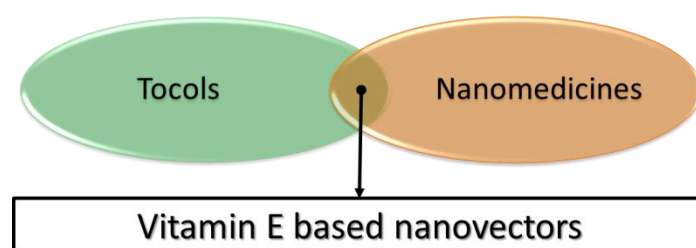


CHAPTER I.
Foreword and Aim of
the Thesis

The aim of this work was to develop novel vitamin E conjugates for the vectorization of active pharmaceutical ingredients through nanotechnologies. The physico-chemical and biological properties of vitamin E derivatives offer multiple advantages in drug delivery like biocompatibility, improvement of drug solubility and anticancer activity. Nanomedicines have shown high potential in drug delivery since (i) they are appropriate to all route of administration, (ii) they may offer better drug biopharmaceutical properties such as longer half-life or better bioavailability and (iii) they have shown benefits in cancer therapy by improving anticancer drug therapeutic index. Vitamin E based nanovectors were developed to combine the pharmaceutical properties of both vitamin E and nanomedicine for two purposes: (i) to improve water solubility of hydrophobic drugs by using vitamin E-based polymeric micelles and (ii) to enhance the therapeutic efficiency of anticancer agents by using vitamin E-based prodrug nanoparticles.



The first part of the thesis aimed at evaluating a new tocol modified chitosan able to self-assemble in micelles and to carry poorly soluble drugs. As most of the new drug candidates are sparingly soluble in water, there is a need to develop efficient and safe solutions to increase their water solubility allowing a better bioavailability. Among these solutions, drug nanocarriers have been investigated due to their ability to be tailored to get specific pharmaceutical and biological properties. Hydrophobically modified chitosan has received increasing attention owing to the biocompatibility, non-toxicity and low-immunogenicity of chitosan. Grafting of vitamin E of chitosan backbone was based on the physico-chemical properties of vitamin E derivatives to improve of drug solubility (tocophily). In this work we have demonstrated that tocopherol succinate glycol chitosan (GC-TOS) conjugates spontaneously formed micelles in aqueous solution at low critical micelle concentration. AFM and TEM analysis showed that spherical micelles were formed. The GC-TOS increased water solubility of 2 model class II drugs. GC-TOS was non-cytotoxic at concentrations up to 10 mg/mL. GC-TOS increased the apparent permeation coefficient of ketoconazole across a Caco-2 cell monolayer.

The second part of the thesis aimed at evaluating whether new vitamin E based anticancer prodrugs could self-organize in nanoparticle to enhance therapeutic index of anticancer drugs. We exploit the numerous advantages of nanotechnologies in cancer therapy, like targeting ability of solid tumor and bypassing resistance, and of the chemosensitizing and anticancer properties of some vitamin E derivatives. To achieve this goal, doxorubicin was chemically conjugated to D- α -tocopherol succinate through an amide bond to form N-doxorubicin- α -D-tocopherol succinate (N-DOX-TOS). The prodrug self-assembled in water into 250 nm nanostructures when stabilized with D- α -tocopherol polyethylene glycol 2000 succinate. Cryo-TEM analysis revealed the formation of nanoparticles with a highly ordered lamellar inner structure. NMR spectra of the N-DOX-TOS nanoparticles indicated that N-DOX-TOS is located in the core of the nanoparticles while PEG chains and part of the tocopherol is in the corona. High drug loading (34% w/w) and low in vitro drug release were achieved. In vitro biological assessment showed significant anticancer activity and cellular uptake of N-DOX-TOS nanoparticles.

CHAPTER II. Introduction

I. NANOMEDICINE AND DRUG DELIVERY*

The European Science Foundation defines nanomedicines as «nanometer size scale complex systems, consisting of at least two components, one of which being the active ingredient». Although mainstream nanotechnology explores particles between 1 and 200 nm in diameter, the size of individual particles tested for drug delivery of therapeutic and imaging agents may range from 2 to 1000 nm (1).

Nanomedicines can increase efficacy, specificity, tolerability and therapeutic index of corresponding drugs (2). They must be stable, non-toxic, non-thrombogenic, non-immunogenic, non-inflammatory, biodegradable, avoid uptake by reticulo-endothelial system (RES) and should be applicable to various molecules such as small drugs, proteins or nucleic acids (3-6).

I.1. NANOMEDICINE STRUCTURE

Most of the nanomedicines used today are (i) nanoparticles, (ii) polymeric micelles, (iii) liposomes, (iv) dendrimers; and (iv) polymer-drug conjugates (Figure 1) (7).

Nanoparticles include nanocapsules and nanospheres. Nanocapsules are vesicular systems in which a drug is confined to a cavity surrounded by a polymer membrane, whereas nanospheres are matrix systems in which the drug is physically and uniformly dispersed. A large panel of biodegradable polymers is available to form nanoparticles. They can be either natural or synthetic. Among natural materials chitosan is the most popular. The main synthetic polymers used for oral drug delivery are: poly(lactide) (PLA), poly(glycolide) (PGA), poly(lactide-co-glycolide) (PLGA), poly(cyano)acrylates (PCA), polyethyleinimine (PEI) or polycaprolactone (PCL) (1). Different methods can be selected to prepare nanoparticles depending on nature of the polymer as well as the nature and purpose of drug to be encapsulated. Most of the methods involve the use of organic solvents, heat or vigorous agitation which may be harmful to biopharmaceuticals. Nanoparticles formation can be also based on electrostatic interactions. These polyelectrolyte complexes do not require

* Partly adapted from a review I wrote with Laurence Plapied et al. (1)

aggressive conditions like organic solvents and sonication or homogenization during preparation, therefore minimizing possible damage to drug during formation (1).

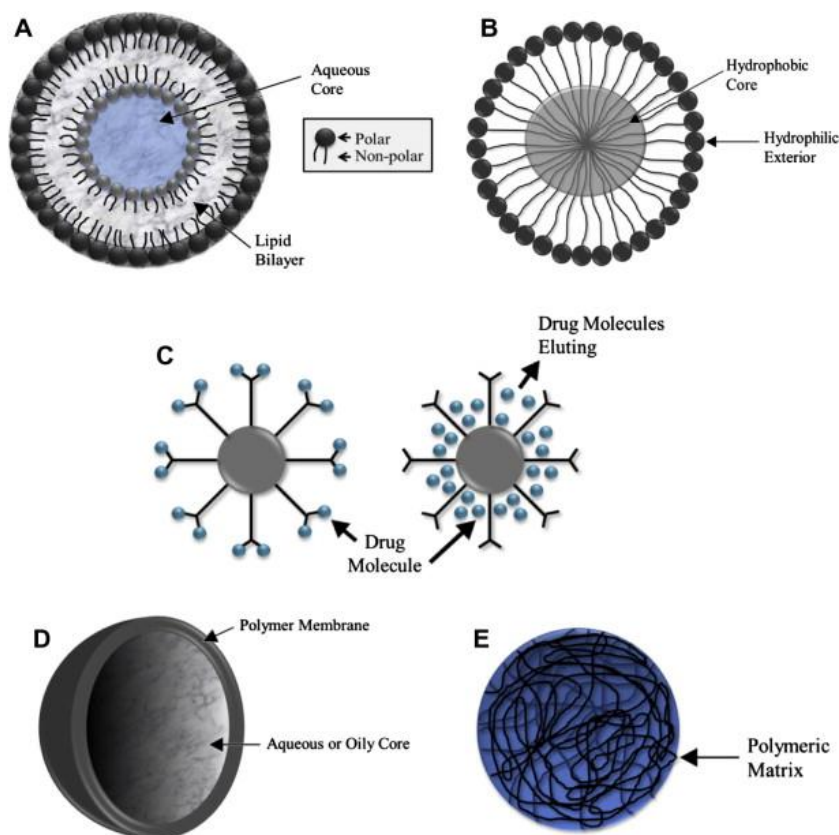


Figure 1. Particle schematics. (A) liposome, (B) micelle, (C) dendrimers functionalized with complexed (left) and encapsulated (right) drug molecules, (D) nanosphere, and (E) nanocapsule (8).

Amphiphilic polymers can self-assemble above the critical micellar concentration (CMC) in colloidal dispersions of molecular aggregates of approximately 20 to 100 nm called polymeric micelles. The hydrophilic moiety, usually polyethylene glycol (PEG), forms the corona of the micelles whereas the hydrophobic moiety forms their core. In contrast to nanoparticles which display a solid static stable structure, polymeric micelles form dynamic structure: amphiphilic copolymers forming the micelles can be exchanged with free unimers. The amphiphilic copolymers provide better kinetic and thermodynamic stability than conventional surfactant. The hydrophobic core of micelles can solubilize poorly soluble drugs and partly protect the drug from the aqueous environment. Therefore, the use of polymeric micelles for drug delivery has been mainly restricted to the delivery of poorly soluble drugs. Depending on the aqueous solubility of the polymers, polymeric micelles can be formed either by simple direct dissolution in water or by dissolving drug and polymer in organic solvents before solvent elimination by dialysis or evaporation (1).

Liposomes are self-assembled artificial vesicles formed by one or several amphiphilic phospholipid bilayers surrounding an aqueous core domain. Their size can vary from 50 nm to several micrometers. Liposomes are attractive delivery systems due to their ability to isolate drugs from the surrounding environment and the ability to entrap both hydrophilic and hydrophobic drugs (7, 9). Liposomes have been extensively studied but only few formulations are available in the market such as Doxil® (9, 10).

Dendrimers are repeatedly branched polymeric macromolecules with numerous arms extending from a center, resulting in a nearly-perfect three-dimensional geometric pattern (10). Polymers grow from a central core by a series of polymerization reactions. Drugs can be encapsulated in the internal cavities of the globular structures formed by the dendrimers and can be released in a controlled way. Drug loading can also be achieved through electrostatic interactions or chemical modifications which enable attachment of drugs to dendrimer surface groups (7, 9).

Polymer-drug conjugates, which have high size variation, are polymeric macromolecules made by the grafting of drugs via linker regions on a polymer backbone. They are used to enhance the blood circulation time of the drugs or to increase the otherwise poor solubility of some drugs. These derivatives are considered as nanoparticles since their size can still be controlled within 100 nm (7, 9).

Nanocarriers can be pegylated to decrease hydrophobicity and escape the RES. The stealth characteristics of the pegylated nanoparticles rely on three important features, (i) the molecular weight of the PEG chain, (ii) the surface chain density and (iii) the conformation. PEG chains located at the surface of the nanoparticles provide them longer blood circulation half-life when injected. They also protect the nanoparticles by creating a hydrophilic protective layer around the nanoparticles and thus steric repulsion forces to repel the absorption of opsonin protein. Nanoparticles can also be liganded to get active targeting properties (1, 7, 9, 11).

I.2. NANOMEDICINES FOR ORAL DRUG DELIVERY

Oral administration is the preferred route for drug delivery. It is patient-friendly, painless and easy for self-medication. Compared to parenteral delivery, it suppresses risk of disease transmission, reduces cost and increases patient compliance. It allows flexible and controlled dosing schedule. It is particularly convenient for chronic therapy (12-14).

Oral bioavailability of drugs is strongly influenced by their properties. Solubility and permeability are two important parameters for their absorption via passive diffusion. The Biopharmaceutic Classification System (BCS) defines four categories of drugs based on their solubility and their permeability (Figure 2). Currently, the solubility of new chemical entities tends to decrease. Hence, the development of formulations able to provide a good oral bioavailability of such drug candidates is one of the new challenge in the pharmaceutical sciences (15). A drug that is administered orally must survive transit through the chemical and enzymatic gastrointestinal (GI) liquids, cross the mucosal layer and the epithelium before being absorbed. If most small molecules are resistant to the harsh environment of the GI tract and can be absorbed, the intestinal barrier limits the oral absorption of macromolecules such as proteins, vaccines or nucleic acids. Hence, protective vehicles to avoid destruction in the GI tract and potentially enhance oral absorption are needed (1).

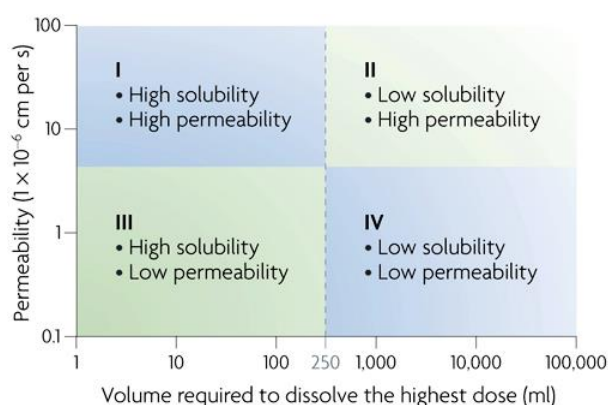


Figure 2. BCS characterization of drugs based on solubility and permeability measures (16).

Nanotechnology brings some advantages to the drug delivery field in general and oral drug delivery in particular. It allows (i) the delivery of poorly water-soluble drugs, (ii) the targeting of drugs to specific part of the gastrointestinal tract, (iii) the transcytosis of drugs across the tight intestinal barrier and (iv) the intracellular and transcellular delivery of large macromolecules (2).

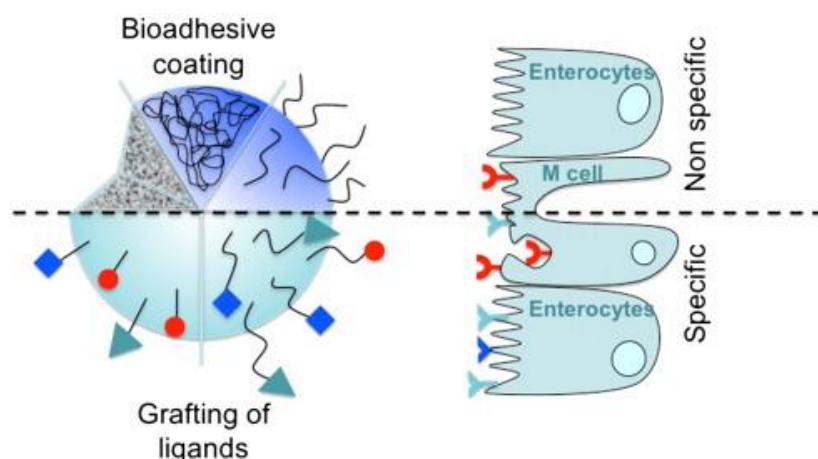


Figure 3. Nanoparticle surface modifications to enhance their uptake by enterocytes and M cells. Non-specific strategies: mucoadhesive polymers coating or forming nanoparticles; PEG chains forming a hydrophilic protective layer stabilizing the colloidal formulation (1).

Moreover, the unique characteristics of the GI tract can be exploited for optimizing formulations aiming at enhancing drug absorption, e.g. changes in pH and microflora for targeting the colon, mucoadhesion for increasing residence time of a drug. In order to increase nanoparticles interactions with the intestinal mucosa, surface can be modified by adsorption or grafting of hydrophilic molecules that confer hydrophilicity (e.g. PEG) or bioadhesivity (e.g. chitosan). Furthermore, delivery of medicines to target specific cells, diseases or areas of the intestine can be achieved by grafting of ligands such as antibodies, glycoproteins or peptides to the surface of nanoparticles (17). Microorganisms derived adhesive factors (flagellin, invasins...), vitamins (18, 19), carbohydrates (20) are also used as targeting ligands. Optimization of ligand density on the nanoparticles surface must allow tissue penetration and cellular uptake resulting in optimal therapeutic efficacy (Figure 3) (2).

I.3. NANOMEDICINES FOR CANCER THERAPY

The administration of anticancer drugs is one of the most powerful options in cancer treatment. However, chemotherapy triggers the death of fast-dividing cells in both healthy and tumor tissue. It can be harmful for healthy cells of liver, GI tract and bone marrow. Thus the balance between the effectiveness of the drug and a patient's ability to tolerate the side effects has to be optimized. Nanoparticles can address some of the limitations of traditional chemotherapy due to their ability to selectively target tumor tissue, overcome biological barriers, and respond to the tumor environment to deliver the anticancer drugs (7, 21, 22).

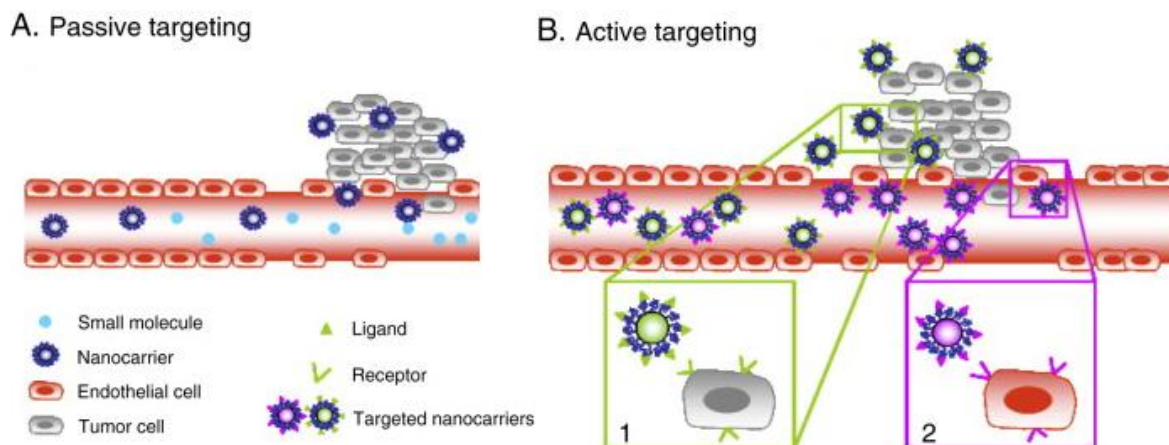


Figure 4. (A) Passive targeting of nanocarriers and (B) Active targeting strategies. Ligands grafted at the surface of nanocarriers bind to receptors (over)expressed by (1) cancer cells or (2) angiogenic endothelial cells (7).

The first rationale to use nanomedicine for cancer therapy is the preferential delivery of nano vectorized drugs to solid tumors due to the enhanced permeability and retention (EPR) effect (Figure 4-A) (23). Tumor vessels surrounding the tumors are leaky due to abnormal basement membranes and incomplete endothelial linings allowing nanomedicine to reach tumor passively through the leaky vasculature. Thus EPR effect enables higher local concentration at the tumor site of the drug when carried with a nanovector (7, 8, 23). To exploit EPR effect nanocarriers must avoid immune surveillance and circulate for a long period. Three parameters are required to achieve this goal: the size and surface characteristics of the nanoparticle and the immune blindness. To efficiently extravasate from the fenestrations in leaky vasculature, particle diameter should be less than 200 nm. Nanocarriers should be larger than 10 nm to avoid clearance by first pass renal filtration. The particle charge should be neutral or anionic to evade efficiently the renal elimination. The nanocarriers have to be unseen from the RES, which destroys any foreign material through opsonization followed by phagocytosis, through pegylation strategies (7, 8). Efficacy of passive targeting depends mainly on (i) the degree of tumor vascularization and angiogenesis which rely on tumor types and anatomical sites and (ii) the high interstitial fluid pressure and heterogenous blood flow limiting the uptake and homogenous distribution of the drug in the tumor (7, 8).

Targeting can be improved by grafting ligands at the surface of the nanomedicines, allowing active targeting by binding to the receptors overexpressed by cancer cells or angiogenic endothelial cells (Figure 4-B) (7, 8, 21).

Nanomedicines have potential to deliver more than one therapeutic agent for combination therapy. Theragnostic nanomedicines which contain imaging and therapeutic agents have also been developed to enable diagnosis and therapy together with monitoring of therapeutic response.

They can also enhance drug-circulation times, controlled drug-release kinetics and allow superior dose scheduling (24). Nanomedicines avoid formulation with synthetic solvents which contribute to side effects for many conventional chemotherapeutics like taxanes. Nanoparticles have also the potential to overcome the development of tumor resistance to conventional chemotherapeutics (9, 23).

II. VITAMIN E

The potential health benefits and therapeutic use of vitamin E derivatives has been extensively studied (25, 26). Physico-chemical and biological properties of vitamin E derivatives have also led to the development of a wide range of drug delivery systems (27). In this chapter I review the chemistry and the biology of vitamin E and focus on the drug delivery application of its derivatives.

II.1. VITAMIN E FAMILY

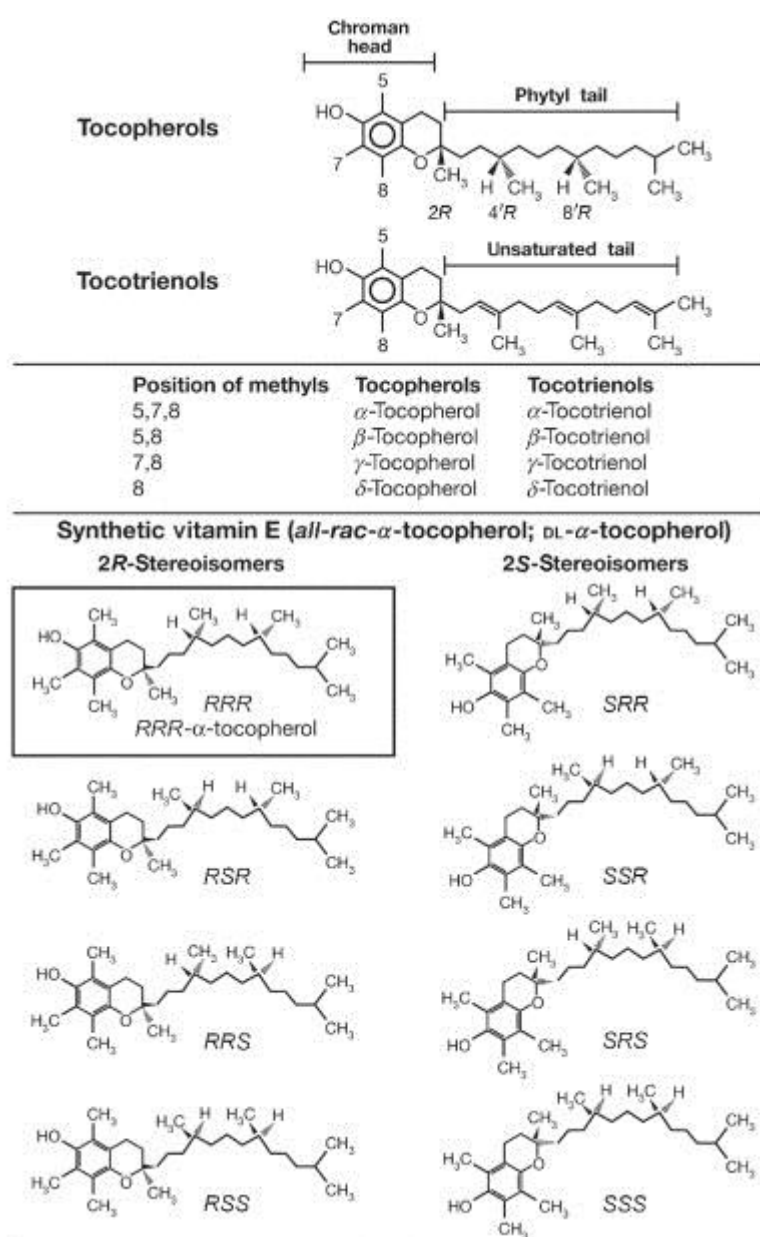


Figure 5. Chemical structures of plant derived, naturally occurring vitamin E compounds (28).

The vitamin E derivatives, also named tocots, belong to the family of tocopherols and tocotrienols. The basic structure of tocots is a 6-hydroxy-2-methyl-phytylchroman. Eight naturally occurring tocots are known as vitamin E, four tocopherols and four tocotrienols existing in alpha (α), beta (β), gamma (γ), and delta (δ) isomers. The tocotrienols have three double bonds in the phytyl tail while tocopherols have a saturated tail. The difference between the vitamers (compounds chemically and metabolically related to vitamin E and with the same biological activity (29)) is in their chromanol ring. The head can have methyl groups attached at up three different locations. The α -isomer has all three sites filled, β -isomer and γ -isomer have two methyl groups attached at different locations, whereas δ -isomer has only one methyl group attached (30, 31). Tocopherols have three chiral centres. RRR- α -tocopherol is the naturally occurring form of α -tocopherol, containing chiral carbons in the R-conformation at positions 2, 4', and 8'. Chemical synthesis of α -tocopherol results in an equal mixture of eight different stereoisomers (RRR, RSR, RRS, RSS, SRR, SSR, SRS, SSS) with half having position 2 in the S-conformation. The synthetic vitamin E is called all-*rac*- α -tocopherol (Figure 5) (25).

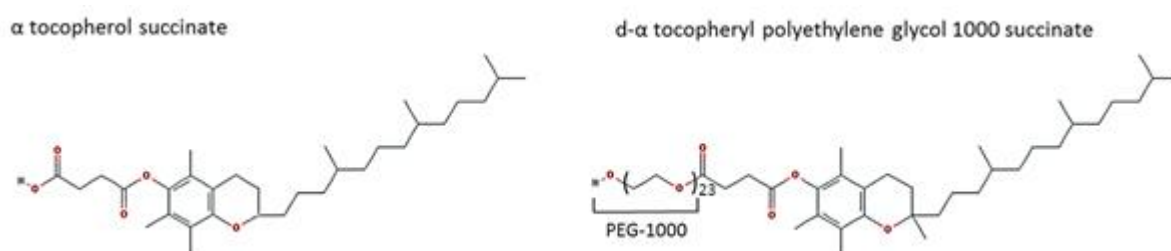


Figure 6. Main synthetic derivatives of vitamin E : TPGS and TOS

Tocol esters have been found to be more stable against oxidation than non-ester form of vitamin E. Vitamin E esters are commercially available, including vitamin E acetate, vitamin E succinate (TOS), and vitamin E polyethylene glycol-1000 succinate (TPGS₁₀₀₀) (Figure 6). *In vivo* ester hydrolysis speed differs depending on the derivative, i.e. hydrolysis is fast for vitamin E acetate but slower for TOS and TPGS₁₀₀₀ (31).

II.2. BIOLOGY OF VITAMIN E

This part on absorption, transport and metabolism is partly adapted from the work of Combs et al and is summarized in Figure 7 (32).

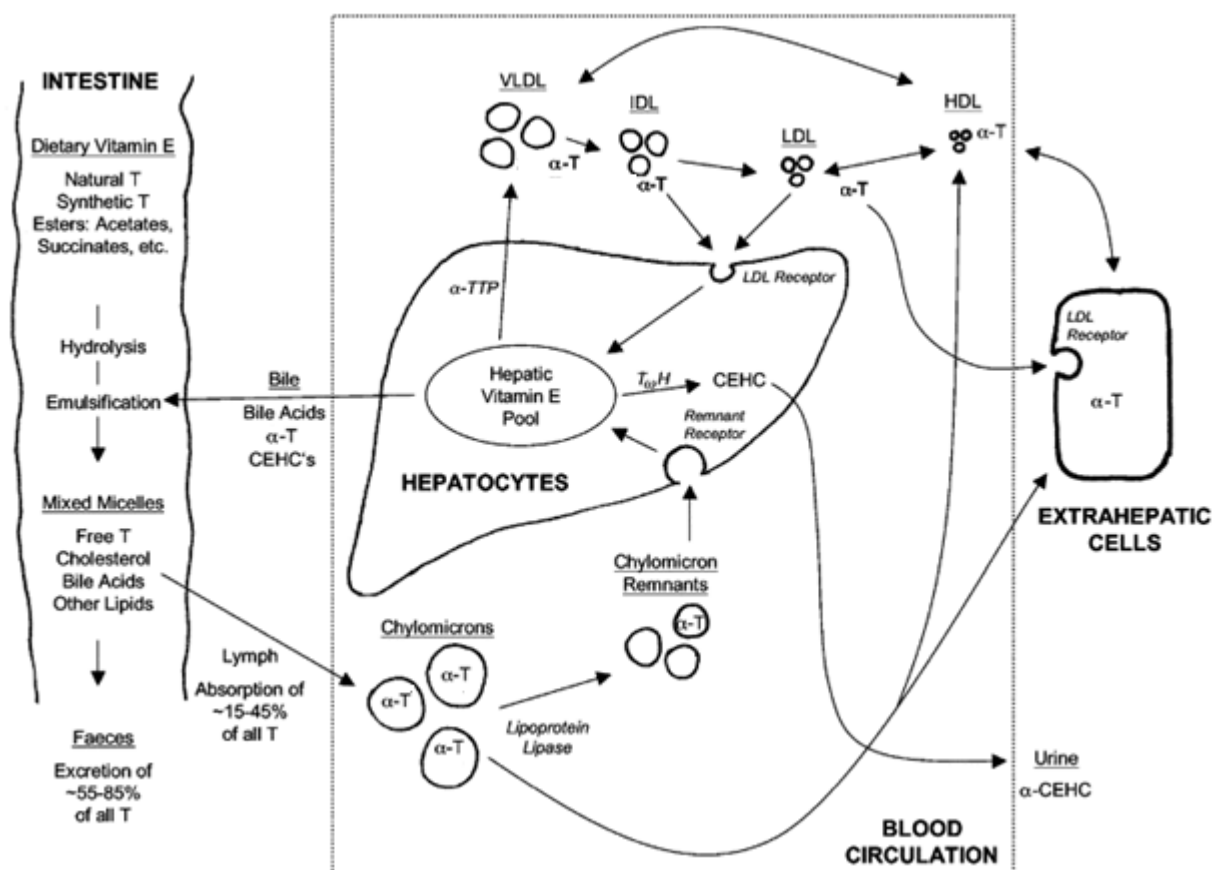


Figure 7. Absorption, transport, and metabolism of α -tocopherol (α -T) in the body. Abbreviations used: CEHC, carboxyethyl hydroxychroman metabolites; α -TTP, α -tocopherol transfer protein; $T_{\alpha}H$, tocopherol- ω -hydroxylase; VLDL, IDL, LDL, and HDL; very low-, intermediate-, low-, and high-density lipoproteins, respectively. Adapted from J. Frank (33).

II.2.1. Absorption, transport and distribution to tissues

Vitamin E is absorbed in the small intestine by passive diffusion dependent on micellar solubilization with no discrimination between vitamers. Pancreatic esterases and bile acids are required for the micellization of vitamin E as for dietary fat. Then, micelles are taken up by intestinal enterocytes. Vitamin E enters the lymphatic circulation with the triglyceride-rich chylomicra which are secreted into lymphatic system to reach into plasma (Figure 7). At normal level of intake, about 20-30% of dietary vitamin E is absorbed (25, 31, 32).

Circulating chylomicrons undergo triglyceride lipolysis by lipoprotein lipase (LPL) to form chylomicron remnants. During this process, some tocopherols are transferred to other lipoproteins, like high density lipoproteins (HDL), and/or taken up by peripheral tissues. Vitamin E is willingly transferred between HDL and other lipoproteins thanks to the phospholipid transfer protein (PLTP) which is also critical for vitamin E intratissular distribution (25). Next, the chylomicron remnants are taken up by the liver and repackaged with dietary fats into nascent very low density lipoproteins (VLDL) for secretion into the

plasma (**Figure 7**). *In vivo* studies in mice demonstrated that VLDL are not critical for peripheral tissue distribution of α -tocopherol, suggesting HDL may be sufficient (25).

The tocopherol contents of tissues tend to be associated to vitamin E intake with no deposition or saturation thresholds. There is a high variability of tocopherol contents of tissues, with no relation between the amount or types of lipids present. A labile, rapidly turning over pool and a fixed, slowly turning over pool of vitamin E are described in tissues. The labile pools stay in plasma and liver as their tocopherol content are depleted rapidly under vitamin E deprivation condition. 90% of vitamin E resides predominantly in the bulk lipid phase of adipose tissue constituting the fixed pool (32).

The average plasma concentration of α -tocopherol is about 23 μM . A plasma level below 12 μM is regarded as deficient. In human plasma of unsupplemented individuals, average α -tocopherol concentrations are about 10 and 100 times higher than γ -tocopherol and δ -tocopherol concentrations, respectively. In tissues the highest contents of α -tocopherol are found in adipose tissue and the adrenal glands. Differences in the relative amounts of the different tocopherols suggest tissue specific mechanism for transport, enrichment and/or storage of tissue tocopherol (34).

II.2.1. Cellular uptake

Cellular uptake of vitamin E occurs by established mechanisms of lipid transfer between lipoproteins and cells. While all vitamins E are taken up by the liver, only RRR- α -tocopherol is released into the circulation. The preferential secretion of RRR- α -tocopherol from the liver into the plasma for distribution to tissues is under control of the α -tocopherol transfer protein (α -TTP) (**Figure 7**) (25, 32). The α -TTP consists of an N-terminal helical domain and a C-terminal domain, which contains a fold that forms a binding pocket for vitamin E. In that pocket binds the ligand and four water molecules, two of which are hydrogen-bonded to the hydroxyl group on the chroman ring (32). Recognition and optimal binding within the ligand pocket requires: (i) a fully methylated chroman ring explaining the affinities of the four isomeric forms: $\alpha > \beta > \gamma > \delta$ -tocopherol; (ii) the ability of the vitamin E tail to fold back in a U-turn, only expected of the tocopherol phytyl tail and not the unsaturated tail; and (3) the stereochemical position of the methyl group in the 2-R position. This third requirement

makes α -TTP selective for 2-R isomers of synthetic α -tocopherol(25, 32). Still, the affinity for the synthetic SRR form is 11% compared with that for RRR- α -tocopherol (35).

The liganded α -TTP takes up the vitamin E from endocytic vesicles and moves it through the cytoplasm to transport vesicles which travel to the plasma membrane. Vitamin E is ultimately secreted complexed to lipoprotein particles in the circulation (32).

II.2.2. Intracellular location

In non-adipose cells, vitamin E is localized almost exclusively in membranes. The highest concentrations are found in the Golgi membranes and lysosomes (32). It has been proposed that vitamin E forms complexes with fatty acids, more likely polyunsaturated fatty acids (PUFA)-rich domains, in phospholipid bilayers. This configuration leads to the vitamin E clustering in membrane locations of greatest need. In this arrangement, vitamin E is oriented at the interface between the aqueous phase and hydrophobic domain with its chromanol group close to the aqueous interface, enable α -tocopherol to trap efficiently radicals created within PUFA chains (32, 36).

Location of vitamin E in membrane is pertinent to understand its biological activity of considering the possibility of mechanism of action independent of antioxidant activity. The effect of α -tocopherol on membrane proteins and enzymes, signaling cascades, and gene expression may be due to its contribution to membrane structure and dynamics, and thus modulation of membrane-dependent signaling mechanism (36).

II.2.3. Metabolism

All forms of vitamin E are degraded along the same pathway involving head group and side chain oxidation but with different metabolic rates (Figure 8). The selective accumulation in tissues and the enrichment of plasma of RRR- α -tocopherol are mediated (i) via α -TTP binding in the liver and (ii) via regulation of hepatic vitamin E metabolism and excretion (25, 32, 35).

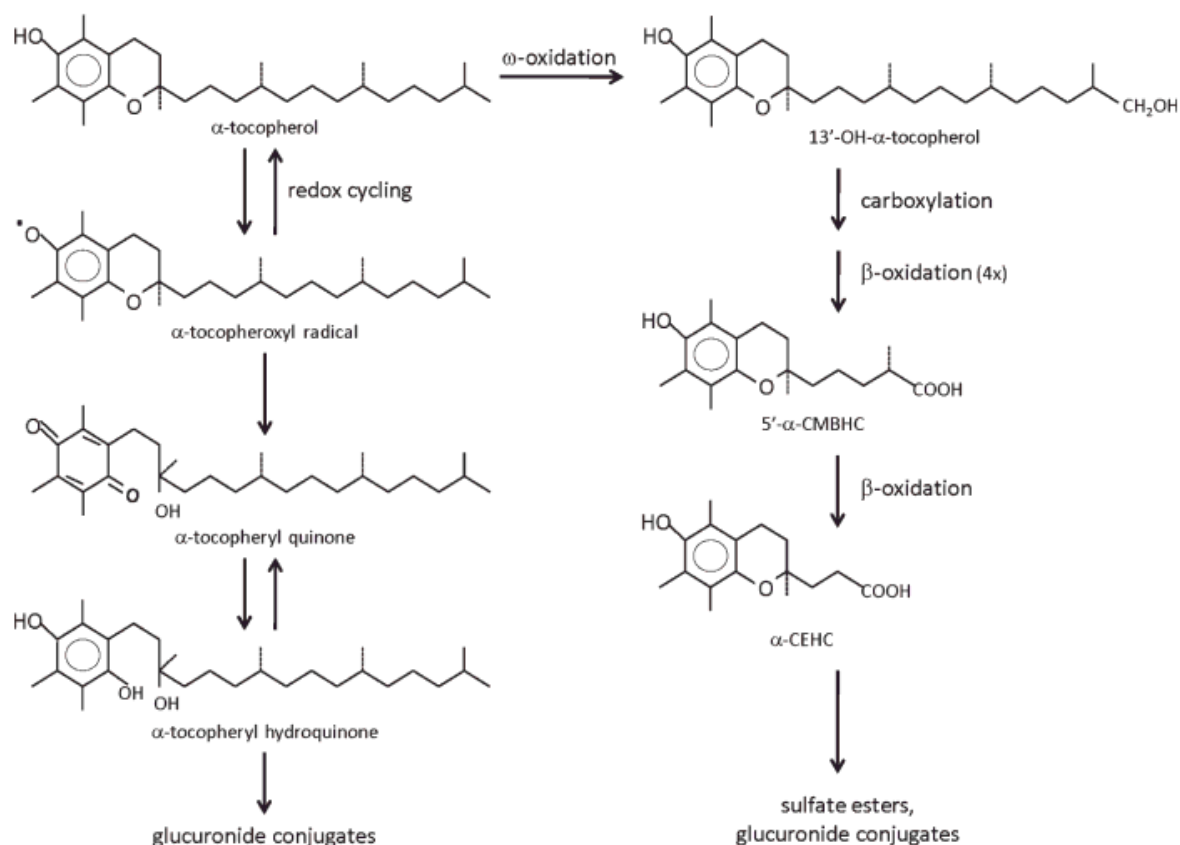


Figure 8. Metabolic pathway of vitamin E (32)

II.2.3.1. Metabolism of the phytyl side chain

Vitamin E is metabolized like xenobiotics through (i) the introduction of a functional group via the phase I enzymes and (ii) the conjugation of the degradation product via phase 2 enzymes (35).

All forms of vitamin E are catabolized by a cytochrome P-450 (CYP)-mediated process, of which CYP3A4 and CYP4F2 are the most likely candidates (35). Initially ω-hydroxylation of terminal methyl group of the phytyl side chain produces 13'-OH-tocopherol metabolites. This is followed by β-oxidation which includes dehydrogenation to the 13'-chromanol and subsequent truncation of the phytyl side chain through the removal of two- and three-carbon fragments. The second and fourth cycles of β-oxidation require 2,4-dienoyl-CoA reductase and 3, 2-enoyl CoA isomerase respectively. The resulting metabolites are 5'-carboxymethylbutylhydroxychroman (CMBHC) and ultimately carboxyethylhydroxychroman (CEHC)(32, 35, 37).

α-, β-, γ-, δ-CEHC are the biologically relevant metabolites. Non-α-tocopherols are much more extensively catabolized than α-tocopherol, resulting in much faster turnover of those

vitamers. No vitamin E form accumulates to toxic levels, suggesting that mechanisms prevent excess accumulation. RRR- α -tocopherol may regulate the metabolism and, possibly excretion, of other forms of vitamin E (25, 32). Some forms of vitamin E may also induce their own metabolism and affect metabolism of other CYP substrates (35).

CEHCs are most of the time conjugated prior their excretion in bile or urine. Conjugation is catalyzed by two enzymes, either sulfotransferases (SULTs) or uridine diphosphate glucosyl transferases (UGTs) which produce sulfate ester and glucuronide conjugation products, respectively. The ratio of the conjugation products differ between species and for the forms of vitamin E (25, 35).

α -tocopherol itself is excreted into bile. Two proteins are involved in this process, Multidrug Resistance 1 (MDR1) and 3 (MDR3) proteins. These ATP-binding cassette (ABC) transporters are known to transport lipophilic compounds to the bile. MDR1 may play a role particularly under conditions of high-dose supplementation. High α -tocopherol doses have been shown to alter the expression of MDR1 which may alter the bioavailability and efficacy of drugs that utilize this transporter (25).

II.2.3.2. Metabolism of the chromanol group and redox cycling

The chromanol hydroxyl group can undergo one- and two- electron oxidations, thus α -tocopherol can be converted to α -tocopheryl quinone and (5,6- or 2,3-)-epoxy- α -tocopherylquinones, respectively. This enables vitamin E to scavenge free radicals such as peroxynitrate and lipid peroxy radicals (32).

Oxidation of the chromanol ring is the basis of the *in vivo* antioxidant function of vitamin E. it involves oxidation primarily to tocopherylquinone, which proceeds through the semistable tocopheryl radical intermediate. A significant amount of vitamin E may be recycled *in vivo* by reduction of tocopheryl radical back to tocopherol (Figure 9). This hypothesis is supported by (i) the low turnover of α -tocopherol, (ii) the slow rate of its depletion in vitamin E-deprived animals, and (iii) the low ratio of vitamin E to PUFA (about 1:850) in most biological membranes (32).

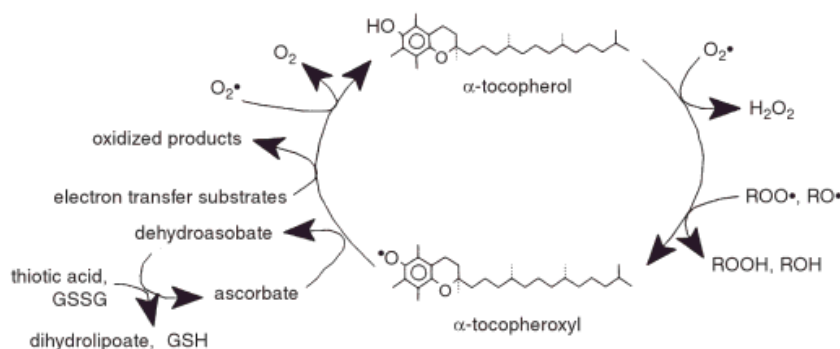


Figure 9. Redox cycling of vitamin E (32)

According to this model, tocopherols and tocotrienols would be retained through recycling until the reduction systems in both aqueous and membrane domains become rate limiting. Whereas the monovalent oxidation of tocopherol to tocopheryl radical is a reversible reaction (at least *in vitro*), further oxidation is unidirectional. Tocopherylquinone lacks vitamin E activity and represents loss of the vitamin from the system. It can be reduced to α -tocopherylhydroquinone, which can be conjugated with glucuronic acid and secreted in the bile. Under conditions of vitamin E intake nutritional levels, less than 1% of the absorbed vitamin is excreted with the urine (32).

II.3. METABOLIC FUNCTIONS OF VITAMIN E

Vitamin E has numerous functions which include antioxidant, anti-inflammatory, antithrombolytic, and other therapeutic effects (31, 33, 38). Vitamin E protects cell membranes, especially in the lung and red blood cells, against damage caused by various pollutants, peroxides, and free radicals formed during metabolic process. It works synergistically with other antioxidant nutrients such as vitamin C, beta-carotene to quench free radicals, or peroxides, and is vital for nerve and muscle cell function. Vitamin E can spare other antioxidants and vice versa. In regard to its anti-inflammatory effects, vitamin E inhibits the enzyme lipoxygenase, responsible of the production of leukotrienes that cause inflammation (31).

II.3.1. Vitamin E as a biological antioxydant

Vitamin E is a major lipid-soluble antioxidant which protects lipids and membranes from oxidative damage *in vitro* and *in vivo* (35). Because of the reactivity of the phenolic

hydrogen on its C-6 hydroxyl group and the ability of the chromanol ring system to stabilize an unpaired electron, vitamin E has antioxidant activity able to end chain reactions among PUFAs in the membranes. This action is named free radical scavenging and involves the donation of the phenolic hydrogen to a fatty acyl free radical (or $O_2^{\cdot-}$) to prevent attack on others PUFAs. Tocopherols have greatest efficacy towards peroxy and phenoxy radicals, but can also quench mutagenic electrophiles as reactive nitrogen oxide species (NO_x) (32).

When tested with organic peroxy radicals, the order of the antioxidant potential of vitamers is $\alpha > \beta \geq \gamma > \delta$ -tocopherol. However NO_x is trapped more effectively by γ -tocopherol than by the α -vitamer and may be important *in vivo* as a trap for electrophilic nitrogen oxides and other electrophilic mutagens (26, 37). This does not correlate with its activity in the conventional bioassays and demonstrates the *in vitro* antioxidant activity does not allow conclusions in respect to the *in vivo* effect (32, 35).

Proof of any relevant antioxidant function *in vivo* is scarce. Most of the clinical trials undertaken to demonstrate a beneficial effect on vitamin E on diseases associated with oxidative stress fails. Nevertheless, almost all vitamin E effects have tentatively been attributed to free radical scavenging (39). Vitamin E might exert its functions in certain domains in membranes, thereby influencing signaling cascades with subsequent effects on the induction/suppression of genes (40).

II.3.2. Non-antioxidant functions of Vitamin E

When Vitamin E was discovered to inhibit cell proliferation and protein kinase C (PKC) activity, it was suggested that this vitamin may act *in vivo* through ways unrelated to its function as biological antioxidant (32, 38, 39) (Table 1).

α -Tocopherol has been shown to play a role in enzyme regulation. It participates in complex membrane-based recruitment processes affecting enzymatic functions. One of the most studied enzyme effect is its role in cellular signaling due to its activity towards PKC. The mechanism of PKC inhibition by α -tocopherol may be attributable in part to its attenuation of the generation of membrane-derived diacylglycerol, a lipid that activates PKC activity. It has also been suggested that the inhibition is likely due to the direct interaction between α -tocopherol and PKC in the membrane (32, 41). Vitamin E also appears to participate in gene

regulation *via* unclear underlying mechanisms. It may involve redox modulation due to free radical scavenging, modulation of translocation in membranes and others may be secondary to effects on factors such as PKC and PPAR- γ . The existence of nuclear receptors for tocopherol has also been shown but the metabolic significance of nuclear-receptors binding vitamin E remains unclear (32).

Table 1. Genes Regulated by Vitamin E (32)

Function	Gene products
Tocopherol uptake	α -TTP
Metabolism	CYP3A, CYP4F2, HMG-CoA reductase, γ -glutamyl cysteine synthase, CRABP-II
Lipid uptake	SR-BI, CD36, SR-AI/II
Extracellular proteins	α -tropomyosin, collagen- α 1, MMP-1, MMP-19, glycoprotein IIb
Cell adhesion	E-selectin, L-selectin, ICAM-1, VCAM-1, integrins
Cell growth	Connective tissue growth factor
Extracellular matrix formation/degradation	Collagen α 1(1)
Inflammation	IL-2, IL-4, IL-1- β , TGF- β
Transcriptional control	PPAR γ
Cell cycle regulation	Cyclins D1 and E, Bcl2-L1, p27, CD95
Apoptosis	CD95L, Bcl2-L1
Lipoprotein receptors	CD36, SR-BI, SR-AI/II, LDL receptor
Other functions	Leptin, tropomyosin, a β -secretase

II.3.3. Vitamin E and cancer

Therapeutic potential of tocopherols have been widely studied. Vitamin E may be beneficial for a variety of disorders including cancer, heart disease and even Alzheimer's disorder (26, 28, 42-45). This part will focus on the potential use of the two vitamin E analogs used in this work, namely TOS and TPGS, as anti-cancer drugs and anticancer adjuvants.

II.3.3.1. Anticancer effect of TOS

Experimental evidence indicated that vitamin E succinate (tocopheryl succinate, TOS) is one of the most effective anticancer compounds of the vitamin E family. TOS has been shown to be highly selective for malignant cells whereas it is largely non-toxic to normal cells (46). The selectivity of TOS towards tumor cells may be related to its chemical properties. Bioavailability of TOS inside cell depends on the ratio of two species: charged and uncharged form, the latter entering cell through free diffusion. With a pKa of 5.84, TOS is a weak acid.

In neutral environment, 99% of TOS are charged while at pH 6.2 uncharged form accounts for up to 25% of TOS. Thus, acidic interstitium of tumor allows free diffusion of TOS into tumor cells while normal cells have neutral environment, limiting the TOS diffusion (46). Nevertheless experiments showed that both cancer cells and normal cells accumulated similar levels of TOS after 24h, suggesting a higher sensitivity of the tumor cells to TOS. The selectivity of α -TOS may result low hydrolytic capacity of malignant cells towards α -TOS (43).

At least 50 types of cancer cell lines have shown apoptosis when incubated with TOS, including different origin (human, murine, avian) and tissue type (breast, prostate, lung, stomach, ovary, monocyte, colon, mesothelium) (45, 47). The effect was dependent upon the TOS concentration, the period of incubation, the form of tumor cells and the culture conditions (Table 2) (43, 48). For example, half maximal inhibitory concentration (IC50) value was 22 μ M for human breast cancer MCF-7 cell lines, 18 μ M for human T lymphoma Jurkat cells and 69 μ M for human cervical cancer cells after 24 h with TOS (49). Nevertheless, IC50 values of TOS are relatively high in comparison to common anticancer drugs (49, 50).

Table 2. TOS apoptosis effect on several cancer cell lines

Cell line	Biological effect	Treatment	Ref.
LNCaP and PC-3 (Prostate carcinoma)	100% and 60% of cells undergoing apoptosis	19 μ M 72h	(51)
MCF7 (breast cancer) BEAS-2B (Bronchocarcinoma)	36% of cells undergoing apoptosis	50 μ M for 12 h	(52)
SGC-7901 (gastric cancer)	90% of cells underwent apoptosis	38 μ M for 48 h	(53)
Cp70 (ovarian cancer)	19% of cells underwent apoptosis	38 μ M for 48 h	(54)

TOS has been shown to enhance selectively the growth-inhibitory effect of several anticancer agents *in vitro*, such as doxorubicin (DOX), cisplatin, tamoxifen, decarbazine on cancer cells (43, 55). Therefore, the combination of TOS with these drugs may be more effective than the individual agents, probably because the mechanism of action on tumor cells is different.

II.3.3.1.1. Structural features for the induction of apoptosis

The anticancer properties depend on the molecular structure of TOS (47). The hydrophobic moiety is responsible for docking the compound in the membrane and circulating lipoproteins. The chromanol moiety is the signaling part acting on the PKC pathways. The

specific functional group of TOS, a charged succinate group, is essential to induce cell apoptosis which occurs through mitochondria apoptotic signaling pathway (47). Succinate group makes TOS redox-silent and endows it with apoptogenic activity. TOS-induced inhibition of cell proliferation *in vitro* requires that TOS remains intact. Thus oral administration of TOS is likely to be ineffective due to its hydrolysis in the GI tract yielding to α -tocopherol and the succinate group which do not display anticancer properties (48).

The size and nature of the ester substituent on the compound is of primary importance. α -TOS and α -tocopheryl maleate have shown apoptogenic properties while this activity falls down for the glutarate analogue and is non-detectable for 2,2-dimethyl and 3,3-dimethylglutaryl monoesters. Lower water solubility or amphiphilicity might be the explanation. The charged group and/or overall amphiphilicity may play a key role in the bioactivity as esters without acid functionality or dicarboxylic diesters show no apoptogenic activity (47). The hydrophobic moiety is also necessary, but not sufficient, to induce apoptosis in cancer cells as succinyl esters without lipid tail have no apoptogenic properties. Modification of the methyl substitution on the aromatic ring also modulates apoptogenic properties with α -TOS being more effective than β -, γ - and δ -vitamers (47).

II.3.3.1.2. Anticancer mechanisms of TOS

TOS acts on tumors through different mechanisms: (i) inhibitory effects on tumor cell proliferation; (ii) induction of apoptosis in tumor cells; (iii) inhibition of metastasis.

TOS inhibits tumor cell proliferation through (i) inhibition of DNA synthesis, (ii) delay of cell cycle and (iii) control of regulatory proteins for cell cycle (45). The latest includes the inhibitory effect of TOS through regulating Ras, an oncogene that when overly expressed can lead to continuous proliferation of tumor cells. The Ras down-regulating effect has been shown in several cell lines including human colon cancer (HCT116) and human breast cancer (MDA-MB) cell lines. TOS also regulates transcriptional factors, such as inhibiting the activities of nuclear factor-kappa B (NF- κ B) and enhancing these of the activator protein 1 (AP-1), which enable inhibition of cell proliferation (46).

The induction of apoptosis in tumor cells by TOS occurs through regulation of multiple signal pathways: (i) an extrinsic receptor-related pathway and (ii) an intrinsic mitochondrial pathway (Figure 10).

The extrinsic pathway initiates apoptosis process through extracellular molecule binding with transmembrane receptor which activates caspase-8. The TOS –regulated apoptosis through extrinsic pathway comprises two main process: (i) Fas pathway and (ii) transforming growth factors (TGF) pathway. Fas is a death receptor, member of the superfamily of neural growth factor and tumor necrosis factor receptor (NFG/TNF), when stimulated leads to apoptosis. It has been shown that TOS was able to activate Fas/Fas-L pathway through increased expression of Fas receptor and Fas-L protein in breast cancer cells. Thus, TOS transformed breast cancer cells from Fas-tolerant to Fas-sensitive cancer cells. Similar observations were made on human gastric cells where Fas, Fas-associating protein with death domain (FADD) and caspase-8 were elevated when treated with TOS (46, 48). TOS can up-regulate TGF genes. TGF is a cytokine binding receptors located in cell membranes associated with inhibition of cell proliferation and induction of epithelial apoptosis. TOS was shown to restore TGF-pathway in human breast cancer cells where the TGF-pathways was non-functional and to increase TGF expression in human gastric tumor cells (46).

TOS primarily induces apoptosis in tumor cells through intrinsic pathway. The intrinsic pathway apoptosis implies dysfunctional mitochondria and is regulated to a certain degree by the extrinsic pathway. The initial triggers in apoptosis induced by TOS are not fully understood. Mechanisms are likely to rely on production by tumor cells of reactive oxygen species (ROS). TOS has been shown to induce ROS in many types of tumor cells. Mitochondria seem to be the site of superoxide generation as well as the target of ROS. The generation of the mitochondrial permeability transition pore may play a central role in TOS induced apoptosis. TOS is likely to modulate mitochondrial pro-apoptotic protein (Bac) and antiapoptotic proteins (bcl-2 and bcl-x_L), which control mitochondrial membrane permeability. The downstream event following mitochondrial destabilization in apoptosis induced by TOS include migration of apoptotic mediators like cytochrome c, the apoptosis-inducing factor (AIF) and Smac/Diablo. When released into cytoplasm by mitochondria, cytochrom C bind the Apoptotic peptidase activating factor 1 (Apaf-1) to form a complex which is further joined by the pro-caspase-9. Then, pro-caspase-9 breaks free and it activates downstream effectors like caspase-3, -6 and -7 to amplify the caspase signal which initiates the apoptosis. Smac/Diablo acts as an antagonist against caspase inhibitor belonging to the inhibitory apoptotic protein (IAPs) family. Since expression of IAPs is under control of NF-kB

which is inhibited by TOS mean, Smac/Diablo may promote inhibition of the survival pathways in apoptosis induced by TOS (43, 46, 47).

Both pathways have a certain degree of interaction through mitogen-activate protein kinase (MAPK) pathway which plays the role of connector during the apoptosis process induced by TOS. A MAPK protein, the c-Jun N-terminal kinases (JNK), regulates phosphorylation of Bcl-2 proteins. It also regulates the migration of Bax, a pro-apoptotic mitochondrial protein forming a heterogeneous tetrapolymer channel to release cytochrome C when apoptotic signal is received, from cytoplasm to mitochondria. TOS can also promote the activities of JNK (46). PKC pathways also play a role between intrinsic and extrinsic pathways by direct and indirect regulation on phosphorylation of Bcl-2 (43, 46).

Some studies have claimed that TOS inhibits angiogenesis. It has been shown that TOS could inhibit the vascular endothelial growth factor (VEGF), a positive control factor for angiogenesis. The regulation of expression by TOS of others proteins implied in the angiogenesis, such as fibroblast growth factor (FGF), NF- κ B, Ras and AP-1, may also be involved (45, 46). TOS may also inhibit tumor metastasis by inhibiting matrix metalloproteinases-9 (MMP-9) which is an enzyme secreted by tumor to degrade extracellular matrix. TOS may reduce expression of MMP-9 secreted by tumor cells which protect the extracellular matrix (46).

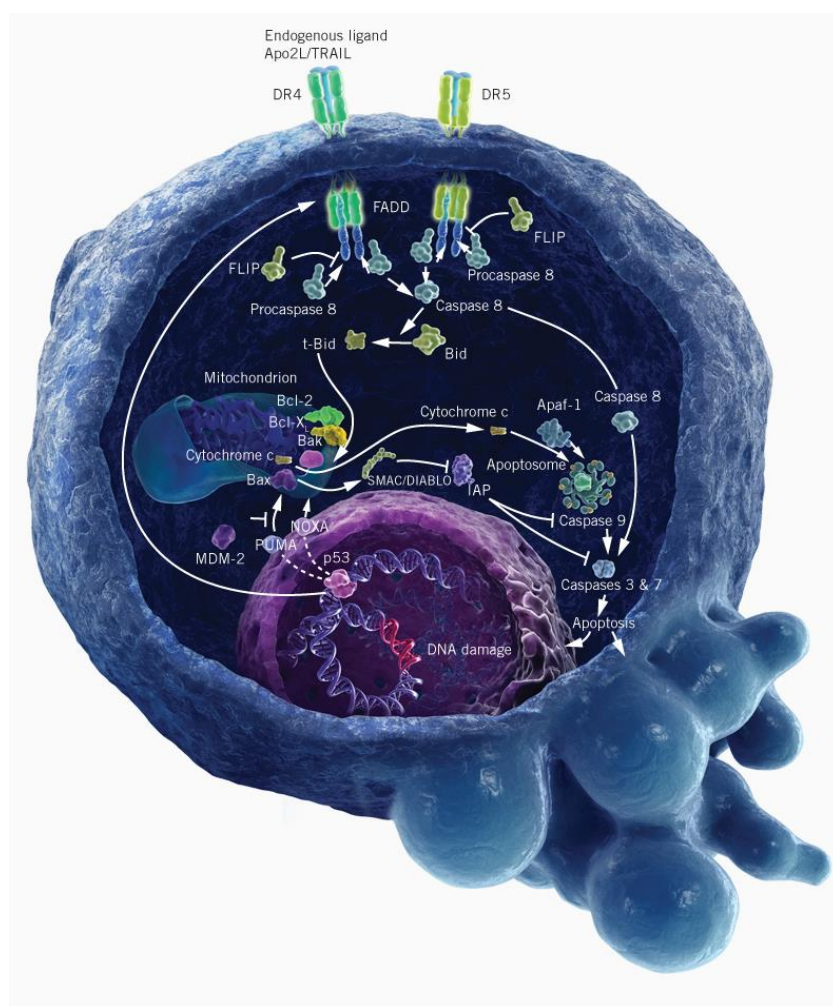


Figure 10. Apoptosis is triggered through 2 main signaling pathways: (i) the extrinsic pathway which is activated in response to external pro-apoptotic signals and other pro-apoptotic receptor agonists and (ii) the intrinsic pathway which is activated by cellular developmental cues or as a result of severe cellular stress (56)

II.3.3.1.3. *In vivo* data on TOS

Exposure to TOS reduced efficiently the incidence of breast, colon, or stomach cancer and melanoma after intraperitoneal (IP) administration (Table 3) (47, 48). Nevertheless, oral administration in rodents was ineffective, suggesting that most TOS may be hydrolyzed by esterases in the intestinal tract before entering in the blood stream (47, 48). Oral administration could be reached by using corresponding ether analogues of tocopherol which are not hydrolysed by esterases (44, 47).

Table 3. Effects of vitamin E succinate in experimental cancer models (mice)(52, 57-64)

Inoculated cell line or tumor inducer	Applied dose	Duration of treatment and effect on tumor growth	Ref.
MDA-MB-231 human breast cancer cells	150 mg/kg/day in sesame oil	2 weeks; 80-90% tumor dormancy	(57)
B16F10 murine melanoma cells	100 mg/kg/day in sesame oil	2 weeks; 80-90% tumor dormancy, inhibition of liver metastasis	(59)
CT-26 colon cancer cells	100 mg/kg/day in 20% DMSO	2 weeks; 75% inhibition of liver metastases	(60)
B16F10 murine melanoma cells	150 mg/kg/day in sesame oil	2 weeks; 70% tumor growth inhibition	(58)
HCT116 human colon cancer cells	100 mg/kg in DMSO every third day	10 days; 75% tumor growth inhibition	(61)
Human mesothelioma Ist-Mes2 cells	100 mg/kg in DMSO every second day	16 days; >90% tumor growth inhibition	(62)
Benzo(a)pyrene induced forestomach tumors	20 mg/kg in corn oil twice per weeks	4 weeks; 85% tumor growth inhibition	(64)
3 LLD122 murine Lewis lung carcinoma cell line	200 mg/kg /day TOS in ethanol or vesiculated TOS	20 days; >70% tumor growth inhibition	(63)

No clinical studies have been achieved with TOS alone or in combination with chemotherapy.

II.3.3.2. Anticancer effects of TPGS

TPGS has been shown to inhibit the function of P-glycoprotein (P-gp), also named MDR-1 protein. This well-known transporter is an ATP-dependent drug efflux-pump which can mediate MDR to cancer cells, thus lowering intracellular drug accumulation through transport across extracellular and intracellular membranes. Substrates of P-gp include many anticancer drugs such as paclitaxel (PTX), etoposide, DOX, vinblastine etc. (27, 65, 66). TPGS enhanced DOX, vinblastine and PTX cytotoxicities in MDR-1 resistant cells which were 27-135 fold more resistant than the parental NIH3T3 cells to these drugs (27, 67). The way TPGS acts on P-gp is not fully understood. TPGS was shown to rigidize cell membranes but this does not appear to be the primary mechanism for inhibition. TPGS property on P-gp could be due to the inhibition of the P-gp ATPase, P-gp energy source of active transport, through its allosteric modulation. TPGS containing a 1kDa PEG chain exhibits better P-gp inhibition than analogs containing PEG 200 to 6000 whereas the optimal TPGS should have a 1100-1500 PEG chain (68).

It has been reported that TPGS has intrinsic anticancer activities. It was able to inhibit growth of human lung carcinoma cells implanted in nude mice more potently than TOS.

TPGS was more potent to induce ROS generation than TOS, apoptosis and growth inhibition, despite similar rate of uptake into cells *in vitro*. These data are suggesting that PEG conjugation may positively affect the interaction of TOS with membrane lipids thereby leading to more extensive ROS generation (69). Studies are needed to understand the underlying mechanism(s) of TPGS cancer properties and its activities on other cell lines.

II.4. TOXICITY OF VITAMIN E

The vitamin E recommended daily allowance is 15 mg of RRR- α -tocopherol for both females and males 14 years of age and older according to the dietary guide published by the United States Department of Agriculture (25). Reports of adverse effects of vitamin E supplements in humans are sufficiently rare that the Food and Nutrition Board set the upper tolerance level for α -tocopherol at 1000 mg per day (25).

Vitamin E toxicity is generally considered to be very rare and is generally observed at very high doses (>3.2 g/kg/day). Potential toxicity may include interference with vitamin K metabolism and elevation in blood pressure. Upon parenteral administration, vitamin E was found to be well tolerated in humans at doses up to 2.3 g m^{-2} for 9 consecutive days (31).

TPGS safety issue has been investigated in details. The acute oral median lethal dose (LD50), i.e. the quantity of an agent that will kill 50% of the test subjects within a designated period, is over 7g/kg for young adult rats of both sexes. The US Food and Drug Administration (FDA) has approved TPGS as a safe pharmaceutical adjuvant used in drug formulation (70).

II.5. VITAMIN E AND DRUG DELIVERY APPLICATIONS

The attractiveness of vitamin E derivatives in drug delivery relies on their biocompatibility, their solvent capacity and the biological properties described previously. This chapter highlights properties and applications for both TOS and TPGS in drug delivery (Table 4).

Table 4. Vitamin E-based nanocarriers.

Carrier Type	Composition	Drug	Loading efficiency (% w/w)	Model	Studies	Ref
Tocopherol						
Nano-sized hydrogels	Polyglutamate-TOS	Proteins		Various	Preclinical or clinical stage	(71)
Polymeric Nanoparticles	mPEG-PLA-Tocopherol	DOX		Breast and uterine cancer	<i>In vitro</i> and <i>In vivo</i> (PK)	(72)
Oligomersomes	α -tocopherol oligochitosan	SiRNA		Human epidermal carcinoma	<i>In vitro</i> and <i>In vivo</i> (efficacy)	(73)
Micelles	chitosan/TOS copolymer	PTX		Cervical cancer	<i>In vitro</i> and <i>In vivo</i> (efficacy)	(74)
	TOS modified pluronic	PTX		Glioma	<i>In vitro</i> and <i>In vivo</i> (PK)	(75)
TPGS						
Mixed micelles	Pluronic P407/TPGS	Gambogic acid		Breast and MDR cancer	<i>In vitro</i>	(76)
	Pluronic P123/TPGS	Quercetin		Breast cancer	<i>In vitro</i>	(77)
	Pluronic P105/TPGS	CPT		Breast cancer	<i>In vitro</i>	(78)
	DSPE-PEG/TPGS	PTX and parthenolide		NSCLC	<i>In vitro</i>	(79)
Micelles	PLV(2K)	DOX		Breast and MDR cancer	<i>In vitro</i> and <i>In vivo</i> (efficacy)	(80)
	FOL-TPGS ₂₀₀₀	DOC		Breast cancer	<i>In vitro</i>	(81)
Liposomes	TPGS coating	DOC and QDs		Breast cancer	<i>In vitro</i>	(82)
		DOC		Glioma	<i>In vitro</i>	(83)
		Emodin		B-cell lymphoma	<i>In vitro</i> and <i>In vivo</i> (PK)	(84)
		Topotecan		Breast and melanoma metastatic cancer	<i>In vitro</i> and <i>In vivo</i> (efficacy)	(85)
	Trastuzumab-conjugated TPGS	DOC		Breast cancer	<i>In vitro</i> and <i>In vivo</i> (PK)	(86)
Complex nanoparticles	Pluronic P85-PEI/TPGS	PTX and shRNA		Lung and breast cancer	<i>In vitro</i> and <i>In vivo</i> (efficacy)	(87)
Nanocrytals	TPGS	PTX		NSCLC	<i>In vitro</i> and <i>In vivo</i> (efficacy)	(89)
Polymeric nanoparticles	TPGS + 4-armed porphyrin-PLA	DOX		Breast cancer	<i>In vitro</i>	(90)
	TPGS + MPEG-SS-PLA	PTX		Lung, breast and uterus cancer	<i>In vitro</i>	(91)
	FOL-TPGS and DOX-PLGA-TPGS	DOX		Breast cancer	<i>In vitro</i>	(92)
	PLA-TPGS + Trastuzumab-TPGS	DOC		Breast cancer	<i>In vitro</i>	(93)
Prodrug	TPGS-DOX	DOX		Breast cancer	<i>In vitro</i> and <i>In vivo</i> (PK)	(94)
	TPGS-DOX-FOL	DOX		Breast cancer	<i>In vitro</i> and <i>In vivo</i> (PK)	(50)
	TPGS-cisplatin	cisplatin		Hepatocarcinoma	<i>In vitro</i>	(95)
	TPGS-cisplatin + PLA-TPGS	cisplatin, DOC and herceptin		Breast cancer	<i>In vitro</i>	(96)

Abbreviation: PK, pharmacokinetics; PTX, paclitaxel; DOX, doxorubicin; PEG-PE, poly(ethylene glycol)-phosphatidyl ethanolamine; PEG-DSPE, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)]; CPT, camptothecin; PLV(2k) lysine-linked di-tocopherol polyethylene glycol 2000 succinate; DOC, docetaxel; QDs, quantum Dots; NSCLC, Non-small cell lung cancer; PLA, poly-lactic acid; PLGA, poly (lactic-co-glycolic acid); FOL, folic acid.

II.5.1. Tocopherol and other water-insoluble tocols

II.5.1.1. Solubilizer and formulation excipient

Tocols are able to solubilize a variety of hydrophobic drugs. A compound or any of its derivatives that exhibits high affinity/solubility in tocol(s) is characterized as being tocophilic, that is “tocol-loving” in a manner analogous to the term “lipophilic” (31). However, there is no correlation between lipophilicity and tocophilicity (97).

An interesting method to predict the solubility of drugs in tocol is performed by examining solubility of the compound in chlorinated organic solvents and in methanol. Drugs that are highly soluble in chlorinated solvents (≥ 6 mg/mL, preferably ≥ 10 mg/mL) have acceptable solubility in vitamin E (≥ 1 mg/mL, ideally ≥ 10 mg/mL) whereas molecules that have good solubility in methanol (≥ 10 mg/mL) have low solubility in vitamin E (≤ 1 mg/mL). Thus, a tool named the “solubility in vitamin E parameter” (SVE) was developed to predict solubility of a compound in vitamin E. SVE is defined as the solubility in chloroform divided by the solubility in methanol expressed in mg/mL for both solvents. A SVE of at least 10, preferably greater than 100, would indicate an acceptable solubility in vitamin E (97, 98).

Table 5. Solubility of some drugs in organic solvents and Vitamin E (31)

Drug	Solubility (mg/mL)				SVE ^a	SP ^b
	Water ^c	Methanol	Chloroform	Vitamin E		
Itraconazole	10 ⁻⁶	Insoluble ^d	500	60	>1,000	10.6
Paclitaxel	0.00034–0.030 ^e	0.03	6	11	200	11.9
Cyclosporine	0.023	0.71	363	100	520	10.7
Ergosterol	Insoluble ^f	1.5	32	50	25	9.6
Cholesterol	0.002	5	200	150	40	9.6
Prednisolone	0.22	33	5.0	Insoluble ^d	0.02	13.6
Amphotericin	0.1 ^g	Soluble ^d	Insoluble ^d	Insoluble ^d	<1	14.4

^a SVE parameter: ratio of drug solubility in chloroform to that in methanol.

^b Solubility parameter.

^c Literature values.

^d Terms not defined in the original references

^e Solubility varies from different sources

^f No value was found in the literature, but it is expected to be similar to that of cholesterol.

^g At pH= 2

Vitamin E emulsions have been studied for poorly soluble drug delivery. A vitamin E based emulsion named Tocosol® was developed for the parenteral delivery of PTX, a poorly water-soluble anticancer drug which is soluble in tocol. This system employs TPGS and poloxamer

407 as emulsifiers and contains PEG400. Tocosol®-PTX was shown to be better tolerated and more efficacious than the clinical formulation of PTX, Taxol® (which contains the undesirable Cremophor EL) using the B16 mouse melanoma model. Tocosol®-PTX has successfully completed Phase I and Phase II clinical investigations (31, 99). Unfortunately Tocosol®-PTX, failed a pivotal phase III study conducted in breast cancer patients. The main reason was the lack of advantages in terms of targeting PTX to the cancer cells (100).

Chemical modification of the drug can be achieved for non-tocophilic compound to ensure high solubility of the modified drug in vitamin E. This strategy was explored by coupling TOS to camptothecin (CPT), 7-ethyl-10-hydroxycamptothecin and other derivatives. After modification, these derivatives can be dissolved in vitamin E and then emulsified in the presence of TPGS, Pluronic 407, and saline to produce a stable nano-emulsion (101).

II.5.1.2. Tocopherol-based polymeric nanoparticles

Vitamine E has been grafted on various polymers to enable drug encapsulation and efficient delivery.

Flamel Technologies has developed a polymer, named Medusa, consisting of a poly-L-glutamate backbone with α -tocopherol randomly grafted to some of the glutamate units through hydrolysable ester bond. This polymer forms a colloidal suspension of nanoparticles due to the self-assembly of the lateral hydrophobic vitamin E groups into hydrophobic nanodomains and the aggregation of the hydrophilic glutamate chains.

The polymer is used for subcutaneous slow release formulations of biopharmaceuticals like interferon alpha-2b (IFN- α_{2b}), Insulin, interleukin-2 (IL-2), human growth hormone, Glucagon-like peptide-1 analog. The controlled release system is obtained by a simple mixing of an aqueous solution of the protein or the peptide with an aqueous solution of the polymer. A clinical trial in patients with hepatitis C has shown that the release of IFN- α_{2b} from Medusa II formulation was extended and that the viral load was lower, in comparison with the IFN commercially available Vitaferon® (71).

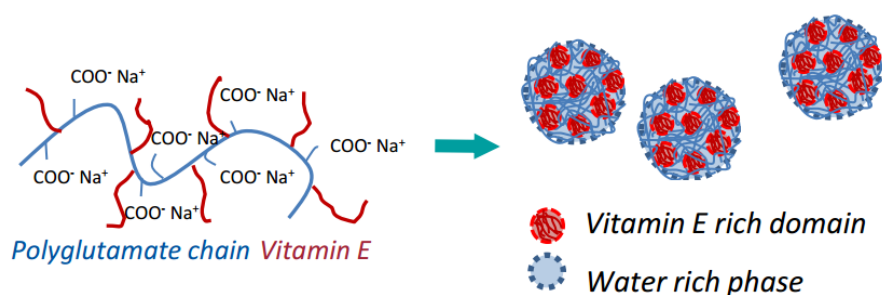


Figure 11. Self-assembly of polyglutamate-TOS in water (71).

A polymeric nanoparticulate drug delivery formulation consisting of the amphiphilic diblock copolymer of mPEG-PLA-Tocopherol and sodium salt of poly(lactic acid-co-mandelic acid) (PLMA-COONa) and incorporating DOX (Dox-PNP) has been developed. Tocopherol moiety was used to increase the stability of the hydrophobic core of the nanoparticle in aqueous medium. The carboxylate end group of the biodegradable polyester allows the formation of ionic complex with DOX. The loading of these 20–25 nm nanoparticles is of 0.92%. Compared to free DOX, Dox-PNP exhibited higher cellular uptake into both human breast cancer cell line (MCF-7) and human uterine cancer cell (MES-SA), especially into doxorubicin-resistant strains. Upon tail-vein injection, Dox-PNP exhibited 70 times higher bioavailability in rats and showed 2 times higher bioavailability in tumor tissue than free DOX (31, 72).

Amphiphilic α -tocopherol oligochitosan conjugates were synthesized by conjugating TOS to oligochitosans with various molecular weights. The tocopherol oligochitosan conjugates self-assembled in water to single layered oligomersomes, named TCOsomes, with size depending of the chain lengths of oligochitosans. TCOsomes were used to encapsulate siRNA by taking advantage of the cationic nature of chitosan derivatives. TCOsomes based on oligochitosan size of 4kDa significantly enhanced the cellular uptake of siRNAs (>98%), and reduced the expression of target proteins more effectively than did Lipofectamine 2000. The mechanisms by which TCOsomes enhanced the delivery of siRNA need to be studied further. In tumor xenografted mice, the intratumoral administration of siRNA using TCOsomes showed a significant reduction of tumor mass after treatment and prevented the growth of tumor (73). A similar amphiphilic chitosan/vitamin E succinate copolymer encapsulating PTX was also studied. *In vivo* efficacy of the polymer encapsulating PTX was higher than Taxol on U14 cervical cancer cells-bearing mice. The side effects after IV administration were also lower for the polymer encapsulating PTX (74).

Vitamin E succinate (TOS) modified pluronic P123 micelles (PF-TOS) were prepared to be used as a vehicle for PTX. Pluronic copolymers have been considered a promising nanocarrier system but have limitations such as low encapsulation efficiency and lack of stability in physiological environment. The rationale for PF-TOS design was based on the expectation that TOS with its lipophilic portion might allow better drug solubilization when conjugated with pluronic P123 (75). Compared to PF-PTX, PF-TOS-PTX-micelles showed similar uptake and superior cytotoxicity of which might be attributed to the combined effect of enhanced encapsulation efficiency and anti-cancer properties of TOS. The PF-TOS-PTX-micelles had longer systemic circulation time and slower plasma elimination rate than those of PF-PTX-micelles after intravenous (IV) administration to mice. This was attributed to the TOS modification, which improved the hydrophobic interactions and the micelles stability (75).

II.5.2. TPGS

The tocol ester TPGS₁₀₀₀ is a water-soluble form of vitamin E (vitamin E content of 260 mg/g) which can self-assemble in 13 nm micelles. It is a waxy solid (melting point: 37-41°C) completely miscible with water (hydrophilic-lipophilic balance (HLB) value about 13) and its CMC at 37°C is 0.02% w/w (31, 70). At physiological temperature, depending on the water content, it forms various phases in aqueous solution that can solubilize a variety of compounds, both water-soluble and water-insoluble. Examples of drugs that are soluble in TPGS include cyclosporines, taxanes steroids, antibiotics and other drugs. When TPGS concentration is above 20% w/w, the polymer forms high-viscosity liquid crystalline phases. The structure of the TPGS/water phase evolves from globular micellar, to hexagonal then reversed micellar, and finally to the lamellar phase when TPGS concentration is increased (Figure 12) (31, 70). In addition to its water miscibility, TPGS is also miscible with oils, other surfactants and cosolvents such as propylene and polyethylene glycols. In aqueous media, TPGS is stable at pH 4.5-7.5. It is also air-stable but reacts with alkali (31).

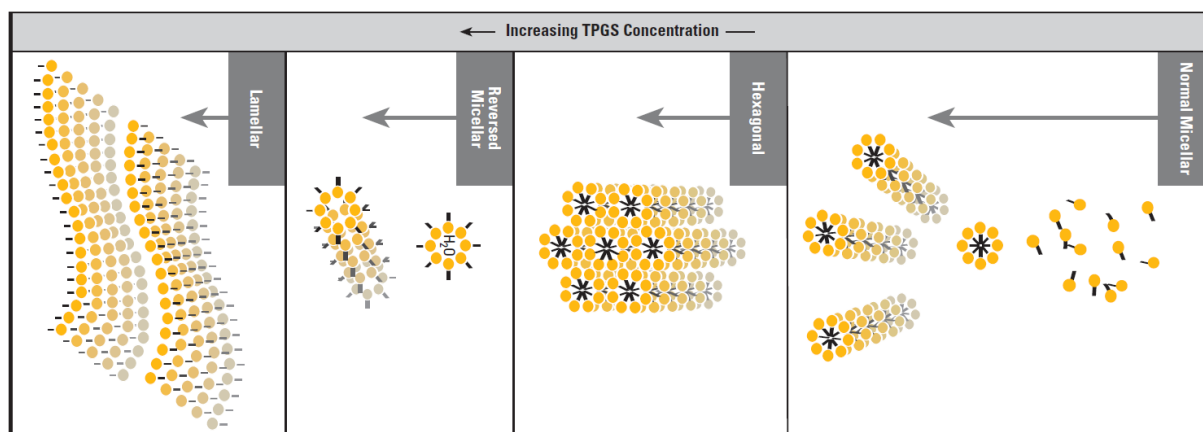


Figure 12. Liquid crystalline phases of TPMS/water systems at 37°C (70).

II.5.2.1. TPMS properties

TPMS has been used as an absorption enhancer, emulsifier, solubilizer, additive, permeation enhancer and stabilizer. TPMS has been used as a carrier for wound care treatment, an oral bioavailability enhancer for poorly absorbed drugs, and a drug solvent and emulsion stabilizer for parenteral administration (27, 31, 42, 70, 102). Examples of drugs that exhibit enhanced bioavailability with TPMS include amprenavir, a poorly water soluble substrate of P-gp, which showed no absorption in dog plasma without TPMS and 69% absorption with 20% TPMS (27, 70).

TPMS has also served as an excipient for overcoming MDR and inhibitor of P-gp for increasing the oral bioavailability of anticancer drugs. TPMS (IC₅₀: 33 μ M) is more effective P-gp inhibitor than excipients with surfactant properties like Cremophor EL (400 μ M) but less potent than P-gp inhibitor like cyclosporine (1 μ M) or tariquidar (20–50 nM) (70).

Still the permeation improvement due to P-gp interaction may be compromised by the micellization during the inclusion of poorly soluble drugs in micelles. Encapsulation of poorly soluble drugs in micelles may impair the passive diffusion of drugs which results in a delicate balance between permeation inhibition due to micelle-association and permeation enhancement due to P-gp interaction (27). TPMS demonstrated the maximum efflux inhibition activity at a concentration of 0.1 mg/mL which may be due to the interplay of concentration dependent P-gp inhibition and the micellar formation. As the concentration of TPMS was increased above 0.1 mg/mL a linear increase in drug solubility was observed, presumably via micellar solubilization (27, 31, 103, 104).

II.5.2.2. TPGS-based nanomedicines

TPGS have been used to develop various nanomedicines including TPGS-based prodrugs, micelles, liposomes, TPGS-emulsified PLGA nanoparticles and nanoparticles of TPGS-based copolymers, which can significantly enhance the solubility, the permeability, and the stability of the formulated drug and realize sustained, controlled and targeted drug delivery. TPGS is an efficient emulsifier for the synthesis of nanoparticles of biodegradable polymers, resulting in high drug encapsulation efficiency, high cellular uptake in vitro and high therapeutic effects in vivo. TPGS-emulsified nanoparticles or TPGS-based nanoparticles increase the cell uptake efficiency (42). This part was adapted from recent reviews about TPGS for drug delivery (27, 42).

TPGS- based micelles are used for drug delivery. Nevertheless, TPGS micelles can easily dissociate in the plasma as the CMC of TPGS is relatively high. Therefore TPGS is often mixed with other materials to form mixed micelles with the ability to increase stability and drug encapsulation. Among these materials, the most studied are poly(ethylene glycol)-phosphatidyl ethanolamine (PEG-PE), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)] (PEG-DSPE), Pluronic and PLGA-PEG (27, 76-78, 105). For example, mixed micelles made of PEG-PE and TPGS showed a CMC comprised within 10^{-6} to 10^{-5} M. The solubility of CPT was enhanced at least 50% compared to the PEG-PE micelles due to the increased inner micelle core volume through the big vitamin E head. The anticancer efficiency of these mixed micelles was high due to their enhanced cCPT solubility, permeability and stability as well as improved cellular uptake ability. This PEG-PE/TPGS system was further used to encapsulate anticancer drugs PTX and gossypol (42). DSPE-PEG/TPGS was more recently used for concurrent delivery of parthenolide and PTX, a promising alternative to single-agent therapies in cancer. The mixed micelles were tested on taxol sensitive and resistant non-small cell lung cancer (NSLC) cell lines. It was shown that efficacy of PTX and parthenolide against both cell lines significantly increased when they were co-administered through mixed micelles (79).

Longer PEG chain derivatives have been synthesized in attempt to improve the stability of TPGS based micelles. Study shown that TPGS₂₀₀₀, with PEG chain of 2kDa, had a lower CMC (0.022 mg/ml) than TPGS (0.2 mg/ml), which improves the micelle stability and could effectively encapsulate docetaxel (DOC) delivery. The cellular uptake and cytotoxicity of

TPGS₂₀₀₀ loaded with DOX by MCF-7 cancer cells *in vitro* were enhanced compared with Taxotere[®]. Moreover, a synergistic effect between TPGS₂₀₀₀ and DOX from cytotoxicity assay was also shown. Thus, the design of drug delivery systems where the carrier materials can also have therapeutic effects, which either modulate the side effects of, or promote a synergistic interaction with the formulated drug could be a promising approach in cancer therapy. Mixed micelles composed of TPGS₂₀₀₀ and a new derivative, TPGS₃₃₅₀-folic acid (TPGS₃₃₅₀-FOL), was also developed to target folate-receptor rich tumors (42, 81).

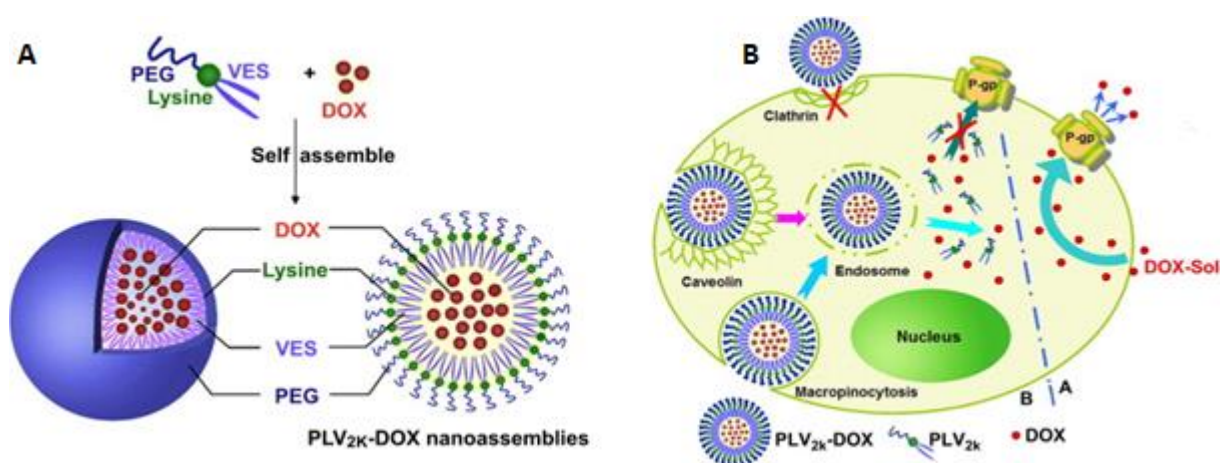


Figure 13. (A) star-shape copolymer of lysine-linked di-TPGS₂₀₀₀ (PLV_{2K}) loaded with DOX micelles and (B) uptake pathway in P-gp overexpressing cells (80).

Another way to improve the TPGS-based micelles stability was achieved by synthesizing a star-shape copolymer of lysine-linked di-TPGS₂₀₀₀ (PLV_{2K}) (Figure 13-A). The system formed micelles with low CMC of 1.14 $\mu\text{g/mL}$. In DOX-resistant cells, MCF-7/Adr, the cytotoxicity was significantly enhanced with PLV_{2K} micelles loaded with DOX (PLV_{2K}-DOX) compared to DOX. The promotion of cellular cytotoxicity and cellular uptake to overcome MDR by PLV_{2K}-DOX micelles in P-gp over-expressing MCF-7/Adr cells was proposed to rely on: (i) enhanced solubility and high encapsulation efficiency of DOX in PLV_{2K} micelles, (ii) uptake of PLV_{2K}-DOX micelles in MCF-7/Adr cells by endocytosis, escaping the efflux induced by P-gp and overcoming the MDR in cancer chemotherapy, (iii) the endocytotic pathway by PLV_{2K}-DOX micelles which is caveolin-dependent, avoiding degradation under lysosomal conditions, (iv) the intracellularly released PLV_{2K} could inhibit P-gp transporter ATPase activity, increasing the DOX cellular accumulation of DOX in the resistant cells (Figure 13-B). *In vivo*, PLV_{2K}-DOX enhanced the antitumor activity and reduced the cytotoxicity compared with free DOX. DOX encapsulated in PLV_{2K} was more effective in inhibiting tumor than TPGS-DOX, which may be due to the lower CMC, better stability and prolonged circulation time by PLV_{2K} micelles (80).

TPGS has been used as surfactant and/or constituent in liposomal formulation since it may provide advantages for the sustained and controlled drug release. TPGS-based liposomes have been used to deliver anticancer agents like DOC, DOX and topotecan (82-85).

Recently, TPGS coated theranostic liposomes containing both DOC and quantum dots (QDs) were developed for combined cancer imaging and therapy. The TPGS coating of the liposomes allowed long-circulation effects, enhanced cellular uptake, and conjugation with folic acid for active targeting effect. A significantly higher cellular uptake and cytotoxicity was demonstrated for the folate receptor targeted liposomes compared to non-targeting liposomes (82).

Other targeted TPGS-based liposomes have been developed successfully like trastuzumab-conjugated TPGS-based liposomes for the delivery of DOC for Human Epidermal Growth Factor Receptor 2 (HER2) overexpressing breast cancer treatment. In vivo, IV administration of trastuzumab-conjugated liposomes showed longer half-life, 1.9 and 10 times more than PEG-coated liposomes and PTX respectively, demonstrating their greater potential for sustained and targeted chemotherapy in the treatment of HER2 overexpressing breast cancer (86).

TPGS-based liposomes may also be used for oral delivery of proteins or drugs with a low oral bioavailability due to gastrointestinal degradation and low permeation (42, 106).

A new co-delivery system, Pluronic 85-PEI/TPGS complex nanoparticle conveying survivin shRNA (shSur) and PTX (PTPNs) for reversing drug resistance was designed and developed. shSur was used to down-regulate survivin gene expression since survivin protein is up-regulated in the most of malignant tumors but rarely in normal differentiated tissues. The 150 nm nanoparticles were shown to be more effective than free PTX *in vitro* in resistant (A549/T) and non-resistant (A549) human lung cancer cells. *In vivo* studies of PTPNs and PTX (Taxol) on nude mice bearing A549/T showed a better efficacy of the PTPNs formulation. Thus, the co-delivery of PTX and shSur by PTPNs could be a powerful approach to improve the therapeutic effect of PTX in resistant lung cancer (87).

Pluronic 85-PEI/TPGS complex nanoparticles were also used for the co-delivery of twist shRNA and PTX to inhibit both metastasis and tumor growth (Figure 14). Twist is a transcript

factor which plays a major role in metastasis of breast cancer. The complex was studied on metastatic 4T1 breast cancer cell line and its pulmonary metastasis mice model. Prolonged circulation and increased accumulation of PTX and shTwi in lung and tumor was demonstrated. The *in vivo* antitumor efficacy showed that PTPNs could inhibit the *in situ* tumor growth effectively and completely restrict the pulmonary metastasis in pulmonary metastatic mice model whereas Taxol did not (88).

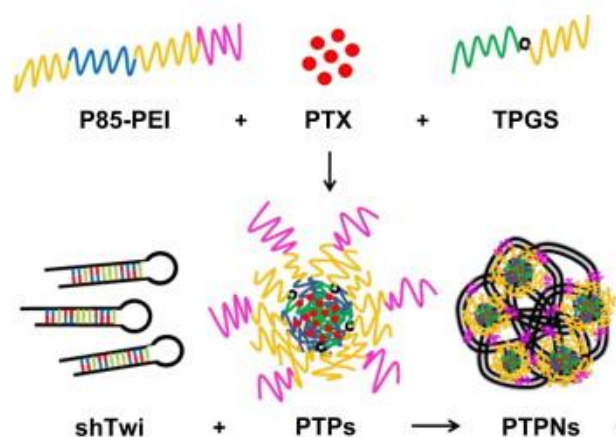


Figure 14. Schematic illustration of preventing tumor metastasis and suppressing tumor growth synchronously by shTwi and PTX co-loaded PTPNs(88)

Drug nanocrystals required surfactant as stabilizers such as TPGS (89, 107). TPGS has been used to develop TPGS-PTX nanocrystals to overcome multidrug resistance in cancer (27, 89). PTX formulations were characterized by crystals with a rod width being 40 nm and length being around 150 nm. The TPGS-PTX nanocrystals showed a significant antiproliferation effect compared with Taxol® when tested in Pg-p overexpressed cells (NCI/ADR-RES). Beyond its surfactant properties which are crucial for the nanocrystal stability, TPGS can act synergistically with the drug as a P-gp inhibitor to reverse MDR in cancer cells (27, 89).

Drug-loaded nanoparticles emulsified with TPGS can achieve higher drug encapsulation efficiency (up to 100%) and cellular uptake, and thus higher therapeutic effects compared with polyvinyl alcohol (PVA) emulsified nanoparticles. TPGS (0.02-0.03%) can have 67 times higher emulsification effects than PVA in the PLGA nanoparticles. TPGS has been used as surfactant for the fabrication of PLGA, PCL, PLA-TPGS and PLGA-PEG nanoparticles. TPGS coated nanoparticles can also take advantage of its P-gp inhibition properties, especially in MDR cancer cells like MCF-7/Adr (90). The main limitation to these nanoparticles is their low loading efficiency (LE), i.e. 2.4% in TPGS-emulsified PLGA (108) and 2.6% in MPEG-PLA (91)

nanoparticles. Higher rates could be reached with TPGS-emulsified NPs made of a new redox-sensitive polymer, namely poly(ethylene glycol)-b-poly(lactic acid) (MPEG-SS-PLA), for which the LE was 9.1%. This stimuli-responsive polymer was prepared to carry PTX. It can release the drug as triggered and mediated by intracellular stimuli, here glutathione (GSH) which reduces disulfide bonds in the cytoplasm (91).

Advantages of TPGS can be utilized to overcome the high hydrophobicity of poly(lactic acid) polymers and their slow degradation. The synthesis of TPGS based copolymers is easily done by ring opening polymerization such as TPGS-PLA, TPGS-PLGA, TPGS-PCL, TPGS-PGA-PCL or TPGS-PLA-PCL (11). TPGS has been used for triggering nanoparticles for faster drug release by improving the biodegradability of PLA/PLGA. PLA-TPGS is the most studied derivative, mainly for anticancer drug encapsulation (Figure 15). The nanoparticles formed by this polymer have been shown to get a higher drug encapsulation efficiency, cellular uptake and cytotoxicity on cancer cells in comparison with the classics PLGA nanoparticles (11, 27, 109). The nanoparticle formulation relies on several classics methods like nanoprecipitation, solvent extraction/evaporation method, dialysis method and double emulsion method (27). PLA-TPGS copolymers have been used for the delivery of DOC, PTX, DOX, curcumin, risperidone, protein, superparamagnetic iron oxides (SPIOs) and QDs. PLA-TPGS were even used for the co-delivery of SPIOs and QDs to develop a multimodal imaging system for concurrent imaging of the magnetic resonance imaging and the fluorescence imaging (11, 42).

Modification of the surface of these nanoparticles was achieved to target specific membrane receptors such as trastuzumab for DOC delivery in HER-2 overexpressed cancer cells and folic acid for DOX delivery in folate-receptor rich tumors (11, 42, 92, 93). Transferrin (Tf)-conjugated nanoparticles of PLA-TPGS loaded with DOC were studied for the targeted therapy across blood-brain-barrier (11).

PLA-TPGS nanoparticles loaded with DOX could also inhibit the P-gp activity which increased intracellular drug accumulation in MCF-7/ADR cells. It was proposed that PLA-TPGS nanoparticles loaded with DOX improved drug efficacy through the combination effect of P-gp inhibition and increase of drug entering into nucleus of drug-resistant MCF/ADR cells (42).

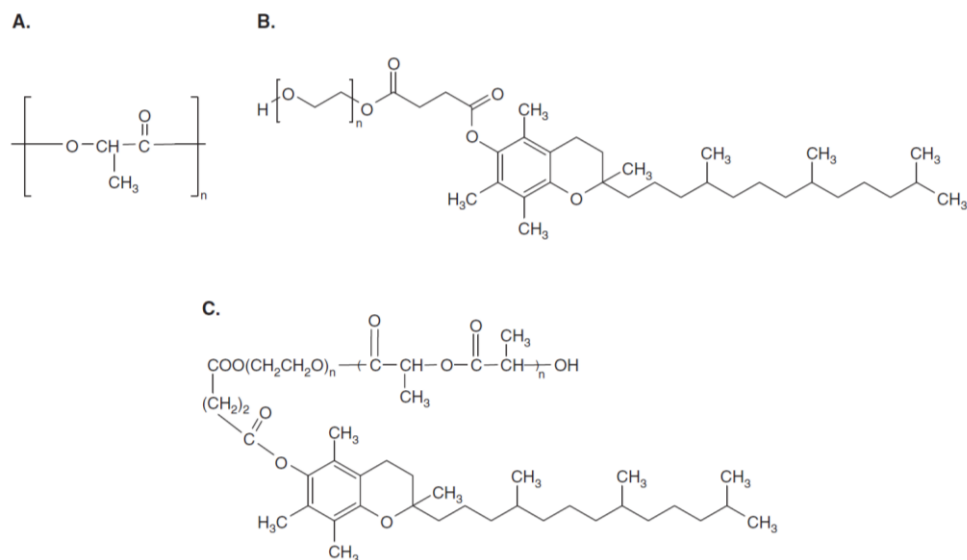


Figure 15. Structural formula of (A) poly(lactic acid) (PLA); (B) D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS);(C) PLA-TPGS.(11)

II.5.2.3. TPGS as prodrug carrier

A prodrug is a pharmaceutical agent which is administered in an inactive form and then bioactivated into active metabolites *in vivo*. The rationale behind a prodrug is to enhance the pharmacokinetics of a drug (42). TPGS has been applied for prodrug design for enhanced chemotherapy since it may induce apoptosis and develop a synergistic effect with other drugs (81).

To reduce side effects from DOX, evade drug resistance and enhance its therapeutic efficiency, DOX was conjugated to TPGS. The prodrug demonstrated higher cellular uptake and better efficiency in MCF7 breast cancer cells and glioma C6 cells compared with the parent drug. The pharmacokinetics parameters such as half-life of the prodrug were improved in comparison with free DOX (42, 94).

TPGS-DOX-folic acid (FOL) conjugate (TPGS-DOX-FOL) for targeted chemotherapy was synthesized and compared with TPGS-DOX conjugate and DOX (Figure 16). Targeting conjugate TPGS-DOX-FOL was 45-fold more effective than DOX in cytotoxicity on MCF-7 cells, while TPGS-DOX conjugate was only 1.19-fold effective than DOX. The $t_{1/2}$ of TPGS-DOX and TPGS-DOX-FOL were extended from 2.69 h (DOX) to 10.2 h and 10.5 h, respectively. Conjugates also significantly decreased the drug distribution in gastric, intestine, and heart suggesting that TPGS conjugate, especially TPGS-DOX-FOL could reduce side effects of the drug (42, 92).

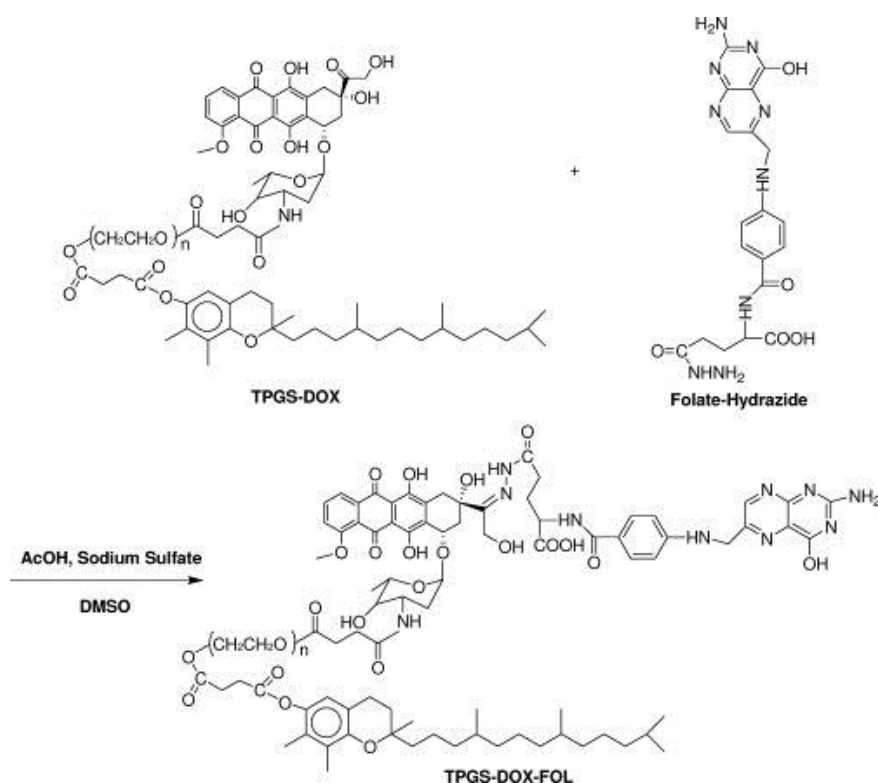


Figure 16. Synthetic scheme of the TPGS–DOX–FOL conjugate (50)

Vitamin E TPGS prodrug micelle strategy was developed for hydrophilic drug formulation with cisplatin as a drug model. The TPGS-cisplatin prodrug micelles show good potential to deliver the hydrophilic cisplatin with a low CMC, a drug load of 4.95% w/w and a controlled release. TPGS-cisplatin micelles showed higher uptake efficiency and better anti-cancer effects than the original cisplatin for HepG2 cancer cells, which may be due to the advantages of nanomedicine and the anti-cancer effect of TPGS (95).

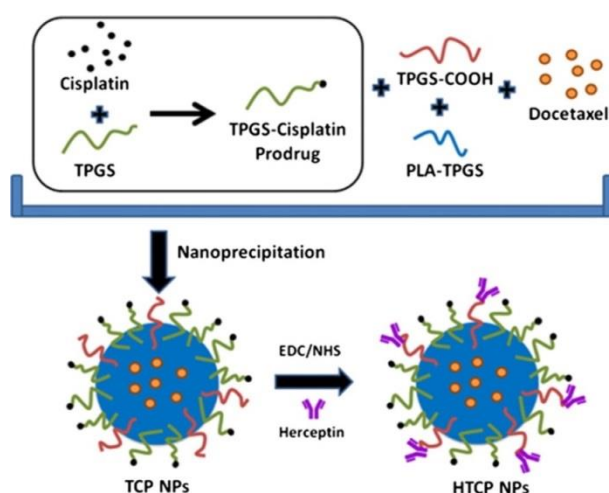


Figure 17. Schematic illustration of the formulation of DOC_loaded TPGS-cisplatin prodrug nanoparticles (TCP NPs) and herceptin-conjugated, DOC-loaded TPGS-cisplatin prodrug nanoparticles (HTCP NPs)(96)

TPGS-cisplatin prodrug was further used for the targeted delivery of cisplatin, DOC and Herceptin (HTCP) for multimodality treatment of breast cancer with HER-2 overexpression (**Figure 17**). Co-polymers PLA-TPGS and TPGS-COOH were added to stabilize the nanoparticles and facilitate Herceptin binding. The targeting effects of HTCP nanoparticles were demonstrated on several cell lines *in vitro* with high and low HER2 overexpression (96).

III. CHITOSAN AND ITS DERIVATIVES[†]

Among all the polymers available to be used for drug delivery systems, (bio)degradable polymers are thus highly recommended. Indeed, one of the key points of this kind of system is the removal of the carrier after the release of the active pharmaceutical ingredients. Moreover, to avoid side effects, in particular when the carrier is injected, the polymer must be biocompatible. For all of these reasons, natural polymers such as polysaccharides, polypeptides or phospholipids are generally used as building blocks for the formulation (111). Among them, chitosan have been extensively in the pharmaceutical fields studied because it presents good physico-chemical and biological properties. Moreover it can be chemically modified to optimize chitosan-based formulations.

This part focuses on amphiphilic chitosan derivatives developed for drug delivery applications and was based on a previous review written by our group and collaborators.

III. 1. PRODUCTION AND PROPERTIES OF CHITOSAN

Chitosan is a biocompatible, semi-crystalline (112), biodegradable (113, 114) and biocompatible (115, 116) linear polysaccharide of randomly distributed *N*-acetyl glucosamine and glucosamine units (Figure 18).

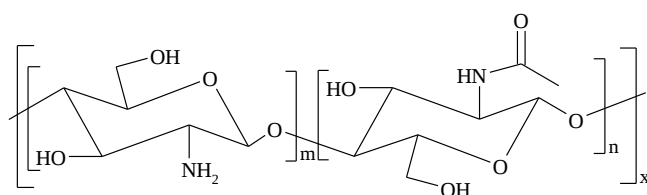


Figure 18. Chemical structure of chitosan.

Chitosan is not largely present as such in the nature and thus cannot be directly extracted from natural resources. Indeed, chitosan is a derivative of natural chitine, the second most abundant polysaccharide in nature after cellulose (112). Typically, chitosan is obtained by deacetylation of the *N*-acetyl glucosamine units of chitine, generally by hydrolysis under alkali conditions at high temperature. The deacetylation of chitine is rarely complete. When

[†] Adapted from a review I wrote with Raphaël Riva(110) Raphael Riva, H. R., Anne des Rieux, Nicolas Duhem, Christine Jerome, and Veronique Preat. (2011) Chitosan and Chitosan Derivatives in Drug Delivery and Tissue Engineering. *Advance in Polymeric Sciences*, 19-44.

the acetylation degree falls under the value of 60 mol%, chitine becomes chitosan. In nature, chitine is present in life forms and more particularly in insect or crustacean where it represents the major component of their exoskeleton. Chitine is also present into the cell wall of some mushrooms (117, 118). Generally, chitosans produced starting from mushrooms present a narrow molecular weight distribution compared to chitosan produced from shrimps.

Chitosan offers remarkable biological properties, which paved the way to its application in the pharmaceutical and biomedical fields (119, 120) as new drug delivery systems (121-123) or a scaffold for tissue engineering (124). Indeed, chitosan has good mucoadhesive properties due to its positive charge (125), which increases the adhesion to mucosa and so the time of contact for a drug penetration. Its haemostatic property makes chitosan a good candidate for wound dressing (126, 127). Moreover, the antibacterial property of chitosan also limits the risk of infection (128, 129).

Chitosan is a polycation whose charge density depends on the degree of acetylation and pH. So, chitosan chains are able to interact by electrostatic interactions with negatively charged molecules. It can form nanoparticles by ionic gelation with polyphosphate (130) and with nucleic acids (131-133).

Nevertheless, chitosan suffers from a poor solubility into water which is a major drawback for drug formulations. Indeed, chitosan is only soluble in acidic solutions of pH below 6.5 required to insure the protonation of the primary amine. In such case, the presence of positive charges onto the chitosan skeleton increases the repulsion between the different polymer chains, facilitating their solubilization. As far as organic solvents are concerned, chitosan is slightly soluble in dimethylsulfoxide (DMSO) and *p*-toluene sulfonic acid (134). This poor solubility is a limitation for the processing of chitosan and is also a brake in its chemical modification. In order to tackle this drawback, chitosan oligomers are sometimes preferred. These oligomers (polymerization degree around 20) are much more soluble into water compared to their polymer counterpart even at physiological pH. Several methods for the synthesis of chitosan oligomers are reported that are mainly based on an acidic hydrolysis at high temperature. Nevertheless, final hydrolysis yields are often low and lead

to a mixture of products (oligomers, glucosamine monomers) that must be purified (135, 136).

An important aspect for the application of chitosan in drug delivery systems is the fate of the chitosan in the body after absorption or injection. Generally, chitosan is eliminated by renal clearance but if the molecular weight is too large a degradation step by enzymes is required (137). In the human body, three chitinases showed an activity leading to the formation of smaller chains (137). Nevertheless, the rate of degradation depends on the molecular weight and the acetylation degree of the starting material (138).

III.2. CHEMICAL GRAFTING OF CHITOSAN WITH HYDROPHOBIC GROUPS

In order to improve or to impart new properties to chitosan, chemical modifications of the chitosan chains generally by either grafting of small molecules or polymer chains onto the chitosan backbone or by quaternization of the amino groups were investigated. Chitosan chains possess three attractive reactive sites for chemical modification: two hydroxyl groups (primary or secondary) and one primary amine by glucosamine units. Because the amino group is more reactive than the hydroxyl groups, most of the research have described development of amphiphilic chitosan based on the chemical grafting of hydrophobic groups on the amine functional group by N-acylation reactions (139).

With the purpose to prepare chitosan based amphiphilic copolymer with only natural and renewable compounds, the grafting of fatty acids was investigated by several research groups (140-143). Generally, the grafting occurred by formation of an amide bond between the primary amine of chitosan and the terminated carboxylic acid of the fatty acid mediated by *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide (EDC) in a water/alcohol mixture under vigorous stirring (Figure 19).

Saturated stearic acid (141, 144-146) and unsaturated lineoic acid (142, 143) are two examples of fatty acids successfully grafted onto chitosan oligomers by this strategy (Figure 19). With the same purpose, Zhang et al preferred to firstly convert the carboxylic acid of oleic acid into acid chloride before to be reacted with chitosan in chloroform in presence of pyridine (147). With a similar strategy, stearic, palmitic or octanoic anhydride were grafted onto chitosan (140).

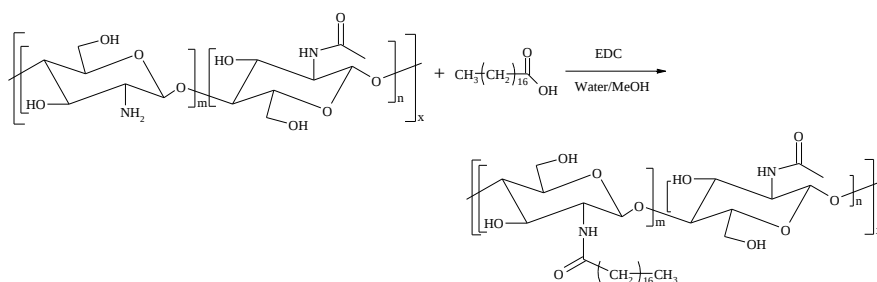


Figure 19. Grafting of stearic acid onto chitosan

Steroid derivatives were also employed as natural compound able to confer amphiphilic property to chitosan. Several examples of grafting of 5 β -cholanic acid (148-151) and cholesterol (152) onto *O*-glycol-chitosan are reported into the literature (Figure 20). For steroids, the strategy relies on the activation of the carboxylic acid by *N*-hydroxysuccinimide (NHS) in order to favor the grafting efficiency on the primary amine of glycol chitosan mediated by EDC.

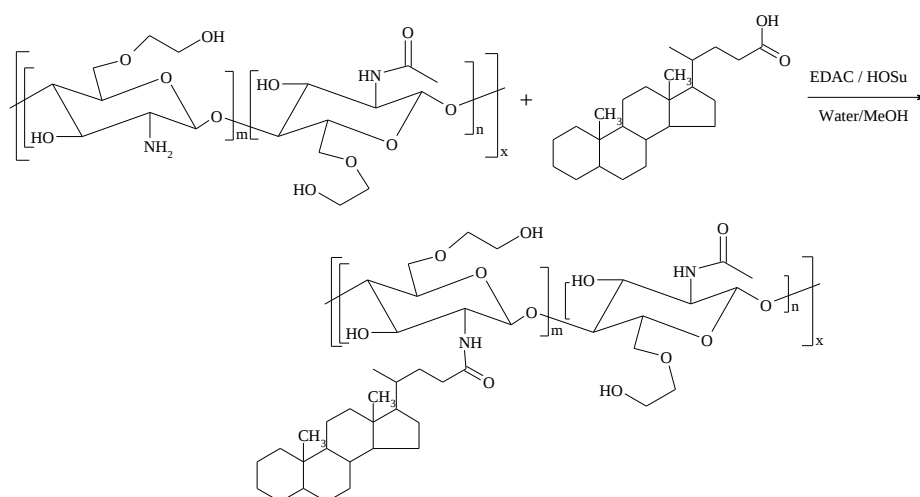


Figure 20. Grafting of cholanic acid onto *O*-glycol-chitosan

Other hydrophobic moieties like aryl, alkyl and polycaprolactone have been grafted to the chitosan backbone. The synthesis of these derivatives relies on complex chemistry and is beyond the scope of this research.

III.3. CHITOSAN DERIVATIVES FOR THE DELIVERY OF POORLY SOLUBLE DRUGS

Chitosan and chitosan derivatives have been extensively studied for drug delivery and other biomedical applications due to (i) their biocompatibility (ii) and their possible formulation in

nanoparticles or in gels (iii) their cationic properties. An overview of their use in biomedical applications will be illustrated for solubilisation of poorly soluble drugs.

Amphiphilic copolymers present a double affinity for both hydrophilic and hydrophobic environments and are then able to self-organize into water to form, in most cases, specific architectures such as micelles, nanoparticles or vesicles, which can be used as carrier in drug delivery systems (Figure 21). The supramolecular organization in water generates small hydrophobic domains well dispersed inside the solution: the self-assembled nanosized colloidal particles display a hydrophobic core surrounded by hydrophilic outer shell in aqueous conditions, which allows the solubilization of hydrophobic drugs. Indeed, the inner core can serve as a nano-container for poorly soluble drugs. Micelles as drug carriers provide a set of advantages: increase water-solubility of sparingly soluble drug, improvement of bioavailability, reduction of toxicity, enhancement of permeability across the physiological barriers, changes in drug biodistribution (153). Because intravenous injections of micellar solution induce extreme dilutions by blood, polymer micelles could disassemble and release the loaded drugs. However, their thermodynamic and kinetic stabilities are usually higher than surfactant ones (144).

Hydrophobically modified chitosan derivatives have been designed to increase the solubility of poorly soluble drugs. However, chitosan is not optimal as the hydrophilic part of an amphiphilic self-assembling polymer because it is only soluble in acidic aqueous solutions with pH values lower than its pKa value (6.5). Hence, glycolchitosan has been used to synthesize new amphiphilic chitosan-based polymers. These amphiphilic glycolchitosan derivatives are expected to self-aggregate and to ensure the solubility of poorly soluble drugs with a better stability in physiological conditions than chitosan derivatives.

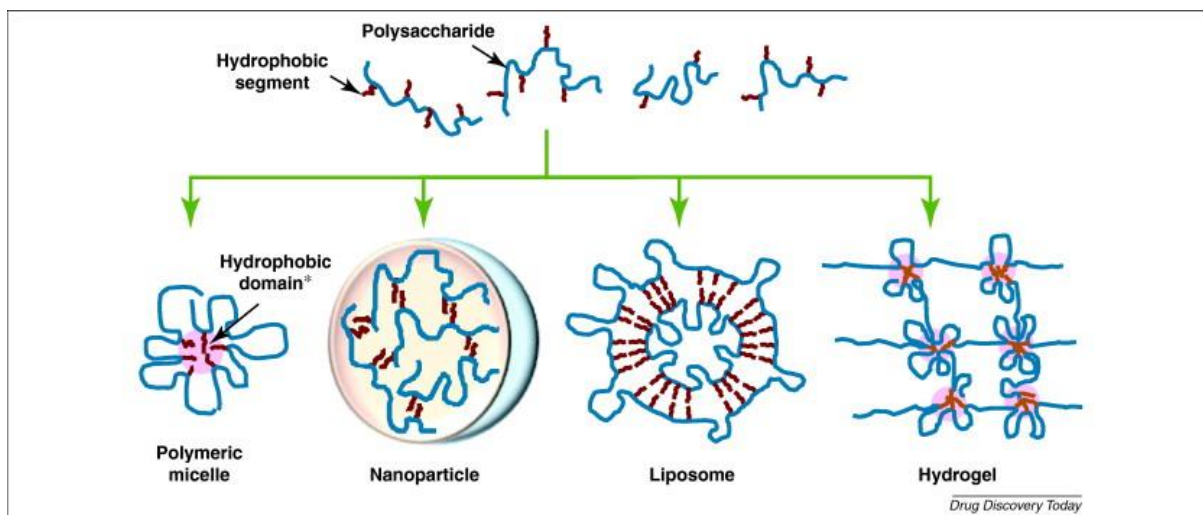


Figure 21. Schematic depiction of different drug delivery systems formed by self-association of amphiphilic polysaccharides in aqueous solution (139)

Four major groups of hydrophobically modified chitosan have been used as potential drug delivery carrier for poorly soluble drugs (i) steroid derivatives; (ii) fatty acids derivatives; (iii) aryl and alkyl derivatives; and (iv) carboxymethyl derivatives of chitosan (Figure 3-6). Others types of modified chitosan have also been synthesized (154, 155).

Many of the poorly soluble drugs included in amphiphilic chitosan based nanocarriers are anticancer drugs e.g., PTX, DOX, CPT or Mytomycin C. Besides increasing their solubility, the polymeric micelles allow passive targeting in the tumor by the EPR effect. This is a form of selective delivery termed as “passive targeting” (153). In addition of the size, the stability of the nanoparticles is an important parameter for a successful passive targeting. If the particles circulate in the bloodstream for longer periods, they can reach tumor sites more effectively. Some other therapeutic agents were also studied like anti-HIV, antifungal, corticosteroids, nonsteroidal anti-inflammatories and proteins.

The drug loading in the polymeric nanocarriers is generally achieved by a method requiring the use of organic solvent to dissolve the drug: dialysis, oil-in-water emulsion solvent evaporation and solid dispersion.

The main steroids used to modify hydrophobically chitosan are the 5- β cholic acid (148, 156), cholic acid (157) and cholesterol (152, 158). Hydrophobically modified glycol chitosan (HGC) with 5- β cholic acid has been extensively studied both in vitro and in vivo. This polymer was developed as a new cremophor EL-free alternative carrier systems for DOC (150) and PTX. Physical characteristics of the nanoparticles such as size, hydrophobic cores

and stability depend on the degree of 5- β cholanic acid substitution. The maximum loading content of PTX into HGC nanoparticles was 10 % wt % and the loading efficiency was above 90 % (156). Cytotoxicity studies on MCF7 breast cancer cells showed that HGC nanoparticles were less toxic than Cremophor EL, and allowed higher dose of PTX administration. The survival rate of mice that received 50 mg/kg PTX in HGC nanoparticles increased substantially compared to 20 mg/kg PTX in Cremophor EL-ethanol solutions (156).

Different fatty acids were used to generate amphiphilic chitosan derivatives: linoleic acid (142, 143), stearic acid (144, 146) and oleic acid (147). The stability of the micellar structure can be controlled by adjusting the balance between hydrophobic acyl groups and hydrophilic chitosan in an N-acyl chitosan. The CMC of chitosan modified with the smaller acyl chain length like octanoyl was weaker than longer chain length like stearyl because their hydrophobicity was poorer (140). Hence, the most studied fatty acid grafted on chitosan is stearic acid, especially stearic acid grafted chitosan oligosaccharides (CSO-SA). CSO-SA has been studied for the solubilization several molecules including lamivudine stearate (145), 10-hydroxycamptothecin (159), mytomicin (146), DOX (147) and DNA (141). As CSO-SA can rapidly release the drug by dilution, stearic acid was solubilized into the core of CSO-SA micelles and significantly reduced DOX release because the enhanced hydrophobic interaction between stearic acid and stearic acid segments in CSO-SA form a tightly packed hydrophobic core, and because the ionic interaction between stearic acid and DOX (144). A second way to reduce the initial burst drug release from CSO-SA micelles was to crosslink the shell of CSO-SA micelles by glutaraldehyde. The drug release could be highly controlled by the shell cross-linking of the micelles without affecting the cellular uptake and drug encapsulation efficiency of CSO-SA micelles (146). Pegylation of CSO-SA used to confer for stealthiness did not affect the cellular uptake of the micelles by cancer cells, and significantly reduced the internalization of the CSO-SA micelles into macrophage (160).

N-m-PEG-*N*-octyl-*O*-sulfate chitosan (mPEGOSC) were synthesized with various PEG chain lengths and various degrees of substitution to entrap PTX. One of the derivatives was able to increase by 3 orders of magnitude of the concentration of PTX. Solubilization performance was influenced by crystallinity: the lower degree of crystallinity, the higher entrapment efficiency (161). The micelle dissociation in plasma proceeded very rapidly for the first 5 min and then slowed down. The micelles based on pegylated chitosan greatly decreased the

accumulation in the liver and the spleen and slowed down the elimination of PTX in the later stage of IV injection (162). PTX-loaded *N*-octyl-*O*-glycol chitosan micelles showed lower toxicity and higher maximum tolerated dose than Taxol (163). Others alkyl chitosan like *N*-succinyl-*N*-octyl chitosan (164) and *N*-octyl-*N*-trimethyl chitosan have been studied (165).

Phthaloylchitosan is a typical aryl modified chitosan developed to improve the solubility of poorly soluble drug like CPT (166), retinoic acid (167) or prednisone acetate (168). Phthaloylchitosan showed concentration dependent cytotoxicity in Hela cells whereas none of the *N*-phthaloylchitosan-grafted poly(ethylene glycol) methyl ether (PLC-*g*-mPEG) micelles were cytotoxic in vitro (166). PLC-*g*-mPEG improved the stability of a light-sensitive drug, all-trans retinoic acid from photodegradation (167).

Functionalization of chitosan with polycaprolactone (PCL) led to the synthesis of chitosan-polycaprolactone (CS-PCL) and ternary derivative, Chitosan-*g*-PCL-mPEG (CPP) (169-171). Spherical micelles were formed through self-assembly of CPP in aqueous media. Encapsulation efficiency higher than 5% could be achieved. The micelles can be subjected to glutaraldehyde treatment to prolong the release of the incorporated drugs (171). The importance of substituent grafting was again highlighted as an important factor for the morphology and the behavior of the nanoparticles (172, 173).

Our group tried to synthesize similar derivatives (chitosan-PCL, cholic acid-chitosan) by using methods based on these publications. Nevertheless, we have not succeeded yet due for several reasons including the degradation of the reactants. Moreover it appears that some of the publications contain protocols errors like using acidic environment to graft hydrophobic moiety through a peptidic bond or using insufficient quantity of reactants in comparison to the substitution degree determined. To my view, the results of these publications are highly speculative and should be interpreted carefully.

IV. SELF-ASSEMBLED PRODRUGS

A prodrug is most often an inactive form of a known drug and is obtained by the conjugation of the parent therapeutic agent and a promoiety. The chemical modification helps to overcome certain inherent hurdles of the parent drug. It can contribute to optimize its pharmacokinetics properties, e.g. targeting-ligand conjugated prodrugs to improve tumor targetability (174, 175). Prodrugs must undergo biotransformation prior to exhibiting their pharmacological effects. Most prodrugs are synthesized through chemical group transformation, which subsequently require a one- or two-step enzyme-mediated or chemical transformation to produce the parent drug (16, 174, 176).

When designing prodrugs, four key factors have to be considered: (i) availability of functional groups in the pristine drug which enables routine functional group transformation reactions under mild-conditions; (ii) a promoiety which should be non-toxic and promote stable and reliable prodrug self-assembly in physiological conditions; (iii) a linker functional-group which releases the pristine drug through either enzyme-mediated or hydrolytic degradation; and (iv) degradation by-products which does not produce ancillary bioactivity or toxicity (174).

Self-assembled prodrugs are designed to overcome the use of carrier. Routinely encountered hurdle related to carriers are low drug loading capacity, drug leakage during encapsulation, inefficient preservation and degradation of the carriers *in vivo* before reaching the targeted site. Self-assembled prodrugs can reach maximum drug loading by forming hierarchical nanostructures like micelles, cubosomes, or vesicles. Self-assembled prodrugs have also the potential to undergo much slower degradation compared to the free molecular prodrug form, enhancing controlled delivery for longer periods of time (174).

Most of the self-assembled prodrugs belong to one of the two following categories: (i) lipid-based and (ii) polymer-based self-assembled prodrugs. The combined approach of prodrugs and nanoparticles has a peculiar attraction for developing chemotherapies because it allows high drug loading and tumor targeting. Thus, most of the self-assembled prodrugs have been studied for potential clinical application in cancer treatment.

IV.1. LIPID-BASED SELF-ASSEMBLED PRODRUGS

The concept of lipid-drug conjugates has gained attention since in some case these bioconjugates have been shown to improve pharmacokinetics, decrease toxicity and increase the therapeutic index (177). Self-assembled lipid-prodrugs remain uncommon drug delivery systems and most of the lipid-drug conjugates need a carrier (176). The main lipid-based self-assembled prodrugs have been designed by using one of the following lipid moiety: (i) squalene; (ii) steroid, and (iii) phytanyl.

IV.1.1. Squalene-based prodrugs

Couvreux et al. have shown that when covalently bind to various drugs, like PTX (178-180), cisplatin (178), DOX (178) and nucleosides (181-189), squalene produced a bioconjugate that self-organized into nanoassemblies of 100-300 nm in water (177). Most of the time the resulting nanoassemblies showed an improved pharmacokinetic profile compared to the parent drug (177). Multifunctional nanocarriers composed of anticancer squalenoyl prodrug and a MRI agent (i.e., ultra small particles of iron oxide or gadolinium complex conjugated with squalene) have also been studied (177, 178).

The primary advantage of these conjugated are their high drug content. For example, a drug loading as high as 41wt/wt may be determined for the gemcitabine-squalene derivative. In comparison, the best 2'-3'-dideoxycytidine (ddC) loading efficiency reported with liposomes was only 8.3% wt/wt (177).

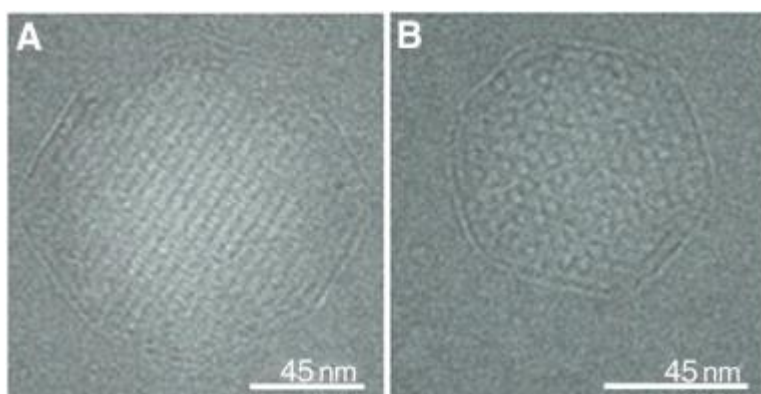


Figure 22. Cryo-TEM images of squalenoyl-gemcitabine (A and B). Nanoassemblies of spherical shape are surrounded by an external shell and display an internal structure (183)

The supramolecular organization of some squalenoyl analogues was studied. For example, Squalenoyl-gemcitabine self-assembled in inverse hexagonal phases in which the central aqueous core was made of water and gemcitabine surrounded by the squalene moieties (Figure 22). Supramolecular organization of squalene nanoassemblies was shown to be dependent on the chemical structure of the drug used (177, 180, 183).

Squalenoylation may be a strategy to overcome different mechanisms of resistance in cancer cells (177, 184, 190). For instance, gemcitabine requires membrane proteins named nucleoside transporters (hENT1) to enter the cells. Squalenoyl-gemcitabine was used to overcome cancer cell resistance due to nucleoside transport deficiency as it is transporter-independent. Squalenoyl-gemcitabine nanoassemblies were shown to be more cytotoxic on the gemcitabine-cross resistant CEM/ARAC8C cell line. Resistance on CEM/ARAC8C cells line decreased by a factor of 66 when treated with squalenoyl-gemcitabine (177).

The anticancer activity of squalenoyl-gemcitabine has been extensively studied *in vivo* (183, 184, 190-194). Squalenoyl-gemcitabine nanomedicines were much more effective than gemcitabine in increasing the survival time of aggressive metastatic leukemia L1210 bearing mice after IV administration. Only the treatment with squalenoyl-gemcitabine led to long-term survivals due to a significantly higher concentration of drug in major metastatic organs like spleen and liver (177, 190, 192). The anticancer activity of squalenoyl-gemcitabine was further tested on an experimental pancreatic adenocarcinoma model. Gem-Sq-treated Panc1 tumor xenografts diminished by 65% compared to saline-treated mice, significantly higher than the 35% decrease attained by gemcitabine (184). Squalenoyl-gemcitabine was also more efficient than gemcitabine in increasing the life span and the number of long-term survivors after oral administration to rats bearing large granular lymphocytic leukemia. Improved anticancer activity of squalenoyl-gemcitabine nanoparticles when administered orally may be due to an improved resistance to deamination, improved pharmacokinetics, and increased accumulation in the lymphoid organs (177, 194). These data are supporting the candidature of squalenoyl-gemcitabine for clinical trials.

However some squalenoyl analogues were less effective or non-effective in comparison with the parent drug. For instance, 5'-trisinorsqualenoyl thymidine nanoassemblies did not display any activity *in vitro* on HIV-1-infected lymphocytes (193). PTX-squalene derivatives with

different PEG linkage showed much lower cytotoxicity than PTX toward M109 cancer cell line (179). Only *cis,cis*-squalenoyl-deca-5,8-dienoate prodrug showed comparable antitumor efficacy to the parent drug with a much lower subacute toxicity based on the body weight loss (180). These examples highlight the importance of cell metabolism to activate the release of the parent drug.

IV.1.2. Steroid-based self-assembled prodrugs

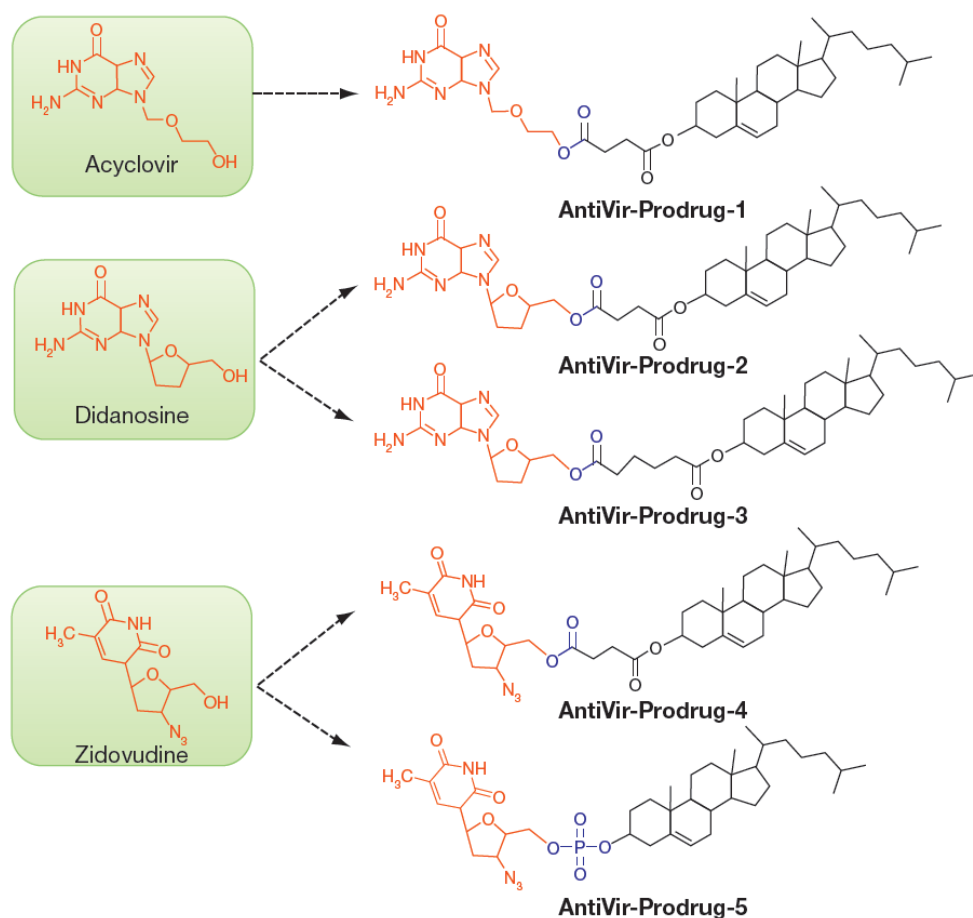


Figure 23. A series of amphiphilic prodrugs prepared from nucleobase-antiviral agents acyclovir, didanosine, and zidovudine (174)

Antiviral nucleoside analogs with either purine-type (i.e., guanine in AntiVir-Prodrug-1, hypoxanthine in AntiVir-Prodrug-2 and AntiVir-Prodrug-3) or pyrimidine-type nucleobase heads (thymine in AntiVir-Prodrug-4 and AntiVir-Prodrug-5) have been conjugated to a steroid-based cholesterol through a dicarboxylic acid that could promote the formation of nanosized monodispersed vesicles (Figure 23) (174, 195-198). The nanoparticles were obtained by dissolving the prodrugs in tetrahydrofuran, and then adding to water to initiate

hydrophobic interactions with promoiety groups and intermolecular hydrogen bonding with nucleobase headgroups. Nevertheless, AntiVir-Prodrug-2 and AntiVir-Prodrug-3 failed to undergo degradation in physiological media to deliver the parent drug and to show therapeutic utility. These data highlight the importance of releasing parent drugs (174, 197, 198).

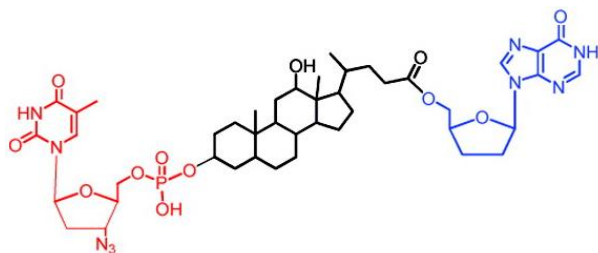


Figure 24. Structure of zidovudine-phosphoryl-deoxycholyl didanosine (199)

More recently a new self-assembled bolaamphiphilic derivative was synthesized (Figure 24). Bolaamphiphilic molecules are one special type of amphiphilic molecules defined as the molecules containing a hydrophobic skeleton and two water-soluble groups on both ends, here zidovudine-phosphoryl-deoxycholyl didanosine (ZPDD). ZPDD combines zidovudine and didanosine in one molecule with deoxycholyl as spacer for combination anti-HIV therapy (199).

IV.1.3. Phytanyl-based self-assembled prodrug

An amphiphilic prodrug, 5'-deoxy-5-fluoro-N4-(phytanyloxycarbonyl) cytidine (5-FCPhy) has been designed to overcome the low absorption rate and low bioavailability of 5-fluorouracil (5-FU) (Figure 25-A). 5-FCPhy successfully self-assembled in a single 250 nm cubosomes after consecutive membrane extrusion (Figure 25-B). The dispersion was stabilized against aggregation by the addition of a nonionic triblock copolymer (Pluronic F127). It has been shown that 5-FCPhy displayed similar toxicity levels to capecitabine, the commercially available 5-FU prodrug, and significantly lower toxicity levels than 5-FU *in vitro*. The formulation of 5-FCPhy in nanoparticles showed some advantages over capecitabine *in vivo*. Enzymatic conversion of 5-FCPhy to its active form occurred at a slower rate than capecitabine which may allow a more sustained delivery and thus may not need to be delivered as often as capecitabine (200).

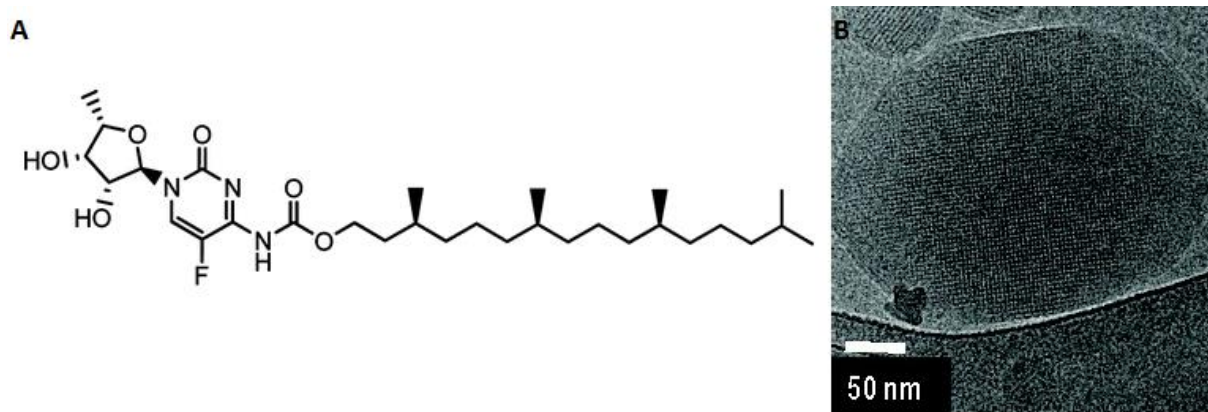


Figure 25. (A) Chemical structure of the amphiphile prodrug 5'-deoxy-5-fluoro-N4-(phytanyloxycarbonyl) cytidine (5-FCPhy) and (B) Cryo-TEM image of 5-FCPhy dispersion (200).

IV.2. POLYMERIC SELF-ASSEMBLED PRODRUGS

Polymer–drug conjugates are drug delivery systems in which a drug is covalently bound to a polymeric carrier, mostly via a biodegradable linker. Most of them have been developed for cancer therapy (201). The main advantages of polymer–drug conjugates compared to the parent drug are: (i) tumour targeting by EPR effect, (ii) decreased toxicity, (iii) better solubility in biological fluids, and (iii) ability to overpass some mechanisms of drug resistance(175, 202).

Table 6. Polymer-drug conjugates (203)

Model Drug	Promoiety	Cancer targeted	Ref.
Paclitaxel	Heparine	Breast	(204)
	Hyaluronic acid	Breast	(205)
	Hyperbranched poly(ether-ester)	Breast	(206)
	Poly(glutamate)	various	(201)
Cisplatin	Poly lactide	Prostate	(207)
Curcumin	Oligo(ethylene glycol)	Ovary	(208)
Doxorubicin	PEG-poly(aspartate)	Various	(209, 210)
	Folate-PEG-poly(aspartate)	Pharyngeal	(211)
	PEO-b-P(CL)	Melanoma	(212)
	RGD4C-attached PEO-b-P(CL)	Breast	(213)
	Au-PEG-NH ₂	Liver	(214)
	Xyloglucan-galactosamine	Liver	(215)
	TPGS	Breast/melanoma	(50, 94)
	HPMA	Liver	(216)
Tubulysin	Beta-cyclodextrin	Colon	(217)

There are two types of polymer conjugates used as nanomedicines: (i) polymer-protein conjugates and (ii) polymer-drug conjugates (201, 203) (Table 6).

Polymer-protein conjugates are already used in cancer therapy like SMANCS, made by covalently linked the anti-tumour protein neocarzinostatin to two styrene maleic anhydride polymer chains, and PEGylated proteins like PEG-L-asparaginase. They are used routinely as anticancer therapeutics, as an adjuvant to chemotherapy and as components of combination therapies (201).

Polymer-drug conjugates have been extensively studied. They can self-organize in micelles, nanoparticles or micelle-like nanoassemblies. Many polymers have been proposed as drug carrier but few have progressed to *in vivo* or clinical studies. Problems prohibiting clinical development include polymer-related toxicity and/or polymer-related immunogenicity, inadequate drug loading or choice of drug with too low potency, use of unsuitable polymer-drug linker. Some polymer–drug conjugates have shown successful results (203, 218). The most relevant example is the polyglutamic acid-paclitaxel conjugate (Opaxio) which is expected to enter the market in the very near future (219).

V. THESIS BACKGROUND

The thesis was based on the utilization of nanomedicines and vitamin E to efficiently deliver drugs. On one hand, nanomedicines present numerous advantages in drug delivery. Among these advantages, the most important are: (i) they are appropriate to all route of administration; (ii) they improve the biopharmaceutical properties of the drugs they carry like increasing the drug solubility, increasing the drug permeability and improving the drug pharmacokinetics; (iii) in cancer therapy, they enhance drug delivery to cancer cells through passive targeting thanks to the EPR effect, through active targeting and through the ability to bypass drug resistance (2). On the other hand, vitamin E, was used, as its properties may be useful for both drug solubilization and cancer therapy. Vitamin E and its derivatives have been studied in drug delivery due to (i) their biocompatibility which is a critical for biomaterials to be used as nanocarrier in drug delivery (ii) their ability to solubilize drugs which are tocophilic like itraconazole, cyclosporine or paclitaxel; (iii) their biological activity as demonstrated by pegylated vitamin E which is able to act on P-gp pump and vitamin E succinate which shows anticancer activity (27, 28, 30).

Therefore, new vitamin E-based nanocarriers have been developed and are described over the following chapters. First, a new amphiphilic vitamin E-modified chitosan able to spontaneously form micelles was synthesized to improve the water solubility of hydrophobic drugs. Second, a new self-assembling doxorubicin-tocopherol succinate prodrug was synthesized as a new drug delivery system to overcome doxorubicin limitations. The last chapters address the conclusions of the vitamin E-based nanocarrier studies as well as the perspectives of development of these nanomedicines.

VI. REFERENCES

- (1) Plapied L., Duhem N., des Rieux A. and Pr  at V. (2011) Fate of polymeric nanocarriers for oral drug delivery. *Current Opinion in Colloid & Interface Science*, 16, 228-237.
- (2) Farokhzad, O. C., and Langer, R. (2009) Impact of nanotechnology on drug delivery. *ACS nano*, 3, 16-20.
- (3) Youan, B. B. (2008) Impact of nanoscience and nanotechnology on controlled drug delivery. *Nanomedicine*, 3, 401-406.
- (4) Singh, R., and Lillard, J. W., Jr. (2009) Nanoparticle-based targeted drug delivery. *Experimental and molecular pathology*, 86, 215-223.
- (5) Hall, J. B., Dobrovolskaia, M. A., Patri, A. K., and McNeil, S. E. (2007) Characterization of nanoparticles for therapeutics. *Nanomedicine*, 2, 789-803.
- (6) Kumari, A., Yadav, S. K., and Yadav, S. C. (2010) Biodegradable polymeric nanoparticles based drug delivery systems. *Colloids and surfaces. B, Biointerfaces*, 75, 1-18.
- (7) Danhier, F., Feron, O., and Preat, V. (2010) To exploit the tumor microenvironment: Passive and active tumor targeting of nanocarriers for anti-cancer drug delivery. *Journal of controlled release*, 148, 135-146.
- (8) Steichen, S. D., Caldorera-Moore, M., and Peppas, N. A. (2012) A review of current nanoparticle and targeting moieties for the delivery of cancer therapeutics. *European journal of pharmaceutical sciences*, 48, 416-427.
- (9) Bamrungsap, S., Zhao, Z., Chen, T., Wang, L., Li, C., Fu, T., and Tan, W. (2012) Nanotechnology in therapeutics: a focus on nanoparticles as a drug delivery system. *Nanomedicine*, 7, 1253-1271.
- (10) Bharali, D. J., Khalil, M., Gurbuz, M., Simone, T. M., and Mousa, S. A. (2009) Nanoparticles and cancer therapy: a concise review with emphasis on dendrimers. *International journal of nanomedicine*, 4, 1-7.
- (11) Vijayakumar, M. R., Muthu, M. S., and Singh, S. (2013) Copolymers of poly(lactic acid) and D-alpha-tocopheryl polyethylene glycol 1000 succinate-based nanomedicines: versatile multifunctional platforms for cancer diagnosis and therapy. *Expert opinion on drug delivery*, 10, 529-543.
- (12) Yamanaka, Y. J., and Leong, K. W. (2008) Engineering strategies to enhance nanoparticle-mediated oral delivery. *Journal of biomaterials science*, 19, 1549-1570.
- (13) Gaucher, G., Satturwar, P., Jones, M. C., Furtos, A., and Leroux, J. C. (2010) Polymeric micelles for oral drug delivery. *European journal of pharmaceuticals and biopharmaceutics*, 76, 147-158.
- (14) Ruenraroengsak, P., Cook, J. M., and Florence, A. T. (2010) Nanosystem drug targeting: Facing up to complex realities. *Journal of controlled release*, 141, 265-276.
- (15) Shugarts, S., and Benet, L. Z. (2009) The role of transporters in the pharmacokinetics of orally administered drugs. *Pharmaceutical research*, 26, 2039-2054.
- (16) Rautio, J., Kumpulainen, H., Heimbach, T., Oliyai, R., Oh, D., Jarvinen, T., and Savolainen, J. (2008) Prodrugs: design and clinical applications. *Nature reviews. Drug discovery*, 7, 255-270.
- (17) Anne des Rieux, Virginie Fievez, Marie Garinot, Yves-Jacques Schneider, V  ronique Pr  at. (2006) Nanoparticles as potential oral delivery systems of proteins and vaccines: a mechanistic approach. *Journal of Controlled Release*, 116, 1-27.

- (18) Salman, H. H., Gamazo, C., Agueros, M., and Irache, J. M. (2007) Bioadhesive capacity and immunoadjuvant properties of thiamine-coated nanoparticles. *Vaccine*, 25, 8123-8132.
- (19) Salman, H. H., Gamazo, C., de Smidt, P. C., Russell-Jones, G., and Irache, J. M. (2008) Evaluation of bioadhesive capacity and immunoadjuvant properties of vitamin B(12)-Gantrez nanoparticles. *Pharmaceutical research*, 25, 2859-2868.
- (20) Borges, O., Borchard, G., Verhoef, J. C., de Sousa, A., and Junginger, H. E. (2005) Preparation of coated nanoparticles for a new mucosal vaccine delivery system. *International journal of pharmaceutics*, 299, 155-166.
- (21) Petros, R. A., and DeSimone, J. M. (2010) Strategies in the design of nanoparticles for therapeutic applications. *Nature reviews. Drug discovery*, 9, 615-627.
- (22) Davis, M. E., Chen, Z. G., and Shin, D. M. (2008) Nanoparticle therapeutics: an emerging treatment modality for cancer. *Nature reviews. Drug discovery*, 7, 771-782.
- (23) Jain, R. K., and Stylianopoulos, T. (2010) Delivering nanomedicine to solid tumors. *Nature reviews. Clinical oncology*, 7, 653-664.
- (24) Torchilin, V. P. (2010) Passive and active drug targeting: drug delivery to tumors as an example. *Handbook of experimental pharmacology*, 3-53.
- (25) Mustacich, D. J., Bruno, R. S., and Traber, M. G. (2007) Vitamin E. *Vitamins and hormones*, 76, 1-21.
- (26) Wong, R. S., and Radhakrishnan, A. K. (2012) Tocotrienol research: past into present. *Nutrition reviews*, 70, 483-490.
- (27) Guo, Y., Luo, J., Tan, S., Otieno, B. O., and Zhang, Z. (2013) The applications of Vitamin E TPGS in drug delivery. *European journal of pharmaceutical sciences*, 49, 175-86
- (28) Kline, K., Lawson, K. A., Yu, W., and Sanders, B. G. (2007) Vitamin E and cancer. *Vitam Horm*, 76, 435-461.
- (29) D.A. White, I. D. F., D.A. Gray. (2006) Characterisation of oat (*Avena sativa* L.) oil bodies and intrinsically associated E-vitamins. *Journal of Cereal Science*, 43, 244-249.
- (30) Constantinides, P. P., Tustian, A., and Kessler, D. R. (2004) Tocol emulsions for drug solubilization and parenteral delivery. *Advanced drug delivery reviews*, 56, 1243-1255.
- (31) Constantinides, P. P., Han, J., and Davis, S. S. (2006) Advances in the use of tocopherols as drug delivery vehicles. *Pharmaceutical research*, 23, 243-255.
- (32) Combs, G. F. J. (2012) Vitamin E, In *The Vitamins, Fourth Edition* (Press, A., Ed.) pp 181-211, Elsevier.
- (33) Frank, J. (2005) Beyond vitamin E supplementation: an alternative strategy to improve vitamin E status. *Journal of plant physiology*, 162, 834-843.
- (34) Zingg, J. M. (2007) Vitamin E: an overview of major research directions. *Molecular aspects of medicine*, 28, 400-422.
- (35) Brigelius-Flohe, R. (2006) Bioactivity of vitamin E. *Nutrition research reviews*, 19, 174-186.
- (36) Atkinson, J., Harroun, T., Wassall, S. R., Stillwell, W., and Katsaras, J. (2010) The location and behavior of alpha-tocopherol in membranes. *Molecular nutrition & food research*, 54, 641-651.
- (37) Wada, S. (2012) Cancer preventive effects of vitamin E. *Current pharmaceutical biotechnology*, 13, 156-164.
- (38) Azzi, A., Ricciarelli, R., and Zingg, J. M. (2002) Non-antioxidant molecular functions of alpha-tocopherol (vitamin E). *FEBS letters*, 519, 8-10.

- (39) Brigelius-Flohe, R., Kelly, F. J., Salonen, J. T., Neuzil, J., Zingg, J. M., and Azzi, A. (2002) The European perspective on vitamin E: current knowledge and future research. *The American journal of clinical nutrition*, 76, 703-716.
- (40) Brigelius-Flohe, R. (2009) Vitamin E: the shrew waiting to be tamed. *Free radical biology & medicine*, 46, 543-554.
- (41) Brigelius-Flohe, R., and Traber, M. G. (1999) Vitamin E: function and metabolism. *FASEB journal*, 13, 1145-1155.
- (42) Zhang, Z., Tan, S., and Feng, S. S. (2012) Vitamin E TPGS as a molecular biomaterial for drug delivery. *Biomaterials*, 33, 4889-4906.
- (43) Prasad, K. N., Kumar, B., Yan, X. D., Hanson, A. J., and Cole, W. C. (2003) Alpha-tocopheryl succinate, the most effective form of vitamin E for adjuvant cancer treatment: a review. *Journal of the American College of Nutrition*, 22, 108-117.
- (44) Kline, K., Yu, W., and Sanders, B. G. (2004) Vitamin E and breast cancer. *The Journal of nutrition*, 134, 3458S-3462S.
- (45) Constantinou, C., Papas, A., and Constantinou, A. I. (2008) Vitamin E and cancer: An insight into the anticancer activities of vitamin E isomers and analogs. *International journal of cancer. Journal international du cancer*, 123, 739-752.
- (46) Yong-Heng Dong, Y.-H. G., Xin-Bin Gu. (2009) Anticancer mechanisms of vitamin e succinate. *Chinese Journal of Cancer*, 28, 1-10.
- (47) Wang, X. F., Dong, L., Zhao, Y., Tomasetti, M., Wu, K., and Neuzil, J. (2006) Vitamin E analogues as anticancer agents: lessons from studies with alpha-tocopheryl succinate. *Molecular nutrition & food research*, 50, 675-685.
- (48) Zingg, J. M. (2007) Modulation of signal transduction by vitamin E. *Molecular aspects of medicine*, 28, 481-506.
- (49) Dong, L. F., Jameson, V. J., Tilly, D., Prochazka, L., Rohlena, J., Valis, K., Truksa, J., Zobalova, R., Mahdavian, E., Kluckova, K., Stantic, M., Stursa, J., Freeman, R., Witting, P. K., Norberg, E., Goodwin, J., Salvatore, B. A., Novotna, J., Turanek, J., Ledvina, M., Hozak, P., Zhivotovsky, B., Coster, M. J., Ralph, S. J., Smith, R. A., and Neuzil, J. (2011) Mitochondrial targeting of alpha-tocopheryl succinate enhances its pro-apoptotic efficacy: a new paradigm for effective cancer therapy. *Free radical biology & medicine*, 50, 1546-1555.
- (50) Anbharasi, V., Cao, N., and Feng, S. S. (2010) Doxorubicin conjugated to D-alpha-tocopheryl polyethylene glycol succinate and folic acid as a prodrug for targeted chemotherapy. *Journal of biomedical materials research. Part A*, 94, 730-743.
- (51) Israel, K., Yu, W., Sanders, B. G., and Kline, K. (2000) Vitamin E succinate induces apoptosis in human prostate cancer cells: role for Fas in vitamin E succinate-triggered apoptosis. *Nutrition and cancer*, 36, 90-100.
- (52) Neuzil, J., Weber, T., Gellert, N., and Weber, C. (2001) Selective cancer cell killing by alpha-tocopheryl succinate. *British journal of cancer*, 84, 87-89.
- (53) Wu, K., Zhao, Y., Liu, B. H., Li, Y., Liu, F., Guo, J., and Yu, W. P. (2002) RRR-alpha-tocopheryl succinate inhibits human gastric cancer SGC-7901 cell growth by inducing apoptosis and DNA synthesis arrest. *World journal of gastroenterology : WJG*, 8, 26-30.
- (54) Anderson, K., Simmons-Menchaca, M., Lawson, K. A., Atkinson, J., Sanders, B. G., and Kline, K. (2004) Differential response of human ovarian cancer cells to induction of apoptosis by vitamin E Succinate and vitamin E analogue, alpha-TEA. *Cancer research*, 64, 4263-4269.

- (55) Zhang, X., Peng, X., Yu, W., Hou, S., Zhao, Y., Zhang, Z., Huang, X., and Wu, K. (2011) Alpha-tocopheryl succinate enhances doxorubicin-induced apoptosis in human gastric cancer cells via promotion of doxorubicin influx and suppression of doxorubicin efflux. *Cancer letters*, 307, 174-181.
- (56) Genentech website (2013) <http://www.biooncology.com/biological-pathways/apoptosis/>.
- (57) Malafa, M. P., and Neitzel, L. T. (2000) Vitamin E succinate promotes breast cancer tumor dormancy. *The Journal of surgical research*, 93, 163-170.
- (58) Malafa, M. P., Fokum, F. D., Smith, L., and Louis, A. (2002) Inhibition of angiogenesis and promotion of melanoma dormancy by vitamin E succinate. *Annals of surgical oncology*, 9, 1023-1032.
- (59) Malafa, M. P., Fokum, F. D., Mowlavi, A., Abusief, M., and King, M. (2002) Vitamin E inhibits melanoma growth in mice. *Surgery*, 131, 85-91.
- (60) Barnett, K. T., Fokum, F. D., and Malafa, M. P. (2002) Vitamin E succinate inhibits colon cancer liver metastases. *The Journal of surgical research*, 106, 292-298.
- (61) Neuzil, J., Weber, T., Schroder, A., Lu, M., Ostermann, G., Gellert, N., Mayne, G. C., Olejnicka, B., Negre-Salvayre, A., Sticha, M., Coffey, R. J., and Weber, C. (2001) Induction of cancer cell apoptosis by alpha-tocopheryl succinate: molecular pathways and structural requirements. *FASEB journal*, 15, 403-415.
- (62) Stapelberg, M., Gellert, N., Swettenham, E., Tomasetti, M., Witting, P. K., Procopio, A., and Neuzil, J. (2005) Alpha-tocopheryl succinate inhibits malignant mesothelioma by disrupting the fibroblast growth factor autocrine loop: mechanism and the role of oxidative stress. *The Journal of biological chemistry*, 280, 25369-25376.
- (63) Ramanathapuram, L. V., Hahn, T., Graner, M. W., Katsanis, E., and Akporiaye, E. T. (2006) Vesiculated alpha-tocopheryl succinate enhances the anti-tumor effect of dendritic cell vaccines. *Cancer immunology, immunotherapy : CII*, 55, 166-177.
- (64) Wu, K., Shan, Y. J., Zhao, Y., Yu, J. W., and Liu, B. H. (2001) Inhibitory effects of RRR-alpha-tocopheryl succinate on benzo(a)pyrene (B(a)P)-induced forestomach carcinogenesis in female mice. *World journal of gastroenterology : WJG*, 7, 60-65.
- (65) Wempe, M. F., Wright, C., Little, J. L., Lightner, J. W., Large, S. E., Caflisch, G. B., Buchanan, C. M., Rice, P. J., Wachter, V. J., Ruble, K. M., and Edgar, K. J. (2009) Inhibiting efflux with novel non-ionic surfactants: Rational design based on vitamin E TPGS. *International journal of pharmaceuticals*, 370, 93-102.
- (66) Collnot, E. M., Baldes, C., Wempe, M. F., Hyatt, J., Navarro, L., Edgar, K. J., Schaefer, U. F., and Lehr, C. M. (2006) Influence of vitamin E TPGS poly(ethylene glycol) chain length on apical efflux transporters in Caco-2 cell monolayers. *Journal of controlled release*, 111, 35-40.
- (67) Bansal, T., Akhtar, N., Jaggi, M., Khar, R. K., and Talegaonkar, S. (2009) Novel formulation approaches for optimising delivery of anticancer drugs based on P-glycoprotein modulation. *Drug discovery today*, 14, 1067-1074.
- (68) Collnot, E. M., Baldes, C., Wempe, M. F., Kappl, R., Huttermann, J., Hyatt, J. A., Edgar, K. J., Schaefer, U. F., and Lehr, C. M. (2007) Mechanism of inhibition of P-glycoprotein mediated efflux by vitamin E TPGS: influence on ATPase activity and membrane fluidity. *Molecular pharmaceuticals*, 4, 465-474.
- (69) Youk, H. J., Lee, E., Choi, M. K., Lee, Y. J., Chung, J. H., Kim, S. H., Lee, C. H., and Lim, S. J. (2005) Enhanced anticancer efficacy of alpha-tocopheryl succinate by conjugation with polyethylene glycol. *Journal of controlled release*, 107, 43-52.

- (70) Eastman. (2005) Eastman Vitamin E TPGS NF Applications and properties.
- (71) Chan, Y. P., Meyrueix, R., Kravtsoff, R., Nicolas, F., and Lundstrom, K. (2007) Review on Medusa: a polymer-based sustained release technology for protein and peptide drugs. *Expert opinion on drug delivery*, 4, 441-451.
- (72) Yi, Y., Kim, J. H., Kang, H. W., Oh, H. S., Kim, S. W., and Seo, M. H. (2005) A polymeric nanoparticle consisting of mPEG-PLA-Toco and PLMA-COONa as a drug carrier: improvements in cellular uptake and biodistribution. *Pharmaceutical research*, 22, 200-208.
- (73) Noh, S. M., Han, S. E., Shim, G., Lee, K. E., Kim, C. W., Han, S. S., Choi, Y., Kim, Y. K., Kim, W. K., and Oh, Y. K. (2011) Tocopheryl oligochitosan-based self assembling oligomersomes for siRNA delivery. *Biomaterials*, 32, 849-857.
- (74) Liang, N., Sun, S., Li, X., Piao, H., Piao, H., Cui, F., and Fang, L. (2012) alpha-Tocopherol succinate-modified chitosan as a micellar delivery system for paclitaxel: preparation, characterization and in vitro/in vivo evaluations. *International journal of pharmaceutics*, 423, 480-488.
- (75) Tao, Y., Han, J., Wang, X., and Dou, H. (2013) Nano-formulation of paclitaxel by vitamin E succinate functionalized pluronic micelles for enhanced encapsulation, stability and cytotoxicity. *Colloids and surfaces. B, Biointerfaces*, 102, 604-610.
- (76) Saxena, V., and Hussain, M. D. (2012) Poloxamer 407/TPGS mixed micelles for delivery of gambogic acid to breast and multidrug-resistant cancer. *International journal of nanomedicine*, 7, 713-721.
- (77) Zhao, L., Shi, Y., Zou, S., Sun, M., Lil, L., and Zhail, G. (2011) Formulation and in vitro evaluation of quercetin loaded polymeric micelles composed of pluronic P123 and D- α -tocopheryl polyethylene glycol succinate. *Journal of biomedical nanotechnology*, 7, 358-365.
- (78) Gao, Y., Li, L. B., and Zhai, G. (2008) Preparation and characterization of Pluronic/TPGS mixed micelles for solubilization of camptothecin. *Colloids and surfaces. B, Biointerfaces*, 64, 194-199.
- (79) Gill, K. K., Kaddoumi, A., and Nazzal, S. (2012) Mixed micelles of PEG(2000)-DSPE and vitamin-E TPGS for concurrent delivery of paclitaxel and parthenolide: enhanced chemosensitization and antitumor efficacy against non-small cell lung cancer (NSCLC) cell lines. *European journal of pharmaceutical sciences*, 46, 64-71.
- (80) Wang, J., Sun, J., Chen, Q., Gao, Y., Li, L., Li, H., Leng, D., Wang, Y., Sun, Y., Jing, Y., Wang, S., and He, Z. (2012) Star-shape copolymer of lysine-linked di-tocopherol polyethylene glycol 2000 succinate for doxorubicin delivery with reversal of multidrug resistance. *Biomaterials*, 33, 6877-6888.
- (81) Mi, Y., Liu, Y., and Feng, S. S. (2011) Formulation of Docetaxel by folic acid-conjugated d- α -tocopheryl polyethylene glycol succinate 2000 (Vitamin E TPGS(2k)) micelles for targeted and synergistic chemotherapy. *Biomaterials*, 32, 4058-4066.
- (82) Muthu, M. S., Kulkarni, S. A., Raju, A., and Feng, S. S. (2012) Theranostic liposomes of TPGS coating for targeted co-delivery of docetaxel and quantum dots. *Biomaterials*, 33, 3494-3501.
- (83) Muthu, M. S., Kulkarni, S. A., Xiong, J., and Feng, S. S. (2011) Vitamin E TPGS coated liposomes enhanced cellular uptake and cytotoxicity of docetaxel in brain cancer cells. *International journal of pharmaceutics*, 421, 332-340.

- (84) Wang, T., Yin, X., Lu, Y., Shan, W., and Xiong, S. (2012) Formulation, antileukemia mechanism, pharmacokinetics, and biodistribution of a novel liposomal emodin. *International journal of nanomedicine*, 7, 2325-2337.
- (85) Yu, Y., Wang, Z. H., Zhang, L., Yao, H. J., Zhang, Y., Li, R. J., Ju, R. J., Wang, X. X., Zhou, J., Li, N., and Lu, W. L. (2012) Mitochondrial targeting topotecan-loaded liposomes for treating drug-resistant breast cancer and inhibiting invasive metastases of melanoma. *Biomaterials*, 33, 1808-1820.
- (86) Raju, A., Muthu, M. S., and Feng, S. S. (2013) Trastuzumab-conjugated vitamin E TPGS liposomes for sustained and targeted delivery of docetaxel. *Expert opinion on drug delivery*.
- (87) Shen, J., Yin, Q., Chen, L., Zhang, Z., and Li, Y. (2012) Co-delivery of paclitaxel and survivin shRNA by pluronic P85-PEI/TPGS complex nanoparticles to overcome drug resistance in lung cancer. *Biomaterials*, 33, 8613-8624.
- (88) Shen, J., Sun, H., Xu, P., Yin, Q., Zhang, Z., Wang, S., Yu, H., and Li, Y. (2013) Simultaneous inhibition of metastasis and growth of breast cancer by co-delivery of twist shRNA and paclitaxel using pluronic P85-PEI/TPGS complex nanoparticles. *Biomaterials*, 34, 1581-1590.
- (89) Liu, Y., Huang, L., and Liu, F. (2010) Paclitaxel nanocrystals for overcoming multidrug resistance in cancer. *Molecular pharmaceutics*, 7, 863-869.
- (90) Shieh, M. J., Hsu, C. Y., Huang, L. Y., Chen, H. Y., Huang, F. H., and Lai, P. S. (2011) Reversal of doxorubicin-resistance by multifunctional nanoparticles in MCF-7/ADR cells. *Journal of controlled release*, 152, 418-425.
- (91) Song, N., Liu, W., Tu, Q., Liu, R., Zhang, Y., and Wang, J. (2011) Preparation and in vitro properties of redox-responsive polymeric nanoparticles for paclitaxel delivery. *Colloids and surfaces. B, Biointerfaces*, 87, 454-463.
- (92) Zhang, Z., Huey Lee, S., and Feng, S. S. (2007) Folate-decorated poly(lactide-co-glycolide)-vitamin E TPGS nanoparticles for targeted drug delivery. *Biomaterials*, 28, 1889-1899.
- (93) Sun, B., and Feng, S. S. (2009) Trastuzumab-functionalized nanoparticles of biodegradable copolymers for targeted delivery of docetaxel. *Nanomedicine*, 4, 431-445.
- (94) Cao, N., and Feng, S. S. (2008) Doxorubicin conjugated to D-alpha-tocopheryl polyethylene glycol 1000 succinate (TPGS): conjugation chemistry, characterization, in vitro and in vivo evaluation. *Biomaterials*, 29, 3856-3865.
- (95) Mi, Y., Zhao, J., and Feng, S. S. (2012) Vitamin E TPGS prodrug micelles for hydrophilic drug delivery with neuroprotective effects. *International Journal of Pharmaceutics*, 438, 98-106.
- (96) Mi, Y., Zhao, J., and Feng, S. S. (2013) Targeted co-delivery of docetaxel, cisplatin and herceptin by vitamin E TPGS-cisplatin prodrug nanoparticles for multimodality treatment of cancer. *Journal of controlled release*, 169, 185-92
- (97) Nielsen, P. B., Mullertz, A., Norling, T., and Kristensen, H. G. (2001) The effect of alpha-tocopherol on the in vitro solubilisation of lipophilic drugs. *International journal of pharmaceutics*, 222, 217-224.
- (98) Constantinides, C. P., Koutentis, P. A., and Schatz, J. (2004) A DFT study of the ground state multiplicities of linear vs angular polyheteroacenes. *Journal of the American Chemical Society*, 126, 16232-16241.

- (99) OncoGenex Pharmaceuticals website (2013)
<http://ir.oncogenex.com/releasedetail.cfm?ReleaseID=265739>.
- (100) Medicalnewstoday website (2013)
<http://www.medicalnewstoday.com/releases/83574.php>, Phase 3 Pivotal Trial Of TOCOSOL(R) Paclitaxel Does Not Meet Primary Endpoint.
- (101) Zhang; Yuehua , G. L. C. (2007) Tocopherol-modified therapeutic drug compounds. *US* 7,223,770.
- (102) Collnot, E. M., Baldes, C., Schaefer, U. F., Edgar, K. J., Wempe, M. F., and Lehr, C. M. (2010) Vitamin E TPGS P-glycoprotein inhibition mechanism: influence on conformational flexibility, intracellular ATP levels, and role of time and site of access. *Molecular pharmaceutics*, 7, 642-651.
- (103) Varma, M. V., and Panchagnula, R. (2005) Enhanced oral paclitaxel absorption with vitamin E-TPGS: effect on solubility and permeability in vitro, in situ and in vivo. *European journal of pharmaceutical sciences*, 25, 445-453.
- (104) Zhang, Z., Lv, H., Jia, X., Lingling, C., Xin, J., Yan, C., Tan, X., and Sun, E. (2012) Influence of vitamin E tocopherol polyethylene glycol succinate 1000 on intestinal absorption of icarisperide II. *Die Pharmazie*, 67, 59-62.
- (105) Muthu, M. S., Kulkarni, S. A., Liu, Y., and Feng, S. S. (2012) Development of docetaxel-loaded vitamin E TPGS micelles: formulation optimization, effects on brain cancer cells and biodistribution in rats. *Nanomedicine*, 7, 353-364.
- (106) Parmentier, J., Hartmann, F. J., and Fricker, G. (2010) In vitro evaluation of liposomes containing bio-enhancers for the oral delivery of macromolecules. *European journal of pharmaceutics and biopharmaceutics*, 76, 394-403.
- (107) Rachmawati, H., Al Shaal, L., Muller, R. H., and Keck, C. M. (2013) Development of curcumin nanocrystal: physical aspects. *Journal of pharmaceutical sciences*, 102, 204-214.
- (108) Mu, L., and Feng, S. S. (2003) A novel controlled release formulation for the anticancer drug paclitaxel (Taxol): PLGA nanoparticles containing vitamin E TPGS. *Journal of controlled release*, 86, 33-48.
- (109) Feng, S. S. (2008) Nanoparticles of biodegradable polymers for cancer treatment. *Biomaterials*, 29, 4146-4147.
- (110) Raphael Riva, H. R., Anne des Rieux, Nicolas Duhem, Christine Jerome, and Veronique Preat. (2011) Chitosan and Chitosan Derivatives in Drug Delivery and Tissue Engineering. *Advance in Polymeric Sciences*, 19-44.
- (111) Park, J. H., Saravanakumar, G., Kim, K., and Kwon, I. C. (2010) Targeted delivery of low molecular drugs using chitosan and its derivatives. *Advanced drug delivery reviews*, 62, 28-41.
- (112) Rinaudo, M. (2006) Chitin and chitosan: Properties and applications. *Progress in Polymer Science*, 31, 603-632.
- (113) Bagheri-Khoulenjani, S., Taghizadeh, S. M., and Mirzadeh, H. (2009) An investigation on the short-term biodegradability of chitosan with various molecular weights and degrees of deacetylation. *Carbohydrate Polymers*, 78, 773-778.
- (114) Varum, K. M., Myhr, M. M., Hjerde, R. J. N., and Smidsrod, O. (1997) In vitro degradation rates of partially N-acetylated chitosans in human serum. *Carbohydrate Research*, 299, 99-101.

- (115) VandeVord, P. J., Matthew, H. W. T., DeSilva, S. P., Mayton, L., Wu, B., and Wooley, P. H. (2002) Evaluation of the biocompatibility of a chitosan scaffold in mice. *Journal of Biomedical Materials Research*, 59, 585-590.
- (116) Sashiwa, H., and Aiba, S. I. (2004) Chemically modified chitin and chitosan as biomaterials. *Progress in Polymer Science*, 29, 887-908.
- (117) Rane, K. D., and Hoover, D. G. (1993) Production of chitosan by fungi. *Food Biotechnology*, 7, 11-33.
- (118) Aranaz, I., Harris, R., and Heras, A. (2010) Chitosan Amphiphilic Derivatives. Chemistry and Applications. *Current Organic Chemistry*, 14, 308-330.
- (119) Illum, L. (1998) Chitosan and its use as a pharmaceutical excipient. *Pharmaceutical Research*, 15, 1326-1331.
- (120) Kumar, M., Muzzarelli, R. A. A., Muzzarelli, C., Sashiwa, H., and Domb, A. J. (2004) Chitosan chemistry and pharmaceutical perspectives. *Chemical Reviews*, 104, 6017-6084.
- (121) Paños I, Niuris Acosta, and Heras, A. (2008) New Drug Delivery Systems Based on Chitosan. *Current Drug Discovery Technologies*, 5, 333-341.
- (122) Varshosaz, J. (2007) The promise of chitosan microspheres in drug delivery systems. *Expert Opinion on Drug Delivery*, 4, 263-273.
- (123) Park, J. H., Saravanakumar, G., Kim, K., and Kwon, I. C. (2010) Targeted delivery of low molecular drugs using chitosan and its derivatives. *Advanced Drug Delivery Reviews*, 62, 28-41.
- (124) Madhally, S. V., and Matthew, H. W. T. (1999) Porous chitosan scaffolds for tissue engineering. *Biomaterials*, 20, 1133-1142.
- (125) Lehr, C.-M., Bouwstra, J. A., Schacht, E. H., and Junginger, H. E. (1992) In vitro evaluation of mucoadhesive properties of chitosan and some other natural polymers. *Int J Pharmaceut*, 78, 43-48.
- (126) Yang, J., Tian, F., Wang, Z., Wang, Q., Zeng, Y. J., and Chen, S. Q. (2008) Effect of chitosan molecular weight and deacetylation degree on Hemostasis. *Journal of Biomedical Materials Research Part B-Applied Biomaterials*, 84B, 131-137.
- (127) Minagawa, T., Okamura, Y., Shigemasa, Y., Minami, S., and Okamoto, Y. (2007) Effects of molecular weight and deacetylation degree of chitin/chitosan on wound healing. *Carbohydrate Polymers*, 67, 640-644.
- (128) Sudarshan, N. R., Hoover, D. G., and Knorr, D. (1992) ANTIBACTERIAL ACTION OF CHITOSAN. *Food Biotechnology*, 6, 257-272.
- (129) Ong, S. Y., Wu, J., Moomchala, S. M., Tan, M. H., and Lu, J. (2008) Development of a chitosan-based wound dressing with improved hemostatic and antimicrobial properties. *Biomaterials*, 29, 4323-4332.
- (130) Calvo, P., RemunanLopez, C., VilaJato, J. L., and Alonso, M. J. (1997) Novel hydrophilic chitosan-polyethylene oxide nanoparticles as protein carriers. *J. Appl. Polym. Sci.*, 63, 125-132.
- (131) Lee, K. Y. (2007) Chitosan and its derivatives for gene delivery. *Macromolecular Research*, 15, 195-201.
- (132) Guliyeva, Ü., Öner, F., Özsoy, S., and Hazirolu, R. (2006) Chitosan microparticles containing plasmid DNA as potential oral gene delivery system. *European Journal of Pharmaceutics and Biopharmaceutics*, 62, 17-25.

- (133) Erbacher, P., Zou, S. M., Bettinger, T., Steffan, A. M., and Remy, J. S. (1998) Chitosan-based vector/DNA complexes for gene delivery: Biophysical characteristics and transfection ability. *Pharmaceutical Research*, 15, 1332-1339.
- (134) Mourya, V. K., and Inamdar, N. N. (2008) Chitosan-modifications and applications: Opportunities galore. *React Funct Polym*, 68, 1013-1051.
- (135) Kim, S.-K., and Rajapakse, N. (2005) Enzymatic production and biological activities of chitosan oligosaccharides (COS): A review. *Carbohydrate Polymers*, 62, 357-368.
- (136) Einbu, A., Grasdalen, H., and Vårum, K. M. (2007) Kinetics of hydrolysis of chitin/chitosan oligomers in concentrated hydrochloric acid. *Carbohydrate Research*, 342, 1055-1062.
- (137) Kean, T., and Thanou, M. (2010) Biodegradation, biodistribution and toxicity of chitosan. *Advanced Drug Delivery Reviews*, 62, 3-11.
- (138) Yang, Y. M., Hu, W., Wang, X. D., and Gu, X. S. (2007) The controlling biodegradation of chitosan fibers by N-acetylation in vitro and in vivo. *Journal of Materials Science-Materials in Medicine*, 18, 2117-2121.
- (139) Leila N. Hassani, F. H., Kawthar Bouchemal. (2012) Auto-associative amphiphilic polysaccharides as drug delivery systems. *Drug Discov Today*, 17, 608-614.
- (140) Jiang, G.-B., Quan, D., Liao, K., and Wang, H. (2006) Preparation of polymeric micelles based on chitosan bearing a small amount of highly hydrophobic groups. *Carbohydrate Polymers*, 66, 514-520.
- (141) Du, Y.-Z., Lu, P., Zhou, J.-P., Yuan, H., and Hu, F.-Q. (2010) Stearic acid grafted chitosan oligosaccharide micelle as a promising vector for gene delivery system: Factors affecting the complexation. *International Journal of Pharmaceutics*, 391, 260-266.
- (142) Du, Y.-Z., Wang, L., Yuan, H., and Hu, F.-Q. Linoleic acid-grafted chitosan oligosaccharide micelles for intracellular drug delivery and reverse drug resistance of tumor cells. *International Journal of Biological Macromolecules*, In Press, Corrected Proof.
- (143) Du, Y.-Z., Wang, L., Yuan, H., Wei, X.-H., and Hu, F.-Q. (2009) Preparation and characteristics of linoleic acid-grafted chitosan oligosaccharide micelles as a carrier for doxorubicin. *Colloids and Surfaces B: Biointerfaces*, 69, 257-263.
- (144) Ye, Y.-Q., Yang, F.-L., Hu, F.-Q., Du, Y.-Z., Yuan, H., and Yu, H.-Y. (2008) Core-modified chitosan-based polymeric micelles for controlled release of doxorubicin. *International Journal of Pharmaceutics*, 352, 294-301.
- (145) Li, Q., Du, Y.-Z., Yuan, H., Zhang, X.-G., Miao, J., Cui, F.-D., and Hu, F.-Q. (2010) Synthesis of Lamivudine stearate and antiviral activity of stearic acid-g-chitosan oligosaccharide polymeric micelles delivery system. *European Journal of Pharmaceutical Sciences*, 41, 498-507.
- (146) Hu, F.-Q., Wu, X.-L., Du, Y.-Z., You, J., and Yuan, H. (2008) Cellular uptake and cytotoxicity of shell crosslinked stearic acid-grafted chitosan oligosaccharide micelles encapsulating doxorubicin. *European Journal of Pharmaceutics and Biopharmaceutics*, 69, 117-125.
- (147) Zhang, J., Chen, X. G., Sun, G. Z., Huang, L., and Cheng, X. J. (2010) Effect of molecular weight on the oleoyl-chitosan nanoparticles as carriers for doxorubicin. *Colloids and Surfaces B: Biointerfaces*, 77, 125-130.
- (148) Park, K., Kim, J. H., Nam, Y. S., Lee, S., Nam, H. Y., Kim, K., Park, J. H., Kim, I. S., Choi, K., Kim, S. Y., and Kwon, I. C. (2007) Effect of polymer molecular weight on the tumor

- targeting characteristics of self-assembled glycol chitosan nanoparticles. *Journal of Controlled Release*, 122, 305-314.
- (149) Kim, J. H., Kim, Y. S., Park, K., Lee, S., Nam, H. Y., Min, K. H., Jo, H. G., Park, J. H., Choi, K., Jeong, S. Y., Park, R. W., Kim, I. S., Kim, K., and Kwon, I. C. (2008) Antitumor efficacy of cisplatin-loaded glycol chitosan nanoparticles in tumor-bearing mice. *Journal of Controlled Release*, 127, 41-49.
- (150) Hwang, H. Y., Kim, I. S., Kwon, I. C., and Kim, Y. H. (2008) Tumor targetability and antitumor effect of docetaxel-loaded hydrophobically modified glycol chitosan nanoparticles. *Journal of Controlled Release*, 128, 23-31.
- (151) Park, Y., Hong, H. Y., Moon, H. J., Lee, B. H., Kim, I. S., Kwon, I. C., and Rhee, I. (2008) A new atherosclerotic lesion probe based on hydrophobically modified chitosan nanoparticles functionalized by the atherosclerotic plaque targeted peptides. *Journal of Controlled Release*, 128, 217-223.
- (152) Yu, J. M., Li, Y. H., Qiu, L. Y., and Jin, Y. (2008) Self-aggregated nanoparticles of cholesterol-modified glycol chitosan conjugate: Preparation, characterization, and preliminary assessment as a new drug delivery carrier. *European Polymer Journal*, 44, 555-565.
- (153) Torchilin, V. P. (2007) Nanocarriers. *Pharmaceutical Research*, 24, 2333-2334.
- (154) Saravanakumar, G., Min, K. H., Min, D. S., Kim, A. Y., Lee, C.-M., Cho, Y. W., Lee, S. C., Kim, K., Jeong, S. Y., Park, K., Park, J. H., and Kwon, I. C. (2009) Hydrotropic oligomer-conjugated glycol chitosan as a carrier of paclitaxel: Synthesis, characterization, and in vivo biodistribution. *Journal of Controlled Release*, 140, 210-217.
- (155) Rekha, M. R., and Sharma, C. P. (2009) Synthesis and evaluation of lauryl succinyl chitosan particles towards oral insulin delivery and absorption. *Journal of Controlled Release*, 135, 144-151.
- (156) Kim, J.-H., Kim, Y.-S., Kim, S., Park, J. H., Kim, K., Choi, K., Chung, H., Jeong, S. Y., Park, R.-W., Kim, I.-S., and Kwon, I. C. (2006) Hydrophobically modified glycol chitosan nanoparticles as carriers for paclitaxel. *Journal of Controlled Release*, 111, 228-234.
- (157) Ngawhirunpat, T., Wonglertnirant, N., Opanasopit, P., Ruktanonchai, U., Yoksan, R., Wasanasuk, K., and Chirachanchai, S. (2009) Incorporation methods for cholic acid chitosan-g-mPEG self-assembly micellar system containing camptothecin. *Colloid Surf. B-Biointerfaces*, 74, 253-259.
- (158) Wang, Y., Tu, S., Li, R., Yang, X., Liu, L., and Zhang, Q. (2010) Cholesterol succinyl chitosan anchored liposomes: preparation, characterization, physical stability, and drug release behavior. *Nanomedicine: Nanotechnology, Biology and Medicine*, 6, 471-477.
- (159) Zhou, Y.-Y., Du, Y.-Z., Wang, L., Yuan, H., Zhou, J.-P., and Hu, F.-Q. (2010) Preparation and pharmacodynamics of stearic acid and poly (lactic-co-glycolic acid) grafted chitosan oligosaccharide micelles for 10-hydroxycamptothecin. *International Journal of Pharmaceutics*, 393, 144-152.
- (160) Hu, F.-Q., Meng, P., Dai, Y.-Q., Du, Y.-Z., You, J., Wei, X.-H., and Yuan, H. (2008) PEGylated chitosan-based polymer micelle as an intracellular delivery carrier for anti-tumor targeting therapy. *European Journal of Pharmaceutics and Biopharmaceutics*, 70, 749-757.
- (161) Yao, Z., Zhang, C., Ping, Q., and Yu, L. (2007) A series of novel chitosan derivatives: Synthesis, characterization and micellar solubilization of paclitaxel. *Carbohydrate Polymers*, 68, 781-792.

- (162) Qu, G., Yao, Z., Zhang, C., Wu, X., and Ping, Q. (2009) PEG conjugated N-octyl-O-sulfate chitosan micelles for delivery of paclitaxel: In vitro characterization and in vivo evaluation. *European Journal of Pharmaceutical Sciences*, 37, 98-105.
- (163) Huo, M., Zhang, Y., Zhou, J., Zou, A., Yu, D., Wu, Y., Li, J., and Li, H. (2010) Synthesis and characterization of low-toxic amphiphilic chitosan derivatives and their application as micelle carrier for antitumor drug. *International Journal of Pharmaceutics*, 394, 162-173.
- (164) Xiangyang, X., Ling, L., Jianping, Z., Shiyue, L., Jie, Y., Xiaojin, Y., and Jinsheng, R. (2007) Preparation and characterization of N-succinyl-N'-octyl chitosan micelles as doxorubicin carriers for effective anti-tumor activity. *Colloids and Surfaces B: Biointerfaces*, 55, 222-228.
- (165) Zhang, C., Ding, Y., Yu, L., and Ping, Q. (2007) Polymeric micelle systems of hydroxycamptothecin based on amphiphilic N-alkyl-N-trimethyl chitosan derivatives. *Colloids and Surfaces B: Biointerfaces*, 55, 192-199.
- (166) Opanasopit, P., Ngawhirunpat, T., Rojanarata, T., Choochottiros, C., and Chirachanchai, S. (2007) Camptothecin-incorporating N-phthaloylchitosan-g-mPEG self-assembly micellar system: Effect of degree of deacetylation. *Colloids and Surfaces B: Biointerfaces*, 60, 117-124.
- (167) Opanasopit, P., Ngawhirunpat, T., Rojanarata, T., Choochottiros, C., and Chirachanchai, S. (2007) N-Phthaloylchitosan-g-mPEG design for all-trans retinoic acid-loaded polymeric micelles. *European Journal of Pharmaceutical Sciences*, 30, 424-431.
- (168) Bian, F., Jia, L., Yu, W., and Liu, M. (2009) Self-assembled micelles of N-phthaloylchitosan-g-polyvinylpyrrolidone for drug delivery. *Carbohydrate Polymers*, 76, 454-459.
- (169) Duan, K., Zhang, X., Tang, X., Yu, J., Liu, S., Wang, D., Li, Y., and Huang, J. (2010) Fabrication of cationic nanomicelle from chitosan-graft-polycaprolactone as the carrier of 7-ethyl-10-hydroxy-camptothecin. *Colloids and Surfaces B: Biointerfaces*, 76, 475-482.
- (170) Wu, H., Wang, S., Fang, H., Zan, X., Zhang, J., and Wan, Y. (2011) Chitosan-polycaprolactone copolymer microspheres for transforming growth factor-[beta]1 delivery. *Colloids and Surfaces B: Biointerfaces*, 82, 602-608.
- (171) Chen, C., Cai, G. Q., Zhang, H. W., Jiang, H. L., and Wang, L. Q. (2011) Chitosan-poly(epsilon-caprolactone)-poly(ethylene glycol) graft copolymers: Synthesis, self-assembly, and drug release behavior. *Journal of Biomedical Materials Research Part A*, 96A, 116-124.
- (172) Cai, G., Jiang, H., Chen, Z., Tu, K., Wang, L., and Zhu, K. (2009) Synthesis, characterization and self-assemble behavior of chitosan-O-poly([epsilon]-caprolactone). *European Polymer Journal*, 45, 1674-1680.
- (173) Lu, Y., Liu, L., and Guo, S. (2007) Novel amphiphilic ternary polysaccharide derivatives chitosan-g- PCL-b-MPEG: Synthesis, characterization, and aggregation in aqueous solution. *Biopolymers*, 86, 403-408.
- (174) P K Vemuda, N. R. C., F Zhao, B Xu, G John, J M Karp. (2011) Self-Assembled Prodrugs In *Comprehensive Biomaterials, 1st Edition* (Kirkpatrick, D. H. H. G., Ed.) pp 339-355, Elsevier Science.
- (175) Mahato, R., Tai, W., and Cheng, K. (2011) Prodrugs for improving tumor targetability and efficiency. *Advanced drug delivery reviews*, 63, 659-670.

- (176) Fang, J. Y., and Al-Suwayeh, S. A. (2012) Nanoparticles as delivery carriers for anticancer prodrugs. *Expert opinion on drug delivery*, 9, 657-669.
- (177) Desmaele, D., Gref, R., and Couvreur, P. (2012) Squalenoylation: a generic platform for nanoparticulate drug delivery. *Journal of controlled release*, 161, 609-618.
- (178) Arias, J. L., Reddy, L. H., Othman, M., Gillet, B., Desmaele, D., Zouhiri, F., Dosio, F., Gref, R., and Couvreur, P. (2011) Squalene based nanocomposites: a new platform for the design of multifunctional pharmaceutical theragnostics. *ACS nano*, 5, 1513-1521.
- (179) Dosio, F., Reddy, L. H., Ferrero, A., Stella, B., Cattel, L., and Couvreur, P. (2010) Novel nanoassemblies composed of squalenoyl-paclitaxel derivatives: synthesis, characterization, and biological evaluation. *Bioconjugate chemistry*, 21, 1349-1361.
- (180) Caron, J., Maksimenko, A., Wack, S., Lepeltier, E., Bourgaux, C., Morvan, E., Leblanc, K., Couvreur, P., and Desmaele, D. (2013) Improving the Antitumor Activity of Squalenoyl-Paclitaxel Conjugate Nanoassemblies by Manipulating the Linker between Paclitaxel and Squalene. *Advanced healthcare materials*, 2, 172-185.
- (181) Caron, J., Reddy, L. H., Lepetre-Mouelhi, S., Wack, S., Clayette, P., Rogez-Kreuz, C., Yousfi, R., Couvreur, P., and Desmaele, D. (2010) Squalenoyl nucleoside monophosphate nanoassemblies: new prodrug strategy for the delivery of nucleotide analogues. *Bioorganic & medicinal chemistry letters*, 20, 2761-2764.
- (182) Hillaireau, H., Dereuddre-Bosquet, N., Skanji, R., Bekkara-Aounallah, F., Caron, J., Lepetre, S., Argote, S., Bauduin, L., Yousfi, R., Rogez-Kreuz, C., Desmaele, D., Rousseau, B., Gref, R., Andrieux, K., Clayette, P., and Couvreur, P. (2013) Anti-HIV efficacy and biodistribution of nucleoside reverse transcriptase inhibitors delivered as squalenoylated prodrug nanoassemblies. *Biomaterials*, 34, 4831-4838.
- (183) Couvreur, P., Reddy, L. H., Mangenot, S., Poupert, J. H., Desmaele, D., Lepetre-Mouelhi, S., Pili, B., Bourgaux, C., Amenitsch, H., and Ollivon, M. (2008) Discovery of new hexagonal supramolecular nanostructures formed by squalenoylation of an anticancer nucleoside analogue. *Small*, 4, 247-253.
- (184) Rejiba, S., Reddy, L. H., Bigand, C., Parmentier, C., Couvreur, P., and Hajri, A. (2011) Squalenoyl gemcitabine nanomedicine overcomes the low efficacy of gemcitabine therapy in pancreatic cancer. *Nanomedicine : nanotechnology, biology, and medicine*, 7, 841-849.
- (185) Sarpietro, M. G., Ottimo, S., Giuffrida, M. C., Rocco, F., Ceruti, M., and Castelli, F. (2011) Synthesis of n-squalenoyl cytarabine and evaluation of its affinity with phospholipid bilayers and monolayers. *International journal of pharmaceuticals*, 406, 69-77.
- (186) Bildstein, L., Dubernet, C., Marsaud, V., Chacun, H., Nicolas, V., Gueutin, C., Sarasin, A., Benech, H., Lepetre-Mouelhi, S., Desmaele, D., and Couvreur, P. (2010) Transmembrane diffusion of gemcitabine by a nanoparticulate squalenoyl prodrug: an original drug delivery pathway. *Journal of controlled release*, 147, 163-170.
- (187) Bildstein, L., Hillaireau, H., Desmaele, D., Lepetre-Mouelhi, S., Dubernet, C., and Couvreur, P. (2009) Freeze-drying of squalenoylated nucleoside analogue nanoparticles. *International journal of pharmaceuticals*, 381, 140-145.
- (188) Couvreur P, S. B., Reddy LH, Hillaireau H, Dubernet C, Desmaële D, Lepêtre-Mouelhi S, Rocco F, Dereuddre-Bosquet N, Clayette P, Rosilio V, Marsaud V, Renoir JM, Cattel L. (2006) Squalenoyl nanomedicines as potential therapeutics. *Nano Letter*, 6, 2544-2548.

- (189) Bildstein, L., Pili, B., Marsaud, V., Wack, S., Meneau, F., Lepetre-Mouelhi, S., Desmaele, D., Bourgaux, C., Couvreur, P., and Dubernet, C. (2011) Interaction of an amphiphilic squalenoyl prodrug of gemcitabine with cellular membranes. *European journal of pharmaceuticals and biopharmaceutics*, 79, 612-620.
- (190) Reddy, L. H., Dubernet, C., Mouelhi, S. L., Marque, P. E., Desmaele, D., and Couvreur, P. (2007) A new nanomedicine of gemcitabine displays enhanced anticancer activity in sensitive and resistant leukemia types. *Journal of controlled release*, 124, 20-27.
- (191) Reddy, L. H., Renoir, J. M., Marsaud, V., Lepetre-Mouelhi, S., Desmaele, D., and Couvreur, P. (2009) Anticancer efficacy of squalenoyl gemcitabine nanomedicine on 60 human tumor cell panel and on experimental tumor. *Molecular pharmaceuticals*, 6, 1526-1535.
- (192) Reddy, L. H., Marque, P. E., Dubernet, C., Mouelhi, S. L., Desmaele, D., and Couvreur, P. (2008) Preclinical toxicology (subacute and acute) and efficacy of a new squalenoyl gemcitabine anticancer nanomedicine. *The Journal of pharmacology and experimental therapeutics*, 325, 484-490.
- (193) Couvreur, P., Stella, B., Reddy, L. H., Hillaireau, H., Dubernet, C., Desmaele, D., Lepetre-Mouelhi, S., Rocco, F., Dereuddre-Bosquet, N., Clayette, P., Rosilio, V., Marsaud, V., Renoir, J. M., and Cattel, L. (2006) Squalenoyl nanomedicines as potential therapeutics. *Nano letters*, 6, 2544-2548.
- (194) Reddy, L. H., Ferreira, H., Dubernet, C., Mouelhi, S. L., Desmaele, D., Rousseau, B., and Couvreur, P. (2008) Squalenoyl nanomedicine of gemcitabine is more potent after oral administration in leukemia-bearing rats: study of mechanisms. *Anti-cancer drugs*, 19, 999-1006.
- (195) Jin, Y., Xin, R., Ai, P., and Chen, D. (2008) Self-assembled drug delivery systems 2. Cholesteryl derivatives of antiviral nucleoside analogues: synthesis, properties and the vesicle formation. *International journal of pharmaceuticals*, 350, 330-337.
- (196) Jin, Y., Tong, L., Ai, P., Li, M., and Hou, X. (2006) Self-assembled drug delivery systems. 1. Properties and in vitro/in vivo behavior of acyclovir self-assembled nanoparticles (SAN). *International journal of pharmaceuticals*, 309, 199-207.
- (197) Jin, Y., Ai, P., Xin, R., Tian, Y., Dong, J., Chen, D., and Wang, W. (2009) Self-assembled drug delivery systems: Part 3. In vitro/in vivo studies of the self-assembled nanoparticulates of cholesteryl acyl didanosine. *International journal of pharmaceuticals*, 368, 207-214.
- (198) Jin, Y., Xing, L., Tian, Y., Li, M., Gao, C., Du, L., Dong, J., and Chen, H. (2009) Self-assembled drug delivery systems. Part 4. In vitro/in vivo studies of the self-assemblies of cholesteryl-phosphonyl zidovudine. *International journal of pharmaceuticals*, 381, 40-48.
- (199) Jin, Y., Xin, R., Tong, L., Du, L., and Li, M. (2011) Combination anti-HIV therapy with the self-assemblies of an asymmetric bolaamphiphilic zidovudine/didanosine prodrug. *Molecular pharmaceuticals*, 8, 867-876.
- (200) Gong, X., Moghaddam, M. J., Sagnella, S. M., Conn, C. E., Danon, S. J., Waddington, L. J., and Drummond, C. J. (2011) Lyotropic liquid crystalline self-assembly material behavior and nanoparticulate dispersions of a phytanyl pro-drug analogue of capecitabine-a chemotherapy agent. *ACS applied materials & interfaces*, 3, 1552-1561.
- (201) Duncan, R. (2006) Polymer conjugates as anticancer nanomedicines. *Nature reviews. Cancer*, 6, 688-701.

- (202) Greco, F., and Vicent, M. J. (2009) Combination therapy: opportunities and challenges for polymer-drug conjugates as anticancer nanomedicines. *Advanced drug delivery reviews*, 61, 1203-1213.
- (203) Vicent, M. J., and Duncan, R. (2006) Polymer conjugates: nanosized medicines for treating cancer. *Trends in biotechnology*, 24, 39-47.
- (204) Wang, Y., Xin, D., Liu, K., Zhu, M., and Xiang, J. (2009) Heparin-paclitaxel conjugates as drug delivery system: synthesis, self-assembly property, drug release, and antitumor activity. *Bioconjugate chemistry*, 20, 2214-2221.
- (205) Sugahara S, K. M., Kuriyama H, Kobayashi TR. (2002) Paclitaxel delivery systems: the use of amino acid linkers in the conjugation of paclitaxel with carboxymethyl dextran to create prodrugs. *Biological Pharma Bulletin*, 25, 632-641.
- (206) Guolin Li, J. L., Yan Pang, Ruibin Wang, Limin Mao, Deyue Yan, Xinyuan Zhu, and Jian Sun. (2011) Polymeric Micelles with Water-Insoluble Drug as Hydrophobic Moiety for Drug Delivery. *Biomacromolecules*, 12, 2016-2026.
- (207) Kolishetti, N., Dhar, S., Valencia, P. M., Lin, L. Q., Karnik, R., Lippard, S. J., Langer, R., and Farokhzad, O. C. (2010) Engineering of self-assembled nanoparticle platform for precisely controlled combination drug therapy. *Proceedings of the National Academy of Sciences of the United States of America*, 107, 17939-17944.
- (208) Tang, H., Murphy, C. J., Zhang, B., Shen, Y., Sui, M., Van Kirk, E. A., Feng, X., and Murdoch, W. J. (2010) Amphiphilic curcumin conjugate-forming nanoparticles as anticancer prodrug and drug carriers: in vitro and in vivo effects. *Nanomedicine*, 5, 855-865.
- (209) Nakanishi, T., Fukushima, S., Okamoto, K., Suzuki, M., Matsumura, Y., Yokoyama, M., Okano, T., Sakurai, Y., and Kataoka, K. (2001) Development of the polymer micelle carrier system for doxorubicin. *Journal of controlled release*, 74, 295-302.
- (210) Bae, Y., Nishiyama, N., Fukushima, S., Koyama, H., Yasuhiro, M., and Kataoka, K. (2005) Preparation and biological characterization of polymeric micelle drug carriers with intracellular pH-triggered drug release property: tumor permeability, controlled subcellular drug distribution, and enhanced in vivo antitumor efficacy. *Bioconjugate chemistry*, 16, 122-130.
- (211) Bae, Y., Nishiyama, N., and Kataoka, K. (2007) In vivo antitumor activity of the folate-conjugated pH-sensitive polymeric micelle selectively releasing adriamycin in the intracellular acidic compartments. *Bioconjugate chemistry*, 18, 1131-1139.
- (212) Mahmud, A., Xiong, X. B., and Lavasanifar, A. (2008) Development of novel polymeric micellar drug conjugates and nano-containers with hydrolyzable core structure for doxorubicin delivery. *European journal of pharmaceuticals and biopharmaceutics*, 69, 923-934.
- (213) Xiong, X. B., Ma, Z., Lai, R., and Lavasanifar, A. (2010) The therapeutic response to multifunctional polymeric nano-conjugates in the targeted cellular and subcellular delivery of doxorubicin. *Biomaterials*, 31, 757-768.
- (214) Gu, Y. J., Cheng, J., Man, C. W., Wong, W. T., and Cheng, S. H. (2012) Gold-doxorubicin nanoconjugates for overcoming multidrug resistance. *Nanomedicine : nanotechnology, biology, and medicine*, 8, 204-211.
- (215) Cao, Y., Gu, Y., Ma, H., Bai, J., Liu, L., Zhao, P., and He, H. (2010) Self-assembled nanoparticle drug delivery systems from galactosylated polysaccharide-doxorubicin conjugate loaded doxorubicin. *International journal of biological macromolecules*, 46, 245-249.

-
- (216) Seymour, L. W., Ferry, D. R., Anderson, D., Hesslewood, S., Julyan, P. J., Poyner, R., Doran, J., Young, A. M., Burtles, S., Kerr, D. J., and Cancer Research Campaign Phase, I. I. I. C. T. c. (2002) Hepatic drug targeting: phase I evaluation of polymer-bound doxorubicin. *Journal of clinical oncology*, 20, 1668-1676.
- (217) Schluep, T., Gunawan, P., Ma, L., Jensen, G. S., Durringer, J., Hinton, S., Richter, W., and Hwang, J. (2009) Polymeric tubulysin-peptide nanoparticles with potent antitumor activity. *Clinical cancer research*, 15, 181-189.
- (218) Liu, Z., Wang, Y., and Zhang, N. (2012) Micelle-like nanoassemblies based on polymer-drug conjugates as an emerging platform for drug delivery. *Expert opinion on drug delivery*, 9, 805-822.
- (219) Cell Therapeutics website (2013) <http://www.celltherapeutics.com/opaxio>.