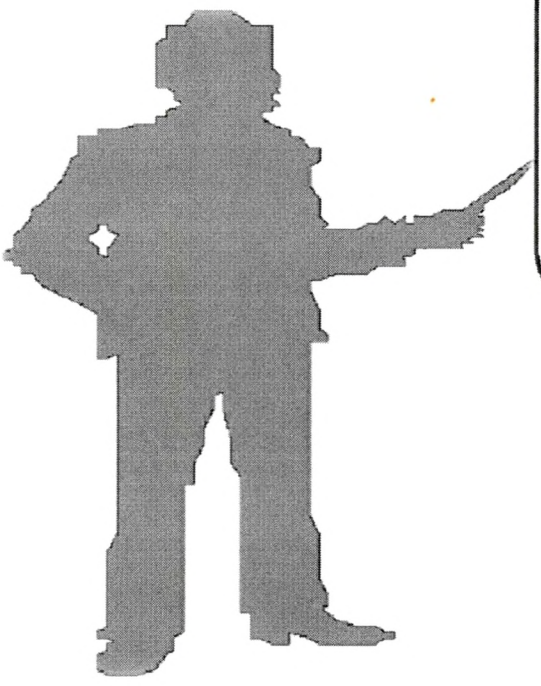
Chapter I



Introduction

1.1 Overview

Nanosciences and nanotechnologies are one of the favorite subjects of today's scientific domain and lead to many speculations. Applying nanotechnology to medicine may bring a new paradigm in healthcare (Duncan 2005). Pharmaceutical scientists and clinicians are working hard to bring these changes in maximizing therapeutic benefits by delivering drugs, genes, and proteins using this new area of science. Shift from traditional drug delivery systems to novel drug delivery systems promises enhanced therapeutic efficacy, reduced dose, low frequency of dosing, and fewer side effects. Formulating medicines by application of nanotechnology via size reduction or physical modification by polymers or chemical moieties to construct nanoparticles or more specifically nanoconstructs, leads to enhancement in diagnosis and management of life-threatening diseases like cancer. From a regulatory point of view, these nanomedicines are more of a new chemical entity then their conventional counterparts in terms of entrapment, solubilization, or controlled drug release without resorting to chemical conjugation (Duncan et al., 2006). Cancer chemotherapy suffers from many constraints such as severe side effects, nonselective cytotoxicity leading to noncompliance, prolonged treatment, drug resistance, incomplete cure, and quality-adjusted life. In a "drug delivery" strategy, the active compound has to be administered in different ways, depending on its physicochemical properties and toxicity. Through spatial and temporal controlled drug delivery, injectable drug carriers have shown the ability to modernize the disease treatment. Spatially controlled drug release acts on the site of action with a reduction of side effects and temporally controlled release of drugs can also reduce the undesired side effects associated with the natural circadian fluctuations of chemical levels throughout the body (Saltzman and Olbricht 2002). The main problem behind the successful delivery of bioactives through intravenous administration is the opsonization process. The drug carriers are preferentially recognized by opsonins, that is, plasmatic proteins and taken up by the cells of reticuloendothelial system (RES) such as liver, spleen, and lung, depending on their size and surface properties. In addition, it has been shown that cells of RES in the liver are not able to directly identify the nanoconstructs but recognize specific opsonin proteins bound to surface of the particles (Owens and Peppas 2006). The unprotected

nanoconstructs are classified as first-generation Nanoconstructs (Gref et al., 1994).They are not very efficient due to the removal from the blood circulation within seconds after intravenous administration through the macrophage of mononuclear phagocytic system (MPS). Nanovectors coated with hydrophilic and flexible polymers such as poly(ethylene oxide) (PEO) are classified as second-generation nanoconstructs. They have been used in order to make nanoconstructs both stealth to plasmatic protein and macrophages (Gref et al., 1994; Gabizon and Papahadjoupolos 1988; Vittaz et al., 1996; Peer and Margalit 2004) and specific to the injured tissues. Indeed, cancer invasion and autoimmune diseases trigger an inflammatory reaction in the tissues, which may cause an increase of their permeability of hundreds of nanometers, thus favoring the extravasations of the drug loaded particles. Matsumura and Maeda referred to this passive targeting phenomenon as the enhanced permeability and retention (EPR) effect (Matsumura and Maeda 1986).

Third-generation nanovectors opened new outlooks by allowing the release of their content selectively, especially to cells that present at their surface specific receptors (Lewin et al., 2000; Torchilin 2007). The receptor-mediated targeting (active targeting) can be achieved by grafting specific biological entities at the surface of the nanoconstructs using functionalized block copolymers (Zeng et al., 2006) or lipids (Saul et al., 2006) as spacers. The receptor-mediated targeting can produce effective drug carriers that enter the cell through the endocytic pathway. Well-known ligands in cancer targeting are folic acid (Leamon and Reddy 2004), RGD peptide (Pasqualini et al., 1997), or specific saccharides (Nagasaki et al., 2001). Attachment of these ligands to next generation of carriers would bring this multifunctionality to overcome the barriers (Table 1.1) and exert their biological activity. This biomimetic approach clearly illustrates the synergetic effect of molecular biology and nanotechnology for drug delivery. Based on the above-cited biological carriers, the requirements of an ideal drug carrier are summarized in Table 1.1.

Table 1.1 Rational Design of an "Ideal" Nanocarrier for Cancer Therapy Taking into Account all the Biological Barriers and Requirements

Biological requirements	Consequences in nano carrier design
Protect drug from degradation	Encapsulation into a carrier
Intravenous injection	Size < 200 nm
Prevent opsonization (increase circulating half-time)	Coating with hydrophilic polymer (PEG, Dextran, poly(L-glutamic acid),...)
Control of biodistribution	Introduction of targeting moieties (antibodies, peptides, carbohydrates)
Control of pharmacokinetics and pharmacodynamics	All previous parameters
Elimination	Use of biocompatible and biodegradable materials

Concerning the tumor targeting, it can be performed by passive targeting using second-generation nanovectors or active targeting (receptor – ligand interaction) using third-generation nanoconstructs. Both ways are very important and drug delivery systems have to be designed for maximum efficacy with minimum side effects of the anticancer drugs. In addition to the EPR effect brought by second-generation nanoconstructs, the poor lymphatic drainage of tumor tissue also favors the retention of nanoconstructs and the drug release into the vicinity of the tumor cells. Several new nanomedicine technologies in cancer therapy have been developed in the last decade and are either evaluated in clinical trials or already on the market (Duncan 2005). Some examples include: liposomes (e.g., DaunoXome®), polymer-coated liposomes (Doxil®, Caelyx®), polymeric drugs (Copaxone®), antibodies (Herceptin®, Avastin®) and antibody conjugates (Mylotarg®), polymer-protein conjugates (Oncaspar®, Neulasta®), and lately, protein nanoparticles containing paclitaxel (Abraxane®). These nanoscale and mostly multicomponent drug delivery systems are the first nanomedicines and have already brought clinical benefits. These successes are at the origin of a good flow of related systems that are currently in development (Figure 1.1). These include natural vectors (antibody-targeted liposomes), pseudo-synthetic vectors (polymer-antibody

hybrids), and synthetic vectors (polymer-drug conjugates, polymer micelles, polymer-based nanoconstructs).

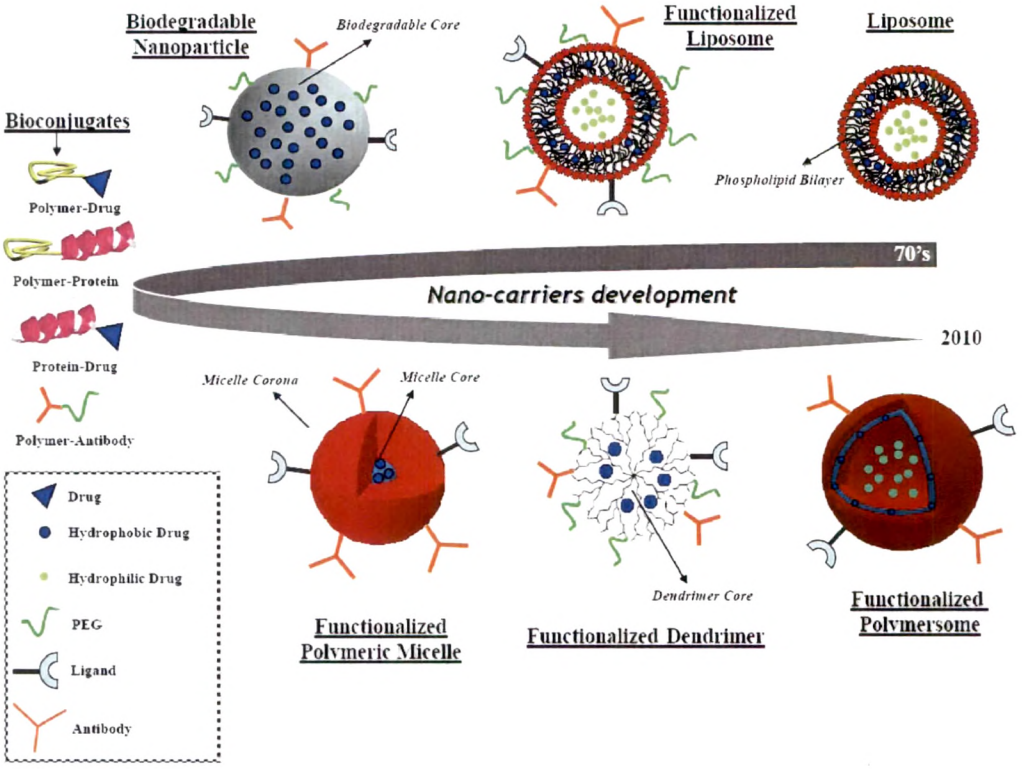


Figure 1.1 Different natural, pseudo synthetic, and synthetic nanocarriers developed in nanomedicine, from the 1970s to nowadays.

In spite of better therapeutic efficacy of marketed nanoconstructs, formulation scientists could not completely eliminate the major side effects of the anticancer drugs. For instance, Abraxane, which is an albumin-bound nanoparticulate formulation of paclitaxel, claimed that it does not require to be dissolved in a toxic solvent (cremophor) prior to administration. However, the product has shown increase in adverse events such as anemia, infections, dyspnea, sensory neuropathy, diarrhea, hepatic changes, and no significant changes in neutropenia, alopecia, thrombocytopenia, and cardiovascular changes (Patient information 2005). Similarly, new findings suggest that repeated dosing with PEGylated liposomes (Doxil, Caelyx) can result in rapid clearance from the blood,

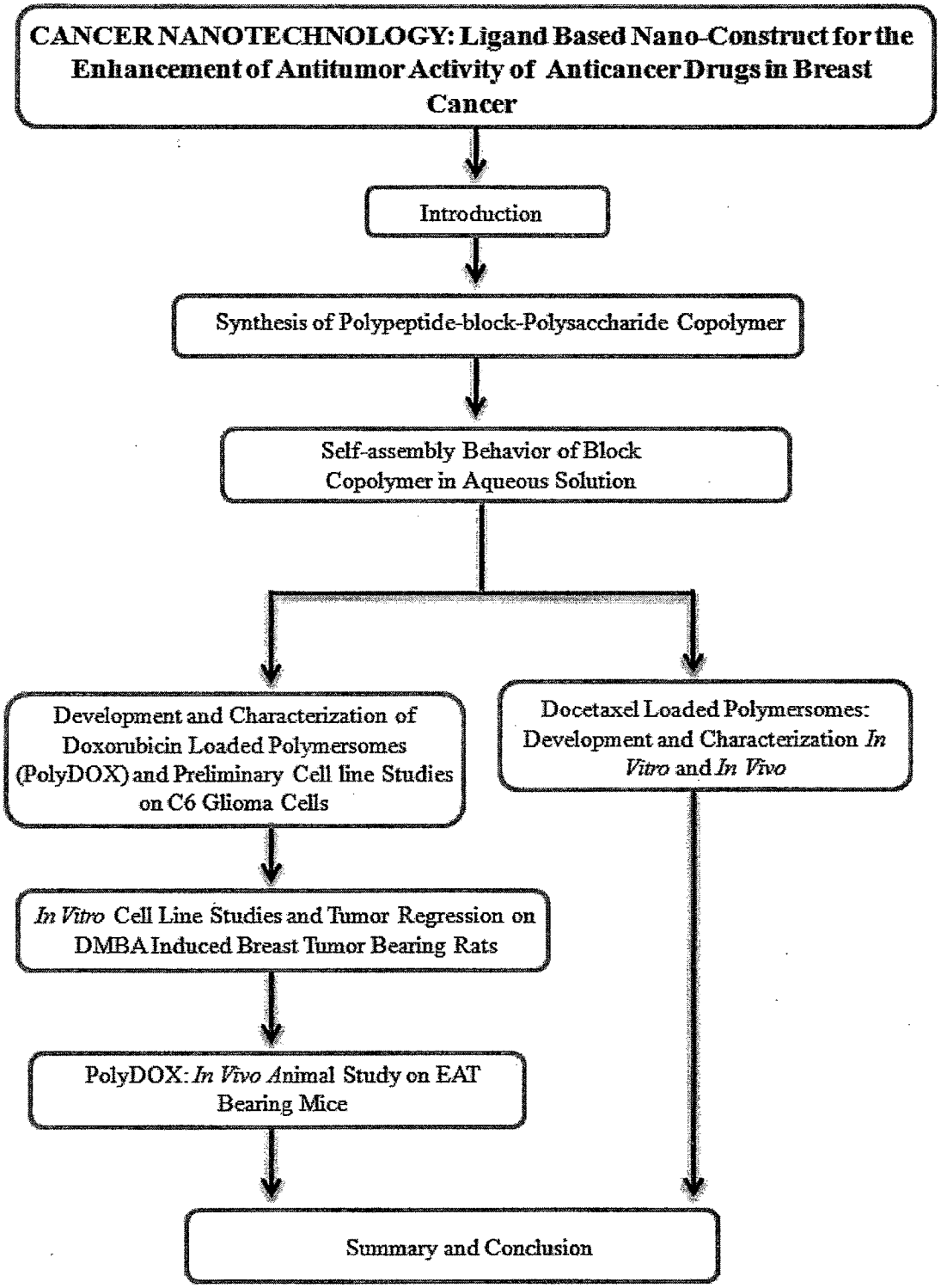
hepatic accumulation, and acute hypersensitivity (Ishida et al., 2005; Judge et al., 2006). Successful applications of liposomal formulations in therapeutics depend upon efforts to enhance the physicochemical stability in the aqueous environment (Brandl 2001; Glavas-Dodov et al., 2005) and ease in functionalizing the nanoconstructs. The limitations and benefits of these multifunctionalized drug carriers have motivated polymer chemists to synthesize new polymer structures forming nanoconstructs with enhanced functionalizing ability and other benefits such as improved drug loading and stability. At this point, a clear distinction between polymer nanoparticles and polymer self-assembled systems, that is, micelles and vesicles, has to be made. Indeed, an impressive number of research articles deal with so-called polymer nanoparticles for various purposes including cancer therapy. Polymer nanoparticles may be defined as polymer aggregates out of thermodynamic equilibrium, whereas self-assembled systems by definition reach this equilibrium. It is very important to assess the extent of equilibrium. Luisi has proposed a few operational criteria that can be used in the case of surfactants (Luisi 2001). The same criteria must apply with polymers, such as dilution experiments below the critical micellar concentration, mixing experiments with two different size populations, or formation of the final state by varying the initial conditions. There are also few morphological differences between these two kinds of systems, with block copolymer micelles having a typical core/shell structure whereas polymer nanoparticles display usually an almost homogeneous structure.

More recently, block copolymer vesicles, which have basically the same structure as liposomes, have been considered as new and promising drug delivery systems. If historically liposomes have been the first synthetic drug carriers used for nanomedicine, they are known to suffer from a lack of membrane stability associated with drug leaking. Because of their relatively high molar mass, block copolymers used for vesicle building overcome this problem by forming a thicker membrane. In addition, the chemical versatility of block copolymers offers almost infinite possibilities for modifying the vesicle surface to accommodate it with different biological targets.

1.2. Research Envisaged

The aim of the studies was to synthesize biomaterial, namely block copolymer, consisting hyaluronan and poly(γ-benzyl-L-glutamate) as hydrophilic and hydrophobic block respectively, characterize them, encapsulate doxorubicin and docetaxel within vesicles, and assess the drug loaded nano-constructs *in vitro* and *in vivo* to justify their role in treatment of breast cancer. Synthesis of block copolymer by use of easy click chemistry will help in avoiding cumbersome procedure used in commercial production of drug loaded vesicular carriers. It was hypothesized that block copolymer will result into nanoconstructs assembly in aqueous solution. These carries are also expected to enhance the stability of vesicular structure due to controllable membrane thickness and self targeted to CD44 over express cancer cells due to hyaluronan ligand presence in the nano constructs. This can be further utilized for encapsulation of anticancer drug/s. Therefore, it will help in improving drug accumulation in the breast tumor visa-a-vise therapeutic response of chemotherapy and will also help in reduction of reported side effects due to non-specific distribution.

1.3. Thesis structure



1.4. Literature Review

1.4.1 Block copolymers and their intrinsic properties

Block copolymers are defined as a combination of different polymer segments in a single polymer chain through various polymerization methods, combining the intrinsic properties of each individual block. Depending on how the monomer repeating units are distributed in the copolymer chains, these copolymers may present a variety of macromolecular architectures such as random, alternating, block, and graft copolymers (Figure 1.2).

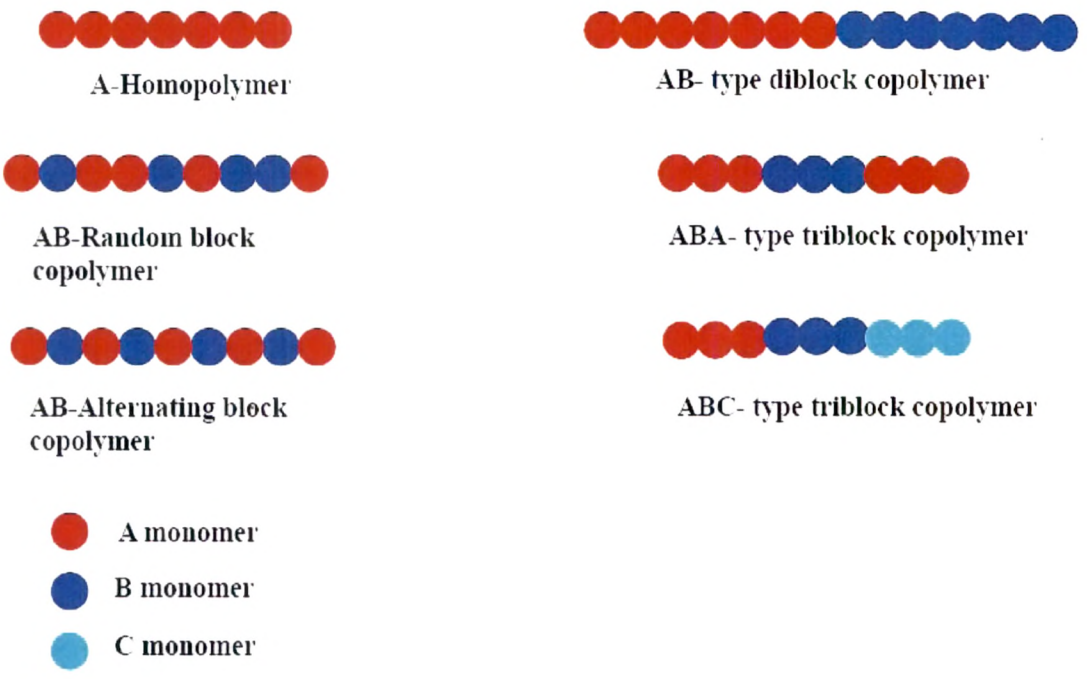


Figure 1.2 Different copolymer architectures resulting from the copolymerization of two or three different monomers.

As a general rule, the most studied and used block copolymers are composed of two chemically incompatible and dissimilar blocks. The incompatibility between the two blocks gives rise to interesting micro domain formation in pure block copolymers through a self-assembly process. The corresponding morphologies (shape and size) depend on the molecular weight, the volume fraction of the block A ($f_A = 1\text{-}f_B$), and the Flory–Huggins interaction parameter χ_{AB} (or χ) related to the enthalpic interaction existing between the

two blocks. Based on these two parameters, bulk phase diagrams have been constructed theoretically (Leibler 1980; Bates 1991; Fredrickson and Bates 1996). As a consequence, self-assembly provides an efficient and rapid pathway for the formation of structures from nanometer to micrometer range that are difficult if not impossible to obtain by conventional chemical reactions. Block copolymers dissolved in a selective solvent, meaning a good solvent for one block, but poor for the other, self-assemble into micellar structures, as observed for conventional low molecular weight surfactants, above a certain concentration called critical micelle concentration (CMC). The shape and the size of the aggregates are controlled by three main forces governing their association process: the stretching of the core forming block, the interaction between the chains in the corona, and the interfacial energy between the core and the solvent (Halperin et al., 1992; Zhang et al., 1998). As a consequence, a large variety of structures, from spherical micelles to vesicles or cylinders, can be obtained. The soluble block will be oriented toward the continuous solvent medium and become the 'corona' of the micelle formed, whereas the insoluble part will be shielded from the solvent in the 'core' of the structure.

As reported by Riess (2003), the structure of amphiphilic block copolymers in aqueous media can be divided into three classes depending on the nature of the hydrophilic block. There are uncharged blocks such as poly(ethylene oxide) (PEO) — also referred to as poly(ethylene glycol) (PEG) — positively charged blocks such as quaternized poly(2- or 4-vinylpyridine), poly(ethyleneimine) or poly(L-lysine), and negatively charged ones such as poly(acrylic acid) (PAA), poly(styrene sulfonate) (PSS), or poly(L-glutamic acid) (PGA). As described later, the characteristics of these systems make them suitable for applications in pharmaceuticals, as vehicles for drug delivery or as separating agents (Gaucher et al., 2005; Torchilin 2005). Of considerable interest and concern are block copolymers containing biocompatible and biodegradable segments, such as poly(lactide)-*b*-poly(ethylene oxide) PLA-*b*-PEO (Rashkov et al., 1996). The glass transition temperature (T_g) of the hydrophobic segment has also been shown to have a direct effect on micelle stability and on the CMC. Indeed, for block copolymers containing high T_g segments, such as polystyrene, thermodynamic considerations are not meaningful below the glass transition temperature (T_g~100°C) because the morphology is frozen at room temperature, contributing to a decrease of CMC. Such systems are called

"frozen micelles". The relative block stiffness, that is, whether one deals with coil-coil or with rod-coil blocks, not only influences the Flory–Huggins segmental interaction parameter, but also directs the microphase separation, which in turn affects the stability of the self-assembled structures (Klok and Lecommandoux 2001). In addition, polyion complexes (PIC) formed in aqueous solution by complexing polyelectrolytes or amphiphilic block copolymers of opposite charge have been extensively used to prepare a variety of self assembled structures in solution (Harada and Kataoka 1999).

1.4.2. General considerations for micelles and vesicles formation

1.4.2.a. Theoretical aspects

In case of amphiphilic block copolymers, according to the common definition, amphiphilic molecules (from Greek, "*amphi*" both and "*philic*" attraction) have affinities for two different environments. A large number of theories have been developed to describe and understand the self-assembly of block copolymers in solution (Leibler et al., 1983; Noolandi and Hong 1983; Gao and Eisenberg 1993; Zhulina and Birshtein 1985; Halperin 1987). Free energy minimization of micelles or mean field expression of the interfacial tension between core and shell regions are the two main approaches used. These theories give prediction about the size, aggregation number and its evolution with the composition and molecular weight of the block copolymer. They also give information about the evolution of the CMC with block copolymer architecture. However, these models are very difficult to use experimentally as each polymer chain has different rigidity, persistence length, bending energy, and so forth, depending on the solvent and environment. In parallel, aiming at predicting surfactant-based morphologies, Israelachvili (1992) developed a simple approach based on geometrical considerations. This method is very robust for low molecular weight surfactants, but cannot describe properly the behavior of block copolymers, mainly because the entropic constraints are not considered. Recently, Disher and Eisenberg (2002) tried to unify the experimental results obtained from different amphiphilic block copolymer systems and proposed an empirical law for neutral and flexible amphiphilic block copolymers in water. Based on a series of examples drawn from the literature, they proposed a unifying rule for the formation of polymersomes (polymer-based vesicles) in water, that is, a ratio f of the

mass of the hydrophilic part to the total mass equal to 35 ± 10%, as in the case of phospholipids (Aranda-Espinoza et al., 2001), whereas block copolymers with $f > 45\%$ are expected to form spherical micelles and those with $f < 25\%$ are expected to self-assemble into inverted structures.

1.4.2.b. Experimental consideration

Micelles and vesicles can be obtained by different processes. One approach is based on the direct dissolution of the block copolymer in a selective solvent for one of the block above its CMC. This procedure is simple and reproducible, but limited to block copolymers with low T_g hydrophobic block to achieve thermodynamic equilibrium. The thin film hydration method, which is essentially used for the preparation of liposomes (Šegota and Težak 2005), has been recently applied to prepare block copolymer vesicles. Here again, the T_g of the hydrophobic segment has to be reached during the process. Nanoprecipitation is another method, especially used when the hydrophobic block has a high T_g or is semi-crystalline, consists in dissolving the copolymer in a common solvent for both blocks. This solution is then subsequently added drop by drop in a selective solvent. Then, the common solvent can be removed from the solution, usually via dialysis or evaporation. A recent review, focused on the preparation of self-assembled block copolymer in solution, can be found elsewhere (Soo and Eisenberg 2004).The dedicated part of Riess' review on micellization of block copolymer can be equally of interest (Riess 2003).

1.4.3. Specific characteristics of micelles and vesicles

As previously described, a variety of morphologies can be obtained, the most commonly reported being spherical micelles, vesicles, or cylindrical micelles (Figure 1.3). Recent reviews analyze in more detail the parameters that afford one or another structure (Chu 1995; Alexandridis 1996; Selb and Gallot 1985; Disher et al., 2000; Rodriguez-Hernandez et al., 2005). Since the literature on this topic is abundant and diverse, our discussion is limited to a few selected examples. Because of our interest in biological applications, we focus on the self-assembled nanostructures formed from amphiphilic block copolymers in aqueous solution.

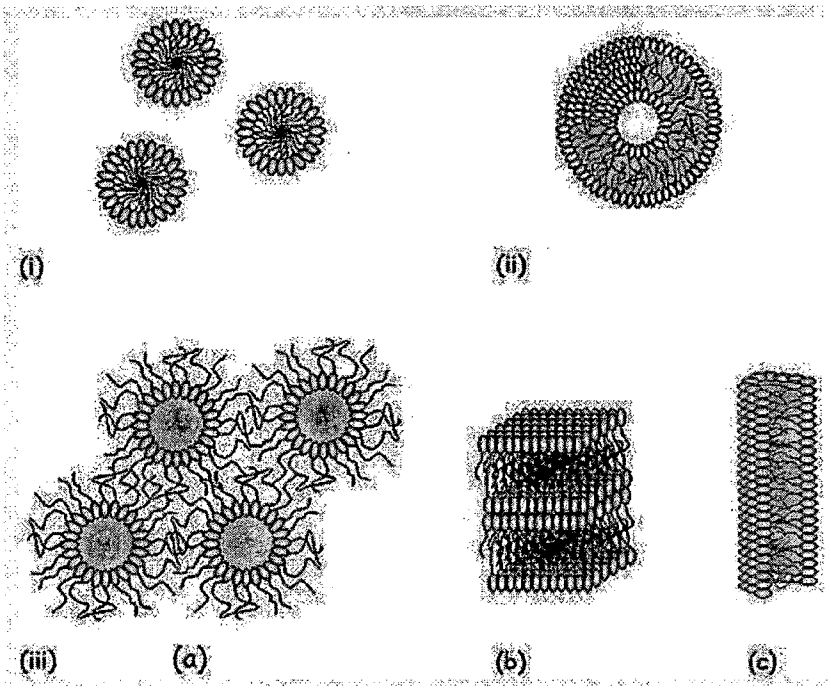


Figure 1.3 Examples of structures obtained from block copolymers: (i) direct micelles, (ii) vesicles, and (iii) other morphologies: (iiia) inverse micelles, (iiib) lamellar structures, and (iiic) cylindrical or tubular micelles.

1.4.3.a. Spherical micelles

Spherical micelles with the so-called 'core-shell' structure have been extensively studied. Formation of spherical micelles via self-assembly of diblock copolymers is directed by an entropically driven association mechanism linked to the contact minimization of water molecules with hydrophobic blocks. In the last two decades, special attention has been paid to aqueous micellar systems, mainly motivated by their applications as emulsifiers, foam stabilizers, or detergents and in biomedicine as stabilizing agents in dermatological creams, lotions, and so forth. PEO is a hydrophilic, biocompatible, nontoxic polymer, which has been widely used as the solubilizing block to form the shell in spherical micelles. Hydrophobic blocks include poly(lactic acid) and polyethers like polypropylene oxide (PPO) or poly(butylene oxide) (PBO). PEO-*b*-PPO or PEO-*b*-PBO block copolymers are commercially available with a diblock or even triblock architecture and, as a consequence, have been extensively investigated in the past. Chu and Zhou (1996) reviewed in detail the characteristic micellization features of these block copolymers. Among the great variety of degradable polymers, linear aliphatic polyesters are

particularly attractive and mostly used in both biomedical and pharmaceutical applications, because of their low toxicity, their hydrolytic and enzymatic degradability, and their versatility regarding physical, chemical, and biological properties (Albertsson et al., 2003). Due to these attractive properties, a large number of micellar structures based on polyesters have been studied in the literature (Kissel et al., 2002). Since α-amino acids are naturally occurring compounds in living systems and in molecules presenting a biological activity, the synthesis of biodegradable, biocompatible, and *a fortiori* nontoxic poly(α-amino acid)s has attracted growing interest. Poly(α-amino acid)s are especially used as natural protein models for the study of different biological processes but more recently, they have found applications in the area of "polymer therapeutics" as biomedical devices and nanoparticles (Duncan 2003; Satchi-Fainaro et al., 2006; Duncan et al., 2006). Cammas-Marion et al., 1999 have shown great interest in the use of polypeptide-based micelles as potential drug carriers using poly(ethylene oxide)-*b*-poly(b-benzyl-L-aspartate) (PEO-*b*-PBLA) block copolymers with PEO as corona. Advantages of this system are related to its small micellar size (diameter ~10–100 nm) and its long-term stability, which is required for a prolonged circulation time. This carrier system possesses acidic functions in the PBLA block that can be used to attach a desired moiety.

1.4.3.b. Block copolymer vesicles or polymersomes

Vesicles are nanometer-sized 'bags' whose double-layer outer membrane encloses an inner volume. Because of the double layer, that recalls the structure of lipids in membrane cells, polymer vesicles are also called polymersomes. The main advantage of polymersomes compared to liposomes is related to the higher membrane stability due to better mechanical properties. Indeed, an important issue is the membrane thickness. In polymer systems, the membrane thickness depends on the polymer size, the ratio between its hydrophilic and hydrophobic block and dimensions from 3 up to 40 nm are usually achievable (Disher et al., 2000; Aranda-Espinoza et al., 2001). Excellent reviews describing the different means to generate vesicles from amphiphilic polymers in various media, and including their properties and applications, have been published recently (Disher and Eisenberg 2002; Soo and Eisenberg 2004; Disher et al., 2000). Lee and colleagues generated vesicles in a broad range of sizes from poly(ethylene oxide)-*b*-

poly(ethylene) (PEO-*b*-PE) and poly(ethylene oxide)-*b*-polybutadiene (PEO-*b*-PB) block copolymers with various block compositions (Lee et al., 2001). With *in vitro* experiments they showed that these vesicles are inert toward various living cells. Stoenescu and Meier (2002) prepared the ABC triblock copolymers of polyethylene-*b*-poly(dimethyl siloxane)-*b*-poly(methyloxazoline) that form vesicles with asymmetric membranes in aqueous media and with sizes ranging between 60–300 nm. Block copolymers that combine advantageous features of synthetic polymers (solubility, processability, rubber elasticity, etc.) with those of polypeptides or polysaccharides (secondary structure, functionality, biocompatibility, etc.) recently found increasing interest. It is well known that secondary structures in synthetic peptides are subject to change from α-helix to coil or to β-sheet morphology by slight modifications of environmental parameters like pH, ionic strength, or temperature. Examples of vesicles obtained from peptide-based diblock copolymers (also called peptosomes) have been early reported by Kukula et al.2002 and Chécot and colleagues (Chécot et al., 2002; 2003; 2005). These systems present the particularity of forming vesicles for a broad range of composition because of the rod-like conformation of polypeptide chains in certain pH and temperature conditions.

1.4.3.c. Other morphologies and recent developments with block copolymer

In addition to common spherical micelles and vesicles, other more complex structures/morphologies based on amphiphilic block copolymers have also been observed. It is outside the scope of this article to present these rare examples in detail, but we want to illustrate some of the most relevant in order to give the reader a flavor. For instance, Yu and Eisenberg reported the formation of cylindrical structures, also known as worm-like micelles, by self-assembly of PS-*b*-PEO diblock copolymers in aqueous solution (Yu and Eisenberg 1998). Recently, Geng et al., 2007 explored the effect of paclitaxel loaded worm-like micelles on cancer therapy. These worm-like micelles were obtained from hydration of PEG-*b*-poly(ethylethylene) or PEG-*b*-poly(ε-caprolactone) block copolymers and were called filomicelles. Seven days postinjection on nude mice has demonstrated interesting advantages of the filomicelle as a paclitaxel carrier. Indeed, promising phase I clinical trials with paclitaxel-loaded spherical micelles of PEG-*b*-(polylactic acid) use approximately an 8-fold higher paclitaxel dosage (Kim et al., 2004).

Low concentration regimes naturally induced during drug administration can provoke disruption of the assemblies. In that case, self-assembled structures obtained from block copolymers can have poor stability. Therefore, several groups have recently focused their attention on the design of novel strategies to increase the stability of such nanostructures. This normally concerns cross-linking methodologies where the core or shell is covalently bonded. Ishizu and Fukutomi (1988) were the first in applying this concept on core-shell micelles and later Liu et al.,1996 prepared block copolymer fibers in this way. Cross-linking increases the range of concentration and temperature where self-assembled aggregates can be used. Any critical micellar concentration (CMC) or temperature (CMT) transitions are identified, so high dilution or heating are not limiting factors in the performance of these systems. Several other reasons also justify the use of these stabilization techniques, for example, the drug side effect. Murthy et al., 2001 extensively studied the role of cross-linking on not only stability but also on controlling permeability and side effects by controlling the degree of cross-linking.

The control of permeability may offer unexplored alternatives for the preparation of novel controlled drug delivery systems. For example, vesicular morphologies based on polybutadiene-*b*-poly(L-glutamic acid) PB-b-PGA block copolymer could be stabilized by cross-linking the 1,2-vinyl double bonds present in the polybutadiene segment into a permanent "shape-persistent stimuli-responsive nanoparticle" (Chécot et al., 2003).

Hybrid self-assembled nanostructures have been developed and studied recently. They are based on the association of inorganic particles (Au, Ag, Fe2O3...) with micelles or vesicles of block copolymers (Lecommandoux et al., 2005; 2006a,b). Magnetic and metallic particles begin to play a significant role in biomedical applications by their capacity to be heated in presence of a magnetic field (hyperthermia) or a near-infrared beam (plasmon resonance), respectively. Such local heating sources are of high interest to treat tumors for example. In addition, magnetic nanoparticles can be used as contrast agents for medical imaging. Solubilization of these nanoparticles within self-assembled block copolymer structures allow both to increase their concentration and to protect them from the external medium.

1.4.4. Characterization techniques

In order to characterize a micellar system, several parameters have to be considered, including the equilibrium constant, the quality of the solvent, CMT and CMC, the overall molar mass (Mw) of the micelle, its aggregation number (Z), and its morphology. These variables affect the hydrodynamic radius (RH), the radius of gyration (RG), the ratio of RH to RG, (which reflects the morphology), the core radius (RC), and the thickness (L) of the corona. For more detailed information, the reader is referred to general books on block copolymers (Hadjichristidis et al., 2003; Hamley 1998; Lazzari et al., 2006; Gnanou and Fontanille 2008). and reviews (Lodge 2003; Kita-Tokarczyk et al., 2005; Lecommandoux and Borsali 2006; Riess 2003; Disher and Eisenberg 2002). The properties of the various block copolymer based self-assemblies have been characterized with the help of experimental methods such as scattering (light, X-rays, neutrons), imaging (AFM, TEM), and other techniques like circular dichroism, Fourier transform infrared, differential scanning calorimetry, polarizing optical microscopy, capillary viscometry, membrane osmometry, ultracentrifugation, size exclusion chromatography, and typical spectroscopic methods such as nuclear magnetic resonance where the disappearance of the core forming block signal is a strong evidence of the micelle formation.

1.4.5. Therapeutic application of self-assembled micelles and vesicles or polymersomes

Scientific interest has been growing fast in the last few years toward the use of block copolymers for therapeutic applications due to their versatility in chemical and physical properties. Block copolymer based micelles and vesicles also have the ability to encapsulate both hydrophilic and hydrophobic compounds with the unique property to graft targeting moieties onto their surface, making them excellent candidates for use in medical, pharmaceutical, and environmental fields. In this portion, we will mainly focus on cancer therapeutic application as this is the most active research domain using block copolymer micellar structures. The following section describes the various types of systems depending on their shape (spherical micelles, vesicles) and their "smartness"

(passive or active targeting, stimuli-responsiveness) with the most relevant examples of literature.

1.4.5.1. Block copolymer micelles

1.4.5.1.a. Drug solubility enhancement

One of the major pharmaceutical applications of polymeric micelles is their use as solubility enhancers (Torchilin 2001; Jones and Leroux 1999). Indeed, solubility is a precondition for intravenous administration of drug carrier and still solubility improvement is an ongoing research by many pharmaceutical companies. Some new chemotherapeutic agents are highly efficient *in vitro* but are often poorly water soluble. (Gelderblom et al., 2001).Micellar architecture of block copolymers can be used to solubilize hydrophobic guest molecules, which are otherwise only sparingly soluble in water, through the stabilization of hydrophobic core of block copolymer, providing a compatible microenvironment for the water-insoluble guest molecules, thereby enhancing its overall solubility in water. Even though the micellar structure is expected to be well adapted to efficiently load most of the hydrophobic drugs, many polymeric micelles have shown only limited loading capacity, regardless of the hydrophobic guest molecule. Since the last few years, considerable efforts have been made to enhance micellar loading capacity. Ideally, the solubility parameters of the guest molecules (probe or drug) and the core-forming polymer block should be the same in order to achieve very high loading into micelles. However, there is no universal core-forming segment, because each probe or drug is unique.

The hydrophobic drug can be encapsulated in micelle-forming block copolymers by two main methods. The first one is based on physical interactions between drug and the core-forming block (hydrophobic, ionic...). The second one is based on a covalent linkage between drugs to one of the blocks of the copolymer as with the prodrug approach.

1.4.5.1.a.1. Solubility enhancement via physical interactions

Numerous studies have been performed on the encapsulation of paclitaxel, a powerful hydrophobic anticancer drug usually formulated in a 50:50 mixture of Cremophor EL: absolute ethanol known as Taxol^{TE}. Indeed, if this formulation is the most successful chemotherapeutic drug, the use of Taxol^{TE} continues to bring with it significant side

effects including dyspnea, flushing, rash, and urticaria, as well as neurotoxicity and nephrotoxicity problems (Onetto et al., 1993;1995). Poly(D,L-lactide)-*b*-(methoxypolyethylene glycol) PDLLA-*b*-MePEG has been successfully used to encapsulate paclitaxel and consequently increase its aqueous solubility (Zhang et al., 1996; Burt et al., 1999). The efficiency of the encapsulation was strongly dependent of the composition and global molecular weight of the copolymer and the method employed (Zhang et al., 1996; Liggins et al., 2002; Kim et al., 2001).This was also observed for other systems like poly(2-ethyl-2-oxazoline)-*b*-poly(ε-caprolactone) PEtOz-*b*-PCL forming micelles in which paclitaxel was loaded using the dialysis method (Cheon et al., 2003), where feed weight ratio of paclitaxel to block copolymer also plays an important role (Shuai et al., 2004a).

Self-assembly of lactone based diblock and triblock copolymers like poly(ε-caprolactone)-*b*-poly(ethylene glycol) PCL-*b*-PEG and poly(ethylene glycol)-*b*-poly(δ-valerolactone) PEG-*b*-PVL have been also successfully exploited to encapsulate paclitaxel. Both the micelle size and the drug loading efficiency increased markedly with increasing the lactone block lengths (Cheon et al., 2003; Lee et al., 2005). The paclitaxel, docetaxel, teniposide, and etoposide have been successfully solubilized by the micellar structure of block copolymer poly(N-vinylpyrrolidone)-*b*-poly(D,L-lactide) PVP-*b*-PDLLA (Le Garrec et al., 2004).

Kwon et al., 1995; 1997 have also encapsulated doxorubicin in the poly(ethylene glycol)-*b*-poly(β-benzyl-L-aspartate) block copolymer (PEG-*b*-PBLA) in a physical manner. This method has been also successfully employed to solubilize epirubicin and doxorubicin in micelles of block copolymers such as pluronics (Batrakova et al., 1996) and poly(ε-caprolactone)-*b*-poly(ethylene glycol) (PCL-*b*-PEG) with various compositions (Shuai et al., 2004b).

In the case of paclitaxel, the most satisfactory results were lately reported by Lee et al., 2007 who reached a loading of about 30% w/w and developed the concept of hydrotropic polymeric micelles. Hydrotropic agents are additives that help solubilization of hydrophobic molecules in aqueous media. Based on experimental evidence that N,N-diethylnicotinamide and N-picolylnicotinamide were excellent hydrotropes for

solubilizing paclitaxel, these authors synthesized an original block copolymer system in which the core-forming block contained a given amount of covalently bound hydrotropic agent.

Another "physical" method to encapsulate drug and enhance their solubility in water is based on complexation between the drug and the copolymer. As an example, cisplatin [cis-dichlorodiammineplatinum (II)] (CDDP), a well-known metal complex that exhibits high antitumor activity (Takahara et al., 2002), presents several problems limiting its clinical use like poor water solubility, significant toxic side effects (Pinzani et al., 1994), and short half-life (Siddik et al., 1987). These problems were solved by incorporating CDDP through the polymer–metal complex formation between CDDP and PEG-*b*-poly(aspartic acid) PEG-*b*-PAsp (Nishiyama et al., 1999; 2001) or PEG-*b*-poly(glutamic acid) PEG-*b*-PGlu copolymers (Nishiyama et al., 2003). CDDP loaded micelle formation is based on the ligand substitution reaction of the Pt(II) from chloride (leaving group) to carboxylate in the block copolymers. They show a remarkable stability in distilled water. Release of the CDDP can be obtained in physiological media (0.15 M NaCl), which induces inverse ligand substitution reaction of Pt (II) from the carboxylate to chloride ions. 1,2-diaminocyclohexane platinum (II), a new class of platinum drugs more potent than CDDP (McKeage 2005; Yokoyama et al., 1991), has also been successfully solubilized in polymer-metal complex micelles of PEG-*b*-PGlu (Cabral et al., 2005).

1.4.5.1.a.2. Solubility enhancement via covalent bridging

Drug entrapment in micellar systems via physical interaction may lead to a poor control in drug loading. Several studies have reported the benefits of an approach based on the covalent attachment of drugs to the polymer. However, as for the construction of polymer-drug conjugates, such developments required relatively complicated synthetic strategies. One of the major examples concerning this approach came from Yokoyama et al., 1991 who increased the hydrophobic drug doxorubicin loading in poly(ethylene oxide)-*b*-poly(aspartic acid) block copolymers, forming polymeric micelles with a diameter of 15–60 nm. Doxorubicin was covalently attached with the side chain of the poly(aspartic acid) using carbodiimide chemistry (approximately 50%). As a result, the "hydrophobized" poly(aspartic acid) segment drives the self-assembly into micelles in an

aqueous environment. Additionally, the self-associating property of doxorubicin increases the cohesive force in the core through π–π interactions favoring both the entrapment of doxorubicin and the micelle stability (Yokoyama et al., 1998). The doxorubicin micelles made of this copolymer and called NK911 are now studied in a phase II clinical trial at the National Cancer Center (NCC) Hospital in Japan (Yokoyama et al., 1990; Kwon et al., 1994).

1.4.5.1.b. Passive drug targeting

All the passive targeting systems have in common the presence of an hydrophilic poly(ethylene oxide) segment known for its stealth effect (Gref et al., 1994; Gabizon et al.,1988; Vittaz et al., 1996; Peer and Margalit 2004). The hydrophobic segment, necessary for micelle formation, is then biodegradable or at least biocompatible. Among all the block copolymer systems that have been developed and so far proved some efficacy *in vivo*, one can describe three main families, based on polypeptides, polyesters, or poloxamers.

1.4.5.1.b.1. Polypeptide-based block copolymer micelle

As we have already described, one of the first anticancer drug loaded polymeric micelle systems was developed by Yokoyama and colleagues and was a doxorubicin conjugated PEG-*b*-PAsp block copolymer micelle (Yokoyama et al., 1990; Kwon et al., 1994). The micelle structure was also stabilized by increasing the amount of physically entrapped doxorubicin in the core, reducing the systemic leakage of doxorubicin and achieving enhanced doxorubicin accumulation into a solid tumor with lesser toxic side effects caused by non-specific organ distribution (Matsumura et al.,2004; Nishiyama et al., 2006; Yokoyama et al., 1994; Yokoyaxna et al., 1993). The benzyl containing core of the micelles and aromatic ring of the drug involved in π–π interaction give a more stable system even in the presence of serum proteins (Cammas et al., 1997) and illustrate higher antitumor activity, compared to free doxorubicin (Kataoka et al., 2000). The anticancer formulation NK105 containing paclitaxel in the modified PEG-*b*-PAsp with 4-phenyl-1-butanolate based polymeric micelle showed 90- and 25-fold higher plasma and tumor areas under the curve (AUC), respectively, with the remarkably enhanced antitumor activity against a human colorectal cancer HT-29 cell xenograft in mice compared to free

paclitaxel, thereby limiting the neurotoxicity, a dose-dependant side effect of paclitaxel (Figure 4A and 4B) (Hamaguchi et al., 2005). This formulation of NK105 is currently being studied in a phase I clinical trial at the NCC in Japan.

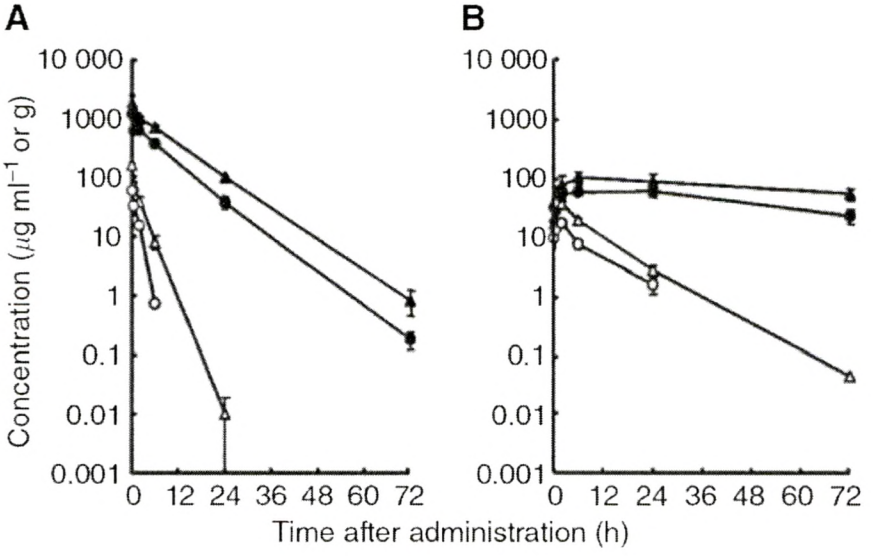


Figure 1.4 PTX concentration in the plasma (A) and tumor (B) after intravenous injection of NK105 or free PTX to Colon 26-bearing mice [PTX-equivalent dose of 50 mg kg-1 (filled circles), NK105 at a PTX-equivalent dose of 100 mg kg-1 (filled triangles), PTX 50 mg kg-1 (open circles), and PTX 100 mg kg-1 (open triangles)].

Chemically conjugated doxorubicin to PEG-*b*-poly(D,L-lactide-co-glycolic acid) [PEO-*b*-PLGA] demonstrated a more cytotoxic activity versus free doxorubicin against HepG2 hepatoma cells due to the enhanced endocytotic uptake of PEO-*b*-PLGA-doxorubicin micelles followed by intracellular transport through a fluid-phase-endocytotic mechanism relative to passive diffusion of free doxorubicin (Yoo and Park 2001).

Yokoyma and colleagues introduced cisplatin into PEG-*b*-PAsp micelles through the ligand substitution reaction at platinum atoms of cisplatin with aspartic acid residues of block copolymer (yokoyma et al., 1996). The micelles showed sustained release behavior of cisplatin in saline media for 50 h and a good stability in distilled water (Nishiyama et al., 2001). However, cisplatin loaded PEG-*b*-PAsp micelles produced *in vivo* antitumor activity slightly higher than free cisplatin for the same dose. This is due to higher accumulation of cisplatin loaded PEG-*b*-PAsp micelles in the tumor cells against free

cisplatin after 8 h. However, it did not produce nephrotoxicity as for the free CDDP that accumulates in the kidney (Nishiyama et al., 2003). Using PEG-*b*-PGlu instead of PEG-*b*-PAsp presents numerous advantages such as increase in sustained release (half-value period: > 90 h instead of 30 h), longer induction period (> 20 h instead of 10 h) under physiological conditions, higher plasma Pt level with a longer persistent time (11% of the injected dose at 24 h instead of 1.5%), and a lower accumulation in the liver and spleen. As with long circulating PEG-*b*-PGlu(CDDP) micelles, it accumulated a 20-fold higher level in tumor than that of the free CDDP and acted as a tumor-selective targeting due to the EPR effect. In the tumor regression study, complete tumor regression is observed for five out of six mice with minimal bodyweight loss (within 5% of the initial weight) with the cisplatin loaded PEG-*b*-PGlu micelle (Figure 1.5), but at the same dose of free cisplatin, tumor regression is observed for only one mouse out of six mice with more bodyweight loss of 20% of the initial weight. The PEG-*b*-PGlu(CDDP) micelles are currently undergoing Phase I clinical trial (UK) (Osada and Kataoka 2006; Uchino et al., 2005).

1.4.5.1.b.2. *Polyester-based block copolymer micelle*

Paclitaxel loaded polymeric micelles have been proved more effective than formulations containing Cremophor to inhibit several tumor types, including ovarian and breast (Kim et al., 2001), lung, (Zhang et al., 1997a; 1997b) colon (Zhang et al., 1997b), and prostate (Leung et al., 2000) cancers. However, in one report, paclitaxel loaded micellar (PEO-*b*-PDLLA) and Cremophor formulations were equally potent against human lung tumors in nude mice at *i.v.* doses of 25 and 20 mg/kg, respectively (Zhang et al., 1997b). Burt et al., 1999 achieved stable paclitaxel loaded micelles by the PDLLA-*b*-MePEG [poly(D,L-lactide)-*b*-methoxypolyethylene glycol] diblock copolymer compared to PGACL-*b*-MePEG [poly(glycolide-co-caprolactone)-*b*-methoxypolyethylene glycol]. *In vivo* data revealed that paclitaxel rapidly dissociated from the micellar compartments in the blood and 95% of the administered dose was eliminated within 15 h with PDLLA-*b*-MePEG micelles. With such copolymers, during phase I study on the loaded paclitaxel micelles, Kim et al., 2004 demonstrated a maximum tolerated dosage of 390 mg/m2. The paclitaxel incorporated poly(N-vinylpyrrolidone)-*b*-poly(D,L-lactide) PVP-*b*-PDLLA (Le Garrec et al., 2004) or P(EtOz)-*b*-PCL (Shuai et al., 2004a) micelles, were potent as Taxol® *in*

vitro and both paclitaxel loaded micelles demonstrated nonhemolytic activity *in vitro* toward human red blood cells (RBC) and rat RBC, respectively.

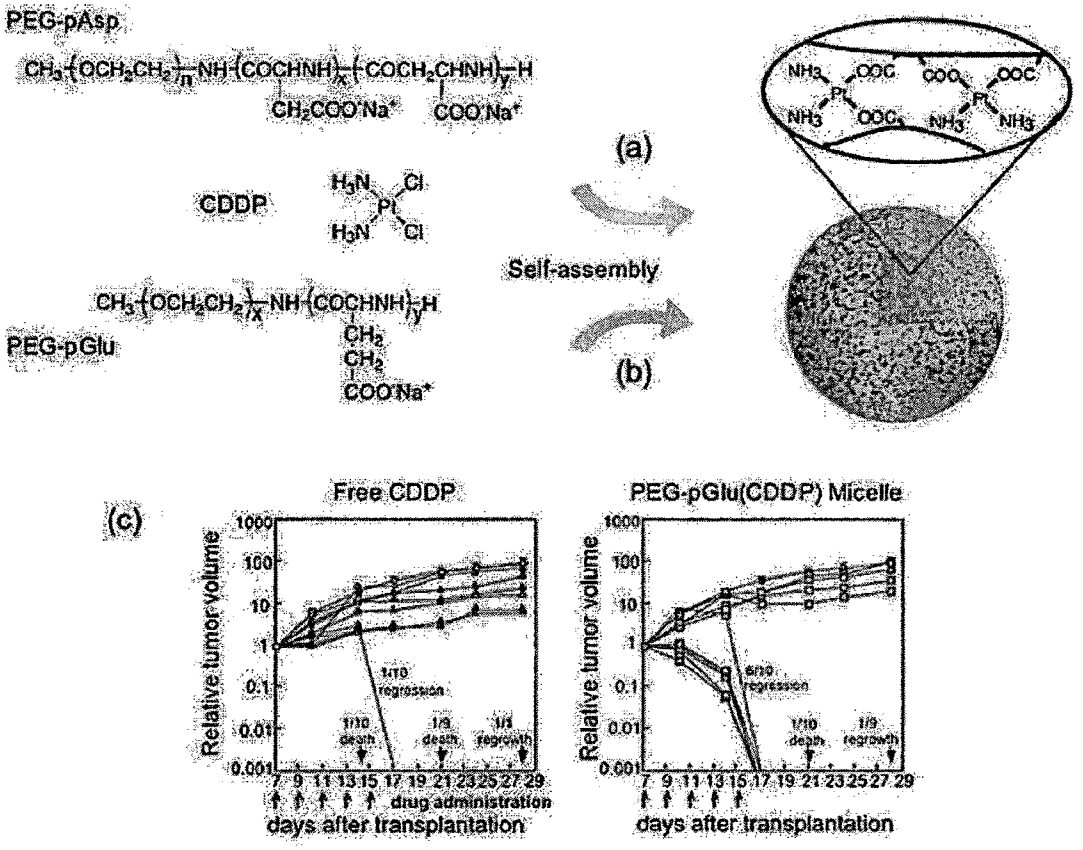


Figure 1.5 Polymeric micelle formation of PEG-PAsp/CDDP (A), and PEG-PGlu/CDDP (B), where carboxylic groups and Pt are linked through coordination bonds. (C) Effect of free CDDP (left-hand side) and PEG-PGlu(CDDP) micelles (right-hand side) on the growth of C26 colon adenocarcinoma subcutaneously transplanted in CDF1 mice (n = 10). Each drug was administrated by i.v. route 5 times at 2-day intervals (arrow) at the dose of 4 mg/kg CDDP eq. The molecular ratio of CDDP to the block copolymer in the micelles was calculated to be 27, and the dose of the injected polymer was 6.3 mg/kg.

1.4.5.1.b.3. Poloxamer-based block copolymer micelle

A major problem in the cancer chemotherapy is the development of multidrug resistance during treatment of many types of tumor cells caused by an increase in the expression of P-glycoprotein (overexpression of MDR1 gene), a transmembrane protein that functions as an ATP-dependent drug efflux pump and development of multidrug resistance–associated protein (MRP). These two pumps are commonly found in tumor cells causing resistance to chemotherapeutic agents. The unique feature of Pluronic® (poloxamers)

micelles against MDR tumor cells, to increase their hypersensitivity, has been used for enhancing antitumor activity of doxorubicin (Venne et al., 1996), epirubicin, mitomycin, vinblastine, and daunorubicin (Alakhov et al., 1996) in MDR tumor cells. This effect has been observed at a lowest concentration than that required for micelles formation of copolymer and MDR repairing properties of poloxamers depend on their block composition (Batrakova et al., 1999).

1.4.5.1.c. Active drug targeting

Tremendous research has been done to bypass the many barriers with novel drug carriers but still indefinable problems, such as fusion and endocytosis of drug carriers within the cell, remain to be solved to achieve maximum response of drug, specifically in cancer therapy. This can be achieved by the modification of drug carriers with specific ligands or leading moieties to cancer cells (active targeting) to improve their retention in the tumor tissue and also to help in their internalization through the receptor-mediated endocytosis with minimum undesired distribution of drug in body. In the following paragraphs, the most relevant strategies used to develop targeted nanocarriers are presented.

1.4.5.1.c.1 Folate funtionalization

Recently, Bae and colleagues developed micelles with receptor selectivity by conjugating folate to the end of the PEO shell of PEO-*b*-PBLA copolymers that self-assemble into micelles (Bae et al., 2005). The obtained results showed that folate-bound polymeric micelle was an excellent intelligent nanodevice for actively delivering drugs inside the cell via selective protein-binding affinity (Figure 1.6).

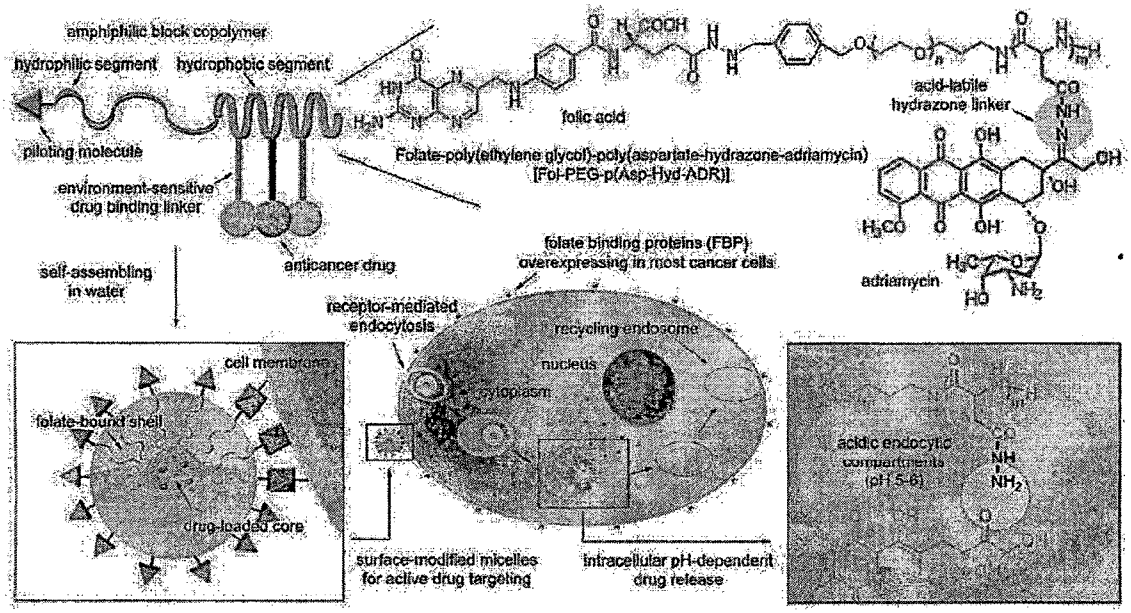


Figure 1.6 Preparation of multifunctional polymeric micelles with tumor selectivity for active drug targeting and pH-sensitivity for intracellular site specific drug transport. Folic acid with high-tumor affinity due to the over expression of its receptors was conjugated onto the surface of the micelle.

A similar approach was used by Lee et al., 2007 who prepared the folate-conjugated pH-sensitive polymeric micelles for targeting doxorubicin-resistant MCF-7 cancer cells. Targeted micelles were made by mixing block copolymers of poly(L-histidine)-*b*-PEG-folate and poly(L-lactic acid)-*b*-PEG-folate in the ratio of 75:25 respectively. The multifunctional micelles showed more than 90% cytotoxicity of doxorubicin-resistant MCF-7 because of active internalization of folate-conjugated micelles via folate receptor-mediated endocytosis. In addition, ionization of the histidine residues results in micelle deformation and disruption of endosomal membranes, with a bypass of P-glycoprotein efflux pump, and release of doxorubicin in acidic intracellular compartments, thus producing high cytotoxicity. *In vivo* studies were carried out on mice bearing MCF-7 or MCF-7 with doxorubicin-resistant xenografts and data revealed that accumulation of doxorubicin from the folate-conjugated micelles was 20 times higher than free doxorubicin and 3 times higher than nonfolate-conjugated micelles.

Yoo and Park (2004) also used folate moiety with biodegradable polymeric micelles and prepared targeted micelles from PEG-*b*-PLGA block copolymers loaded with doxorubicin. Folate was separately conjugated at the PEG terminal end of PEG-*b*-PLGA

diblock copolymer. Tumor regression with higher cytotoxicity in mice model was observed with doxorubicin loaded micelles when folate is bounded onto the PEG shell because of a combined effect of the passive targeting (through EPR) and enhanced cellular uptake (folate receptor-mediated endocytosis).

1.4.5.1.c.2. Peptide functionalization

Zeng et al., 2006 investigated the epidermal growth factor (EGF) as ligand for the EGF receptor (EGFR) overexpressing on cancer cells, attached with the polymeric micelles of block copolymer of poly(ethylene glycol)-*b*-poly(δ-valerolactone) PEG-*b*-PVL. The targeted micelles containing CM-DiI (hydrophobic fluorescent probe) accumulated intracellularly in EGFR-overexpressing MDA-MB-468 breast cancer cells following a 2-h incubation period, while no detectable cell uptake was observed for the nontargeted micelles. Lee et al., 2007 developed an apoptotic EGF-conjugated micelle of PEO-*b*-PCL block copolymer system for targeted combination therapy against EGFR-overexpressing cancers. EGFin this study acts as both a targeting ligand for the drug carrier and an apoptotic factor against EGFR-overexpressing breast cancer cells. EGF micelles were found to be more potent than free EGF at inhibiting EGFR-overexpressing breast cancer cell growth.

PEO-*b*-PCL block copolymers conjugated with RGD (arginine-glycine-aspartic acid) containing model peptide, which acts as a ligand able to recognize integrins overexpressed on the surface of metastatic cancer cells, show pronounced cellular uptake by melanoma B16-F10 cells compared to unconjugated PEO-*b*-PCL micelles and in that sense are promising ligand targeted carriers for drug delivery to metastatic tumor cells (Xiong et al., 2007).

Recently, cell-penetrating peptide like HIV TAT peptide has been used as an anticancer agent to acidic solid tumors. The system is based on the complexation of block copolymer of (methacryloyl sulfadimethoxine) (PSD) and PEG (PSD-*b*-PEG) to cationic TAT micelle constituted of poly(L-lactic acid) (PLLA) core and a hydrophilic shell consisting of polyethylene glycol (PEG) conjugated to TAT (TAT micelle). These systems could target tumor areas that provide an acidic profile. The designed micellar system was able to effectively distinguish a small difference in pH and internalize into

cells. Interestingly, the TAT functionalization helped the micelles' translocation not only into the cells but also near the nucleus (Sethuraman and Bae 2007).

1.4.5.1.c.3. Antibody functionalization

One of the first works in this field by Kabanov et al., 1992 has been realized to target brain tumor. Kabanov and colleagues (Kabanov et al., 1992) used pluronic micelles conjugated with antibodies to the antigen of brain glial cells (α2 glycoprotein), leading to an improvement of the distribution of neuroleptic (haloperidol) in the brain . Decorating micelle surface by antibodies to tumor-specific antigens also results in effective molecular targeting. Selective tumor accumulation of PTX encapsulated in PEO–distearyl phosphatidylethanolamine micelles conjugated to tumor-specific antinucleosome antibody 2C5 (''immunomicelles'') was reported by Lukyanov et al. 2003for the Lewis lung carcinoma mouse model. Recently, Schmidt et al., 2008 have developed spherical micelles with amphiphilic block copolymer polystyrene-*b*-poly(2-phosphatethyl methacrylate-stat-2-hydroxyethyl methacrylate) (PS-*b*-P(PEMA-*stat*-HEMA)) containing Annexin A5, a protein platform for antibody grafting, at their periphery. Other work has been devoted to synthesis of pegylated immunonanoparticles by conjugation of an anti-transferrin receptor monoclonal antibody (MAb) to maleimide-grafted pegylated nanoparticles obtained from emulsion/solvent evaporation technique using poly(lactic acid) (PLA)-*b*-poly(ethyleneglycol) (PEG) block copolymer (Olivier et al., 2002). On these two last examples, the efficiency of the targeting has not yet been tested up to now but we can anticipate interesting properties.

1.4.5.1.c.4. Carbohydrates functionalization

Targeting efficacy of anticancer drugs can also be improved by nanocarrier conjugation with biorecognizable groups such as carbohydrates. Hydroxypropyl methacrylate copolymers bearing galactosamine (GalN), lactose (Lac), or multivalent galactose residues (TriGal) to produce targetable polymeric drug carriers have been synthesized by David et al., 2004. The efficiency of these copolymers evaluated by coupling doxorubicin showed an increase of its cytotoxicity toward human colon-adenocarcinoma cells, which was caused by their bio-recognition and effective internalization via receptor-mediated endocytosis.

Very recently, micelles obtained from the micellization of poly(ethyl ethylene phosphate) and poly(ε-caprolactone) block copolymers were conjugated at their periphery with galactosamine to target asialoglycoprotein receptor (ASGP-R) of HepG2 cells (Wang et al., 2008). Through recognition of galactose ligands with asialoglycoprotein receptor of HepG2 cells, cell surface binding and internalization of galactosamine-conjugated micelles were significantly promoted, which were demonstrated by flow cytometric analyses using rhodamine 123 fluorescent dye. These results indicate a high potential for specific anticancer drug transportation and intracellular drug release.

1.4.5.2. Block copolymer vesicles or polymersomes

The stealthness introduced to liposomes through PEGylation is extended with completely synthetic polymersomes by Discher's group and has been successfully used for cancer chemotherapy. They found a two-fold longer circulation *in vivo* (20–30 h in rats) of polymersomes made of poly(ethyl ethylene) (PEE) or polybutadiene (PBD) as hydrophobic block and polyethylene glycol (PEG) as hydrophilic block than PEGylated liposomes (Photos et al., 2003). Next, they extended the system for controlled release of doxorubicin from polymersomes, composed of block copolymers with pH-sensitive polyesters such as poly(lactic acid) (PLA) or poly(ε-caprolactone) (PCL) mixed with or without inert PEG-*b*-PBD. They observed that vesicles containing PEG-*b*-PCL or PEG-*b*-PLA acted as time-evolving molecular triggers that altered both release kinetics of encapsulant and vesicle disintegration (Ahmed et al., 2004). The features of these polymersomes — such as a thick vesicle membrane, an aqueous lumen, as well as a pH-triggered release — have recently been investigated for loading the two most commonly used anticancer drugs, paclitaxel and doxorubicin, within these degradable polymersomes for tumor growth arrest. Paclitaxel was loaded into the polymersome membranes with 10-fold more efficiency (per mass) than for reported liposomes due to the much thicker membranes of polymersomes (Figure 1.7) compared to that of liposomes (with ~3-nm-thick membrane). These polymer vesicles were transformable into the membrane-lytic micelles within hours at 37°C and low pH. Its entry into acidic endolysosomes accelerates hydrolytic scission of degradable PLA chains and triggers endolysosomal rupture with release of cytotoxic drugs (Ahmed et al., 2006a; 2006b; Discher and Ahmed

2006). This is certainly one of the most promising and exciting approaches that is expected to bring a real breakthrough in the future.

Also recently, ABA triblock copolymers (poly(2-methyloxazoline)–*b*-poly(dimethylsiloxane)–*b*-poly(2-methyloxazoline) PMOXA-*b*-PDMS-*b*-PMOXA based vesicles containing ligand at the surface for specific targeting of cells have been introduced for diagnostic or therapeutic medical use (Brož et al., 2005). In this approach, biotin-functionalized PMOXA-*b*-PDMS-*b*-PMOXA triblock copolymers were self-assembled to form nanocontainers and they were functionalized with the oligonucleotide poly(guanylic acid) (poly-G), which is a specific ligand for the SRA1 receptor (scavenger receptor A1 from macrophages, an important cell in human disease). Linkage of the ligand to vesicle membrane was accomplished via a biotin-streptavidin complex. Further loading of the nanocontainers with fluorescent labels allowed the microscopic observation of the binding and uptake of the vesicles by the cells with high receptor specificity, while unwanted uptake of the targeted vesicles by cell was avoided due to very low interaction of proteins with polymer chains.

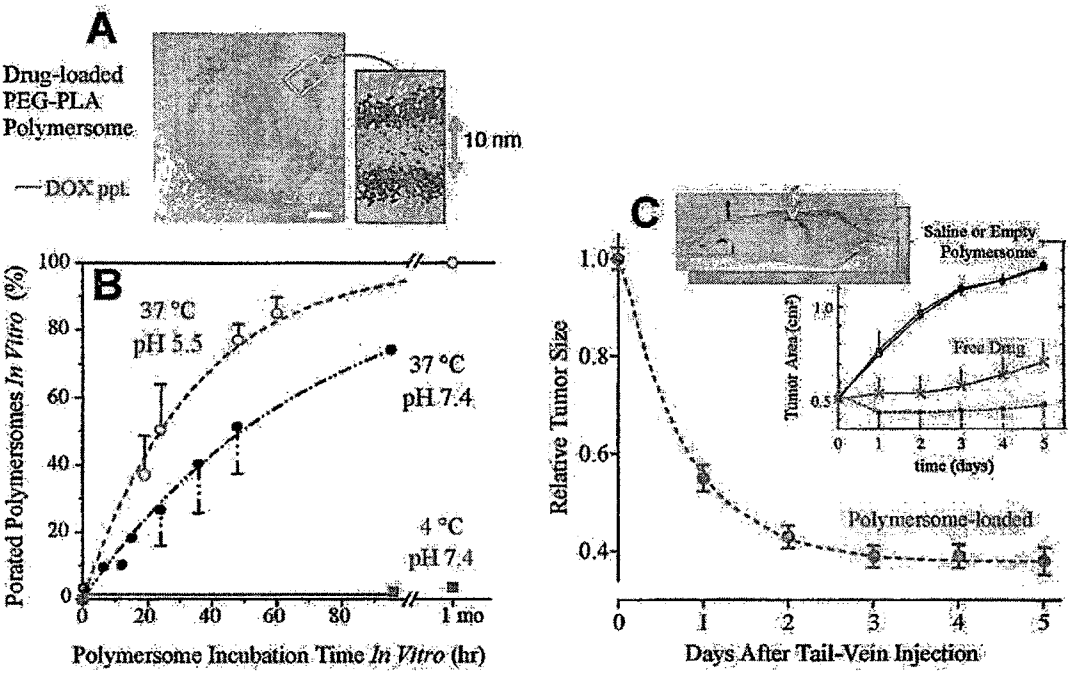


Figure 1.7 Drug loading, release, and antitumor activity of degradable polymersomes. (A) Cryo-TEM image of doxorubicin and paclitaxel loaded polymersome. Doxorubicin permeates and precipitates as an aggregate within the PEG-*b*-PLA–based vesicles, whereas paclitaxel intercalates into the ~10-nm thick hydrophobic core. (B) Degradable giant vesicles visibly porate and release encapsulants in isotonic PBS, pH 7.4 at 37°C and even faster in isotonic HEPES, pH 5.5 at 37°C; however, the vesicles are stable in PBS at 4°C. (C) Solid tumors shrink after a single injection of (doxorubicin + paclitaxel)-loaded polymersomes.

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