

Review

Polysorbate-Based Drug Formulations for Brain-Targeted Drug Delivery and Anticancer Therapy

Vasanthan Ravichandran ¹, Minjong Lee ^{2,3} , Thuy Giang Nguyen Cao ¹ and Min Suk Shim ^{1,*} 

¹ Division of Bioengineering, Incheon National University, Incheon 22012, Korea; vasanthan.ravi@gmail.com (V.R.); nctgiang2405@gmail.com (T.G.N.C.)

² Department of Internal Medicine, Ewha Womans University College of Medicine, Seoul 07804, Korea; minjonglee2@naver.com

³ Department of Internal Medicine, Ewha Womans University Medical Center, Seoul 07804, Korea

* Correspondence: msshim@inu.ac.kr; Tel.: +82-32-835-8268

Abstract: Polysorbates (PSs) are synthetic nonionic surfactants consisting of polyethoxy sorbitan fatty acid esters. PSs have been widely employed as emulsifiers and stabilizers in various drug formulations and food additives. Recently, various PS-based formulations have been developed for safe and efficient drug delivery. This review introduces the general features of PSs and PS-based drug carriers, summarizes recent progress in the development of PS-based drug formulations, and discusses the physicochemical properties, biological safety, P-glycoprotein inhibitory properties, and therapeutic applications of PS-based drug formulations. Additionally, recent advances in brain-targeted drug delivery using PS-based drug formulations have been highlighted. This review will help researchers understand the potential of PSs as effective drug formulation agents.

Keywords: polysorbate; surfactants; blood–brain barrier; cancer therapy; drug delivery



Citation: Ravichandran, V.; Lee, M.; Nguyen Cao, T.G.; Shim, M.S.

Polysorbate-Based Drug Formulations for Brain-Targeted Drug Delivery and Anticancer Therapy. *Appl. Sci.* **2021**, *11*, 9336. <https://doi.org/10.3390/app11199336>

Academic Editors: Manuela Pintado and Gianpiero Calabrese

Received: 3 September 2021

Accepted: 28 September 2021

Published: 8 October 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Drug delivery systems are one of the mainstream strategies for achieving targeted delivery and controlled release of therapeutic drugs in the body. Various drug carriers composed of polymers, dendrimers, metal oxides, hydrogels, cell-derived membranes, exosomes, and surfactants have been developed to improve the therapeutic efficacy of drugs and alter their pharmacokinetics [1]. Despite the several advantages of drug carriers, some drug carriers have limitations, including toxicity, low physicochemical stability, immune responses, poor cell specificity, unwanted drug leakage, and high manufacturing cost, which make them unsuitable for practical applications [2]. Extensive research efforts have been made to develop drug carriers for safe, efficient, and targeted drug delivery by addressing the aforementioned issues [2]. Recently, drug carriers composed of surfactants have garnered great attention owing to their enhanced bioavailability and high biocompatibility [3]. Surfactants have been widely used in pharmaceutical formulations because they can enhance drug solubility and prevent protein aggregation [4]. Nonionic surfactants with charge-free head groups are an important subclass of surfactants. They have been intensively utilized as emulsifiers and stabilizers in the cosmetic industry [5]. Nonionic surfactants are commercially available in various chemical structures. Various types of nonionic surfactants have been used to form emulsions, micelles, and niosomes, all of which can efficiently encapsulate hydrophilic and hydrophobic molecules [6,7]. The features of nonionic surfactant-based formulations are similar to those of liposomes, and thus, they are suitable alternatives to phospholipids. Low cost, bulk abundance, high physical stability, and ease of storage are advantages of using nonionic surfactants as pharmaceutical formulations. Moreover, the rich phase separation behavior and low critical micellization temperature values are important features of nonionic surfactants that make them attractive for pharmaceutical applications. Polysorbates (PSs), which are commercially

known as Tween, are synthetic nonionic surfactants that consist of polyethoxy sorbitan fatty acid esters. PSs have numerous advantages, such as commercial abundance and ease of chemical modification, which render them suitable for various biomedical applications. Therefore, PSs have been widely employed as emulsifiers and stabilizers in various drug formulations and food additives [6,7]. PSs have also served as wetting agents, foaming agents, and dispersants [8–11]. Recently, various PS–drug conjugates and PS-conjugated drug carriers have been developed for safe and efficient drug delivery [12–14]. PSs have also attracted great attention as brain-targeting coating materials that can facilitate the transport of drug carriers across the blood–brain barrier (BBB) [15]. This review introduces the general features of PSs and PS-based drug carriers and summarizes recent progress in the development of PS-based drug carriers for the treatment of various diseases. Special emphasis is placed on PS-based drug carriers for brain-targeted drug delivery. In addition, the mechanism of action, current limitations, and perspectives of PS-based drug carriers are presented.

2. Basic Characteristics of PSs

2.1. Chemical Structures and Physicochemical Properties of PSs

The common backbone structure of PSs is a sorbitan ring (sugar alcohol) with poly (ethylene oxide) (PEO) conjugated to hydroxyl groups. The different numbers of ethylene oxide subunits are conjugated to the four hydroxyl groups of the sorbitan ring, but the overall number of repeating units of ethylene oxide is approximately 20. The chemical preparation of PSs involves a two-step process: sorbitan is ethoxylated, followed by esterification with fatty acids (e.g., lauric acid, palmitic acid, stearic acid, and oleic acid). The synthesis scheme of PSs is shown in Figure 1.

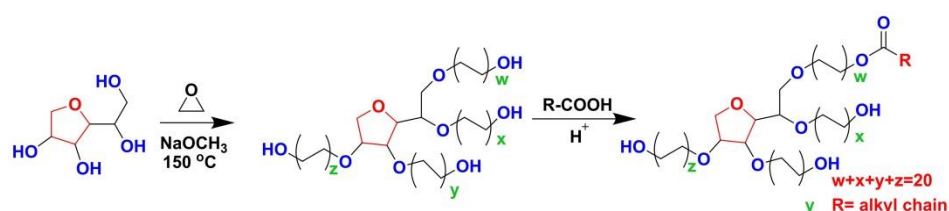


Figure 1. Schematic illustration for the synthesis of polysorbates from sorbitan.

The number following the “polysorbate” refers to the type of fatty acid ester associated with the polyoxyethylene sorbitan portion of the molecule. Fatty acid ester moieties of PS 20, PS 40, PS 60, and PS 80 are laurate, palmitate, stearate, and oleate, respectively. Figure 2 illustrates the chemical structures of various PSs (i.e., PS 20, PS 40, PS 60, and PS 80). The PEO segments in PS are hydrophilic, and the fatty acid segments are hydrophobic. Therefore, amphiphilic PSs can serve as effective surfactants. The amphiphilic nature of PSs allows them to self-assemble into micelles in aqueous solutions after reaching critical micellar concentrations. PS-based micelles have been widely employed for drug delivery because of their nanoscale size and high drug-loading capacity [16–18]. PSs have been used as emulsifiers and solubilizers for hydrophobic drugs and as protein-stabilizing agents [19]. For example, PS 80 was used as a stabilizer in the Janssen COVID-19 vaccine formulation [20]. Emulsion-based formulations containing PSs as emulsifiers have been intensively developed and have shown potential as effective drug carriers for both hydrophobic and hydrophilic drugs [21–23]. The behavior of protein aggregation in the absence or presence of PS 80 as an excipient was evaluated [24]. The addition of PS 80 substantially inhibited protein aggregation during agitation [24]. PS 80 and PS 20 have been used in the formulation of therapeutic monoclonal antibodies owing to their biocompatibility, low toxicity, and good protein-stabilizing properties [25]. PSs have been utilized to increase the permeability of various drugs in vivo and in vitro [26–29]. Additionally, PSs exhibit high biocompatibility and low toxicity and are thus advantageous for use as drug carriers.

In particular, nonionic surfactants are less toxic and more biocompatible and have lower hemolytic potential than charged surfactants (cationic, anionic, or amphoteric) [30].

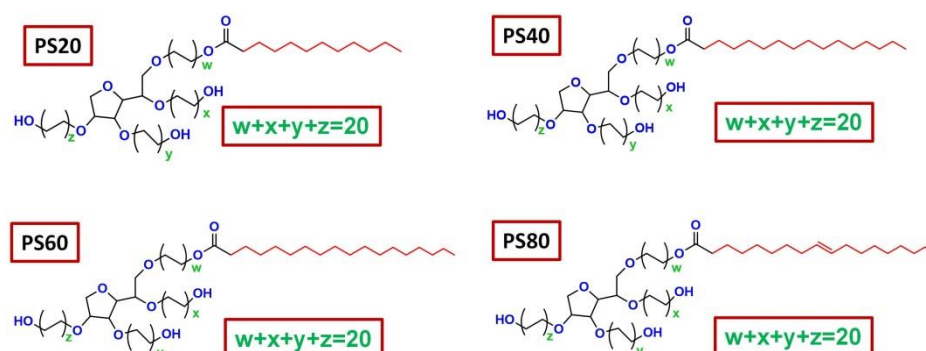


Figure 2. Chemical structures of various polysorbates.

2.2. Biosafety Studies of PSs

Biosafety is an umbrella term that reflects the damage of the material to biological integrity [31]. Any material used for biomedical applications should possess desirable biosafety. To achieve desirable biosafety, materials should be degraded in biological environments without causing any adverse effects after their therapeutic use. Enzymatic degradation is a crucial function of the human body that involves the breakdown or elimination of unnecessary materials. Food additives are generally excreted from the body without causing adverse effects. PSs have been utilized as emulsifiers in a variety of food and cosmetic products [32]. PSs were approved as a food additive in the USA in 1960 and the EU in 1995 [33]. The Joint Food and Agriculture Organization of the United Nations/World Health Organization Expert Committee on Food Additives established an acceptable oral daily intake of PSs (e.g., PS 20, PS 60, PS 65, and PS 80) of 25 mg/kg by adults [34,35]. Recently, the pediatric safety of PSs in drug formulations has also been investigated [36]. The amount of parenterally administered PSs in formulations was calculated using a progressive pediatric safety factor through an age- and weight-based estimation. For example, the maximum total daily doses of parenterally administered PS 20 and PS 80 for 2-year-old children are 3.37 and 50.6 mg, respectively [36].

Over the past few decades, the cytotoxicity and metabolism of various PSs have been investigated to demonstrate their potential for biomedical applications. Upon parenteral administration, PSs are degraded readily via hydrolysis by esterases in the plasma [37]. The fatty acids generated by the hydrolysis of PSs are expected to be further metabolized via beta-oxidation, leading to the formation of carbon dioxide, which can be exhaled. Polyoxyethylene sorbitan, which is generated by the hydrolysis of PSs, is excreted via urine and feces [38]. An in vivo study showed that PSs were efficiently hydrolyzed by esterase in the digestive tract after oral administration in a rat model [39,40]. Free fatty acids are then readily absorbed from the gastrointestinal tract and further metabolized to carbon dioxide in exhaled breath, similar to the phenomena observed in normal fatty acid metabolism [39]. The absorption of polyoxyethylene sorbitan into the digestive tract was found to be very low. Approximately 87% of PS 20 and 91% of PS 80 were excreted in the feces [40]. Negligible systemic toxicity was observed when PS 80 (22 g/kg body weight) was orally administered to rats. In addition, liver or kidney dysfunction was not observed [34]. An in vivo rabbit eye model was used to evaluate the irritation effect of aqueous and oily solutions (Miglyol 812) of PS 20 and PS 80. In vitro tests using fibroblast cells demonstrated that cytotoxicity of PSs depended on surfactant concentrations (0.1, 1, or 5%) and types of solvents (water or oil). Treatment with 1% aqueous solutions of PS 20 and PS 80 induced the cell death of approximately 20%. Only 10% of cells were dead when the oily solution of the surfactant was added. The difference in cell viability between aqueous and oily solutions was larger when the concentration of PSs increased from 1 to 5%. In vivo tests showed that aqueous (0.9% NaCl) solutions of PSs were nonirritant,

whereas small changes in conjunctiva were observed after administration of oily PSs [41]. The in vitro cytotoxicity of PSs against human fibroblast cells has also been studied. The half-maximal inhibitory concentration (IC_{50}) values of aqueous solutions of PS 60 and PS 80 were found to be 70.8 and 65.5 mg/mL, respectively [42].

Although PS 20 and PS 80 have been recognized as safe and nonirritant food additives and drug-formulating agents [36], some rare cases of hypersensitivity [43] and hepatotoxicity [44] have been reported, possibly owing to highly dosed PSs as excipients. These reports suggest that safe and acceptable doses of PSs in food additives and drug formulations should be regulated or defined. Various toxicological studies of PSs are highly required to develop safe PS-based drug formulations.

2.3. *P-glycoprotein (P-gp) Inhibitory Property of PS*

P-gp is a membrane transport protein present in normal tissues, such as the intestine, brain, liver, placenta, kidney, and cancer cells [45]. The crucial role of P-gp is to maintain intracellular drug concentrations via influx and efflux pumps. Therefore, P-gp plays a key role in the absorption, localization, and elimination of various drugs. Drug-resistant tumors overexpress P-gp, which can eliminate cancer chemotherapeutic agents. Multidrug resistance (MDR) is a major issue in cancer treatment [46]. The inhibition of P-gp is one of the ideal steps to reverse MDR.

Recently, nonionic surfactants were found to be effective P-gp inhibitors to overcome P-gp-mediated MDR in various types of cancer cells [47]. Working mechanisms of P-gp inhibition by PSs have been investigated using fluorescence polarization techniques [47]. The results indicated that PSs can alter the membrane fluidity of cells, thereby changing the conformation of transporters in the membranes [47]. Effects of nonionic surfactants such as PS 20 and Cremophor EL on breast cancer resistance protein (BCRP) transporters in Caco-2 cell monolayers were analyzed [48]. PS 20 increased the membrane fluidity of the inner lipid bilayers of the intestine. Therefore, PS 20 effectively improved the intestinal absorption of drugs topotecan in rats via the transcellular pathway by inhibiting the function of BCRP.

The effects of PS as a pharmaceutical excipient on the intracellular localization, transport kinetics, and intestinal absorption of epirubicin were evaluated in both human colon adenocarcinoma (Caco-2) cells and rat jejunum and ileum [49]. PS 20 and PS 80 substantially increased apical to basolateral absorption and reduced the basolateral to apical efflux of epirubicin across Caco-2 cell monolayers [49]. In addition, PS drastically enhanced the mucosal to serosal absorption of epirubicin in the rat jejunum and ileum. These results suggest that the inhibition of intestinal P-gp by PSs significantly improves the oral absorption of drugs in multidrug-resistant cancer cells.

The effect of PS 80 on the absorption of lipophilic digoxin was also investigated in vitro and in vivo. PS 80 enhanced the bioavailability of digoxin by modulating P-gp systems in cancer cells [29]. When rats were orally administered digoxin (0.2 mg/kg) containing 1% (*v/v*) and 10% (*v/v*) PS 80, the in vivo oral absorption of digoxin increased by 30% and 61%, respectively, compared to the control groups [26]. The significantly increased absorption of digoxin after oral administration was attributed to the inhibition of P-gp in the gut.

To inhibit the effect of P-gp, a clay-based nanocomposite using montmorillonite and PS 20 was developed [50]. The particles consisted of montmorillonite, the nonionic surfactant PS 20, and the P-gp substrate digoxin. In vivo results showed that compared to administration of free digoxin, administration of montmorillonite–surfactant hybrid particles resulted in an increased digoxin concentration in the plasma. This increase in digoxin concentration might be attributed to the increase in mucosa-localized concentrations of both digoxin and PS 20.

3. PS-Based Formulations for Various Routes of Drug Administration

Drugs are administered to human bodies through various routes, depending on their formulations and disease sites [51]. For instance, ointment, emulsion, and solutions are

suitable for ocular and intranasal delivery; pills and capsules are suitable for oral delivery; and patches, gels, and ointments are effective for transdermal delivery. For all formulations, surfactants play a key role in enhancing the therapeutic index of drugs. In particular, various PS-based formulations, including emulsions [52], micro/nanoemulsions [53], suspensions [54], and niosomes [55], have been utilized as drug carriers and delivered into the body via various routes of drug administration.

3.1. PS-Based Formulations for Ocular Delivery

Most eye-related problems, including dry eye, fungal infection, inflammation, ocular hypertension, and glaucoma, are generally treated with topical ophthalmic drugs [56]. Ophthalmic drug formulations include nanosomes, micelles, and emulsions; these formulations show high drug-loading efficiency, ocular bioavailability, and prolonged drug effects in the eyes [57]. Compared to other charged surfactants, PSs offer more efficient ocular drug delivery owing to their properties such as smooth penetration, drug-solubilizing capability, and effective drug protection. Moreover, nonionic surfactants are known to be less harmful and irritating than charged surfactants [58]. PSs increase ocular bioavailability because they can act as penetration enhancers that can remove mucus layers and destroy junctional complexes [59].

DUREZOL[®] is a commercially available corticosteroid formulation used for the treatment of inflammation and pain associated with ocular surgery. This Food and Drug Administration (FDA)-approved formulation contains PS 80 as a nonionic emulsifier (4% *w/v*) [60]. Similarly, cyclosporine emulsions containing 1% PS 80 were developed and sold under the brand name Restasis[®] [61]. Many PS-based formulations with various ocular drugs, including flurbiprofen [62], ibuprofen [63], indomethacin [64], dexamethasone [65], chloramphenicol [66], and hydrocortisone [67], have been developed and evaluated in preclinical studies. These PS-based formulations exhibit low irritation, high drug solubility, efficient penetration across cell membranes, and high bioavailability.

Recently, niosomes composed of cationic lipids and PSs have been developed for nonviral gene delivery. Niosomes are nonionic surfactant-based vesicles formed via self-assembly in an aqueous solution [68,69]. Niosomes have bilayer structures that can encapsulate both hydrophilic and hydrophobic drugs, such as liposomes. Because niosomes are biodegradable, stable, and inexpensive, they have great potential as alternatives to liposomes [70,71]. Niosome formulations comprising the cationic lipid *N*-[1-(2,3-dioleoyloxy) propyl]-*N,N,N*-trimethylammonium chloride (DOTMA) and nonionic PS 60 were developed for retinal gene delivery [72]. The incorporation of natural lipids (i.e., lycopene) resulted in high transfection efficiency in the human retinal pigment epithelial cells (ARPE-19) through the caveolae-mediated endocytic pathway. The DOTMA/PS 60/lipid niosomes administered into the rat retina via subretinal and intravitreal routes efficiently transfected the outer segments of the retina. The same group studied the effects of PS (PS 20, PS 80, and PS 85) on the transfection efficiency of niosomes in the rat retina [73]. The graphical representation of niosomes is shown in Figure 3. Niosomes formulated with PS 20 showed more efficient transfection in retinal cells than the niosomes containing PS 85. The greater hydrophilicity of PS 20-containing niosomes than the PS 85-containing niosomes facilitated caveolae-mediated endocytosis in retinal cells, thereby preventing lysosomal degradation [73].

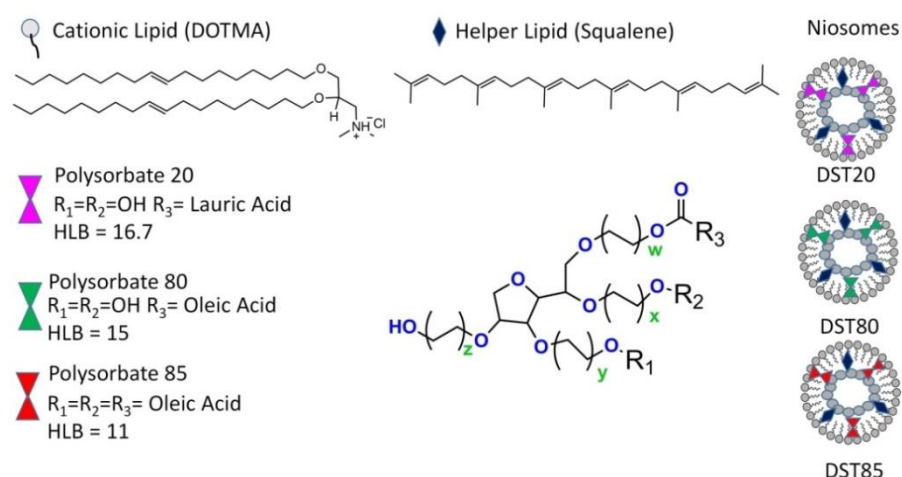


Figure 3. Chemical structures of niosome components (DOTMA: 1,2-di-O-octadecenyl-3-trimethylammonium propane, HLB: Hydrophilic lipophilic balance). Reproduced with permission from Reference [73]. Copyright 2018, Elsevier B.V.

3.2. PS-Based Formulations for Transdermal Delivery

Transdermal drug delivery involves the topical administration of drugs across the skin barrier. The topical delivery of active compounds is hampered by the limited skin permeability [74]. Surfactants, such as PSs, enhance the permeation of drugs through the skin [75]. Microemulsions composed of eucalyptol and PS 80 were fabricated as transdermal delivery vehicles for curcumin [76]. The curcumin permeation rate of the microemulsion was 15.7-fold higher than that of the control (eucalyptol only). The microemulsions containing 25% water, 25% ethanol, 37.5% ethanol, and 12.5% PS 80 exhibited the highest curcumin permeation rate [77]. Microemulsions composed of PS 80, medium-chain glycerides, and propylene glycol were developed for the efficient transdermal delivery of hydrophilic (i.e., adenosine) and hydrophobic (i.e., progesterone) drugs [78]. Microemulsions with 47% (*w/v*) PS and 63% (*w/v*) PS were prepared. Microemulsions with 63% (*w/v*) PS exhibited higher efficacy in transdermal delivery of hydrophilic agents than those with 47% (*w/v*) PS. In addition, various PS-based microemulsions have been developed for the transdermal delivery of testosterone [79], meloxicam [80], capsaicin [81], fluorouracil [82], and ketoprofen [83].

3.3. PS-Based Formulations for Oral Delivery

Intestine, brain, liver, placenta, kidney, and cancer tissues express ATP-binding cassettes and solute carrier transporters (e.g., P-gp, MDR1, and ABCB1) [84,85]. These membrane transport proteins avoid the uptake of toxins and xenobiotics from the cells and maintain the bioavailability of active drug ingredients [86]. Previous studies have demonstrated that PS-based formulations enhance the intestinal absorption and bioavailability of drugs by modulating membrane transport proteins [87,88] and etoposide [89,90].

To improve the bioavailability of erythromycin for an *in vivo* fish model, three types of microemulsified erythromycin formulations were prepared and administered to fish over seven consecutive days. The results showed that emulsified erythromycin improved oral bioavailability and achieved a higher therapeutic plasma concentration than erythromycin powder [91]. Salmon calcitonin has low bioavailability and stability in the gastrointestinal tract [92]. PS-based microemulsions containing salmon calcitonin improved its oral bioavailability as well as pharmacological activity compared to those of free drugs [92].

The oral bioavailability of curcumin is poor owing to its low aqueous solubility, alkaline instability, and rapid elimination. Curcumin was encapsulated into ionic-gelated alginate–PS 80 nanoparticles (NPs) to increase its oral bioavailability. In healthy human volunteers, administration of the NPs showed five-fold higher bioavailability and higher plasma concentrations of curcumin than administration of curcumin suspensions [93].

9-Nitrocamptothecin (9-NC) is a potent topoisomerase I inhibitor for the treatment of pancreatic cancer. However, its bioavailability after oral administration is poor. To address the low bioavailability of 9-NC, 9-NC microemulsions were prepared using a self-microemulsifying drug delivery system (SMEDDS). Different types of SMEDDS formulations of 9-NC were prepared using ethyl oleate as an oil phase, cremophor EL or PS 80 as a surfactant, and PEG-400/ethanol as cosurfactants. The antitumor effects of the resulting microemulsions were evaluated against human ovarian cancer cells in vitro and in vivo. In SKOV-3 cells, PS 80-based nanoemulsions showed the most efficient growth inhibition. Orally administered PS 80-based nanoemulsions also showed efficient tumor growth suppression in mice bearing human ovarian cancer xenografts [94].

Rosuvastatin is a prescription medicine used to prevent cardiovascular diseases. Microemulsion formulations were used to improve the oral bioavailability of rosuvastatin calcium. The microemulsion of rosuvastatin calcium was prepared using a mixture of peceol oil, PS 20 as a surfactant, and transcutool as a cosurfactant. Stability studies showed that the emulsion was stable for at least 3 months, with a high drug encapsulation efficiency of 91.6%. The microemulsion increased the oral bioavailability of rosuvastatin calcium by increasing the effective surface area of drug exposure in the physiological medium [95].

Fenofibrate is a lipid-regulating agent that controls the levels of cholesterol and triglycerides (fatty acids) in the blood. A fenofibrate-loaded emulsion was formulated by mixing olive oil, surfactants (a mixture of PS 20 and PS 80), and distilled water; a small-sized emulsion was prepared with a 5% olive oil concentration, and the mixture of PS 20 and PS 80 at 1:2 ratios showed high fenofibrate solubility [96]. The formulations were physically stable for up to 4 weeks, with no significant change in particle size. In contrast to the limited dissolution rate of the drug, the SMEDDS formulation showed effective and rapid drug release. The comparative pharmacodynamic evaluation was investigated in terms of lipid-lowering efficacy using a Triton-induced hypercholesterolemia model in rats [97]. The SMEDDS formulation substantially lowered serum lipid levels in phases I and II of the Triton test compared with plain fenofibrate. This result demonstrates that the SMEDDS formulation is an effective alternative to traditional oral formulations of fenofibrate to improve its bioavailability [97].

3.4. PS-Based Formulations for Intranasal Delivery

Intranasal delivery is a noninvasive administration that can bypass the BBB and thus facilitate the transport of drugs to the brain. However, intrinsic drug accumulation in the brain after intranasal delivery may not be sufficient to achieve clinical efficacy. PS 80 was used to increase the drug accumulation in the brain. In vivo rat model studies showed that the intranasal delivery of 2,3,5,6-tetramethylpyrazine (TMPP) along with PS 80 increased the concentration of TMPP in the brain [98]. The nasal mucosa is known to be the entry site of respiratory syncytial virus (RSV) into the body. The results showed that intranasal administration of a formulation containing virucidal lipids along with PS 20 significantly lowered the RSV load in the nasal mucosa of infected rat models compared to saline-treated groups [99].

Neurotoxin-II (NT-II) is an analgesic peptide, which has limited permeability across the BBB via intravenous injection. Intranasal delivery of NT-II into brain using PS 80-coated poly(lactic acid) (PLA) NPs was demonstrated. In vivo mouse model studies revealed PS 80-coated PLA NPs promoted the biodistribution of NT-II into brain via intranasal delivery [100].

4. PS-Conjugated Drugs or Drug Carriers

Substituents conjugated to functional groups of polymeric drug carriers can alter their physicochemical properties and drug loading capacity, consequently affecting their drug delivery efficiency [101–105]. For example, polyglycerols (PGs) possessing multiple hydroxyl groups, which can be functionalized with other polymers and drugs, have been utilized as versatile drug carriers [103]. Drug-conjugated polymers have been developed

to enhance drug stability in systemic circulation and increase drug encapsulation [103]. Conjugation of drugs to polymers via stimuli-responsive linkers has also achieved controlled drug release [104,105]. For example, hyperbranched PG-polycaprolactone block copolymer (PG-co-PCL)-based NPs were developed for controlled delivery of gemcitabine (GEM) [104]. GEM was conjugated to the PG-co-PCL through polycarbonate blocks modified with pH-sensitive tertiary amine. The GEM-conjugated NPs were stable at pH 7.4 and released the GEM in pH-dependent manner. Amine-functionalized dendritic PG was also prepared and conjugated to paclitaxel (PTX) via bioresponsive ester linkages [105]. PTX was rapidly released under hydrolytic or esterase-rich conditions.

The examples mentioned above regarding the functionalization of polymeric drug carriers suggest the strategies for chemical modifications of PSs [103–105]. PSs bear three hydroxyl groups at the end of the ethylene oxide chains. These hydroxyl groups can be easily functionalized or transformed into various functional groups, including esters, ethers, carbonates, and carbamates. The possible chemical modifications of the PSs are summarized in Figure 4. These functional groups can be used to incorporate chemical drugs and proteins to increase the therapeutic effects and pharmacokinetics of PS-based formulations. Some bioresponsive linkers have also been utilized to achieve controlled drug release. Herein, we summarize recent progress regarding PS-conjugated drugs or drug carriers for biomedical applications.

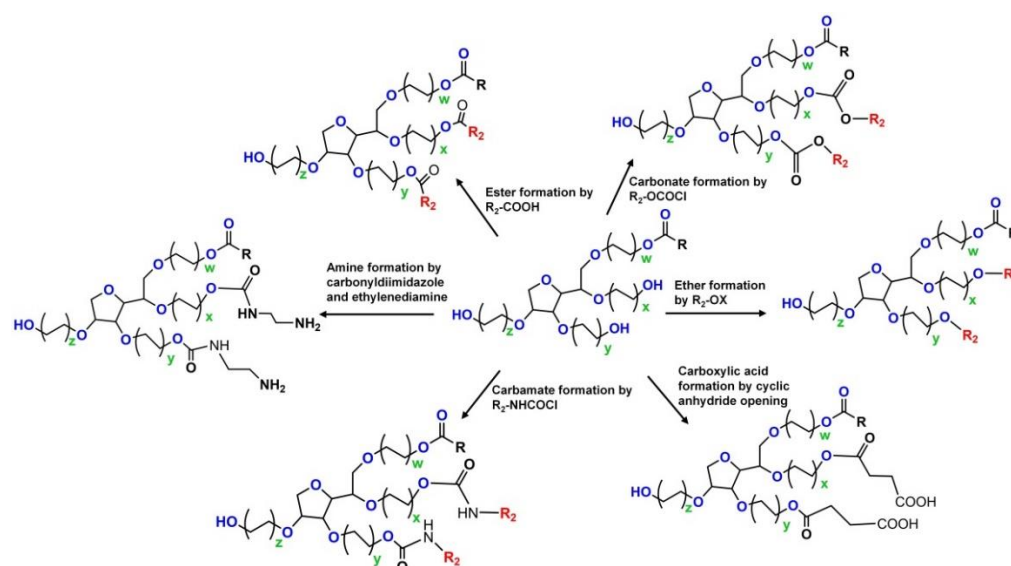


Figure 4. Possible chemical modifications of PSs. Hydroxyl groups of PS can be functionalized (e.g., esters, carbonates, ethers, carboxylic acids, carbamate, and amines).

Photo-responsive microgels composed of polymeric β -cyclodextrin (P β -CD) and PS 20–coumarin conjugates (TCCs) have been developed [106]. TCC was prepared via covalent conjugation of coumarin to the hydroxyl groups of PS 20. In contrast, P β -CD was prepared by reacting epichlorohydrins with the hydroxyl groups of β -CDs. The addition of TCC to aqueous solutions of P β -CD led to the formation of microgels. The coumarin residue of TCC underwent dimerization and de-dimerization processes upon irradiation, which caused size variation in the particles. The size of the microgels was controlled by UV irradiation at different wavelengths. Irradiation with $\lambda = 365$ nm decreased the microgel size, whereas the size of the microgels was increased in response to UV irradiation at 254 nm. This photo-responsive microgel can be used as a drug carrier, and the drug release could be controlled using UV irradiation [107].

Cinnamic acid (CA) was identified as a UV protecting molecule because it absorbs a broad range of UV light. CA was covalently attached to a nonionic PS to obtain a UV absorbing emulsifier [108]. After covalent conjugation with a PS, the nature of molar extinction coefficient of CA residue did not change. This result indicates that CA could absorb

the UV light after conjugation to the PS. In vitro cytotoxicity of various surfactant–CA conjugates were evaluated, and the cell viability was higher than 80% at the concentration of 0.2% for all the conjugates tested [108,109].

Amphotericin B (AmB), a polyene antibiotic for the treatment of fungal and leishmanial infections, is insoluble in water and exhibits severe toxicity. PS 20 was activated by *p*-nitrophenyl chloroformate to form a stable carbonate intermediate, followed by the direct nucleophile-catalyzed reaction with mycosamine of AmB to form PS 20-AmB conjugates [110]. Chemical synthesis of PS 20-AmB conjugates is illustrated in Figure 5. PS 20-AmB conjugates demonstrated good water-solubility and negligible hemolytic potentials. The conjugates showed potent antifungal activity against *C. albicans*, *C. parapsilosis*, and *C. neoformans*. They also showed anti-leishmanial activity against promastigotes of *L. donovani* in vitro [110].

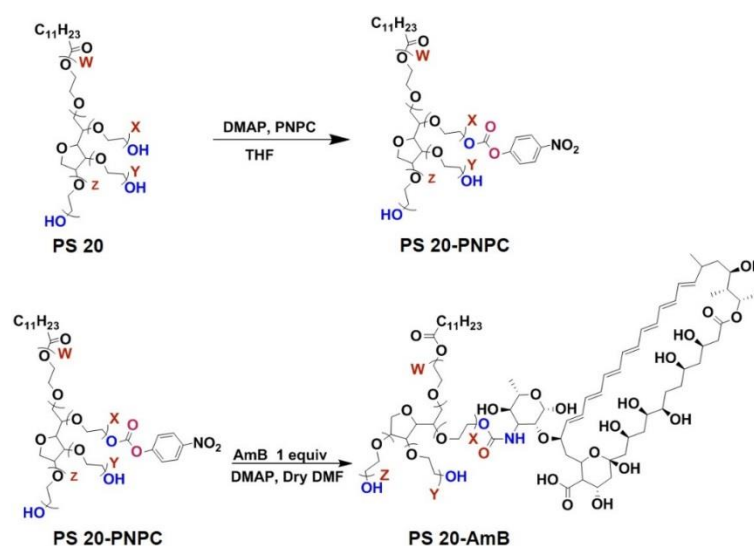


Figure 5. Synthesis of PS 20-AmB conjugates. Hydroxyl groups of PS were activated with para-nitrophenyl chloroformate (PNPC), followed by conjugation with amphotericin B (AmB) in the presence of dimethylaminopyridine (DMAP). THF: Tetrahydrofuran, DMF: Dimethylformamide. Reproduced with permission from Reference [110]. Copyright 2018, Bentham Science.

Hydrogels can be used as drug delivery carriers owing to their high drug-loading capacity. PS 20–trimethacrylate was synthesized and used as a flexible crosslinker for photopolymerizable hydrogels. The aqueous solutions of PS 20–trimethacrylate were photo-polymerized within 30 min of exposure to UV light. Aqueous PS 20–trimethacrylate was then coupled with N-vinyl-2-pyrrolidone to form hydrogels whose swelling ratios and swelling rates were tuned by varying the amount of PS 20–trimethacrylate. These hydrogels showed potential for controlled drug release [111].

Hydrogel NPs containing poly(ethylenimine) (PEI)–graft–PS (PEIP) copolymers have been developed as drug carriers. A schematic of the preparation of PEIP and hydrogel formation is shown in Figure 6. The PEIP-based hydrogel NPs showed good stability and efficient cellular internalization owing to the stabilizing ability and drug-absorption-enhancing activity of PS. The cytotoxicity of PEIP was considerably lower than that of unmodified PEI in retinal ganglion cell 5 and human embryonic kidney HEK293 cells. The highly branched structure of PEIP enhances the sustainability of protein release by increasing the degree of chain entanglement. These results demonstrate that PEIP-based hydrogel NPs are promising drug carriers for protein delivery [112].

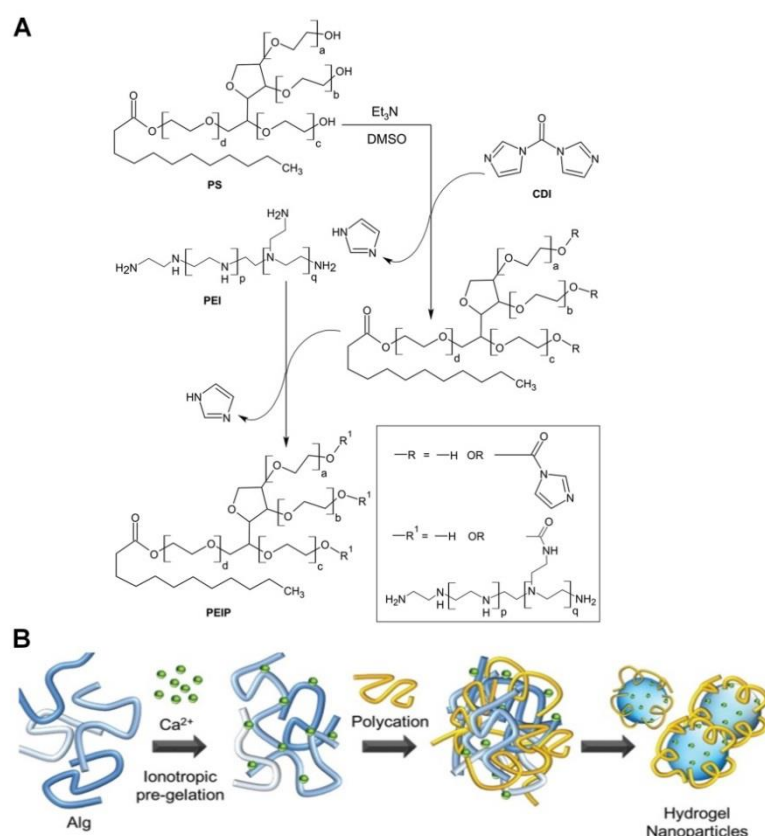


Figure 6. (A) Schematic illustration for the synthesis of poly(ethyleneimine-*graft*-PS (PEIP) copolymer. (B) Schematic representation for the fabrication of hydrogel NPs using PEIP and alginate (TEA: Triethylamine, DMSO: Dimethyl sulfoxide, CDI: Carbonyldiimidazole). Reproduced with permission from Reference [112]. Copyright 2016, Royal Society of Chemistry.

5. PS-Coated NPs for Efficient BBB Transport

PS-coated NPs are known to efficiently deliver therapeutic molecules to the brain by crossing the BBB [113–115]. PS-coated NPs can interact strongly with the brain blood vessel endothelial cells. PS 80 enables the adsorption of apolipoproteins (e.g., apolipoprotein E (ApoE)) onto NPs [116], and the NPs interact with lipoprotein receptors on the luminal surface of the BBB, triggering the receptor-mediated transcytosis of the PS-coated NPs (Figure 7). Moreover, PS-coated particles reduce nonspecific uptake by the reticuloendothelial system [117].

In recent years, various PS-coated NPs have been developed to improve brain-specific delivery of drugs, such as tacrine [118], doxorubicin [119], hexapeptide dalargin [120], loperamide [121], and tubocurarine [122], by increasing LDL-mediated endocytosis [123]. Kreuter et al. extensively investigated PS-coated nanoparticle systems for the brain-targeted delivery of drugs [113–115]. Transport of dalargin across the BBB was facilitated using PS 80-coated poly(butyl cyanoacrylate) (PBCA) NPs. Intravenous injection of PS 80-coated PBCA NPs encapsulating dalargin in mice showed an analgesic effect [113]. PBCA NPs encapsulating the anti-nociceptive peptide dalargin were coated with apolipoproteins for efficient brain targeting [115]; additionally, the NPs were coated with PS 80. The formulations were administered intravenously into mice, and the mean percentage of the maximum possible effect (MPE) was calculated. It was shown that PS 80 pre-coating achieved a significantly higher MPE than that in the control groups. PS 80-coated NPs adsorb apolipoproteins from the blood after injection, thus mimicking lipoprotein particles that can be efficiently internalized by the brain capillary endothelial cells via receptor-mediated endocytosis. Additionally, tail-flick test results using ApoE-deficient mice demonstrated that ApoE plays a critical role in the transport of NPs across the BBB.

These findings explain the mechanism of PS 80-coated drug carriers for brain-targeted delivery [115]. Recent progress in the use of PS-coated NPs for brain-targeted delivery is summarized in Table 1.

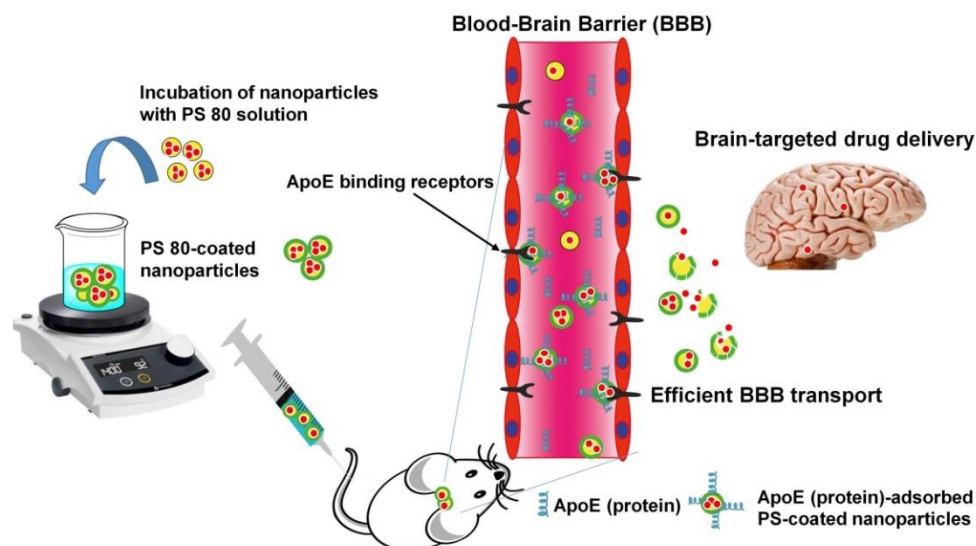


Figure 7. Schematic representation of PS 80-mediated drug delivery to brain. PS 80 enables the adsorption of ApoE onto NPs, and the PS 80-coated NPs interact with lipoprotein receptors on the BBB's luminal surface, leading to receptor-mediated transcytosis of the PS-coated NPs.

Several studies have demonstrated that PS 80-coated NPs can improve drug accumulation in the brain [124–131]. It has been reported that brain targeting of PS 80-coated NPs is closely associated with the interaction between PS 80 coating and brain microvessel endothelial cells [132]. Efficient penetration of the hexapeptide dalargin across the BBB was achieved using PS 80-coated PBCA NPs [124]. Intravenous administration of this formulation to mice exerted analgesic effects due to efficient phagocytic uptake by the brain blood vessel endothelial cells. This study demonstrated that PS-coated NPs facilitate the transport of large peptides that cannot penetrate the BBB in their native form. When dalargin bound to PS 80-coated NPs was intravenously administered to mice, a strong analgesic effect was observed; however, its administration with other surfactants, such as poloxamers 184, 188, 338, 407, poloxamine 908, cremophor EZ, cremophor RH 40, and Brij 35, showed negligible effects [125]. Rivastigmine-loaded PBCA NPs have been developed for the treatment of Alzheimer's disease [126]. Animal model results showed that drug uptake in the brain increased four-fold for PBCA NPs coated with 1% PS 80 compared to free drugs.

PS 80-bound poly(lactic-co-glycolic acid) (PLGA) NPs were prepared to increase the localization of acetylpuerarin (AP) in the brain of mice [133]. AP-loaded PLGA NPs showed more efficient protective effects against cerebral ischemia-reperfusion injury in rats compared to the free drug. The antitumor effects of PS 80-coated PBCA NPs encapsulating GEM were demonstrated using a rat brain tumor model. The 1% PS 80-coated GEM-loaded PBCA NPs were able to cross the BBB, leading to necrosis. As a result, the NPs significantly inhibited the growth of C6 glioma cells in the rat brain tumor model. Furthermore, they significantly extended the survival time compared to the saline-treated control groups [134].

Gelperina et al. evaluated the toxicity of PS 80-coated NPs encapsulating doxorubicin (DOX) in glioblastoma-bearing rat models. PS 80-coated NPs did not show any changes in toxicity compared to free DOX or DOX-encapsulated NPs. The in vivo toxicity results showed similar effects in both healthy and tumor-bearing rats [135].

Ren et al. synthesized PS 80-coated AmB/PLA-b-PEG NPs for transport across the BBB. The BBB efficiency was significantly enhanced when NPs were coated with PS 80.

The drug concentration in mouse brain was greatly enhanced compared to non-coated NPs [136].

Table 1. Various PS-coated NPs for brain-targeted delivery.

Drug Carriers	Therapeutic Agents	Diseases	Surface Coating	Study Model	Reference
PCBA	Dalargin	Analgesic	PS 80	In vivo mice	[113]
PCBA	Dalargin	Analgesic	PS 80	In vivo mice	[115]
PCBA	Dalargin	Analgesic	PS 80	In vivo mice	[120]
PCBA	Dalargin	Analgesic	PS 80	In vivo mice	[125]
PCBA	Rivastigmine	Alzheimer	PS 80	In vivo Wistar rats	[126]
PLA	FITC-dextran	BBB	PS 80	In vivo Kunming mice	[132]
PLGA	Acetylpuerarin	Cerebral ischaemia-reperfusion injury	PS 80	In vivo Wistar rats and Kunming mice	[133]
PCBA	Gemcitabine	Glioblastoma	PS 80	In vivo rats	[134]
PCBA	Doxorubicin	Glioblastoma	PS 80	In vivo white male non-inbred rats	[135]
PLA-b-PEG	Amphotericin B	Cryptococcal Meningitis	PS 80	In vivo BALB/c mice	[136]
Iron oxide		Brain targeting	PS 80	In vivo Sprague–Dawley rats	[137]
Hyaluronic acid	Curcumin	Targeting glioma	PS 80	In vitro B.End3 cells and G422 cells	[138]
PBCA	Fluorophore and anti-A β antibody	Brine targeting/Alzheimer detection	PS 80	In vivo mice	[139]
PLGA	Methotrexate-transferrin	Brain cancer	PS 80	In vivo Wistar rats	[140]
PLGA	Thymoquinone	Alzheimer	PS 80	In vivo albino mice	[141]
PLGA	siRNA	Traumatic brain injury	PS 80	In vivo C57BL/6J mice	[142]

Super paramagnetic iron oxide NPs (SPIONs) coated with polyethylene glycol (PEG), PEI, and PS 80 were synthesized for effective drug delivery to the brain [137]. A detailed illustration of the BBB transport of PS 80-coated SPIONs is presented in Figure 8. An in vivo study using rat models demonstrated that PS 80-coated SPIONs efficiently crossed normal BBB under an external magnetic field (EMF). The energy dispersive spectroscopy (EDS) results showed that SPIONs accumulated in the cortex near the magnet. Transmission electron microscope (TEM) images and elemental identification through EDS showed efficient accumulation of PS 80-coated SPIONs in the brain. This study demonstrates that both PS conjugation and EMF play crucial roles in the effective passage of SPIONs across the intact BBB [137].

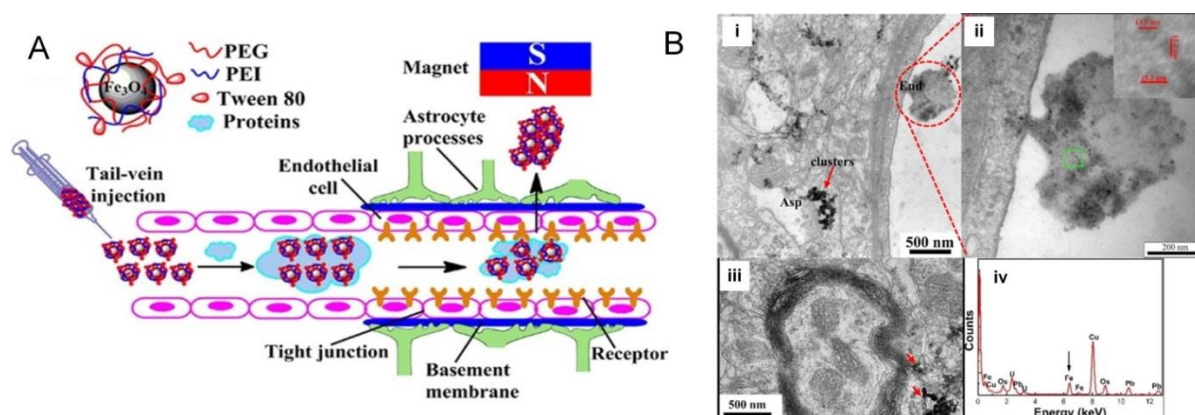


Figure 8. (A) PS-coated superparamagnetic iron oxide NPs (PS-SPIONs) pass through the intact BBB in rats under magnetic field. (B) Intracellular distribution of the PS-SPIONs in the frontal cortex in the presence of EMF. (i, ii) TEM images of PS-SPIONs that enter the brain by crossing BBB. (iii) Nanoparticle clusters were found near the axons of neurons. (iv) EDS analysis of electron-dense black clusters indicates the presence of Fe. Reproduced with permission from Reference [137]. Copyright 2016, American Chemical Society.

PS 80-coated redox-sensitive micelles were fabricated using hydrophilic hyaluronic acid-conjugated curcumin through disulfide linkers [138]. The micelles were coated with PS 80 to achieve effective brain targeting. The PS 80-coated redox-sensitive micelles exhibited redox sensitivity in the presence of glutathione. CD44 is a cell surface hyaluronic acid-binding glycoprotein that is overexpressed in cancer cells. Therefore, cellular uptake of hyaluronic acid-curcumin conjugates encapsulated into PS 80-coated redox-sensitive micelles was higher than that of free curcumin in CD44 receptor-expressing G422 glioblastoma cells. The increased cellular internalization of PS 80-coated redox-sensitive micelles incorporating curcumin induced higher cytotoxicity against G422 cells than free curcumin [138]. The redox-sensitive micelles also exhibited high plasma stability and did not induce hemolysis in erythrocytes.

PS 80-coated PBCA NPs were developed for the delivery of brain-impermeable imaging agents into the brain. The results showed that PS 80-coated PBCA NPs delivered imaging agents into the brain of mice for in vivo imaging of neuronal and glial nuclei. Neuropathological changes in neurodegenerative diseases can be visualized using NPs. Notably, BBB-permeable NPs did not induce nonspecific disruption of the BBB [139].

PS 80-coated PLGA NPs loaded with methotrexate–transferrin conjugates were developed for efficient BBB migration [140]. Figure 9 shows the BBB crossing details in the pictorial view. The nanoformulation coated with PS-80 crossed the BBB through the endocytosis pathway and inhibited the P-gp efflux pump to avoid drug elimination [140]. PS 80-coated particles showed higher anticancer efficacy than free drugs or PS 80-free formulations. Alzheimer’s disease-induced albino mice were treated with PS 80-coated PLGA NPs encapsulating thymoquinone [141]. These NPs enhanced the bioavailability of thymoquinone, reduced the immobility time (39.45 ± 3.32 s), and increased superoxide dismutase functioning (8.33 ± 2.61 units/mg) upon injection into mice (5 mg/kg concentrations). Thymoquinone reduced oxidative stress and thus restricted the accumulation of A β and hyperphosphorylated τ -protein tangles via the antioxidant cascade [141].

PS 80-coated PLGA NPs were developed for the efficient transport of small interfering RNA (siRNA) across the BBB in traumatic brain injury (TBI) [142] (Figure 10). siRNA-based therapeutics can mitigate disease progression in TBI treatment, but siRNA suffers from poor BBB transport. The surface of siRNA-loaded PLGA NPs was coated with five different coating materials (e.g., PS 80, Pluronic F-68, DSPE-PEG, DSPE-PEG-glutathione (GSH), DSPE-PEG-transferrin (Tf)) to identify a surface coating material and coating density that can maximize the penetration of siRNA-loaded NPs across intact BBB. Among the NPs with different coatings materials, PS-coated NPs showed the most efficient in vitro gene silencing efficiency and BBB penetration. As a result, tau siRNA-loaded PS 80-coated PLGA NPs

significantly suppressed tau expression in cultured primary neural cells. Additionally, they achieved efficient tau silencing in TBI mice with no systemic toxicity when administered during the early or late injury period [142].

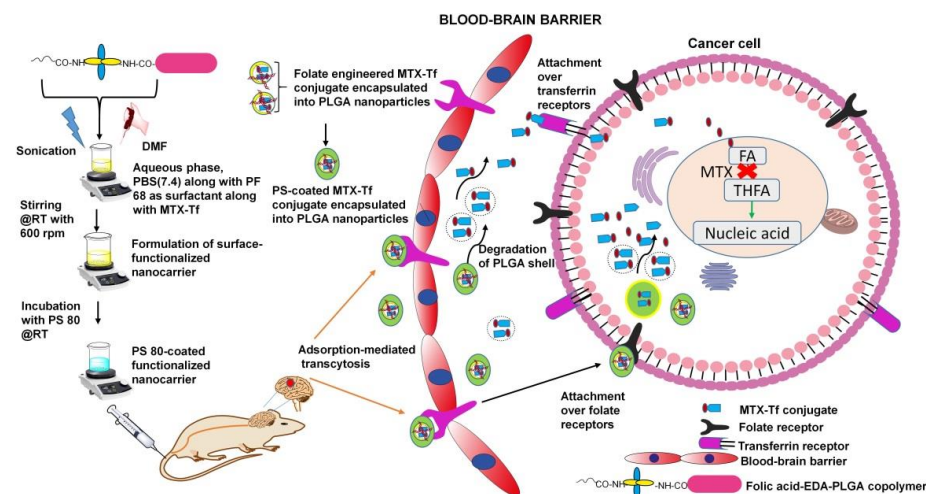


Figure 9. BBB crossing mechanism of PS 80-coated PLGA NPs loaded with methotrexate–transferrin prodrug conjugates. Redrawn and reproduced with permission from Reference [140].

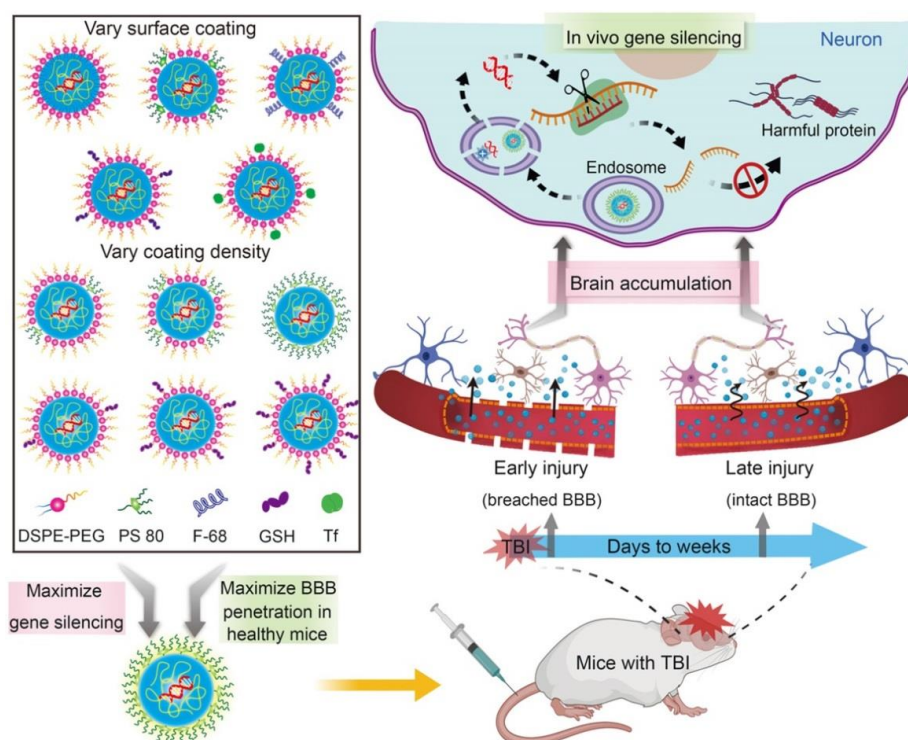


Figure 10. Schematic illustrating the design and mechanism of BBB pathophysiology-independent delivery of siRNA in TBI using siRNA-loaded PLGA NPs. Various siRNA-loaded PLGA NPs with five different surface coating materials (e.g., PS 80, Pluronic F-68, DSPE-PEG, DSPE-PE G-GSH, and DSPE-PEG-transferrin (DSPE-PEG-Tf)) and different coating densities were prepared. The gene silencing efficiency and BBB permeability of siRNA-loaded PLGA NPs with various surface coatings were assessed in TBI mice when administered during early injury or late injury periods, corresponding to physically breached BBB and intact BBB, respectively. Upon neuronal uptake of NPs, siRNA is released and silences the harmful proteins involved in TBI pathophysiology. Reproduced with permission from Reference [142].

6. Anticancer Therapy Using PS-Based Formulations

Cisplatin is widely used for treating bladder cancer [143,144]. Because of its high toxicity, direct therapeutic usage is avoided. Cisplatin formulation developed with nonionic surfactants for i.v. administration. The nanoemulsions showed sustained release of the drugs and better activity against bladder cancer cells.

GEM, a chemotherapeutic agent, has limitations to in vivo cancer therapy owing to its hydrophilic nature. The mixture of Tween 80, Span 80, 0.9 % sodium chloride solution was used to prepare GEM-loaded nanoemulsions [145]. The results showed that the release of GEM at pH 6.5 (45.19%) was higher than that at pH 7.4 (13.62%). The cytotoxicity study showed that the optimized nanoemulsion containing GEM induced cytotoxicity towards A549 cells compared to a control group (i.e., GEM solution).

Docetaxel (DTX) is a widely used anticancer drug for the treatment of several types of cancers, including metastatic breast cancer, metastatic non-small cell lung cancer, hormone-refractory prostate cancer, gastric adenocarcinoma, and squamous cell carcinoma of the head and neck [146]. DTX-encapsulated, rod-like micelles were fabricated using PS 80 and phospholipids. The size of micelles was 13 nm with narrow size distribution. Pharmacokinetics results suggested that the micelles increased the residence amount of DTX in kidney, spleen, ovary, uterus, heart, and liver. The hemolytic potentials study revealed that the mixed micelles were safe for intravenous injection [147].

PS-based nanoemulsions were developed for efficient intracellular delivery of photosensitizers [148]. Indocyanine green (ICG) is a near-infrared light-absorbing dye approved by the FDA [149]. ICG has been employed as an NIR fluorescence-imaging tracer and photosensitizer. ICG was loaded into nanoemulsions composed of cationic lipid stearylamine (SA) and surfactant mixtures of PS 80 and Span 85. Loading ICG into SA-incorporated nanoemulsions more effectively reduced the aggregation and degradation of ICG compared to loading in SA-free nanoemulsions. SA incorporation also enhanced tumor cell uptake of ICG-loaded nanoemulsions due to positive charges of the SA-incorporated nanoemulsions. As a result, compared to SA-free nanoemulsions encapsulating ICG, SA-incorporated nanoemulsions encapsulating ICG exhibited higher phototoxicity upon NIR light irradiation. After subcutaneous injection into the footpad of mice, SA-incorporated nanoemulsions exhibited higher concentrations of ICG in popliteal lymph nodes compared to SA-free nanoemulsions [148].

PS 80-based micelles were prepared for combination chemo/photothermal/photodynamic cancer therapy via effective intracellular delivery of piperlongumine (PL) and ICG [150] (Figure 11). PL was used as a cancer-specific anticancer agent by inducing selective oxidative stress in cancer cells. ICG was utilized as an NIR light-absorbing photothermal and photodynamic agent. PS 80-based micelles demonstrated successful dual drug encapsulation. The micelle formulations enhanced the cellular uptake, photothermal effects, and photodynamic effects of ICG. ICG-loaded PS 80-based micelles demonstrated NIR light-triggered photothermal and photodynamic effects. ICG- and PL-loaded PS 80-based micelles exhibited cancer-specific cytotoxicity because PL selectively induces oxidative stress in cancer cells. This study confirmed that PL-ICG-T80 micelles are an effective platform to achieve efficient, cancer-targeted chemo-photodynamic combination therapy [150].

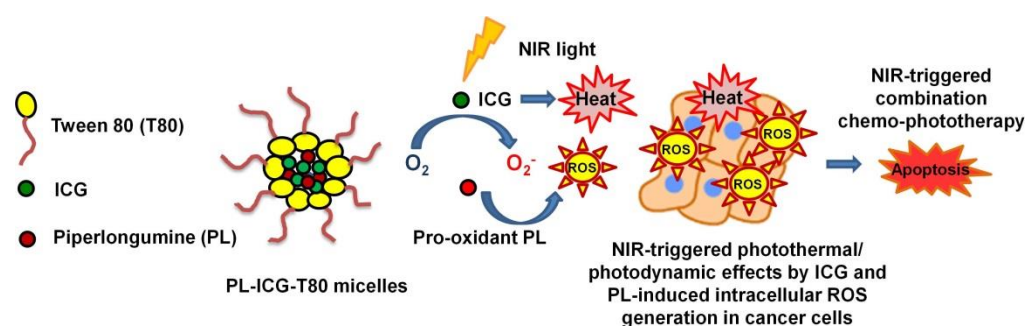


Figure 11. PL- and ICG-loaded PS 80-based NPs for combination chemo/photothermal/photodynamic therapy. Reproduced with permission from Reference [150]. Copyright 2020, Elsevier B.V.

7. Conclusions and Perspectives

PSs are considered important excipients in pharmaceutical formulations. Several studies and reviews have reported the multimodal use of PSs as solubilizers, emulsifiers, stabilizers, microemulsions, nanosomes, self-assembled micelles, and drug carriers for pharmaceutical applications. In this review, we summarized recent progress in PS-based drug formulations and drug delivery systems. We also summarized various PS-based drug formulations in terms of different routes of administration, such as ocular, transdermal, oral, and nasal delivery. PS was found to be safe and effective for all the administration routes. PSs are used as emulsifiers and solubilizers for hydrophobic drugs and proteins. PS-based drug formulations improve the solubility, stability, and pharmacokinetic properties of drugs, as well as control drug release and drug dissolution rates. In addition, PS can inhibit the P-gp efflux transporter, resulting in increased drug accumulation. Notably, PS-coated drug carriers can cross the BBB via receptor-mediated transcytosis, resulting in brain-targeted drug delivery. The biocompatible nature of PSs is an additional advantage for drug delivery systems.

PSs have been widely used in formulations and have demonstrated high therapeutic performance in preclinical studies [3]. In addition to the emulsifying and stabilizing properties, the advantages of using PSs as drug formulation agents are low cost, commercial availability, and ease of handling. Additionally, PSs are relatively low in toxicity, and their characteristic features can be achieved at low concentrations. However, complex mixtures of PSs, lack of spectral/quantitative characterization, and chemical/enzymatic degradation have hampered the use of PSs as active drug formulations. Commercial PSs are heterogeneous mixtures of structurally related compounds. PSs are available in three different grades according to their purities, including multi-compendial, super-refined, and ultrapure grades. The multi-compendial-grade PS 80 is a mixture of various fatty acid esters, with $\geq 58\%$ oleic acid content. In contrast, super-refined and ultrapure PS grades are esterified with at least 98% of their corresponding fatty acids. Ultrapure PS 80 is a highly purified PS that was prepared to meet the standards of Chinese pharmacopeia for human use [151,152]. Commercial PS 80 contains impurities such as 12-tricosanone, which leads to the occurrence of visible 12-tricosanone particles in drug formulations and decreases the activity of drugs [153]. The impurities of PSs can affect their performance in drug formulations. However, the challenges in characterization of PS heterogeneity have aroused significant interest in the development of new analytical methods to characterize PS heterogeneity. A recent study addressed the limitations in the characterization of PS heterogeneity using high-resolution mass spectrometry (HRMS) [154]. The molecular heterogeneity of PS 80 was analyzed using HRMS coupled with hydrogen/deuterium (H/D) exchange in deuterated methanol. The terminal hydroxyl groups of PSs have labile protons that can undergo H/D exchange in deuterated solvents. The resultant mass shifts indicate structural differences of isomers. Mass variation was identified to predict the structure and heterogeneity of PSs. This method was successfully applied to profile PS 80 samples from different suppliers or of different purity grades [154].

One of the major concerns involved in PS use is the degradation of PSs. The two main routes of PS degradation are oxidation and hydrolysis [155]. Degradation of PSs leads to protein aggregation, particle formation, lowered efficacy of drugs, and immune responses. Therefore, formulators need to characterize and understand PS degradation because impurities and degradation products strongly influence the physicochemical properties and therapeutic efficacies of PS-based drug formulations. Reversed-phase ultrahigh-performance liquid chromatography was also used to collect and characterize the ester fractions of PSs such as sorbitan-polyoxyethylene (POE)-monoester, isosorbide-POE-monoester, and sorbitan-POE-diester [155]. Various physicochemical properties of the ester fractions of PSs, including surface tension, micelle size, critical micelle concentration, and agitation protection for a monoclonal antibody, have been evaluated [155]. This method can provide information regarding the pattern of PS degradation, thus enabling formulators to better understand the risk of particle formation caused by degradation.

The purity of PS plays a crucial role in the physicochemical properties of PS-based drug formulations. Therefore, new synthesis strategies to minimize impurities and impurity-induced PS degradation should be exploited. The bulk synthesis of PSs should be monitored carefully using recently developed characterization techniques such as HRMS. The generation of byproducts from PS synthesis should be minimized by using suitable purification methods. Precise and less time/cost-consuming characterization techniques should be investigated to easily identify impurities and degradation products of PSs. To avoid PS degradation, the temperature, types of buffers, and types of coexcipients should be carefully considered.

Owing to the amphiphilic nature, PSs have been used as solubilizers of hydrophobic drugs, proteins, and inorganic nanoparticles. Therefore, PS-based drug carriers are able to carry multiple drugs for combination therapy. By incorporating inorganic nanoparticles as molecular imaging probes, PS-based drug carriers can be used as theranostic nanoplatforams for combined imaging and therapy. Particularly, PS-drug conjugates and PS-coated drug carriers have great potential to treat central nervous system-related diseases because PS coatings can improve the crossing of the BBB. In addition to the development of PS-based drug formulations, biosafety evaluations, such as systemic clearance, must be performed prior to their clinical use. These efforts will facilitate the successful translation of PS-based drug formulations into clinical settings.

Author Contributions: Conceptualization, V.R. and M.S.S.; methodology, V.R.; investigation, V.R. and T.G.N.C.; resources, M.L.; writing—original draft preparation, V.R. and M.S.S.; writing—review and editing, V.R. and M.S.S.; supervision, M.S.S.; project administration, V.R.; funding acquisition, M.S.S. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Research Assistance Program (2019) in the Incheon National University. This work was also supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2020R1I1A1A01071074).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Allen, T.M.; Cullis, P.R. Drug delivery systems: Entering the mainstream. *Science* **2004**, *303*, 1818–1822. [\[CrossRef\]](#)
2. Trucillo, P. Drug carriers: Classification, administration, release profiles, and industrial approach. *Processes* **2021**, *9*, 470. [\[CrossRef\]](#)
3. Kaur, G.; Mehta, S.K. Developments of Polysorbate (Tween) based microemulsions: Preclinical drug delivery, toxicity and antimicrobial applications. *Int. J. Pharm.* **2017**, *529*, 134–160. [\[CrossRef\]](#)
4. Milton, J.R.; Joy, T.K. Surfactants and interfacial phenomena. In *Surfactants in Biology*; John Wiley & Sons: Hoboken, NJ, USA, 2012; ISBN 978-0-470-54194-4.

5. Uchegbu, I.F.; Vyas, S.P. Non-ionic surfactant based vesicles (niosomes) in drug delivery. *Int. J. Pharm.* **1998**, *172*, 33–70. [CrossRef]
6. Tao, X.; Li, Y.; Hu, Q.; Zhu, L.; Huang, Z.; Yi, J.; Yang, X.; Hu, J.; Feng, X. Preparation and drug release study of novel nanopharmaceuticals with polysorbate 80 surface adsorption. *J. Nanomater.* **2018**, *2018*, 4718045. [CrossRef]
7. Prieto, C.; Calvo, L. Performance of the biocompatible surfactant tween 80, for the formation of microemulsions suitable for new pharmaceutical processing. *J. Appl. Chem.* **2013**, *2013*, 930356. [CrossRef]
8. Szymczyk, K.; Zdziennicka, A.; Jańczuk, B. Effect of polysorbates on solids wettability and their adsorption properties. *Colloids Interfaces* **2018**, *2*, 26. [CrossRef]
9. Lu, Y.; Wang, Y.Y.; Yang, N.; Zhang, D.; Zhang, F.Y.; Gao, H.T.; Rong, W.T.; Yu, S.Q.; Xu, Q. Food emulsifier polysorbate 80 increases intestinal absorption of di-(2-ethylhexyl) phthalate in rats. *Toxicol. Sci.* **2014**, *139*, 317–327. [CrossRef]
10. Ding, S. Quantitation of hydroperoxides in the aqueous solutions of non-ionic surfactants using polysorbate 80 as the model surfactant. *J. Pharm. Biomed. Anal.* **1993**, *11*, 95–101. [CrossRef]
11. Porter, M.R. *Handbook of Surfactants*; Chapman and Hall: London, UK, 1994; Volume 0751401706, pp. 126–168.
12. Wu, H.; Zhu, H.; Zhuang, J.; Yang, S.; Liu, C.; Cao, Y.C. Water-soluble nanocrystals through dual-interaction ligands. *Angew. Chem.* **2008**, *47*, 3730–3734. [CrossRef]
13. Xiao, J.; Duan, X.; Meng, Q.; Yin, Q.; Zhang, Z.; Yu, H.; Chen, L.; Gu, W.; Li, Y. Effective delivery of p65 shRNA by optimized Tween 85-polyethyleneimine conjugate for inhibition of tumor growth and lymphatic metastasis. *Acta Biomater.* **2014**, *10*, 2674–2683. [CrossRef]
14. Lim, E.K.; Yang, J.; Suh, J.S.; Huh, Y.M.; Haam, S. Self-labeled magneto nanoprobe using tri-aminated polysorbate 80 for detection of human mesenchymal stem cells. *J. Mater. Chem.* **2009**, *19*, 8958–8963. [CrossRef]
15. Kreuter, J.; Ramge, P.; Petrov, V.; Hamm, S.; Gelperina, S.E.; Engelhardt, B.; Alyautdin, R.; von Briesen, H.; Begley, D.J. Direct evidence that polysorbate-80-coated poly(butylcyanoacrylate) nanoparticles deliver drugs to the CNS via specific mechanisms requiring prior binding of drug to the nanoparticles. *Pharm. Res.* **2003**, *20*, 409–416. [CrossRef]
16. Sahu, A.K.; Mishra, J.; Mishra, A.K. Introducing Tween-curcumin niosomes: Preparation, characterization and microenvironment study. *Soft Matter* **2020**, *16*, 1779–1791. [CrossRef]
17. Deng, L.L.; Taxipalati, M.; Que, F.; Zhang, H. Physical characterization and antioxidant activity of thymol solubilized Tween 80 micelles. *Sci. Rep.* **2016**, *6*, 38160. [CrossRef] [PubMed]
18. Hekmat, A.; Attar, H.; Seyf Kordi, A.A.; Iman, M.; Jaafari, M.R. New oral formulation and in vitro evaluation of docetaxel-loaded nanomicelles. *Molecules* **2016**, *21*, 1265. [CrossRef] [PubMed]
19. Garidel, P.; Hoffmann, C.; Blume, A. A thermodynamic analysis of the binding interaction between polysorbate 20 and 80 with human serum albumins and immunoglobulins: A contribution to understand colloidal protein stabilisation. *Biophys. Chem.* **2009**, *143*, 70–78. [CrossRef] [PubMed]
20. Interim Clinical Considerations for Use of COVID-19 Vaccines Currently Approved or Authorized in the United States. Available online: <https://www.cdc.gov/vaccines/covid-19/clinical-considerations/covid-19-vaccines-us.html#Appendix-C> (accessed on 3 September 2021).
21. Jiao, J. Polyoxyethylated nonionic surfactants and their applications in topical ocular drug delivery. *Adv. Drug Deliv. Rev.* **2008**, *60*, 1663–1673. [CrossRef] [PubMed]
22. El-Setouhy, D.A.; Basalious, E.B.; Abdelmalak, N.S. Bioenhanced sublingual tablet of drug with limited permeability using novel surfactant binder and microencapsulated polysorbate: In vitro/in vivo evaluation. *Eur. J. Pharm. Biopharm.* **2015**, *94*, 386–392. [CrossRef] [PubMed]
23. Su, R.; Yang, L.; Wang, Y.; Yu, S.; Guo, Y.; Deng, J.; Zhao, Q.; Jin, X. Formulation, development, and optimization of a novel octyldodecanol-based nanoemulsion for transdermal delivery of ceramide IIIB. *Int. J. Nanomed.* **2017**, *12*, 5203–5221. [CrossRef]
24. Liu, L.; Qi, W.; Schwartz, D.K.; Randolph, T.W.; Carpenter, J.F. The effects of excipients on protein aggregation during agitation: An interfacial shear rheology study. *J. Pharm. Sci.* **2013**, *102*, 2460–2470. [CrossRef] [PubMed]
25. Bam, N.B.; Cleland, J.L.; Yang, J.; Manning, M.C.; Carpenter, J.F.; Kelley, R.F.; Randolph, T.W. Tween protects recombinant human growth hormone against agitation-induced damage via hydrophobic interactions. *J. Pharm. Sci.* **1998**, *87*, 1554–1559. [CrossRef]
26. Zhang, H.; Yao, M.; Morrison, R.A.; Chong, S. Commonly used surfactant, Tween 80, improves absorption of P-glycoprotein substrate, digoxin, in rats. *Arch. Pharmacol. Res.* **2003**, *26*, 768–772. [CrossRef] [PubMed]
27. Hugger, E.D.; Novak, B.L.; Burton, P.S.; Audus, K.L.; Borchardt, R.T. A comparison of commonly used polyethoxylated pharmaceutical excipients on their ability to inhibit P-glycoprotein activity in vitro. *J. Pharm. Sci.* **2002**, *91*, 1991–2002. [CrossRef]
28. Shono, Y.; Nishihara, H.; Matsuda, Y.; Furukawa, S.; Okada, N.; Fujita, T.; Yamamoto, A. Modulation of intestinal p-glycoprotein function by Cremophor EL and other surfactants by an in vitro diffusion chamber method using the isolated rat intestinal membranes. *J. Pharm. Sci.* **2004**, *93*, 877–885. [CrossRef]
29. Cornaire, G.; Woodley, J.F.; Saivin, S.; Legendre, J.Y.; Decourt, S.; Cloarec, A.; Houin, G. Effect of polyoxyl 35 castor oil and polysorbate 80 on the intestinal absorption of digoxin in vitro. *Arzneimittelforschung* **2000**, *50*, 576–579. [CrossRef] [PubMed]
30. Kerwin, B.A. Polysorbates 20 and 80 used in the formulation of protein biotherapeutics: Structure and degradation pathways. *J. Pharm. Sci.* **2008**, *97*, 2924–2935. [CrossRef]
31. Yu, Y.; Bu, F.; Zhou, H.; Wang, Y.; Cui, J.; Wang, X.; Nie, G.; Xiao, H. Biosafety materials: An emerging new research direction of materials science from the COVID-19 outbreak. *Mater. Chem. Front.* **2020**, *4*, 1930–1953. [CrossRef]

32. Safety Assessment of Polysorbates as Used in Cosmetics. Available online: <https://www.cir-safety.org/sites/default/files/polysorbates.pdf> (accessed on 3 September 2021).
33. Kazuaki, W.; Haruyoshi, K.; Noriko, F.; Chiye, T.; Kyoko, S.; Hiroshi, A. A comparative study of the hydroxyl and saponification values of polysorbate 60 in international food additive specifications. *AJAC* **2014**, *5*, 199–204.
34. Cox, S.; Sandall, A.; Smith, L.; Rossi, M.; Whelan, K. Food additive emulsifiers: A review of their role in foods, legislation and classifications, presence in food supply, dietary exposure, and safety assessment. *Nutr. Rev.* **2020**, *79*, 726–741. [\[CrossRef\]](#)
35. Aguilar, F.; Crebelli, R.; Di Domenico, A.; Dusemund, B.; Jose Frutos, M.; Galtier, P.; Gott, D.; Gundert-Remy, U.; Lambre, C.; Leblanc, J.C.; et al. Scientific opinion on the re-evaluation of polyoxyethylene sorbitan monolaurate (E 432), polyoxyethylene sorbitan monooleate (E 433), polyoxyethylene sorbitan monopalmitate (E 434), polyoxyethylene sorbitan monostearate (E 435) and polyoxyethylene sorbitan tristearate (E 436) as food additives. *EFSA J.* **2015**, *13*, 1–74.
36. Kriegel, C.; Festag, M.; Kishore, R.S.K.; Roethlisberger, D.; Schmitt, G. Pediatric safety of polysorbates in drug formulations. *Children* **2020**, *7*, 1. [\[CrossRef\]](#) [\[PubMed\]](#)
37. Van Tellingen, O.; Beijnen, J.H.; Verweij, J.; Scherrenburg, E.J.; Nooijen, W.J.; Sparreboom, A. Rapid esterase-sensitive breakdown of polysorbate 80 and its impact on the plasma pharmacokinetics of docetaxel and metabolites in mice. *Clin. Cancer Res.* **1999**, *5*, 2918–2924.
38. Mary, A.L. Final report on the safety assessment of polysorbates 20, 21, 40, 60, 61, 65, 80, 81, and 85. *J. Am. Coll. Toxicol.* **1984**, *3*, 1–82.
39. Nelson, M.F.; Poulos, T.A.; Gongwer, L.E.; Kirschman, J.C. Preparations of carbon-14-labeled polyoxyethylene (20) sorbitan monolaurate and their metabolic fate in rats. *J. Food Sci.* **1966**, *31*, 253–258. [\[CrossRef\]](#)
40. Oser, B.L.; Oser, M. Nutritional studies on rats on diets containing high levels of partial ester emulsifiers. III. Clinical and metabolic observations. *J. Nutr.* **1957**, *61*, 149–166. [\[CrossRef\]](#)
41. Czajkowska-Kośnik, A.; Wolska, E.; Chorazewicz, J.; Sznitowska, M. Comparison of cytotoxicity in vitro and irritation in vivo for aqueous and oily solutions of surfactants. *Drug Dev. Ind. Pharm.* **2015**, *41*, 1232–1236. [\[CrossRef\]](#)
42. Arechabala, B.; Coiffard, C.; Rivalland, P.; Coiffard, L.J.; de Roeck-Holtzhauer, Y. Comparison of cytotoxicity of various surfactants tested on normal human fibroblast cultures using the neutral red test, MTT assay and LDH release. *J. Appl. Toxicol.* **1999**, *19*, 163–165. [\[CrossRef\]](#)
43. Norris, L.; Qureshi, Z.; Bookstaver, B.; Raisch, D.; Sartor, O.; Chen, H.; Chen, F.; Bennett, C. Polysorbate 80 hypersensitivity reactions: A renewed call to action. *Community Oncol.* **2010**, *7*, 425–428. [\[CrossRef\]](#)
44. Kicker, J.S.; Haizlip, J.A.; Buck, M.L. Hepatotoxicity after continuous amiodarone infusion in a postoperative cardiac infant. *J. Pediatr. Pharmacol. Ther.* **2012**, *17*, 189–195. [\[CrossRef\]](#)
45. Gameiro, M.; Silva, R.; Rocha-Pereira, C.; Carmo, H.; Carvalho, F.; Bastos, M.L.; Remião, F. Cellular models and in vitro assays for the screening of modulators of P-gp, MRP1 and BCRP. *Molecules* **2017**, *22*, 600. [\[CrossRef\]](#) [\[PubMed\]](#)
46. Ferreira, R.J.; dos Santos, D.J.; Ferreira, M.J. P-glycoprotein and membrane roles in multidrug resistance. *Future Med. Chem.* **2015**, *7*, 929–946. [\[CrossRef\]](#) [\[PubMed\]](#)
47. Dudeja, P.K.; Anderson, K.M.; Harris, J.S.; Buckingham, L.; Coon, J.S. Reversal of multidrug resistance phenotype by surfactants: Relationship to membrane lipid fluidity. *Arch. Biochem. Biophys.* **1995**, *319*, 309–315. [\[CrossRef\]](#) [\[PubMed\]](#)
48. Sawangrat, K.; Yamashita, S.; Tanaka, A.; Morishita, M.; Kusamori, K.; Katsumi, H.; Sakane, T.; Yamamoto, A. Modulation of intestinal transport and absorption of topotecan, a BCRP substrate, by various pharmaceutical excipients and their inhibitory mechanisms of BCRP transporter. *J. Pharm. Sci.* **2019**, *108*, 1315–1325. [\[CrossRef\]](#) [\[PubMed\]](#)
49. Lo, Y.L. Relationships between the hydrophilic-lipophilic balance values of pharmaceutical excipients and their multidrug resistance modulating effect in Caco-2 cells and rat intestines. *J. Control. Release* **2003**, *90*, 37–48. [\[CrossRef\]](#)
50. Nielsen, R.B.; Kahnt, A.; Dillen, L.; Wuyts, K.; Snoeys, J.; Nielsen, U.G.; Holm, R.; Nielsen, C.U. Montmorillonite-surfactant hybrid particles for modulating intestinal P-glycoprotein-mediated transport. *Int. J. Pharm.* **2019**, *571*, 118696. [\[CrossRef\]](#) [\[PubMed\]](#)
51. Chenthamara, D.; Subramaniam, S.; Ramakrishnan, S.G.; Krishnaswamy, S.; Essa, M.M.; Lin, F.-H.; Qoronfleh, M.W. Therapeutic efficacy of nanoparticles and routes of administration. *Biomater. Res.* **2019**, *23*, 20. [\[CrossRef\]](#) [\[PubMed\]](#)
52. Tamilvanan, S.; Benita, S. The potential of lipid emulsion for ocular delivery of lipophilic drugs. *Eur. J. Pharm. Biopharm.* **2004**, *58*, 357–368. [\[CrossRef\]](#)
53. Lv, F.F.; Zheng, L.Q.; Tung, C.H. Phase behavior of the microemulsions and the stability of the chloramphenicol in the microemulsion-based ocular drug delivery system. *Int. J. Pharm.* **2005**, *301*, 237–246. [\[CrossRef\]](#)
54. Ali, Y.; Lehmuusaari, K. Industrial perspective in ocular drug delivery. *Adv. Drug Delivery Rev.* **2006**, *58*, 1258–1268. [\[CrossRef\]](#)
55. Murdan, S.; Gregoriadis, G.; Florence, A. Non-ionic surfactant based organogels incorporating niosomes. *Stp. Pharm. Sci.* **1996**, *6*, 44–48.
56. Alonso, M.J.; Sánchez, A. The potential of chitosan in ocular drug delivery. *J. Pharm. Pharmacol.* **2010**, *55*, 1451–1463. [\[CrossRef\]](#) [\[PubMed\]](#)
57. Desiredy, R.B.; Taraka, L.K.C.; Naga, S.G.; Sowjanya, T.; Lavanya, L.R.K. Ocular drug delivery system—Emerging trends. *Int. J. Pharm. Sci. Rev. Res.* **2012**, *16*, 29–33.
58. Draize, J.H.; Kelley, E.A. Surface-active agents and the eye. *Drug Cosmet. Ind.* **1952**, *71*, 118–120.
59. Furrer, P.; Mayer, J.M.; Gurny, R. Ocular tolerance of preservatives and alternatives. *Eur. J. Pharm. Biopharm.* **2002**, *53*, 263–280. [\[CrossRef\]](#)

60. Carmignani, C.; Rossi, S.; Saettone, M.F.; Burgalassi, S. Ophthalmic vehicles containing polymer-solubilized tropicamide: “in vitro/in vivo” evaluation. *Drug Dev. Ind. Pharm.* **2002**, *28*, 101–105. [\[CrossRef\]](#)
61. Rodriguez-Aller, M.; Kaufmann, B.; Guillarme, D.; Stella, C.; Furrer, P.; Rudaz, S.; El Zaoui, I.; Valamanesh, F.; Di, T.C.; Behar-Cohen, F.; et al. In vivo characterisation of a novel water-soluble cyclosporine a prodrug for the treatment of dry eye disease. *Eur. J. Pharm. Biopharm.* **2012**, *80*, 544–552. [\[CrossRef\]](#)
62. Gonzalez-Mira, E.; Egea, M.A.; Souto, E.B.; Calpena, A.C.; García, M.L. Optimizing flurbiprofen-loaded NLC by central composite factorial design for ocular delivery. *Nanotechnology* **2011**, *22*, 045101. [\[CrossRef\]](#)
63. Bucolo, C.; Maltese, A.; Puglisi, G.; Pignatello, R. Enhanced ocular anti-inflammatory activity of ibuprofen carried by an Eudragit RS100 nanoparticle suspension. *Ophthalmic Res.* **2002**, *34*, 319–323. [\[CrossRef\]](#)
64. Hippalgaonkar, K.; Adelli, G.R.; Hippalgaonkar, K.; Repka, M.A.; Majumdar, S. Indomethacin-loaded solid lipid nanoparticles for ocular delivery: Development, characterization, and in vitro evaluation. *J. Ocul Pharmacol Ther.* **2013**, *29*, 216–228. [\[CrossRef\]](#)
65. Patel, S.; Garapati, C.; Chowdhury, P.; Gupta, H.; Nesamony, J.; Nauli, S.; Boddu, S.H. Development and evaluation of dexamethasone nanomicelles with potential for treating posterior uveitis after topical application. *J. Ocul. Pharmacol. Ther.* **2015**, *31*, 215–227. [\[CrossRef\]](#)
66. Lv, F.F.; Li, N.; Zheng, L.Q.; Tung, C.H. Studies on the stability of the chloramphenicol in the microemulsion free of alcohols. *Eur. J. Pharm. Biopharm.* **2006**, *62*, 288–294. [\[CrossRef\]](#)
67. Zimmer, A.K.; Maincent, P.; Thouvenot, P.; Kreuter, J. Hydrocortisone delivery to healthy and inflamed eyes using a micellar polysorbate 80 solution or albumin nanoparticles. *Int. J. Pharm.* **1994**, *110*, 211–222. [\[CrossRef\]](#)
68. Abdelkader, H.; Alani, A.W.G.; Alany, R.G. Recent advances in non-ionic surfactant vesicles (niosomes): Self-assembly, fabrication, characterization, drug delivery applications and limitations. *Drug Deliv.* **2014**, *21*, 87–100. [\[CrossRef\]](#)
69. Ag Seleci, D.; Seleci, M.; Walter, J.G.; Stahl, F.; Scheper, T. Niosomes as nanoparticulate drug carriers: Fundamentals and recent applications. *J. Nanomater.* **2016**, *2016*, 7372306. [\[CrossRef\]](#)
70. Bartelds, R.; Nematollahi, M.H.; Pols, T.; Stuart, M.C.A.; Pardakhty, A.; Asadikaram, G.; Poolman, B. Niosomes, an alternative for liposomal delivery. *PLoS ONE* **2018**, *13*, e0194179. [\[CrossRef\]](#) [\[PubMed\]](#)
71. Mazet, R.; Yaméogo, J.B.G.; Wouessidjewe, D.; Choisnard, L.; Gèze, A. Recent advances in the design of topical ophthalmic delivery systems in the treatment of ocular surface inflammation and their biopharmaceutical evaluation. *Pharmaceutics* **2020**, *12*, 570. [\[CrossRef\]](#) [\[PubMed\]](#)
72. Mashal, M.; Attia, N.; Puras, G.; Martinez-Navarrete, G.; Fernandez, E.; Pedraz, J.L. Retinal gene delivery enhancement by lycopene incorporation into cationic niosomes based on DOTMA and polysorbate 60. *J. Controlled Release* **2017**, *254*, 55–64. [\[CrossRef\]](#) [\[PubMed\]](#)
73. Villate-Beitia, I.; Gallego, I.; Martinez-Navarrete, G.; Zarate, J.; Lopez-Mendez, T.; Soto-Sanchez, C.; Santos-Vizcaino, E.; Puras, G.; Fernandez, E.; Pedraz, J.L. Polysorbate 20 non-ionic surfactant enhances retinal gene delivery efficiency of cationic niosomes after intravitreal and subretinal administration. *Int. J. Pharm.* **2018**, *550*, 388–397. [\[CrossRef\]](#) [\[PubMed\]](#)
74. Shukla, T.; Upmanyu, N.; Agrawal, M.; Saraf, S.; Saraf, S.; Alexander, A. Biomedical applications of microemulsion through dermal and transdermal route. *Biomed. Pharmacother.* **2018**, *108*, 1477–1494. [\[CrossRef\]](#)
75. Michel, C.; Purmann, T.; Mentrup, E.; Seiller, E.; Kreuter, J. Effect of liposomes on percutaneous penetration of lipophilic materials. *Int. J. Pharm.* **1992**, *84*, 93–105. [\[CrossRef\]](#)
76. Liu, C.H.; Chang, F.Y. Development and characterization of eucalyptol microemulsions for topical delivery of curcumin. *Chem. Pharm. Bull.* **2011**, *59*, 172–178. [\[CrossRef\]](#)
77. Liu, C.-H.; Chang, F.-Y.; Hung, D.-K. Terpene microemulsions for transdermal curcumin delivery: Effects of terpenes and cosurfactants. *Colloids Surf. B* **2011**, *82*, 63–70. [\[CrossRef\]](#) [\[PubMed\]](#)
78. Hosmer, J.; Reed, R.; Bentley, M.V.L.B.; Nornoo, A.; Lopes, L.B. Microemulsions containing medium-chain glycerides as transdermal delivery systems for hydrophilic and hydrophobic drugs. *AAPS Pharm. Sci. Tech.* **2009**, *10*, 589–596. [\[CrossRef\]](#) [\[PubMed\]](#)
79. Hathout, R.M.; Woodman, T.J.; Mansour, S.; Mortada, N.D.; Geneidi, A.S.; Guy, R.H. Microemulsion formulations for the transdermal delivery of testosterone. *Eur. J. Pharm. Sci.* **2010**, *40*, 188–196. [\[CrossRef\]](#)
80. Yuan, Y.; Li, S.M.; Mo, F.K.; Zhong, D.F. Investigation of microemulsion system for transdermal delivery of meloxicam. *Int. J. Pharm.* **2006**, *321*, 117–123. [\[CrossRef\]](#)
81. Tavano, L.; Alfano, P.; Muzzalupo, R.; de Cindio, B. Niosomes vs microemulsions: New carriers for topical delivery of Capsaicin. *Colloids Surf. B* **2011**, *87*, 333–339. [\[CrossRef\]](#) [\[PubMed\]](#)
82. Xiao, Y.Y.; Liu, F.; Chen, Z.P.; Ping, Q.N. Water in oil microemulsions containing NaCl for transdermal delivery of fluorouracil. *Yao Xue Xue Bao* **2011**, *46*, 720–726.
83. Dhamankar, A.K.; Manwar, J.V.; Kumbhar, D.D. The novel formulation design of O/W microemulsion of ketoprofen for improving transdermal absorption. *Int. J. Pharm. Tech. Res.* **2009**, *1*, 1449–1457.
84. Thiebaut, F.; Tsuruo, T.; Hamada, H.; Gottesman, M.M.; Pastan, I.; Willingham, M.C. Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. *Proc. Natl. Acad. Sci. USA* **1987**, *84*, 7735–7738. [\[CrossRef\]](#)
85. Giacomini, K.M.; Huang, S.M.; Tweedie, D.J.; Benet, L.Z.; Brouwer, K.L.; Chu, X.; Dahlin, A.; Evers, R.; Fischer, V.; Hillgren, K.M.; et al. Membrane transporters in drug development. *Nat. Rev. Drug Discov.* **2010**, *9*, 215–236.

86. Leslie, E.M.; Deeley, R.G.; Cole, S.P. Multidrug resistance proteins: Role of P-glycoprotein, MRP1, MRP2, and BCRP (ABCG2) in tissue defense. *Toxicol. Appl. Pharmacol.* **2005**, *204*, 216–237. [\[CrossRef\]](#)
87. Cornaire, G.; Woodley, J.; Hermann, P.; Cloarec, A.; Arellano, C.; Houin, G. Impact of excipients on the absorption of P-glycoprotein substrates in vitro and in vivo. *Int. J. Pharm.* **2004**, *278*, 119–131. [\[CrossRef\]](#)
88. Nielsen, C.U.; Abdulhussein, A.A.; Colak, D.; Holm, R. Polysorbate 20 increases oral absorption of digoxin in wild-type Sprague Dawley rats, but not in mdr1a(-/-) Sprague Dawley rats. *Int. J. Pharm.* **2016**, *513*, 78–87. [\[CrossRef\]](#)
89. Al-Ali, A.A.A.; Quach, J.R.C.; Bundgaard, C.; Steffansen, B.; Holm, R.; Nielsen, C.U. Polysorbate 20 alters the oral bioavailability of etoposide in wild type and mdr1a deficient Sprague-Dawley rats. *Int. J. Pharm.* **2018**, *543*, 352–360. [\[CrossRef\]](#)
90. Al-Ali, A.A.A.; Nielsen, R.B.; Steffansen, B.; Holm, R.; Nielsen, C.U. Nonionic surfactants modulate the transport activity of ATP-binding cassette (ABC) transporters and solute carriers (SLC): Relevance to oral drug absorption. *Int. J. Pharm.* **2019**, *566*, 410–433. [\[CrossRef\]](#)
91. Serdoz, F.; Voinovich, D.; Perissutti, B.; Grabnar, I.; Hasa, D.; Ballestrazzi, R.; Coni, E.; Pellegrini, E. Development and pharmacokinetic evaluation of erythromycin lipidic formulations for oral administration in rainbow trout (*Oncorhynchus mykiss*). *Eur. J. Pharm. Biopharm.* **2011**, *78*, 401–407. [\[CrossRef\]](#)
92. Fan, Y.; Li, X.; Zhou, Y.; Fan, C.; Wang, X.; Huang, Y.; Liu, Y. Improved intestinal delivery of salmon calcitonin by water-in-oil microemulsions. *Int. J. Pharm.* **2011**, *416*, 323–330. [\[CrossRef\]](#)
93. Govindaraju, R.; Karki, R.; Chandrashekarappa, J.; Santhanam, M.; Shankar, A.K.K.; Joshi, H.K.; Divakar, G. Enhanced water dispersibility of curcumin encapsulated in alginate-polysorbate 80 nano particles and bioavailability in healthy human volunteers. *Pharm. Nanotechnol.* **2019**, *7*, 39–56. [\[CrossRef\]](#)
94. Lu, J.L.; Wang, J.C.; Zhao, S.X.; Liu, X.Y.; Zhao, H.; Zhang, X.; Zhou, S.F.; Zhang, Q. Self-microemulsifying drug delivery system (SMEDDS) improves anticancer effect of oral 9-nitrocamptothecin on human cancer xenografts in nude mice. *Eur. J. Pharm. Biopharm.* **2008**, *69*, 899–907. [\[CrossRef\]](#)
95. Mestry, M.; Rane, M.; Kadu, P.; More, S. Self-emulsifying drug delivery system of rosuvastatin calcium. *Int. J. Pharm.* **2017**, *7*, 130–139.
96. Seo, J.K.; Sang, E.L.; Choon, L.N.; Joon, K.L.; Tae, H.K.; Cheong, W.C.; Jeong, S.P. Preformulation and in vitro physicochemical characterization of fenofibrate-loaded emulsion. *J. Pharm. Investig.* **2015**, *45*, 669–674.
97. Patel, A.R.; Vavia, P.R. Preparation and in vivo evaluation of SMEDDS (self-microemulsifying drug delivery system) containing fenofibrate. *AAPS J.* **2007**, *9*, E344–E352. [\[CrossRef\]](#)
98. Gao, M.; Mei, D.; Huo, Y.; Mao, S. Effect of polysorbate 80 on the intranasal absorption and brain distribution of tetramethylpyrazine phosphate in rats. *Drug Deliv. Transl.* **2019**, *9*, 311–318. [\[CrossRef\]](#)
99. Hilmarsson, H.; Kristmundsdottir, T.; Gunnarsson, E.; Thormar, H. Intranasal delivery of formulations containing virucidal lipids for treatment of respiratory syncytial virus (RSV) infection in rats. *J. Drug Deliv. Sci. Technol.* **2013**, *23*, 455–457. [\[CrossRef\]](#)
100. Ruan, Y.; Yao, L.; Zhang, B.; Zhang, S.; Guo, J. Nanoparticle-mediated delivery of neurotoxin-II to the brain with intranasal administration: An effective strategy to improve antinociceptive activity of neurotoxin. *Drug Dev. Ind. Pharm.* **2012**, *38*, 123–128. [\[CrossRef\]](#)
101. Deshpande, N.U.; Jayakannan, M. Cisplatin-stitched polysaccharide vesicles for synergistic cancer therapy of triple antagonistic drugs. *Biomacromolecules* **2017**, *18*, 113–126. [\[CrossRef\]](#)
102. Jensen, A.I.; Binderup, T.; Kumar, E.P.; Kjær, A.; Rasmussen, P.H.; Andresen, T.L. Positron emission tomography based analysis of long-circulating cross-linked triblock polymeric micelles in a U87MG mouse xenograft model and comparison of DOTA and CB-TE2A as chelators of copper-64. *Biomacromolecules* **2014**, *15*, 1625–1633. [\[CrossRef\]](#)
103. Kumar, S.; Alnasif, N.; Fleige, E.; Kurniasih, I.; Kral, V.; Haase, A.; Luch, A.; Weindl, G.; Haag, R.; Schäfer-Korting, M.; et al. Impact of structural differences in hyperbranched polyglycerol-polyethylene glycol nanoparticles on dermal drug delivery and biocompatibility. *Eur. J. Pharm. Biopharm.* **2014**, *88*, 625–634. [\[CrossRef\]](#)
104. Sousa-Herves, A.; Würfel, P.; Wegner, N.; Khandare, J.; Licha, K.; Haag, R.; Welker, P.; Calderón, M. Dendritic polyglycerol sulfate as a novel platform for paclitaxel delivery: Pitfalls of ester linkage. *Nanoscale* **2015**, *7*, 3923–3932. [\[CrossRef\]](#)
105. Ray, P.; Ferraro, M.; Haag, R.; Quadir, M. Dendritic polyglycerol-derived nano-architectures as delivery platforms of gemcitabine for pancreatic cancer. *Macromol. Biosci.* **2019**, *19*, e1900073. [\[CrossRef\]](#) [\[PubMed\]](#)
106. Dai, J.; Kim, J.C. Photo responsive monoolein cubic phase containing coumarin-Tween 20 conjugates. *Drug Dev. Ind. Pharm.* **2013**, *39*, 1457–1463. [\[CrossRef\]](#)
107. Seo, H.J.; Dai, J.; Kim, J.C. The effect of UV irradiation on air/water interfacial activity of Tween 20-coumarin conjugates. *Colloid Polym. Sci.* **2013**, *291*, 2311–2318. [\[CrossRef\]](#)
108. Wang, M.; Kim, J.C. Tween 20-cinnamic acid conjugate as a UV-absorbing emulsifier. *Colloids Surf. A Physicochem. Eng. Asp.* **2014**, *453*, 62–67. [\[CrossRef\]](#)
109. Kim, J.C. Characterization of cinnamic acid-attached nonionic amphiphiles in UV extinction, emulsification, and in vitro toxicity. *J. Dispers. Sci. Technol.* **2016**, *37*, 104–112. [\[CrossRef\]](#)
110. Vasanthan, R.; Venkitasamy, K.; Sandrine, C.; Philippe, M.L.; Jayakrishnan, A. Polysorbate surfactants as drug carriers: Tween 20-Amphotericin B conjugates as anti-fungal and anti-leishmanial agents. *Curr. Drug Deliv.* **2018**, *15*, 1028–1037.
111. Borges, A.C.; Jayakrishnan, A.; Bourban, P.E.; Plummer, C.J.G.; Pioletti, D.P.; Manson, J.-A.E. Synthesis and photopolymerization of tween 20 methacrylate/n-vinyl-2-pyrrolidone blends. *Mater. Sci. Eng. C* **2012**, *32*, 2235–2241. [\[CrossRef\]](#)

112. Lai, W.F.; Shum, H.C. A stimuli-responsive nanoparticulate system using poly(ethylenimine)-graft-polysorbate for controlled protein release. *Nanoscale* **2016**, *8*, 517–528. [\[CrossRef\]](#)
113. Kreuter, J.; Alyautdin, R.N.; Kharkevich, D.A.; Ivanov, A.A. Passage of peptides through the blood-brain barrier with colloidal polymer particles (nanoparticles). *Brain Res.* **1995**, *674*, 171–174. [\[CrossRef\]](#)
114. Kreuter, J. Nanoparticulate systems for brain delivery of drugs. *Adv. Drug Deliv. Rev.* **2012**, *64*, 213–222. [\[CrossRef\]](#)
115. Kreuter, J.; Shamenkov, D.; Petrov, V.; Ramge, P.; Cychutek, K.; Koch-Brandt, C.; Alyautdin, R. Apolipoprotein-mediated transport of nanoparticle-bound drugs across the blood-brain barrier. *J. Drug Target.* **2002**, *10*, 317–325. [\[CrossRef\]](#)
116. Hartl, N.; Adams, F.; Merkel, O.M. From adsorption to covalent bonding: Apolipoprotein E functionalization of polymeric nanoparticles for drug delivery across the blood–brain barrier. *Adv. Ther.* **2021**, *4*, 2000092. [\[CrossRef\]](#) [\[PubMed\]](#)
117. Jose, S.; Anju, S.S.; Cinu, T.A.; Aleykutty, N.A.; Thomas, S.; Souto, E.B. In vivo pharmacokinetics and biodistribution of resveratrol-loaded solid lipid nanoparticles for brain delivery. *Int. J. Pharm.* **2014**, *474*, 6–13. [\[CrossRef\]](#) [\[PubMed\]](#)
118. Wilson, B.; Samanta, M.K.; Santhi, K.; Kumar, K.P.S.; Paramakrishnan, N.; Suresh, B. Targeted delivery of tacrine into the brain with polysorbate 80-coated poly(n-butylcyanoacrylate) nanoparticles. *Eur. J. Pharm. Biopharm.* **2008**, *70*, 75–84. [\[CrossRef\]](#) [\[PubMed\]](#)
119. Gulyaev, A.E.; Gelperina, S.E.; Skidan, I.N.; Antropov, A.S.; Kivman, G.Y.; Kreuter, J. Significant transport of doxorubicin into the brain with polysorbate 80-coated nanoparticles. *Pharm. Res.* **1999**, *16*, 1564–1569. [\[CrossRef\]](#)
120. Alyautdin, R.; Gothier, D.; Ve, P.; Kharkevich, D.A.; Kreuter, J. Analgesic activity of the hexapeptide dalargin adsorbed on the surface of polysorbate 80-coated poly(butyl cyanoacrylate) nanoparticles. *Eur. J. Pharm. Biopharm.* **1995**, *41*, 44–48.
121. Alyautdin, R.N.; Petrov, V.E.; Langer, K.; Berthold, A.; Kharkevich, D.A.; Kreuter, J. Delivery of loperamide across the blood-brain barrier with polysorbate 80-coated polybutylcyanoacrylate nanoparticles. *Pharm. Res.* **1997**, *14*, 325–328. [\[CrossRef\]](#)
122. Alyautdin, R.N.; Tezikov, E.B.; Ramge, P.; Kharkevich, D.A.; Begley, D.J.; Kreuter, J. Significant entry of tubocurarine into the brain of rats by adsorption to polysorbate 80-coated polybutylcyanoacrylate nanoparticles: An in situ brain perfusion study. *J. Microencapsul.* **1998**, *15*, 67–74. [\[CrossRef\]](#)
123. Prabhakar, K.; Afzal, S.M.; Surender, G.; Kishan, V. Tween 80 containing lipid nanoemulsions for delivery of indinavir to brain. *Acta Pharm. Sin. B.* **2013**, *3*, 345–353. [\[CrossRef\]](#)
124. Schröder, U.; Sabel, B.A. Nanoparticles, a drug carrier system to pass the blood-brain barrier, permit central analgesic effects of i.v. dalargin injections. *Brain Res.* **1996**, *710*, 121–124. [\[CrossRef\]](#)
125. Kreuter, J.; Petrov, V.E.; Kharkevich, D.A.; Alyautdin, R.N. Influence of the type of surfactant on the analgesic effects induced by the peptide dalargin after its delivery across the blood–brain barrier using surfactant-coated nanoparticles. *J. Control. Release* **1997**, *49*, 81–87. [\[CrossRef\]](#)
126. Wilson, B.; Samanta, M.K.; Santhi, K.; Kumar, K.P.S.; Paramakrishnan, N.; Suresh, B. Poly(n-butyl cyanoacrylate) nanoparticles coated with Polysorbate 80 for the targeted delivery of rivastigmine into the brain to treat Alzheimer’s disease. *Brain Res.* **2008**, *1200*, 159–168. [\[CrossRef\]](#)
127. Chintamaneni, P.K.; Krishnamurthy, P.T.; Pindiprolu, S.K.S.S. Polysorbate-80 surface modified nano-stearylamine BQCA conjugate for the management of Alzheimer’s disease. *RSC Adv.* **2021**, *11*, 5325–5334. [\[CrossRef\]](#)
128. Kreuter, J. Influence of the surface properties on nanoparticle-mediated transport of drugs to the brain. *J. Nanosci. Nanotechnol.* **2004**, *4*, 484–488. [\[CrossRef\]](#)
129. Wilson, B. Brain targeting PBCA nanoparticles and the blood-brain barrier. *Nanomedicine* **2009**, *4*, 499–502. [\[CrossRef\]](#)
130. Kreuter, J. Application of nanoparticles for the delivery of drugs to the brain. *Int. Congr. Ser.* **2005**, *1277*, 85–94. [\[CrossRef\]](#)
131. Zhang, W.; Mehta, A.; Tong, Z.; Esser, L.; Voelcker, N.H. Development of polymeric nanoparticles for blood–brain barrier transfer—strategies and challenges. *Adv. Sci.* **2021**, *8*, 2003937. [\[CrossRef\]](#) [\[PubMed\]](#)
132. Sun, W.; Xie, C.; Wang, H.; Hu, Y. Specific role of polysorbate 80 coating on the targeting of nanoparticles to the brain. *Biomaterials* **2004**, *25*, 3065–3071. [\[CrossRef\]](#) [\[PubMed\]](#)
133. Sun, D.; Xue, A.; Zhang, B.; Lou, H.; Shi, H.; Zhang, X. Polysorbate 80-coated PLGA nanoparticles improve the permeability of acetylpuerarin and enhance its brain-protective effects in rats. *J. Pharm. Pharmacol.* **2015**, *67*, 1650–1662. [\[CrossRef\]](#)
134. Wang, C.X.; Huang, L.S.; Hou, L.B.; Jiang, L.; Yan, Z.T.; Wang, Y.L.; Chen, Z.L. Antitumor effects of polysorbate-80 coated gemcitabine polybutylcyanoacrylate nanoparticles in vitro and its pharmacodynamics in vivo on C6 glioma cells of a brain tumor model. *Brain Res.* **2009**, *1261*, 91–99. [\[CrossRef\]](#) [\[PubMed\]](#)
135. Gelperina, S.E.; Khalansky, A.S.; Skidan, I.N.; Smirnova, Z.S.; Bobruskin, A.I.; Severin, S.E.; Turowski, B.; Zanella, F.E.; Kreuter, J. Toxicological studies of doxorubicin bound to polysorbate 80-coated poly(butyl cyanoacrylate) nanoparticles in healthy rats and rats with intracranial glioblastoma. *Toxicol. Lett.* **2002**, *126*, 131–141. [\[CrossRef\]](#)
136. Ren, T.; Xu, N.; Cao, C.; Yuan, W.; Yu, X.; Chen, J.; Ren, J. Preparation and therapeutic efficacy of polysorbate-80-coated amphotericin B/PLA-b-PEG nanoparticles. *J. Biomater. Sci. Polym. Ed.* **2009**, *20*, 1369–1380. [\[CrossRef\]](#)
137. Huang, Y.; Zhang, B.; Xie, S.; Yang, B.; Xu, Q.; Tan, J. Superparamagnetic iron oxide nanoparticles modified with tween 80 pass through the intact blood-brain barrier in rats under magnetic field. *ACS Appl. Mater. Interfaces* **2016**, *8*, 11336–11341. [\[CrossRef\]](#)
138. Tian, C.; Asghar, S.; Xu, Y.; Chen, Z.; Zhang, J.; Ping, Q.; Xiao, Y. Tween 80-modified hyaluronic acid-ss-curcumin micelles for targeting glioma: Synthesis, characterization and their in vitro evaluation. *Int. J. Biol. Macromol.* **2018**, *120*, 2579–2588. [\[CrossRef\]](#) [\[PubMed\]](#)

139. Koffie, R.M.; Farrar, C.T.; Saidi, L.J.; William, C.M.; Hyman, B.T.; Spires-Jones, T.L. Nanoparticles enhance brain delivery of blood-brain barrier-impermeable probes for in vivo optical and magnetic resonance imaging. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 18837–18842. [[CrossRef](#)]
140. Parmar, A.; Jain, A.; Uppal, S.; Mehta, S.K.; Kaur, K.; Singh, B.; Sandhir, R.; Sharma, S. Anti-proliferate and apoptosis triggering potential of methotrexate-transferrin conjugate encapsulated PLGA nanoparticles with enhanced cellular uptake by high-affinity folate receptors. *Artif. Cells Nanomed. Biotechnol.* **2018**, *46*, 704–719. [[CrossRef](#)] [[PubMed](#)]
141. Yusuf, M.; Khan, M.; Alrobaian, M.M.; Alghamdi, S.A.; Warsi, M.H.; Sultana, S.; Khan, R.A. Brain targeted Polysorbate-80 coated PLGA thymoquinone nanoparticles for the treatment of Alzheimer's disease, with biomechanistic insights. *J. Drug. Deliv. Sci. Technol.* **2021**, *61*, 102214. [[CrossRef](#)]
142. Li, W.; Qiu, J.; Li, X.L.; Aday, S.; Zhang, J.; Conley, G.; Xu, J.; Joseph, J.; Lan, H.; Langer, R.; et al. BBB pathophysiology-independent delivery of siRNA in traumatic brain injury. *Sci. Adv.* **2021**, *7*, eabd6889.
143. Hwang, T.L.; Fang, C.L.; Chen, C.H.; Fang, J.Y. Permeation enhancer-containing water-in-oil nanoemulsions as carriers for intravesical cisplatin delivery. *Pharm. Res.* **2009**, *26*, 2314–2323. [[CrossRef](#)] [[PubMed](#)]
144. Pectasides, D.; Pectasides, M.; Economopoulos, T. Systemic chemotherapy in locally advanced and/or metastatic bladder cancer. *Cancer Treat. Rev.* **2006**, *32*, 456–470. [[CrossRef](#)]
145. Wahgiman, N.A.; Salim, N.; Abdul Rahman, M.B.; Ashari, S.E. Optimization of nanoemulsion containing gemcitabine and evaluation of its cytotoxicity towards human fetal lung fibroblast (MRC5) and human lung carcinoma (A549) cells. *Int. J. Nanomed.* **2019**, *14*, 7323–7338. [[CrossRef](#)]
146. Clarke, S.J.; Rivory, L.P. Clinical pharmacokinetics of docetaxel. *Clin. Pharmacokinet.* **1999**, *36*, 99–114. [[CrossRef](#)]
147. Song, H.; Geng, H.; Ruan, J.; Wang, K.; Bao, C.; Wang, J.; Peng, X.; Zhang, X.; Cui, D. Development of polysorbate 80/Phospholipid mixed micellar formation for docetaxel and assessment of its in vivo distribution in animal models. *Nanoscale Res. Lett.* **2011**, *6*, 354. [[CrossRef](#)] [[PubMed](#)]
148. Lee, E.H.; Kim, J.K.; Lim, J.S.; Lim, S.J. Enhancement of indocyanine green stability and cellular uptake by incorporating cationic lipid into indocyanine green-loaded nanoemulsions. *Colloids Surf. B* **2015**, *136*, 305–313. [[CrossRef](#)]
149. Boni, L.; David, G.; Mangano, A.; Dionigi, G.; Rausei, S.; Spampatti, S.; Cassinotti, E.; Fingerhut, A. Clinical applications of indocyanine green (ICG) enhanced fluorescence in laparoscopic surgery. *Surg. Endosc.* **2015**, *29*, 2046–2055. [[CrossRef](#)] [[PubMed](#)]
150. Ravichandran, V.; Nguyen Cao, T.G.; Choi, D.G.; Kang, H.C.; Shim, M.S. Non-ionic polysorbate-based nanoparticles for efficient combination chemo/photothermal/photodynamic therapy. *J. Ind. Eng. Chem.* **2020**, *88*, 260–267. [[CrossRef](#)]
151. Martos, A.; Koch, W.; Jiskoot, W.; Wuchner, K.; Winter, G.; Friess, W.; Hawe, A. Trends on analytical characterization of polysorbates and their degradation products in biopharmaceutical formulations. *J. Pharm. Sci.* **2017**, *106*, 1722–1735. [[CrossRef](#)] [[PubMed](#)]
152. Singh, S.R.; Zhang, J.; O'Dell, C.; Hsieh, M.C.; Goldstein, J.; Liu, J.; Srivastava, A. Effect of polysorbate 80 quality on photostability of a monoclonal antibody. *AAPS Pharm. Sci. Tech.* **2012**, *13*, 422–430. [[CrossRef](#)] [[PubMed](#)]
153. Hampl, V.; Guo, X.; Ehrenstrasser, C.; Viertler, M.; Rayner, L.; Campanelli, G.; Schipflinger, R.; Thewes, K.; Cerreti, A.; Boehm, S.; et al. A newly identified impurity in polysorbate 80, the long-chain ketone 12-tricosanone, forms visible particles in a biopharmaceutical drug product. *J. Pharm. Sci.* **2018**, *107*, 1552–1561. [[CrossRef](#)] [[PubMed](#)]
154. Yang, K.; Hewarathna, A.; Geerlof-Vidavsky, I.; Rao, V.A.; Gryniewicz-Ruzicka, C.; Keire, D. Screening of polysorbate-80 composition by high resolution mass spectrometry with rapid H/D exchange. *Anal. Chem.* **2019**, *91*, 14649–14656. [[CrossRef](#)]
155. Tomlinson, A.; Zarraga, I.E.; Demeule, B. Characterization of polysorbate ester fractions and implications in protein drug product stability. *Mol. Pharm.* **2020**, *17*, 2345–2353. [[CrossRef](#)] [[PubMed](#)]