CTAB = Cetyl Trimethylammonium Bromide, DC-Chol = 3beta- [N-(N0,N0-dimethylaminoethane) carbamoyl] cholesterol, DOPE = L-α-dioleoyl phosphatidylethanolamine, DDAB = Dimethyl Dioctadecyl Ammonium Bromide, Dlin-MC3-DMA = 2,2-dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane, DMF = Dimethyl Formamide, DMPC = 1,2-dimyristoyl-sn-glycero-3-phosphocholine, DMSO = Dimethyl Sulfoxide, DOGS-NTA-Ni = 1,2-Dioleoylsn-glycero-3-[(N-(5-amino-1-carboxypentyl) imidodiacetic acid) succinyl nickel salt], DOTMA = N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethyl-ammonium chloride, EGFR = Endothelial Growth Factor, EUT = Emulsification-Ultrasonication Technique, FA = Folic Acid, FACT = Fatty Acid Coacervation Technique, FUM = Film Ultrasound Method, GB = Glyceryl Behenate, GBM = Glioblastoma Multiforme, $GemC_{12}$ = lauroyl-gemcitabine, GL = Gene Loading, GNLCs = Gelatin Nanostructured Lipid Carriers, GMO = Glyceryl monooleate, GMS = Glyceryl Monostearate, GRA = Glycyrrhetinic acid, GSH = Glutathione, GTP = Glyceryl Tripalmitate, HA-LNPs = Hyaluronic Acid Lipid-Based Nanoparticles HCC = Hepatocellular Carcinoma, HPMC = Hydroxy Propyl Methyl Cellulose, HPH = High Pressure Homogenization, HMET = Hot Melt Emulsion Technique, HMHT = Hot Melt Homogenization Technique, HST = Homogenization Sonication Technique, HSHT = High-Speed Homogenization Technique, HSPC = Hydrogenated Soybean Phosphatidyl Choline, HTMAB = Hexadecyl Trimethylammonium Bromide, ICV-STZ = Intra-cerebroventricular injection of streptozotocin, IDP = Interfacial Deposition of Polymer, LE = Lipid Emulsion, Lf = Lactoferrin, LHLN = 6-lauroxyhexyl lysinate, LNC = Lipid Nanocapsule, LPNP = Lipo-Polymeric Nanoparticles, LSM = Liposome, Mannan-PE = Mannan-Phosphatidyl Ethanolamine, MCT, = Medium Chain Triglycerides, ME = Melt Emulsification, MEM = Microemulsion, ME-SEM = Multiple Emulsification Solvent Evaporation Method, MG = Malignant Glioma, Mtf = Melanotransferrin, N/A = Non-Applicable, Nfasc = Neurofascin, MS=Multiple Sclerosis, NLCs = Nanostructured Lipid Carrier, Prer = Nanoprecipitation, NVU=Neuro Vascular Unit, PA = Dimyristoylphosphatidic acid, PAc = Palmitic Acid, PC = L-phosphatidyl choline, PCL = Poly (ε-Caprolactone), pCPP = Photo-responsive Cell Penetrating Peptide, PCLS = Precision-Cut Lung Slices, PD=Parkinson`s Disease, PEG = Poly Ethylene Glycol, PEG_{2000}-SA = Poly Ethylene $Glycol_{2000}$ – Stearic Acid, PE = Phosphatidylethanolamine, PG = Diphosphatidyl Glycerol, PLGA = Poly (Lactide-*co*-Glycolide), PMS = Polyethylene glycol Monostearate, PNPs = Polymeric

Nanoparticles, PTX = Paclitaxel, PVA = Poly Vinyl Alcohol, QESD = Quasi-Emulsion Solvent Diffusion, RGD = Arginine-glycine-aspartic acid,ROS=Reactive oxygen species, rtPA=recombinant tissue Plasminogen Activator, S1BRSA = serotonergic 1B receptor subtype antagonist SDM = Solvent Displacement Method, SFN =Sulforaphane, SESEM = Single Emulsification-Solvent Evaporation, SEEM = Solvent Emulsification/Evaporation Method, SEvaM = Solvent Evaporation Method, SDS = Sodium Dodecyl Sulfate, SDT = Solvent Displacement Technique, SIT = Solvent Injection Technique SLNs= Solid Lipid Nanoparticle, Sm = Sphingomyelin, SOD = Super-oxide dismutase, SPC = Soybean phosphatidylcholine, SPIONs = Super-Paramagnetic Iron Oxide Nanoparticles, ST = Shear Technique, StA = Stearic Acid, Trf = Transferrin, TMSP = Tumor Microenvironment-Sensitive Polypeptides, TMX = Tamoxifen, TTMAB = Tetradecy Trimethylammonium Bromide , UHT = Ultrasonic Homogenization Technique ZO=Zonula Occludens

Therapeutic Molecules Abbreviations

APO = Apomorphine, ASN = Asenapine, BCF = Baclofen, bFGF = basic Fibroblast Growth Factor, BVZ = Bevacizumab, CRB = Cytarabine, CUR = Curcumin, CRN = Carmustine, CTP = Camptothecin, DAA = Diacetyl Apomorphine, DCB = Decarbazine, DOX = Doxorubicin, DLX = Duloxetin, EDB = Erlotinib, EDF = Edelfosine, ETP = Etoposide, Gem = Gemcitabine, HupA = Huperzine A, IBU = Ibuprofen, IDB = Idebenone, ILO = Iloperidone IndOH = Indomethacin, LA-MEM = Lipoyl–Memantine, LMT = Lamotrigin, LNA = Locked Nucleic Acid, MEP = Methylprednisolone, NBP = 3-n-Butylphthalide, PPR = Piperine, PTX = Paclitaxel, QRT = Quercetin, R123 = Rhodamine 123, R800 = Rhodamine 800, RLZ = Riluzole, RPN = Ropinirole, RVR = Resveratrol, RSP = Risperidone, siRNA = Small Interference RNA, SMV = Simvastatin, SRB = Sulforhodamine B, TMZ = Temozolomide, TXL = Taxol, VCR = Vincristine, VIN = Vinpocetine, VPA = Valproic acid, ZRB = Zerumbone

Cell Abbreviations

A172 = Human Brain Cancer cell line A549 = human pulmonary adenocarcinoma cell line, ARPE-19 = Human retinal epithelium pigment, BCEC = Brain Capillary Endothelial Cells, bEnd3 = Brain endothelial cells, BxPC-3 = Pancreatic cancer cell line, C8D1A = Mouse astrocytes CaCo-2 = heterogeneous human epithelial colorectal adenocarcinoma, CHO = Chinese Hamster Ovary cells, Ea.hy926 = Human umbilical vein, HBMEC = Human brain microvascular endothelial cells, HEK-293 = Human embryonic kidney, HepG2 = Hepatocellular Carcinoma cell line, HT-1080 = human fibrosarcoma cells, HUVEC = Human Umbilical Vein Endothelial Cells, KB = human mouth epidermoid carcinoma cells, LLC = Lewis Lung Carcinoma cells, MCF-7 = human breast adenocarcinoma cell line, MES83 = Mesenchymal glioma stem cells, MGG8 = Human glioblastoma cell line, Mia PaCa-2 = Pancreatic cancer cell line, N2a NB = N2a Neuroblastoma cells, NCl-H460 = Human non-small

cell lung carcinoma cell line, NSCLC = Non-small cell lung cancer, Panc-1 = Pancreatic cancer cell line, PHWB = Primary Human Whole Blood, U87 MG = U87 Malignant glioma cells

Bibliography

[1] W. Stöber, A. Fink, E. Bohn, Controlled growth of monodisperse silica spheres in the micron size range, J. Colloid Interface Sci. 26 (1968) 62–69. doi:10.1016/0021-9797(68)90272-5.

[2] S. Wilhelm, A.J. Tavares, Q. Dai, S. Ohta, J. Audet, H.F. Dvorak, W.C.W. Chan, Analysis of nanoparticle delivery to tumours, Nat. Rev. Mater. 1 (2016) 16014. doi:10.1038/natrevmats.2016.14.

[3] J. Shi, P.W. Kantoff, R. Wooster, O.C. Farokhzad, Cancer nanomedicine: progress, challenges and opportunities, Nat. Rev. Cancer. 17 (2016) 20–37. doi:10.1038/nrc.2016.108.

[4] A. Jayagopal, M.F. Linton, S. Fazio, F.R. Haselton, Insights into Atherosclerosis Using Nanotechnology, Curr. Atheroscler. Rep. 12 (2010) 209–215. doi:10.1007/s11883-010-0106-7.

[5] M.E. Lobatto, V. Fuster, Z.A. Fayad, W.J.M. Mulder, Perspectives and opportunities for nanomedicine in the management of atherosclerosis, Nat. Rev. Drug Discov. 10 (2011) 835–852. doi:10.1038/nrd3578.

[6] J. Sargent, Cardiovascular disease: New nanomedicines for treating atherosclerotic plaques, Nat. Rev. Endocrinol. 11 (2015) 256–256. doi:10.1038/nrendo.2015.35.

[7] X. Yang, L. Jin, L. Yao, F.H. Shen, A. Shimer, X. Li, Antioxidative nanofullerol prevents intervertebral disk degeneration, Int. J. Nanomedicine. (2014) 2419. doi:10.2147/IJN.S60853.

[8] L. Jin, M. Ding, A. Oklopcic, B. Aghdasi, L. Xiao, Z. Li, V. Jevtovic-Todorovic, X. Li, Nanoparticle fullerol alleviates radiculopathy via NLRP3 inflammasome and neuropeptides., Nanomedicine. 13 (2017) 2049–2059. doi:10.1016/j.nano.2017.03.015.

[9] T. Dvir, M. Bauer, A. Schroeder, J.H. Tsui, D.G. Anderson, R. Langer, R. Liao, D.S. Kohane, Nanoparticles Targeting the Infarcted Heart, Nano Lett. 11 (2011) 4411–4414. doi:10.1021/nl2025882.

[10] S. Suarez, A. Almutairi, K.L. Christman, Micro- and nanoparticles for treating cardiovascular disease, Biomater. Sci. 3 (2015) 564–580. doi:10.1039/C4BM00441H.

[11] S. Wohlfart, S. Gelperina, J. Kreuter, Transport of drugs across the blood–brain barrier by nanoparticles, J. Control. Release. 161 (2012) 264–273. doi:10.1016/j.jconrel.2011.08.017.

[12] C. Saraiva, C. Praça, R. Ferreira, T. Santos, L. Ferreira, L. Bernardino, Nanoparticle-mediated brain drug delivery: Overcoming blood–brain barrier to treat neurodegenerative diseases, J. Control. Release. 235 (2016) 34–47. doi:10.1016/j.jconrel.2016.05.044.

[13] J. Kreuter, Drug delivery to the central nervous system by polymeric nanoparticles: What do we know?, Adv. Drug Deliv. Rev. 71 (2014) 2–14. doi:10.1016/j.addr.2013.08.008.

[14] T. Patel, J. Zhou, J.M. Piepmeier, W.M. Saltzman, Polymeric nanoparticles for drug

delivery to the central nervous system, Adv. Drug Deliv. Rev. 64 (2012) 701–705. doi:10.1016/j.addr.2011.12.006.

[15] R. Rajera, K. Nagpal, S.K. Singh, D.N. Mishra, Niosomes: A Controlled and Novel Drug Delivery System, Biol. Pharm. Bull. 34 (2011) 945–953. doi:10.1248/bpb.34.945.

[16] D. Ag Seleci, M. Seleci, J.G. Walter, F. Stahl, T. Scheper, Niosomes as nanoparticular drug carriers: Fundamentals and recent applications, J. Nanomater. 2016 (2016). doi:10.1155/2016/7372306.

[17] S. Chaurasia, S.S. Dogra, European Journal of Ejpmr Transfersomes : Novel Approach for Intranasal Delivery, Eur. J. Pharm. Rev. Artic. Eur. J. Pharm. Med. Res. Med. Res. 4 (2017) 192–203.

[18] B.S. Pattni, V. V. Chupin, V.P. Torchilin, New Developments in Liposomal Drug Delivery, Chem. Rev. 115 (2015) 10938–10966. doi:10.1021/acs.chemrev.5b00046.

[19] R.R. Sawant, V.P. Torchilin, Challenges in Development of Targeted Liposomal Therapeutics, AAPS J. 14 (2012) 303–315. doi:10.1208/s12248-012-9330-0.

[20] J. Ezzati Nazhad Dolatabadi, Y. Omidi, Solid lipid-based nanocarriers as efficient targeted drug and gene delivery systems, TrAC - Trends Anal. Chem. 77 (2016) 100–108. doi:10.1016/j.trac.2015.12.016.

[21] A. Beloqui, M. ?ngeles Solin?s, A. Rodr?guez-Gasc?n, A.J. Almeida, V. Pr?at, Nanostructured lipid carriers: Promising drug delivery systems for future clinics, Nanomedicine Nanotechnology, Biol. Med. 12 (2016) 143–161. doi:10.1016/j.nano.2015.09.004.

[22] M. Agrawal, Ajazuddin, D.K. Tripathi, S. Saraf, S. Saraf, S.G. Antimisiaris, S. Mourtas, M. Hammarlund-Udenaes, A. Alexander, Recent advancements in liposomes targeting strategies to cross blood-brain barrier (BBB) for the treatment of Alzheimer's disease., J. Control. Release. 260 (2017) 61–77. doi:10.1016/j.jconrel.2017.05.019.

[23] L.F. Gamarra, Getting into the brain : liposome-based strategies for effective drug delivery across the blood – brain barrier, Int. J. Nanomedicine. (2016) 5381–5414.

[24] J. Varshosaz, S. Taymouri, A. Pardakhty, M. Asadi-Shekaari, A. Babaee, Niosomes of Ascorbic Acid and ??-Tocopherol in the Cerebral Ischemia-Reperfusion Model in Male Rats, Biomed Res. Int. 2014 (2014). doi:10.1155/2014/816103.

[25] S. Haque, S. Md, M.I. Alam, J.K. Sahni, J. Ali, S. Baboota, Nanostructure-based drug delivery systems for brain targeting, Drug Dev. Ind. Pharm. 38 (2012) 387–411. doi:10.3109/03639045.2011.608191.

[26] C. Spuch, C. Navarro, Liposomes for Targeted Delivery of Active Agents against Neurodegenerative Diseases (Alzheimer's Disease and Parkinson's Disease), J. Drug Deliv. 2011 (2011) 1–12. doi:10.1155/2011/469679.

[27] M.R. Gasco, Method for producing solid lipid microspheres having a naroww size distribution, US005250236A, 1993.

[28] M.R. Gasco, Lipid nanoparticles: perspectives and challenges, Adv. Drug Deliv. Rev. 59 (2007) 377–378. doi:10.1016/j.addr.2007.05.004.

[29] S. Müller, R. H., Mäder, K., Gohla, Solid lipid nanoparticles (SLN) for controlled drug delivery - a review of the state of the art, Eur. J. Pharmaxeutics Biopharm. 50 (2000) 161–177.

[30] N. Naseri, H. Valizadeh, P. Zakeri-Milani, Solid lipid nanoparticles and nanostructured lipid carriers: Structure preparation and application, Adv. Pharm. Bull. 5 (2015) 305–313. doi:10.15171/apb.2015.043.

[31] L. Feng, R.J. Mumper, A critical review of lipid-based nanoparticles for taxane delivery, Cancer Lett. 334 (2013) 157–175. doi:10.1016/j.canlet.2012.07.006.

[32] S.A.W. R.H. Muller, M. Radtke, Nanostructured lipid matrices for improved microencapsulation of drugs, Int. J. Pharm. 242 (2002) 121–128.

[33] S. Das, W.K. Ng, R.B.H. Tan, Are nanostructured lipid carriers (NLCs) better than solid lipid nanoparticles (SLNs): development, characterizations and comparative evaluations of clotrimazole-loaded SLNs and NLCs?, Eur J Pharm Sci. 47 (2012) 139–151. doi:10.1016/j.ejps.2012.05.010.

[34] F. Li, Y. Wang, Z. Liu, X. Lin, H. He, X. Tang, Formulation and characterization of bufadienolides-loaded nanostructured lipid carriers, Drug Dev. Ind. Pharm. 36 (2010) 508–517. doi:10.3109/03639040903264397.

[35] T. Alam, J. Pandit, D. Vohora, M. Aqil, A. Ali, Y. Sultana, Optimization of nanostructured lipid carriers of lamotrigine for brain delivery: in vitro characterization and in vivo efficacy in epilepsy, Expert Opin. Drug Deliv. 12 (2015) 181–194. doi:10.1517/17425247.2014.945416.

[36] J. Pardeike, S. Weber, T. Haber, J. Wagner, H.P. Zarfl, H. Plank, A. Zimmer, Development of an itraconazole-loaded nanostructured lipid carrier (NLC) formulation for pulmonary application, Int J Pharm. 419 (2011) 329–338. doi:10.1016/j.ijpharm.2011.07.040.

[37] W. Mehnert, K. Mäder, Solid lipid nanoparticles, Adv. Drug Deliv. Rev. 64 (2012) 83–101. doi:10.1016/j.addr.2012.09.021.

[38] R. Parhi, P. Suresh, Production of Solid Lipid Nanoparticles-Drug Loading and Release Mechanism, J. Chem. Pharmacuetical Res. 2 (2010) 211–227.

[39] M.A. Karami, B. Sharif Makhmal Zadeh, M. Kouchak, E. Moghimipour, Superoxide Dismutase-Loaded Solid Lipid Nanoparticles Prepared by Cold Homogenization Method: Characterization and Permeation Study Through Burned Rat Skin, Jundishapur J. Nat. Pharm. Prod. 11 (2016). doi:10.17795/jjnpp-33968.

[40] C. Fornaguera, A. Dols-Perez, G. Calderó, M.J. García-Celma, J. Camarasa, C. Solans, PLGA nanoparticles prepared by nano-emulsion templating using low-energy methods as efficient nanocarriers for drug delivery across the blood–brain barrier, J. Control. Release. 211 (2015) 134–143. doi:10.1016/j.jconrel.2015.06.002.

[41] S. Gelperina, O. Maksimenko, A. Khalansky, L. Vanchugova, E. Shipulo, K. Abbasova, R. Berdiev, S. Wohlfart, N. Chepurnova, J. Kreuter, Drug delivery to the brain using surfactant-coated poly(lactide-co-glycolide) nanoparticles: Influence of the formulation parameters, Eur. J. Pharm. Biopharm. 74 (2010) 157–163. doi:10.1016/j.ejpb.2009.09.003.

[42] C. Tapeinos, A. Larrañaga, J.-R. Sarasua, A. Pandit, A. Larranaga, J.-R. Sarasua, A.

Pandit, Functionalised collagen spheres reduce H2O2 mediated apoptosis by scavenging overexpressed ROS, Nanomedicine Nanotechnology, Biol. Med. (2017). doi:10.1016/j.nano.2017.03.022.

[43] V.K. Venishetty, R. Komuravelli, M. Kuncha, R. Sistla, P. V. Diwan, Increased brain uptake of docetaxel and ketoconazole loaded folate-grafted solid lipid nanoparticles, Nanomedicine Nanotechnology, Biol. Med. 9 (2013) 111–121. doi:10.1016/j.nano.2012.03.003.

[44] M. Wang, L. Qin, K. Li, R. Zhu, W. Wang, S. Wang, The improvement of the anticancer effect of a novel compound benzoic acid, 2-hydroxy-, 2-D-ribofuranosylhydrazide (BHR) loaded in solid lipid nanoparticles, AAPS PharmSciTech. 13 (2012) 1348–1354. doi:10.1208/s12249-012-9862-8.

[45] D. Pooja, L. Tunki, H. Kulhari, B.B. Reddy, R. Sistla, Optimization of solid lipid nanoparticles prepared by a single emulsification-solvent evaporation method, Data Br. 6 (2016) 15–19. doi:10.1016/j.dib.2015.11.038.

[46] S. Song, G. Mao, J. Du, X. Zhu, Novel RGD containing, temozolomide-loading nanostructured lipid carriers for glioblastoma multiforme chemotherapy, Drug Deliv. 23 (2016) 1404–1408. doi:10.3109/10717544.2015.1064186.

[47] Z. Chen, X. Lai, S. Song, X. Zhu, J. Zhu, Nanostructured lipid carriers based temozolomide and gene co-encapsulated nanomedicine for gliomatosis cerebri combination therapy, Drug Deliv. 23 (2016) 1369–1373. doi:10.3109/10717544.2015.1038857.

[48] M. Wu, Y. Fan, S. Lv, B. Xiao, M. Ye, X. Zhu, Vincristine and temozolomide combined chemotherapy for the treatment of glioma: a comparison of solid lipid nanoparticles and nanostructured lipid carriers for dual drugs delivery, Drug Deliv. 23 (2016) 2720–2725. doi:10.3109/10717544.2015.1058434.

[49] J. Qu, L. Zhang, Z. Chen, G. Mao, Z. Gao, X. Lai, X. Zhu, J. Zhu, Nanostructured lipid carriers, solid lipid nanoparticles, and polymeric nanoparticles: which kind of drug delivery system is better for glioblastoma chemotherapy?, Drug Deliv. 23 (2016) 3408–3416. doi:10.1080/10717544.2016.1189465.

[50] N. V. Shah, A.K. Seth, R. Balaraman, C.J. Aundhia, R.A. Maheshwari, G.R. Parmar, Nanostructured lipid carriers for oral bioavailability enhancement of raloxifene: Design and in vivo study, J Adv Res. 7 (2016) 423–434. doi:10.1016/j.jare.2016.03.002.

[51] D. Xia, N. Shrestha, J. Van De Streek, H. Mu, M. Yang, Spray drying of fenofibrate loaded nanostructured lipid carriers, Asian J. Pharm. Sci. 11 (2016) 507–515. doi:10.1016/j.ajps.2016.01.001.

[52] M.R. Freitas C, Spray-drying of Solid lipid nanoparticles (SLNTM), Eur J Pharm Biopharm. 46 (1998) 145–151.

[53] S. Weber, A. Zimmer, J. Pardeike, European Journal of Pharmaceutics and Biopharmaceutics Solid Lipid Nanoparticles (SLN) and Nanostructured Lipid Carriers (NLC) for pulmonary application : A review of the state of the art, Eur. J. Pharm. Biopharm. (2013). doi:10.1016/j.ejpb.2013.08.013.

[54] R. Campardelli, M. Cherain, C. Perfetti, C. Iorio, M. Scognamiglio, E. Reverchon,

G. Della, The Journal of Supercritical Fluids Lipid nanoparticles production by supercritical fluid assisted emulsion – diffusion, J. Supercrit. Fluids. 82 (2013) 34–40. doi:10.1016/j.supflu.2013.05.020.

[55] D. Chirio, M. Gallarate, E. Peira, L. Battaglia, L. Serpe, M. Trotta, Formulation of curcumin-loaded solid lipid nanoparticles produced by fatty acids coacervation technique, J. Microencapsul. 28 (2011) 537–548. doi:10.3109/02652048.2011.590615.

[56] C. Tapeinos, E.K. Efthimiadou, N. Boukos, G. Kordas, Sustained release profile of quatro stimuli nanocontainers as a multi sensitive vehicle exploiting cancer characteristics, Colloids Surfaces B Biointerfaces. 148 (2016) 95–103. doi:10.1016/j.colsurfb.2016.08.019.

[57] E.K. Efthimiadou, C. Tapeinos, A. Chatzipavlidis, N. Boukos, E. Fragogeorgi, L. Palamaris, G. Loudos, G. Kordas, Dynamic in vivo imaging of dual-triggered microspheres for sustained release applications: Synthesis, characterization and cytotoxicity study, Int. J. Pharm. 461 (2014). doi:10.1016/j.ijpharm.2013.11.037.

[58] C. Tapeinos, I. Kartsonakis, P. Liatsi, I. Daniilidis, G. Kordas, Synthesis and characterization of magnetic nanocontainers, J. Am. Ceram. Soc. 91 (2008). doi:10.1111/j.1551-2916.2007.02240.x.

[59] S. Mura, J. Nicolas, P. Couvreur, Stimuli-responsive nanocarriers for drug delivery, Nat. Mater. 12 (2013) 991–1003. doi:10.1038/nmat3776.

[60] H. Cao, Y. Dong, L. Bre, C. Tapeinos, W. Wang, A. Pandit, An acetal-based polymeric crosslinker with controlled pH-sensitivity, RSC Adv. 6 (2016) 9604–9611. doi:10.1039/C6RA00423G.

[61] E.K. Efthimiadou, C. Tapeinos, P. Bilalis, G. Kordas, New approach in synthesis, characterization and release study of pH-sensitive polymeric micelles, based on PLA-Lys-b-PEGm, conjugated with doxorubicin, J. Nanoparticle Res. 13 (2011). doi:10.1007/s11051-011-0579-5.

[62] C. Tapeinos, A. Pandit, Physical, Chemical, and Biological Structures based on ROS-Sensitive Moieties that are Able to Respond to Oxidative Microenvironments, Adv. Mater. 28 (2016) 5553–5585. doi:10.1002/adma.201505376.

[63] E.K. Efthimiadou, C. Tapeinos, L.A. Tziveleka, N. Boukos, G. Kordas, PH- and thermo-responsive microcontainers as potential drug delivery systems: Morphological characteristic, release and cytotoxicity studies, Mater. Sci. Eng. C. 37 (2014) 271–277. doi:10.1016/j.msec.2014.01.024.

[64] C. Tapeinos, E.K. Efthimiadou, N. Boukos, C. a. Charitidis, M. Koklioti, G. Kordas, Microspheres as therapeutic delivery agents: synthesis and biological evaluation of pH responsiveness, J. Mater. Chem. B. (2013) 194–203. doi:10.1039/c2tb00013j.

[65] J. Bizeau, C. Tapeinos, C. Marella, A. Larrañaga, A. Pandit, Synthesis and characterization of hyaluronic acid coated manganese dioxide microparticles that act as ROS scavengers, Colloids Surfaces B Biointerfaces. 159 (2017) 30–38. doi:10.1016/j.colsurfb.2017.07.081.

[66] M. Masserini, Nanoparticles for Brain Drug Delivery, ISRN Biochem. 2013 (2013)

1–18. doi:10.1155/2013/238428.

[67] E.F. Craparo, M.L. Bondì, G. Pitarresi, Cavallaro Gennara, Nanoparticulate Systems for Drug Delivery and Targeting to the Central Nervous System, CNS Neurosci. Ther. 17 (2011) 670–677. doi:10.1111/j.1755-5949.2010.00199.x.

[68] H. Yang, Nanoparticle-mediated brain-specific drug delivery, imaging, and diagnosis, Pharm. Res. 27 (2010) 1759–1771. doi:10.1007/s11095-010-0141-7.

[69] R.A. Morshed, Y. Cheng, B. Auffinger, M.L. Wegscheid, M.S. Lesniak, The potential of polymeric micelles in the context of glioblastoma therapy, Front. Pharmacol. 4 (2013). doi:10.3389/fphar.2013.00157.

[70] V.K. Thakur, M.K. Thakur, eds., Handbook of Polymers for Pharmaceutical Technologies, John Wiley & Sons, Inc., Hoboken, NJ, USA, 2015. doi:10.1002/9781119041450.

[71] M. Esteves, A.C. Cristóvão, T. Saraiva, S.M. Rocha, G. Baltazar, L. Ferreira, L. Bernardino, Retinoic acid-loaded polymeric nanoparticles induce neuroprotection in a mouse model for parkinson's disease, Front. Aging Neurosci. 7 (2015) 1–10. doi:10.3389/fnagi.2015.00020.

[72] C.Y. Lee, I.H. Ooi, Preparation of temozolomide-loaded nanoparticles for glioblastoma multiforme targeting-ideal versus reality, Pharmaceuticals. 9 (2016). doi:10.3390/ph9030054.

[73] T. Führmann, M. Ghosh, A. Otero, B. Goss, T.R. Dargaville, D.D. Pearse, P.D. Dalton, Peptide-functionalized polymeric nanoparticles for active targeting of damaged tissue in animals with experimental autoimmune encephalomyelitis, Neurosci. Lett. 602 (2015) 126–132. doi:10.1016/j.neulet.2015.06.049.

[74] B. Fonseca-santos, M. Daflon palmira, Gremiao, M. Chorilli, Nanotechnology-based drug delivery systems for the treatment of Alzheimer ' s disease, Int. J. Nanomedicine. 10 (2015) 4981–5003. doi:10.2147/IJN.S87148.

[75] D. Hadavi, A.A. Poot, Biomaterials for the Treatment of Alzheimer's Disease, Front. Bioeng. Biotechnol. 4 (2016). doi:10.3389/fbioe.2016.00049.

[76] S. Somani, C. Dufès, Applications of dendrimers for brain delivery and cancer therapy, Nanomedicine. 9 (2014) 2403–2414. doi:10.2217/nnm.14.130.

[77] D. Pandita, N. Poonia, S. Kumar, V. Lather, K. Madaan, Dendrimers in drug delivery and targeting: Drug-dendrimer interactions and toxicity issues, J. Pharm. Bioallied Sci. 6 (2014) 139. doi:10.4103/0975-7406.130965.

[78] L. Xu, H. Zhang, Y. Wu, Dendrimer advances for the central nervous system delivery of therapeutics, ACS Chem. Neurosci. 5 (2014) 2–13. doi:10.1021/cn400182z.

[79] J. Zhao, B. Zhang, S. Shen, J. Chen, Q. Zhang, X. Jiang, Z. Pang, CREKA peptide-conjugated dendrimer nanoparticles for glioblastoma multiforme delivery, J. Colloid Interface Sci. 450 (2015) 396–403. doi:10.1016/j.jcis.2015.03.019.

[80] S. Beg, A. Samad, M.I. Alam, I. Nazish, Dendrimers as novel systems for delivery of neuropharmaceuticals to the brain., CNS Neurol. Disord. Drug Targets. 10 (2011) 576–88. http://www.ncbi.nlm.nih.gov/pubmed/21631401.

[81] T. Glaser, I. Han, L. Wu, X. Zeng, Targeted nanotechnology in glioblastoma multiforme, Front. Pharmacol. 8 (2017) 1–14. doi:10.3389/fphar.2017.00166.

[82] K. Cherukula, K. Manickavasagam Lekshmi, S. Uthaman, K. Cho, C.-S. Cho, I.-K. Park, Multifunctional Inorganic Nanoparticles: Recent Progress in Thermal Therapy and Imaging, Nanomaterials. 6 (2016) 76. doi:10.3390/nano6040076.

[83] A.C. Anselmo, S. Mitragotri, A Review of Clinical Translation of Inorganic Nanoparticles, AAPS J. 17 (2015) 1041–1054. doi:10.1208/s12248-015-9780-2.

[84] K. Mahmoudi, C.G. Hadjipanayis, The application of magnetic nanoparticles for the treatment of brain tumors, Front. Chem. 2 (2014) 1–5. doi:10.3389/fchem.2014.00109.

[85] M. Busquets, R. Sabaté, J. Estelrich, Potential applications of magnetic particles to detect and treat Alzheimer's disease, Nanoscale Res. Lett. 9 (2014) 538. doi:10.1186/1556-276X-9-538.

[86] D.Y. Joh, L. Sun, M. Stangl, A. Al Zaki, S. Murty, P.P. Santoiemma, J.J. Davis, B.C. Baumann, M. Alonso-Basanta, D. Bhang, G.D. Kao, A. Tsourkas, J.F. Dorsey, Selective Targeting of Brain Tumors with Gold Nanoparticle-Induced Radiosensitization, PLoS One. 8 (2013). doi:10.1371/journal.pone.0062425.

[87] N. Gao, H. Sun, K. Dong, J. Ren, X. Qu, Gold-nanoparticle-based multifunctional amyloid-β inhibitor against Alzheimer's disease, Chem. - A Eur. J. 21 (2015) 829–835. doi:10.1002/chem.201404562.

[88] C.K. Kim, T. Kim, I.Y. Choi, M. Soh, D. Kim, Y.J. Kim, H. Jang, H.S. Yang, J.Y. Kim, H.K. Park, S.P. Park, S. Park, T. Yu, B.W. Yoon, S.H. Lee, T. Hyeon, Ceria nanoparticles that can protect against ischemic stroke, Angew. Chemie - Int. Ed. 51 (2012) 11039–11043. doi:10.1002/anie.201203780.

[89] X.-X. Zhang, T.J. McIntosh, M.W. Grinstaff, Functional lipids and lipoplexes for improved gene delivery, Biochimie. 94 (2012) 42–58. doi:10.1016/j.biochi.2011.05.005.

[90] A. Schroeder, C.G. Levins, C. Cortez, R. Langer, D.G. Anderson, Lipid-based nanotherapeutics for siRNA delivery, J. Intern. Med. 267 (2010) 9–21. doi:10.1111/j.1365-2796.2009.02189.x.

[91] W. Chen, H. Li, Z. Liu, W. Yuan, Lipopolyplex for therapeutic gene delivery and its application for the treatment of Parkinson's disease, Front. Aging Neurosci. 8 (2016) 1–10. doi:10.3389/fnagi.2016.00068.

[92] P.M. Costa, A.L. Cardoso, C. Nóbrega, L.F. Pereira de almeida, J.N. Bruce, P. Canoll, M.C. Pedroso de Lima, MicroRNA-21 silencing enhances the cytotoxic effect of the antiangiogenic drug sunitinib in glioblastoma, Hum. Mol. Genet. 22 (2013) 904–918. doi:10.1093/hmg/dds496.

[93] J. Zhou, K.-B. Atsina, B.T. Himes, G.W. Strohbehn, W.M. Saltzman, Novel Delivery Strategies for Glioblastoma, Cancer J. 18 (2012) 89–99. doi:10.1097/PPO.0b013e318244d8ae.

[94] C. Tros de Ilarduya, Y. Sun, N. Düzgüneş, Gene delivery by lipoplexes and polyplexes, Eur. J. Pharm. Sci. 40 (2010) 159–170. doi:10.1016/j.ejps.2010.03.019.

[95] N. Nayerossadat, P. Ali, T. Maedeh, Viral and nonviral delivery systems for gene delivery, Adv. Biomed. Res. 1 (2012) 27. doi:10.4103/2277-9175.98152.

[96] M. Huang, M. Hu, Q. Song, H. Song, J. Huang, X. Gu, X. Wang, J. Chen, T. Kang, X. Feng, D. Jiang, G. Zheng, H. Chen, X. Gao, GM1-Modified Lipoprotein-like Nanoparticle: Multifunctional Nanoplatform for the Combination Therapy of Alzheimer's Disease, ACS Nano. 9 (2015) 10801–10816. doi:10.1021/acsnano.5b03124.

[97] J. Varkey, N. Mizuno, B.G. Hegde, N. Cheng, A.C. Steven, R. Langen, A-Synuclein Oligomers With Broken Helical Conformation Form Lipoprotein Nanoparticles, J. Biol. Chem. 288 (2013) 17620–17630. doi:10.1074/jbc.M113.476697.

[98] K.M. McMahon, L. Foit, N.L. Angeloni, F.J. Giles, L.I. Gordon, C.S. Thaxton, Synthetic High-Density Lipoprotein-Like Nanoparticles as Cancer Therapy, in: 2015: pp. 129–150. doi:10.1007/978-3-319-16555-4_6.

[99] Q. Song, M. Huang, L. Yao, X. Wang, X. Gu, J. Chen, J. Chen, J. Huang, Q. Hu, T. Kang, Z. Rong, H. Qi, G. Zheng, H. Chen, X. Gao, Lipoprotein-based nanoparticles rescue the memory loss of mice with alzheimer's disease by accelerating the clearance of amyloid-beta, ACS Nano. 8 (2014) 2345–2359. doi:10.1021/nn4058215.

[100] H. Huang, W. Cruz, J. Chen, G. Zheng, Learning from biology: synthetic lipoproteins for drug delivery, Wiley Interdiscip. Rev. Nanomedicine Nanobiotechnology. 7 (2015) 298–314. doi:10.1002/wnan.1308.

[101] F.L. Cardoso, D. Brites, M.A. Brito, Looking at the blood-brain barrier: Molecular anatomy and possible investigation approaches, Brain Res. Rev. 64 (2010) 328–363. doi:10.1016/j.brainresrev.2010.05.003.

[102] D. Ricard, A. Idbaih, F. Ducray, M. Lahutte, K. Hoang-Xuan, J.Y. Delattre, Primary brain tumours in adults, Lancet. 379 (2012) 1984–1996. doi:10.1016/S0140-6736(11)61346-9.

[103] Y. Koo, G. Reddy, M. Bhojani, R. Schneider, M. Philbert, A. Rehemtulla, B. Ross, R. Kopelman, Brain cancer diagnosis and therapy with nanoplatforms☆, Adv. Drug Deliv. Rev. 58 (2006) 1556–1577. doi:10.1016/j.addr.2006.09.012.

[104] G. LB, C. Bertels, D. JN, Interrater reliability of the nih stroke scale, Arch. Neurol. 46 (1989) 660–662.

[105] J.L. Hinkle, M.M. Guanci, Acute ischemic stroke review., J. Neurosci. Nurs. 39 (2007) 285–293, 310. doi:10.1097/01376517-200710000-00005.

[106] Connolly Barbara S., Lang Anthony E., Pharmacological Treatment of Parkinson Disease, Jama. 311 (2014) 442–449. doi:10.1111/bcp.12170.

[107] M. Ferri C.P., Prince, M.P., Brayne. C., Brodaty, H., Fratiglioni, L., Ganguli, M., Hall, K., Hasegawa, K., Hendrie, H., Huang, Y., Jorm, a., Mathers, C., Menezes, P.R., Rimmer, E., & Scazufca, Global prevalence of dementia: a Delphi consensus study, Lancet. 366 (2005) 2112–2117. doi:10.1016/S0140-6736(05)67889-0.Global.

[108] S. Dolati, Z. Babaloo, F. Jadidi-Niaragh, H. Ayromlou, S. Sadreddini, M. Yousefi,

Multiple sclerosis: Therapeutic applications of advancing drug delivery systems, Biomed. Pharmacother. 86 (2017) 343–353. doi:10.1016/j.biopha.2016.12.010.

[109] Y.-C. Kuo, C.-T. Liang, Inhibition of human brain malignant glioblastoma cells using carmustine-loaded catanionic solid lipid nanoparticles with surface anti-epithelial growth factor receptor, Biomaterials. 32 (2011) 3340–3350. doi:10.1016/j.biomaterials.2011.01.048.

[110] Y.C. Kuo, C. Te Liang, Catanionic solid lipid nanoparticles carrying doxorubicin for inhibiting the growth of U87MG cells, Colloids Surfaces B Biointerfaces. 85 (2011) 131–137. doi:10.1016/j.colsurfb.2011.02.011.

[111] Y.C. Kuo, I.H. Lee, Delivery of doxorubicin to glioblastoma multiforme in vitro using solid lipid nanoparticles with surface aprotinin and melanotransferrin antibody for enhanced chemotherapy, J. Taiwan Inst. Chem. Eng. 61 (2015) 32–45. doi:10.1016/j.jtice.2015.12.012.

[112] Y.C. Kuo, C.H. Lee, Inhibition against growth of glioblastoma multiforme in vitro using etoposide-loaded solid lipid nanoparticles with p-aminophenyl-α-D-manno-pyranoside and folic acid, J. Pharm. Sci. 104 (2015) 1804–1814. doi:10.1002/jps.24388.

[113] Y.C. Kuo, C.C. Wang, Carmustine-loaded catanionic solid lipid nanoparticles with serotonergic 1B receptor subtype antagonist for in vitro targeted delivery to inhibit brain cancer growth, J. Taiwan Inst. Chem. Eng. 46 (2015) 1–14. doi:10.1016/j.jtice.2014.08.035.

[114] Y.C. Kuo, C.H. Lee, Dual targeting of solid lipid nanoparticles grafted with 83-14 MAb and anti-EGF receptor for malignant brain tumor therapy, Life Sci. 146 (2016) 222–231. doi:10.1016/j.lfs.2016.01.025.

[115] Y.C. Kuo, I.W. Chao, Conjugation of melanotransferrin antibody on solid lipid nanoparticles for mediating brain cancer malignancy, Biotechnol. Prog. 32 (2016) 480–490. doi:10.1002/btpr.2214.

[116] Y.C. Kuo, S.J. Cheng, Brain targeted delivery of carmustine using solid lipid nanoparticles modified with tamoxifen and lactoferrin for antitumor proliferation, Int. J. Pharm. 499 (2016) 10–19. doi:10.1016/j.ijpharm.2015.12.054.

[117] Y.-C. Kuo, I.-H. Wang, Enhanced delivery of etoposide across the blood-brain barrier to restrain brain tumor growth using melanotransferrin antibody- and tamoxifen-conjugated solid lipid nanoparticles., 2016. doi:10.3109/1061186X.2015.1132223.

[118] A. Estella-Hermoso De Mendoza, V. Préat, F. Mollinedo, M.J. Blanco-Prieto, In vitro and in vivo efficacy of edelfosine-loaded lipid nanoparticles against glioma, J. Control. Release. 156 (2011) 421–426. doi:10.1016/j.jconrel.2011.07.030.

[119] P. Sharma, B. Dube, K. Sawant, Development and evaluation of nanostructured lipid carriers of cytarabine for treatment of meningeal leukemia, J Nanosci Nanotechnol. 11 (2011) 6676–6682. doi:10.1166/jnn.2011.4235.

[120] J. Jin, K.H. Bae, H. Yang, S.J. Lee, H. Kim, Y. Kim, K.M. Joo, S.W. Seo, T.G. Park, D.-H. Nam, In Vivo Specific Delivery of c-Met siRNA to Glioblastoma Using Cationic Solid Lipid Nanoparticles, Bioconjug. Chem. 22 (2011) 2568–2572.

doi:10.1021/bc200406n.

[121] S. Martins, S. Costa-Lima, T. Carneiro, A. Cordeiro-Da-Silva, E.B. Souto, D.C. Ferreira, Solid lipid nanoparticles as intracellular drug transporters: An investigation of the uptake mechanism and pathway, Int. J. Pharm. 430 (2012) 216–227. doi:10.1016/j.ijpharm.2012.03.032.

[122] S. Martins, I. Tho, I. Reimold, G. Fricker, E. Souto, D. Ferreira, M. Brandl, Brain delivery of camptothecin by means of solid lipid nanoparticles: Formulation design, in vitro and in vivo studies, Int. J. Pharm. 439 (2012) 49–62. doi:10.1016/j.ijpharm.2012.09.054.

[123] S.M. Martins, B. Sarmento, C. Nunes, M. Lúcio, S. Reis, D.C. Ferreira, Brain targeting effect of camptothecin-loaded solid lipid nanoparticles in rat after intravenous administration, Eur. J. Pharm. Biopharm. 85 (2013) 488–502. doi:10.1016/j.ejpb.2013.08.011.

[124] A. Khajavinia, J. Varshosaz, A.J. Dehkordi, Targeting etoposide to acute myelogenous leukaemia cells using nanostructured lipid carriers coated with transferrin, Nanotechnology. 23 (2012) 405101. doi:10.1088/0957-4484/23/40/405101.

[125] A. Griveau, J. Bejaud, S. Anthiya, S. Avril, D. Autret, E. Garcion, Silencing of miR-21 by locked nucleic acid-lipid nanocapsule complexes sensitize human glioblastoma cells to radiation-induced cell death, Int. J. Pharm. 454 (2013) 765–774. doi:10.1016/j.ijpharm.2013.05.049.

[126] A. Zanotto-Filho, K. Coradini, E. Braganhol, R. Schröder, C.M. de Oliveira, A. Simões-Pires, A.M.O. Battastini, A.R. Pohlmann, C.M. Guterres, Sílvia Stanisisçuaski Guterres Forcelini, R.C.R. Beck, J.C.F. Moreira, Curcumin-loaded lipid-core nanocapsules as a strategy to improve pharmacological efficacy of curcumin in glioma treatment, Eur. J. Pharm. Biopharm. 83 (2013) 156–167. doi:10.1016/j.ejpb.2012.10.019.

[127] A. Bernardi, R.L. Frozza, J.B. Hoppe, C. Salbego, A.R. Pohlmann, A.M.O. Battastini, S.S. Guterres, The antiproliferative effect of indomethacin-loaded lipid-core nanocapsules in glioma cells is mediated by cell cycle regulation, differentiation, and the inhibition of survival pathways., Int. J. Nanomedicine. 8 (2013) 711–28. doi:10.2147/IJN.S40284.

[128] S. Jose, S.S. Anju, T.A. Cinu, N.A. Aleykutty, S. Thomas, E.B. Souto, In vivo pharmacokinetics and biodistribution of resveratrol-loaded solid lipid nanoparticles for brain delivery, Int. J. Pharm. 474 (2014) 6–13. doi:10.1016/j.ijpharm.2014.08.003.

[129] L. Battaglia, M. Gallarate, E. Peira, D. Chirio, E. Muntoni, E. Biasibetti, M.T. Capucchio, A. Valazza, P.P. Panciani, M. Lanotte, D. Schiffer, L. Annovazzi, V. Caldera, M. Mellai, C. Riganti, Solid lipid nanoparticles for potential doxorubicin delivery in glioblastoma treatment: Preliminary in vitro studies, J. Pharm. Sci. 103 (2014) 2157–2165. doi:10.1002/jps.24002.

[130] D. Chirio, M. Gallarate, E. Peira, L. Battaglia, E. Muntoni, C. Riganti, E. Biasibetti, M.T. Capucchio, A. Valazza, P. Panciani, M. Lanotte, L. Annovazzi, V. Caldera, M. Mellai, G. Filice, S. Corona, D. Schiffer, Positive-charged solid lipid

nanoparticles as paclitaxel drug delivery system in glioblastoma treatment, Eur. J. Pharm. Biopharm. 88 (2014) 746–758. doi:10.1016/j.ejpb.2014.10.017.

[131] R.G. Madane, H.S. Mahajan, Curcumin-loaded nanostructured lipid carriers (NLCs) for nasal administration: design, characterization, and in vivo study, Drug Deliv. 7544 (2014) 1–9. doi:10.3109/10717544.2014.975382.

[132] Z.R. Cohen, S. Ramishetti, N. Peshes-Yaloz, M. Goldsmith, A. Wohl, Z. Zibly, D. Peer, Localized RNAi Therapeutics of Chemoresistant Grade IV Glioma Using Hyaluronan-Grafted Lipid-Based Nanoparticles, ACS Nano. 9 (2015) 1581–1591. doi:10.1021/nn506248s.

[133] L. Battaglia, M. Gallarate, E. Peira, D. Chirio, I. Solazzi, S.M.A. Giordano, C.L. Gigliotti, C. Riganti, C. Dianzani, Bevacizumab loaded solid lipid nanoparticles prepared by the coacervation technique: preliminary in vitro studies, Nanotechnology. 26 (2015) 255102. doi:10.1088/0957-4484/26/25/255102.

[134] Z. Chen, X. Lai, S. Song, X. Zhu, J. Zhu, Nanostructured lipid carriers based temozolomide and gene co-encapsulated nanomedicine for gliomatosis cerebri combination therapy., Drug Deliv. 23 (2015) 1369–73. doi:10.3109/10717544.2015.1038857.

[135] S. Song, G. Mao, J. Du, X. Zhu, Novel RGD containing , temozolomide-loading nanostructured lipid carriers for glioblastoma multiforme chemotherapy, Drug Deliv. 0 (2015) 1–5. doi:10.3109/10717544.2015.1064186.

[136] M. Wu, Y. Fan, S. Lv, B. Xiao, M. Ye, X. Zhu, Vincristine and temozolomide combined chemotherapy for the treatment of glioma: a comparison of solid lipid nanoparticles and nanostructured lipid carriers for dual drugs delivery, Drug Deliv. 23 (2016) 2720–2725. doi:10.3109/10717544.2015.1058434.

[137] Y.H. Chen, L.Z. Pan, M. Jiang, D. Li, L.J. Jin, Nanostructured lipid carriers enhance the bioavailability and brain cancer inhibitory efficacy of curcumin both in vitro and in vivo, Drug Deliv. 23 (2016) 1383–1392. doi:10.3109/10717544.2015.1049719.

[138] J. Emami, M. Rezazadeh, H. Sadeghi, K. Khadivar, Development and optimization of transferrin-conjugated nanostructured lipid carriers for brain delivery of paclitaxel using Box–Behnken design, Pharm. Dev. Technol. 22 (2017) 370–382. doi:10.1080/10837450.2016.1189933.

[139] D. Yu, O.F. Khan, M.L. Suvà, B. Dong, W.K. Panek, T. Xiao, M. Wu, Y. Han, A.U. Ahmed, I. V. Balyasnikova, H.F. Zhang, C. Sun, R. Langer, D.G. Anderson, M.S. Lesniak, Multiplexed RNAi therapy against brain tumor-initiating cells via lipopolymeric nanoparticle infusion delays glioblastoma progression, Proc. Natl. Acad. Sci. (2017) 201701911. doi:10.1073/pnas.1701911114.

[140] J.E. Dahlman, C. Barnes, O.F. Khan, A. Thiriot, S. Jhunjunwala, T.E. Shaw, Y. Xing, H.B. Sager, G. Sahay, L. Speciner, A. Bader, R.L. Bogorad, H. Yin, T. Racie, Y. Dong, S. Jiang, D. Seedorf, A. Dave, K. Singh Sandhu, M.J. Webber, T. Novobrantseva, V.M. Ruda, A.K.R. Lytton-Jean, C.G. Levins, B. Kalish, D.K. Mudge, M. Perez, L. Abezgauz, P. Dutta, L. Smith, K. Charisse, M.W. Kieran, K. Fitzgerald, M. Nahrendorf, D. Danino, R.M. Tuder, U.H. von Andrian, A. Akinc, D. Panigrahy, A. Schroeder, V. Koteliansky, R. Langer, D.G. Anderson, In vivo

endothelial siRNA delivery using polymeric nanoparticles with low molecular weight., Nat. Nanotechnol. 9 (2014) 648–655. doi:10.1038/nnano.2014.84.

[141] C.Y. Zhuang, N. Li, M. Wang, X.N. Zhang, W.S. Pan, J.J. Peng, Y.S. Pan, X. Tang, Preparation and characterization of vinpocetine loaded nanostructured lipid carriers (NLC) for improved oral bioavailability, Int. J. Pharm. 394 (2010) 179–185. doi:10.1016/j.ijpharm.2010.05.005.

[142] N.M. Morsi, D.M. Ghorab, H.A. Badie, Brain targeted solid lipid nanoparticles for brain ischemia: preparation and in vitro characterization, Pharm. Dev. Technol. 18 (2013) 736–744. doi:10.3109/10837450.2012.734513.

[143] C. Lin, F. Chen, T. Ye, L. Zhang, W. Zhang, D. Liu, W. Xiong, X. Yang, W. Pan, A novel oral delivery system consisting in "drug-in cyclodextrin-in nanostructured lipid carriers" for poorly water-soluble drug: Vinpocetine, Int. J. Pharm. 465 (2014) 90–96. doi:10.1016/j.ijpharm.2014.02.013.

[144] V. Kakkar, S.K. Muppu, K. Chopra, I.P. Kaur, Curcumin loaded solid lipid nanoparticles: An efficient formulation approach for cerebral ischemic reperfusion injury in rats, Eur. J. Pharm. Biopharm. 85 (2013) 339–345. doi:10.1016/j.ejpb.2013.02.005.

[145] Y. mei Lu, J. yun Huang, H. Wang, X. fang Lou, M. hua Liao, L. juan Hong, R. rong Tao, M.M. Ahmed, C. lei Shan, X. liang Wang, K. Fukunaga, Y. zhong Du, F. Han, Targeted therapy of brain ischaemia using Fas ligand antibody conjugated PEG-lipid nanoparticles, Biomaterials. 35 (2014) 530–537. doi:10.1016/j.biomaterials.2013.09.093.

[146] M.J. Tsai, P.C. Wu, Y. Bin Huang, J.S. Chang, C.L. Lin, Y.H. Tsai, J.Y. Fang, Baicalein loaded in tocol nanostructured lipid carriers (tocol NLCs) for enhanced stability and brain targeting, Int. J. Pharm. 423 (2012) 461–470. doi:10.1016/j.ijpharm.2011.12.009.

[147] Z. Liu, L. Zhang, Q. He, X. Liu, O. Chukwunweike Ikechukwu, L. Tong, L. Guo, H. Yang, Q. Zhang, H. Zhao, X. Gu, Effect of Baicalin-loaded PEGylated cationic solid lipid nanoparticles modified by OX26 antibody on regulating the levels of baicalin and amino acids during cerebral ischemia-reperfusion in rats, Int. J. Pharm. 489 (2015) 131–138. doi:10.1016/j.ijpharm.2015.04.049.

[148] Z. Liu, H. Zhao, L. Shu, Y. Zhang, C. Okeke, L. Zhang, J. Li, N. Li, Preparation and evaluation of Baicalin-loaded cationic solid lipid nanoparticles conjugated with OX26 for improved delivery across the BBB, Drug Dev. Ind. Pharm. 41 (2015) 353–361. doi:10.3109/03639045.2013.861478.

[149] S.-H. Hsu, C.-J. Wen, S.A. Al-Suwayeh, H.-W. Chang, T.-C. Yen, J.-Y. Fang, Physicochemical characterization and in vivo bioluminescence imaging of nanostructured lipid carriers for targeting the brain: apomorphine as a model drug., Nanotechnology. 21 (2010) 405101. doi:10.1088/0957-4484/21/40/405101.

[150] M.-J. Tsai, Y.-B. Huang, P.-C. Wu, Y.-S. Fu, Y.-R. Kao, J.-Y. Fang, Y.-H. Tsai, Oral Apomorphine Delivery from Solid Lipid Nanoparticles with Different Monostearate Emulsifiers: Pharmacokinetic and Behavioral Evaluations, J. Pharm. Sci. 100 (2010) 547–557. doi:10.1002/jps.22285.

[151] K.-S. Liu, C.-J. Wen, T.-C. Yen, K.C. Sung, M.-C. Ku, J.-J. Wang, J.-Y. Fang,

Combined strategies of apomorphine diester prodrugs and nanostructured lipid carriers for efficient brain targeting, Nanotechnology. 23 (2012) 95103. doi:10.1088/0957-4484/23/9/095103.

[152] C. V. Pardeshi, V.S. Belgamwar, A.R. Tekade, S.J. Surana, Novel surface modified polymer-lipid hybrid nanoparticles as intranasal carriers for ropinirole hydrochloride: In vitro, ex vivo and in vivo pharmacodynamic evaluation, J. Mater. Sci. Mater. Med. 24 (2013) 2101–2115. doi:10.1007/s10856-013-4965-7.

[153] Y.M. Gabal, A.O. Kamel, O.A. Sammour, A.H. Elshafeey, Effect of surface charge on the brain delivery of nanostructured lipid carriers in situ gels via the nasal route, Int. J. Pharm. 473 (2014) 442–457. doi:10.1016/j.ijpharm.2014.07.025.

[154] Y.Z. Zhao, X. Li, C.T. Lu, M. Lin, L.J. Chen, Q. Xiang, M. Zhang, R.R. Jin, X. Jiang, X.T. Shen, X.K. Li, J. Cai, Gelatin nanostructured lipid carriers-mediated intranasal delivery of basic fibroblast growth factor enhances functional recovery in hemiparkinsonian rats, Nanomedicine Nanotechnology, Biol. Med. 10 (2014) 755–764. doi:10.1016/j.nano.2013.10.009.

[155] C.-R. Yang, X.-L. Zhao, H.-Y. Hu, K.-X. Li, X. Sun, L. Li, D.-W. Chen, Preparation, Optimization and Characteristic of Huperzine A Loaded Nanostructured Lipid Carriers, Chem. Pharm. Bull. (Tokyo). 58 (2010) 656–661. doi:10.1248/cpb.58.656.

[156] P.A. Patel, S.C. Patil, D.R. Kalaria, Y.N. Kalia, V.B. Patravale, Comparative in vitro and in vivo evaluation of lipid based nanocarriers of Huperzine A, Int. J. Pharm. 446 (2013) 16–23. doi:10.1016/j.ijpharm.2013.02.014.

[157] S. Dhawan, R. Kapil, B. Singh, Formulation development and systematic optimization of solid lipid nanoparticles of quercetin for improved brain delivery, J. Pharm. Pharmacol. 63 (2011) 342–351. doi:10.1111/j.2042-7158.2010.01225.x.

[158] M. Gobbi, F. Re, M. Canovi, M. Beeg, M. Gregori, S. Sesana, S. Sonnino, D. Brogioli, C. Musicanti, P. Gasco, M. Salmona, M.E. Masserini, Lipid-based nanoparticles with high binding affinity for amyloid-β1-42 peptide, Biomaterials. 31 (2010) 6519–6529. doi:10.1016/j.biomaterials.2010.04.044.

[159] M. Yusuf, M. Khan, R.A. Khan, B. Ahmed, Preparation, characterization, in vivo and biochemical evaluation of brain targeted Piperine solid lipid nanoparticles in an experimentally induced Alzheimer's disease model, J. Drug Target. 21 (2013) 300–311. doi:10.3109/1061186X.2012.747529.

[160] F. Meng, S. Asghar, S. Gao, Z. Su, J. Song, M. Huo, W. Meng, Q. Ping, Y. Xiao, A novel LDL-mimic nanocarrier for the targeted delivery of curcumin into the brain to treat Alzheimer's disease, Colloids Surfaces B Biointerfaces. 134 (2015) 88–97. doi:10.1016/j.colsurfb.2015.06.025.

[161] S. Laserra, A. Basit, P. Sozio, L. Marinelli, E. Fornasari, I. Cacciatore, M. Ciulla, H. Türkez, F. Geyikoglu, A. Di Stefano, Solid lipid nanoparticles loaded with lipoyl-memantine codrug: Preparation and characterization, Int. J. Pharm. 485 (2015) 183–191. doi:10.1016/j.ijpharm.2015.03.001.

[162] A.K. Sachdeva, S. Misra, I. Pal Kaur, K. Chopra, Neuroprotective potential of sesamol and its loaded solid lipid nanoparticles in ICV-STZ-induced cognitive deficits: Behavioral and biochemical evidence, Eur. J. Pharmacol. 747 (2015) 132–

140. doi:10.1016/j.ejphar.2014.11.014.

[163] A. Vedagiri, S. Thangarajan, Mitigating effect of chrysin loaded solid lipid nanoparticles against Amyloid β25–35 induced oxidative stress in rat hippocampal region: An efficient formulation approach for Alzheimer's disease, Neuropeptides. 58 (2016) 111–125. doi:10.1016/j.npep.2016.03.002.

[164] M.L. Bondì, E.F. Craparo, G. Giammona, F. Drago, Brain-targeted solid lipid nanoparticles containing riluzole: preparation, characterization and biodistribution., Nanomedicine (Lond). 5 (2010) 25–32. doi:10.2217/nnm.09.67.

[165] N. Gandomi, R. Varshochian, F. Atyabi, M.H. Ghahremani, M. Sharifzadeh, M. Amini, R. Dinarvand, Solid lipid nanoparticles surface modified with anti-Contactin-2 or anti-Neurofascin for brain-targeted delivery of medicines, Pharm. Dev. Technol. 22 (2017). doi:10.1080/10837450.2016.1226901.

[166] R. Tiwari, K. Pathak, Nanostructured lipid carrier versus solid lipid nanoparticles of simvastatin: Comparative analysis of characteristics, pharmacokinetics and tissue uptake, Int. J. Pharm. 415 (2011) 232–243. doi:10.1016/j.ijpharm.2011.05.044.

[167] S. Eskandari, J. Varshosaz, M. Minaiyan, M. Tabbakhian, Brain delivery of valproic acid via intranasal administration of nanostructured lipid carriers: in vivo pharmacodynamic studies using rat electroshock model., Int. J. Nanomedicine. 6 (2011) 363–371. doi:10.2147/IJN.S15881.

[168] C.Y. Guo, C.F. Yang, Q.L. Li, Q. Tan, Y.W. Xi, W.N. Liu, G.X. Zhai, Development of a Quercetin-loaded nanostructured lipid carrier formulation for topical delivery, Int. J. Pharm. 430 (2012) 292–298. doi:10.1016/j.ijpharm.2012.03.042.

[169] S. Bose, Y. Du, P. Takhistov, B. Michniak-Kohn, Formulation optimization and topical delivery of quercetin from solid lipid based nanosystems, Int. J. Pharm. 441 (2013) 56–66. doi:10.1016/j.ijpharm.2012.12.013.

[170] L.L. Liu, Y. Tang, C. Gao, Y. Li, S. Chen, T. Xiong, J. Li, M. Du, Z. Gong, H. Chen, L.L. Liu, P. Yao, Characterization and biodistribution in vivo of quercetin-loaded cationic nanostructured lipid carriers, Colloids Surfaces B Biointerfaces. 115 (2014) 125–131. doi:10.1016/j.colsurfb.2013.11.029.

[171] M. Fang, Y. Jin, W. Bao, H. Gao, M. Xu, D. Wang, X. Wang, P. Yao, L. Liu, In vitro characterization and in vivo evaluation of nanostructured lipid curcumin carriers for intragastric administration Background : Curcumin has a variety of pharmacological effects . However , poor water, Int. J. Nanomedicine. 7 (2012) 5395–5404. doi:10.2147/IJN.S36257.

[172] V. Kakkar, A.K. Mishra, K. Chuttani, I.P. Kaur, Proof of concept studies to confirm the delivery of curcumin loaded solid lipid nanoparticles (C-SLNs) to brain, Int. J. Pharm. 448 (2013) 354–359. doi:10.1016/j.ijpharm.2013.03.046.

[173] F. Meng, S. Asghar, Y. Xu, J. Wang, X. Jin, Z. Wang, J. Wang, Q. Ping, J. Zhou, Y. Xiao, Design and evaluation of lipoprotein resembling curcumin-encapsulated protein-free nanostructured lipid carrier for brain targeting, Int. J. Pharm. 506 (2016) 46–56. doi:10.1016/j.ijpharm.2016.04.033.

[174] L. Montenegro, A. Campisi, M.G. Sarpietro, C. Carbone, R. Acquaviva, G. Raciti,

G. Puglisi, In vitro evaluation of idebenone-loaded solid lipid nanoparticles for drug delivery to the brain., Drug Dev. Ind. Pharm. 37 (2011) 737–746. doi:10.3109/03639045.2010.539231.

[175] L. Montenegro, S. Ottimo, G. Puglisi, F. Castelli, M.G. Sarpietro, Idebenone loaded solid lipid nanoparticles interact with biomembrane models: Calorimetric evidence, Mol. Pharm. 9 (2012) 2534–2541. doi:10.1021/mp300149w.

[176] L. Mandpe, A. Kyadarkunte, V. Pokharkar, Assessment of novel iloperidone- and idebenone-loaded nanostructured lipid carriers: brain targeting efficiency and neuroprotective potential., Ther. Deliv. 4 (2013) 1365–83. doi:10.4155/tde.13.101.

[177] A. Leonardi, L. Crasci', A. Panico, R. Pignatello, Antioxidant activity of idebenone-loaded neutral and cationic solid–lipid nanoparticles, Pharm. Dev. Technol. 20 (2015) 716–723. doi:10.3109/10837450.2014.915572.

[178] L. Priano, G.P. Zara, N. El-Assawy, S. Cattaldo, E. Muntoni, E. Milano, L. Serpe, C. Musicanti, C. P??rot, M.R. Gasco, G. Miscio, A. Mauro, Baclofen-loaded solid lipid nanoparticles: Preparation, electrophysiological assessment of efficacy, pharmacokinetic and tissue distribution in rats after intraperitoneal administration, Eur. J. Pharm. Biopharm. 79 (2011) 135–141. doi:10.1016/j.ejpb.2011.02.009.

[179] S. Patel, S. Chavhan, H. Soni, A.K. Babbar, R. Mathur, A.K. Mishra, K. Sawant, Brain targeting of risperidone-loaded solid lipid nanoparticles by intranasal route, J. Drug Target. 19 (2011) 468–474. doi:10.3109/1061186X.2010.523787.

[180] A.C. Silva, A. Kumar, W. Wild, D. Ferreira, D. Santos, B. Forbes, Long-term stability, biocompatibility and oral delivery potential of risperidone-loaded solid lipid nanoparticles, Int. J. Pharm. 436 (2012) 798–805. doi:10.1016/j.ijpharm.2012.07.058.

[181] S.K. Singh, P. Dadhania, P.R. Vuddanda, A. Jain, S. Velaga, S. Singh, Intranasal delivery of asenapine loaded nanostructured lipid carriers: formulation, characterization, pharmacokinetic and behavioural assessment, RSC Adv. 6 (2016) 2032–2045. doi:10.1039/C5RA19793G.

[182] M.I. Alam, S. Baboota, A. Ahuja, M. Ali, J. Ali, J.K. Sahni, Intranasal administration of nanostructured lipid carriers containing CNS acting drug: Pharmacodynamic studies and estimation in blood and brain, J. Psychiatr. Res. 46 (2012) 1133–1138. doi:10.1016/j.jpsychires.2012.05.014.

[183] Y. Yang, Y. Yang, X. Xie, X. Cai, X. Mei, Preparation and characterization of photo-responsive cell-penetrating peptide-mediated nanostructured lipid carrier, J. Drug Target. 22 (2014) 891–900. doi:10.3109/1061186X.2014.940589.

[184] M. Almousallam, C. Moia, H. Zhu, Development of nanostructured lipid carrier for dacarbazine delivery, Int. Nano Lett. 5 (2015) 241–248. doi:10.1007/s40089-015-0161-8.