Block copolymer micelles: preparation, characterization and application in drug delivery

Geneviève Gaucher a, Marie-Hélène Dufresne a, Vinayak P. Sant a, Ning Kang a, Dusica Maysinger b, Jean-Christophe Leroux a,*

a Canada Research Chair in Drug Delivery, Faculty of Pharmacy, University of Montreal, P.O. Box 6128, Downtown Station, Montreal (PQ), Canada H3C 3J7
b Department of Pharmacology and Therapeutics, Faculty of Medicine, McGill University, 3655 Promenade Sir William Osler, Montreal (PQ), Canada H3G 1Y6

Received 1 March 2005; accepted 15 August 2005

Abstract

Block copolymer micelles are generally formed by the self-assembly of either amphiphilic or oppositely charged copolymers in aqueous medium. The hydrophilic and hydrophobic blocks form the corona and the core of the micelles, respectively. The presence of a nonionic water-soluble shell as well as the scale (10–100 nm) of polymeric micelles are expected to restrict their uptake by the mononuclear phagocyte system and allow for passive targeting of cancerous or inflamed tissues through the enhanced permeation and retention effect. Research in the field has been increasingly focused on achieving enhanced stability of the micellar assembly, prolonged circulation times and controlled release of the drug for optimal targeting. With that in mind, our group has developed a range of block copolymers for various applications, including amphiphilic micelles for passive targeting of chemotherapeutic agents and environment-sensitive micelles for the oral delivery of poorly bioavailable compounds. Here, we propose to review the innovations in block copolymer synthesis, polymeric micelle preparation and characterization, as well as the relevance of these developments to the field of biomedical research.

© 2005 Elsevier B.V. All rights reserved.

Keywords: Block copolymer micelles; Polyion complex micelles; Drug solubilization; Micelle stability; Targeting

1. Introduction

The delivery of highly efficacious therapeutic compounds can be hindered by their poor water solubility. Recent advances in drug formulation have obviated the potential of colloidal vectors to act as efficient solubilizing agents in such cases. The capacity of block copolymer micelles to increase the
solubility of hydrophobic molecules stems from their unique structural composition, which is characterized by a hydrophobic core sterically stabilized by a hydrophilic corona. The former serves as a reservoir in which the drug molecules can be incorporated by means of chemical, physical or electrostatic interactions, depending on their physicochemical properties.

Beyond solubilizing hydrophobic drugs, block copolymer micelles can also target their payload to specific tissues through either passive or active means. Prolonged in vivo circulation times and adequate retention of the drug within the carrier are prerequisites to successful drug targeting. Long circulation times ensue from the steric hindrance awarded by the presence of a hydrophilic shell and the small scale (10–100 nm) of polymeric micelles. Indeed, micelles are sufficiently large to avoid renal excretion (> 50 kDa), yet small enough (< 200 nm) to bypass filtration by interendothelial cell slits in the spleen [1]. Drug retention, in turn, is dependent on micelle stability and polymer–drug interactions. Many approaches are being employed to enhance the physical stability of the carrier, improve its resistance towards dissociation upon entering the bloodstream, and tailor its properties to better suit those of the incorporated drug.

Our group has focused on improving polymeric micelles as drug delivery systems, with research spanning from amphiphilic block copolymer micelles for the solubilization of anti-cancer agents to environment-responsive systems for the oral delivery of insoluble drugs. The purpose of this review is to provide a concise description of the properties of block copolymer micelles as well as to highlight the past and current applications of these systems in the area of drug delivery. We also intend to outline certain challenges facing researchers developing micellar systems, with emphasis on the stability and drug retention capabilities of the carriers both in vitro and in vivo.

2. Polymeric micelles: composition and structure

Block copolymer micelles can be classified according to the type of intermolecular forces driving the segregation of the core segment from the aqueous milieu. In the past few decades, at least three main categories were identified, viz. amphiphilic micelles (formed by hydrophobic interactions), polion complex micelles (PICM; resulting from electrostatic interactions), and micelles stemming from metal complexation [2,3]. Generally, when the hydrophilic segment is longer than the core block, the shape of the resulting micelles is spherical. Conversely, increasing the length of the core segment beyond that of the corona-forming chains may generate various non-spherical structures, including rods and lamellae [4].

The self-assembly of amphiphilic block copolymers in water is based on non-polar and hydrophobic interactions between the lipophilic core-forming polymer chains. The process is concomitantly driven by a gain in entropy of the solvent molecules as the hydrophobic components withdraw from the aqueous media [5]. Most amphiphilic copolymers employed for drug delivery purposes contain either a polyester or a poly(amide acid) derivative as the hydrophobic segment. Poly(lactic acid) (PLA), poly(ε-caprolactone) (PCL), and poly(glycolic acid) are all biocompatible and biodegradable polysters approved by the FDA for biomedical applications in humans. Poly(L-amino acid)s (PAA) commonly used in drug delivery include poly(aspartic acid) (PAsp), poly(glutamic acid) (PGlu), poly(L-lysine) (PLys) and poly(histidine) (PHis). However, for these polymers to self-assemble into amphiphilic micelles, the PAA segment must either be electrostatically neutral or conjugated to hydrophobic moieties. Amino acid-based block copolymers are being studied extensively in the field of drug delivery because of their biodegradability, biocompatibility and structural versatility. By varying the chemical structure of PAA, it is possible to tailor their enzymatic degradability and degree of immunogenicity [6]. Polyethers constitute another class of polymers that can be employed to prepare amphiphilic micelles. Most of the polyethers of pharmaceutical interest belong to the poloxamer family, i.e. copolymers of poly(ethylene glycol)-b-poly(propylene oxide)-b-poly(ethylene glycol) (PEG-b-PPO-b-PEG) [7].

The self-assembly of PICM, in turn, proceeds through the neutralization and segregation of oppositely charged polions in a way that combines features of amphiphilic micelles and interpolyelectrolyte complexes. The latter are formed from electrostatic interactions between polycations and polyanions, and precipitate in solution. PICM distin-
guish themselves from these complexes in that they possess a hydrophilic segment which ensures the solubility of the condensates in aqueous media [8]. Polymers having protonated amines at physiological pH may be considered good candidates for the preparation of PICM incorporating polyanionic molecules such as plasmid DNA [9], oligodeoxyribonucleotides (ODN) [10], polysaccharides [11] and enzymes [12]. Examples of such polymers are poly(ethyleneimine) (PEI), PLys, polyamidoamide and poly(2-(N,N-dimethylamino)ethyl methacrylate) (PDMAEMA) [11]. To form PICM with polycationic drugs, polymers exhibiting negatively charged units, including poly(methacrylic acid) (PMAA) and PAsp [13], are required.

3. Polymer synthesis

To better predict and control the properties of a micellar system, it is crucial that the copolymers utilized be well-defined. The nature of the polymer to be synthesized will determine the most appropriate polymerization reaction to use (viz. anionic, ring-opening polymerization reaction to use (viz. anionic, ring-opening polymerization, radical polymerization). Since the end methoxy is inert, there is growing interest in customizing this chain end to facilitate the preparation of diblock copolymers bearing a targeting moiety. This can be achieved by initiating the anionic polymerization of ethylene oxide with a functionalized initiator. NH$_2$-PEG-OH [14,15], COOH-PEG-OH [16], aldehyde-PEG-OH [17], and monosaccharide-PEG-OH [18] were successfully prepared by this synthetic approach.

α-Methoxy-ω-hydroxyl-PEG, in turn, can serve as a macroinitiator in the preparation of polyester block copolymers (Fig. 1A). Polyesters of D,L-lactide, glycolide, ε-caprolactone, and δ-valerolactone are all very attractive because of their safety profile and solubilizing capabilities [24,25]. These copolymers are prepared by ROP at melt temperatures (i.e. from 115 to 180 °C) in the presence of stannous octoate (0.05-0.5 wt.%) [26–28]. Under these conditions, Riley et al. were able to control the length of the PDLLA segment of PEG-b-PDLLA from 2000 to as much as 110,900 [29]. The same procedure was adopted by our group to prepare the isotactic stereoisomers PEG-b-P(D-lactide) (PEG-b-PDLA) and PEG-b-P(L-lactide) (PEG-b-PLLA) with control over PLA chain length and optical purity [30]. Catalysts other than stannous octoate can also be used. For instance, Piao et al. reported the ROP of ε-caprolactone in xylene with calcium ammoniate [31]. This nontoxic catalyst was argued to be safer than other transition metal catalysts for the preparation of copolymers with pharmaceutical applications. Alternatively, the group of Kataoka applied potassium naphthalene to generate a PEG alkoxide and initiate the anionic ROP of DLLA at room temperature [32].

In addition to PAA and polyesters, vinylic polymers are widespread as core-forming segments given that they can easily be tailored for hydrophobic
Fig. 1. Approaches to the synthesis of (A) diblock and (B) multiblock copolymers for drug delivery applications.
interactions, ionic association, or the preparation of smart drug delivery systems. A shortcoming of vinylic polymers is that they are not biodegradable. Hence, when administered parenterally, care should be taken to maintain their molecular weight below the renal excretion limit to ensure their systemic elimination. Our group exploited atom transfer radical polymerization (ATRP) to prepare well-defined block copolymers with hydrophobic as well as ionizable units (Fig. 1A). α-Methoxy-ω-hydroxyl-PEG was first reacted with 2-bromoisobutyryl bromide to yield a macroinitiator suitable for ATRP. The polymerization reaction was then conducted in bulk or in tetrahydrofuran (THF) in the presence of the Cu(I)Br/N,N,N′,N″-pentamethyldiethylenetriamine catalytic system (1/1 molar ratio) at 65 °C. Such reaction conditions permitted the controlled copolymerization of ethyl acrylate (EA), ethyl methacrylate (EMA), methyl methacrylate (MMA), n-butyl acrylate (nBA), tert-butyl methacrylate (tBMA), DMAEMA, and 2-(N,N-diethylamino)ethyl methacrylate (DEAEMA) [33–36]. Cleavage of the tert-butyl group of tBMA provided ionizable MAA units whereas alkylation of the amino groups of DMAEMA and DEAEMA afforded permanent positive charges. Moreover, we showed for the first time that it was possible to use ATRP for the preparation of polymers containing primary amino groups (namely, aminoethyl methacrylate (AEMA)) [36].

Besides ATRP, other radical polymerization procedures have been exploited to prepare vinylic block copolymers. For instance, α-methoxy-ω-hydroxyl-PEG was capped with a dithiobenzoyl group to generate a macro-transfer agent which was used in the reversible addition-fragmentation transfer (RAFT) polymerization of N-isopropylacrylamide (NIPAM) (Fig. 1A) [37]. Copolymers with molecular weight distributions as low as 1.1 were thereby obtained. Alternatively, α-methoxy-ω-hydroxyl-PEG was coupled to an azo-initiator to promote the free radical polymerization of NIPAM and oligolactate esters of 2-hydroxypropyl methacrylamide (Fig. 1A) [38,39]. The composition of the copolymers conferred unique thermostensitive properties to the resulting micelles and was adjusted by simply varying the monomer to macroinitiator ratio.

While PEG remains the gold standard for the steric stabilization of colloidal drug carriers, other nonionic and hydrophilic polymers can be used. This is the case for poly(N-vinyl-2-pyrrolidone) (PVP), a nonionic, biocompatible, and water-soluble synthetic polymer. Due to its lyoprotectant and cryoprotectant properties, PVP may be preferred to PEG for preparation methods involving freeze-drying. Furthermore, PVP was shown to interact with a variety of hydrophilic and hydrophobic pharmaceutical agents, thus potentially increasing the solubilizing capacity of micelles [40]. PVP is usually obtained via free radical polymerization with poor control over molecular weight and chain end functionalization. Hence, the synthesis of well-defined PVP-OH macroinitiators to prepare PVP block copolymers remains challenging. Our first attempts at preparing a PVP-OH macroinitiator were conducted with 1,1′-azobis(cyclohexane-carbonitrile) as the radical source and 2-isopropanol as both the chain transfer agent and solvent [40]. PVP-b-PDLLA copolymers prepared from this macroinitiator were contaminated with PDLLA homopolymers and unreacted PVP. Possible causes are the presence of residual solvent (high boiling point, capable of forming H-bonds with PVP) which can coinitiate the polymerization of PDLLA and a poor control over the chain end functionalization of PVP (i.e. not all of the chains were terminated with 2-isopropanol). Luo et al. showed that it is possible to solve these issues by using a hydroxyl-bearing azo-initiator, substituting the solvent for isopropyl alcohol (lower boiling point), and adding 2-mercaptoethanol as a second chain transfer agent [41]. Control over the molecular weight of PVP was achieved by varying either the solvent/monomer or mercaptoethanol/monomer ratios. Matrix-assisted laser desorption ionization-time-of-flight analysis revealed that, while most of the polymer chains were terminated by isopropyl alcohol, weight distribution also accounted for chains terminated by 2-mercaptoethanol and the azo-initiator. Under these conditions, PVP-OH macroinitiators with not less than 95% end hydroxyl groups were prepared and productively employed in the preparation of PVP-b-PDLLA.

Anionic ROP was applied to prepare PVP-b-PDLLA copolymers (Fig. 1A). This procedure conferred greater reactivity to the hydroxyl-terminated PVP macroinitiator than ROP mediated by conventional tin catalysts [40,41]. Briefly, PVP-OH was
thoroughly dried, added to a potassium hydride slurry, and dissolved in anhydrous THF. The polymerization reaction was conducted at 60 °C after the addition of DLLA and 18-crown-6. Copolymers with PDLLA chain lengths of ~2000 and molecular weight distributions as low as 1.14 were obtained [41].

3.2. Multiblock copolymers

A first approach for the preparation of multiblock copolymers consists of coupling two or more diblock copolymers. For instance, Hwang et al. prepared PEG-b-PCL-b-PEG using hexamethylene disocyanate to conjugate the end hydroxyl groups of the PCL segments [42]. However, such reactions are usually associated with the formation of side products and should be avoided. The preferred scheme is the sequential synthesis of a multifunctional core segment which is then applied to initiate polymerization of the shell block. This strategy is employed commercially in the synthesis of poloxamers where PPO initiates the anionic polymerization of ethylene oxide (Fig. 1B). Similarly, we used a PCL core to promote the free radical polymerization of triblock and star copolymers of poly(N-(2-hydroxypropyl)methacrylamide) (PHPMA) (Fig. 1B) [43,44]. PHPMA is a nonionic, biocompatible, and nonimmunogenic polymer which presents functionalities for tethering ligands in a micellar system. In short, the hydroxyl groups of OH-PCL-OH and star-(PCL-OH)₄ were derivatized with dithiodipropionic acid. Cleavage of the disulfide bonds with dithiothreitol yielded the corresponding thiolated PCLs, which then served as macromolecular chain transfer agents in the radical polymerization of HPMA. The synthetic approach was soon after adapted by Kang and Leroux for the preparation of PHPMA-b-PDLLA-b-PHPMA, star-(PDLLA-b-PHPMA)₄, PVP-b-PDLLA-b-PVP, and star-(PDLLA-PVP)₄ copolymers [26].

4. Micelle preparation

Depending on the physicochemical properties of the block copolymer, two main classes of drug-loading procedures can be applied [45]. The first class, direct dissolution, involves dissolving the block copolymer along with the drug in an aqueous solvent. This procedure is mostly employed for moderately hydrophobic copolymers, such as poloxamers, and may require heating of the aqueous solution to bring about micellization via the dehydration of the core-forming segments. The direct dissolution method is also used to prepare PICM. Here, the copolymer and drug are dissolved separately in an injectable aqueous vehicle. Micelle formation is induced by combining the two solutions to appropriate drug–polymer charge ratios.

The second category of drug-loading procedures applies to amphiphilic copolymers which are not readily soluble in water and for which an organic solvent common to both the copolymer and the drug (such as dimethylsulfoxide, N,N-dimethylformamide, acetonitrile, THF, acetone or dimethylacetamide) is needed. The mechanism by which micelle formation is induced depends on the solvent-removal procedure. For water-miscible organic solvents, the copolymer mixture can be dialyzed against water, whereby slow removal of the organic phase triggers micellization. Alternatively, the solution-casting method entails evaporation of the organic phase to yield a polymeric film where polymer–drug interactions are favored. Rehydration of the film with a heated aqueous solvent produces drug-loaded micelles. Physical entrapment of a hydrophobic drug may be further achieved through an oil-in-water (O/W) emulsion process which involves the use of a non-water-miscible organic solvent (viz. dichloromethane, ethyl acetate). The above-mentioned techniques all require sterilization and freeze-drying steps to produce injectable formulations with an adequate shelf-life. Fig. 2 illustrates these commonly employed drug incorporation methods.

Recently, an innovative one-step procedure was described, based on the dissolution of both the polymer and the drug in a water/tert-butanol (TBA) mixture with subsequent lyophilization of the solvents. Drug-loaded micelles are formed spontaneously upon reconstitution of the freeze-dried polymer–drug cake in an injectable vehicle (Fig. 2E) [5]. TBA, a Class 3 category water-miscible solvent, was chosen as the co-solvent because of its high vapor pressure which accelerates its sublimation and, hence, the lyophilization process [46]. Moreover, TBA induces the formation of fine, needle-like ice crystals that sublime rapidly, leaving behind freeze-dried cakes
with a high degree of porosity. With this procedure, two hydrophobic anticancer agents, paclitaxel and docetaxel, were loaded successfully into a PVP-b-PDLLA copolymer, yielding stable spherical micelles with a monodisperse size distribution and a mean diameter of 30–60 nm. The influence of TBA on the dynamics of micellization prior to the lyophilization step was studied by dynamic light scattering (DLS) and $^1$H-NMR spectroscopy. Higher amounts of TBA amplified the intensity of the PDLLA core $^1$H-NMR signals and led to an increase in micelle size. This phenomenon was ascribed to the improved solubilizing capacity of TBA towards the PDLLA core chains compared to water, as predicted by their similar solubility parameters. Moreover, larger proportions of TBA in the feed resulted in an increase in the mean micelle diameter and the aggregation number after lyophilization. For instance, the diameter of PVP-b-PDLLA micelles went from 52 to 86 nm when the water/TBA ratio was raised from 80/20 to 50/50 (v/v). It was speculated that micelles formed at higher proportions of TBA contained swollen PDLLA cores which were “frozen-in” upon lyophilization, accounting for their larger diameters. Overall, this straightforward procedure allows for the preparation of freeze-dried, drug-loaded micelles which possess adequate shelf-life while being easily redispersed in water [47].

As evoked by our results, process parameters such as the nature and proportion of the organic phase, as well as the latter’s affinity for the core-forming segment, can affect the preparation of drug-loaded polymeric micelles and alter the properties of the end product. In addition, the incorporation method itself can modulate the attributes of the yielded micelles. For instance, Sant et al. determined that O/W emulsion yielded a 1.5-fold increase in the drug encapsulation efficiencies of several hydrophobic drugs into PEG-b-poly(alkyl acrylate-co-MAA) (PEG-b-P(AlA-co-MAA)) micelles compared to the dialysis method [35]. It was argued that, using this procedure, drug molecules are entrapped within the droplets of organic solvent stabilized by polymer chains and are retained accordingly upon evaporation of the organic phase. Disparities among micelles prepared by different methods were also encountered by Vangeyte et al. [48]. They demonstrated that, in the case of PEG-b-PCL micelles, the dialysis procedure did not offer adequate size control, whereas stable assemblies with unimodal size distributions

---

**Fig. 2.** Common drug-loading procedures: (A) simple equilibrium, (B) dialysis, (C) O/W emulsion, (D) solution casting, and (E) freeze-drying.
were formed by rapid precipitation under stirring (addition of the organic phase containing the copolymer to the aqueous phase or vice versa, followed by dialysis against water). The nature of the organic solvent, the order of addition of the liquid phases and the concentration of the copolymer in the organic phase were found to significantly impact the size and polydispersity index of the formulations.

5. Micellar drug solubilization

Surfactants and amphiphilic block copolymers can greatly affect the aqueous solubility of compounds by providing a hydrophobic reservoir where they can partition. Allen et al. showed that the water solubility of dihydrotestosterone was enhanced by a factor of 300 when incorporated into the core of PEG-\(b\)-PCL micelles [49]. We established the partition coefficients of paclitaxel and docetaxel into PVP22-\(b\)-PDLLA14 micelles to be \(5.07 \times 10^4\) and \(2.46 \times 10^4\), respectively, suggesting that partitioning of the drugs, in particular paclitaxel, into the hydrophobic PDLLA phase was highly favored [47].

The body of literature on polymeric micelles as solubilizers underlines the importance of selecting an appropriate hydrophobic core-forming polymer. The degree of compatibility or interaction between the polymer and the drug is expected to influence many important features, including stability, encapsulation efficiency and drug release kinetics. For instance, Shuai et al. determined the level of doxorubicin encapsulated within PEG-\(b\)-PCL micelles to be highly dependent on polymer–drug hydrophobic interactions, hydrogen-bonding interactions and PCL crystallinity, as monitored by Fourier transform infrared spectroscopy [50]. Lin et al., in turn, found the difference in the hydrophobicity of various poly(lactones) (PLLA, PCL and (poly(\(\delta\)-valerolactone)) to have a bearing on the loading efficiency of indomethacin and its release profile from corresponding micelles [24]. Likewise, Burt et al. suggested that the stability of polymeric micelles carrying paclitaxel varied according to the nature of the core-forming polyester [25]. It follows that polymer–drug compatibility is of prime relevance in the design of colloidal vectors and should be addressed methodically. Empirical as well as theoretical approaches have been proposed to evaluate and predict the compatibility between a micellar system and a given drug molecule. As such, Liu et al. demonstrated that a good correlation could be established between the polymer–drug compatibility predicted by total and partial solubility parameters (calculated by a group contribution theory) and actual formulation performance (encapsulation efficiency, drug release profiles) [51].

6. Micelle stability

Physical stability is fundamental for a micelle drug delivery system to withstand dissociation and premature release of its cargo after entry into the bloodstream. It is well-established that blood proteins are likely to adsorb onto the surface of charged or hydrophobic foreign matter and hasten its clearance from the blood circulation. Moreover, protein binding may disrupt micelle cohesion as well as trigger premature drug release from the carrier in vivo. Protein–carrier interactions are therefore of prime relevance when evaluating the targeting potential of a given drug delivery system. Heller et al. applied dynamic light scattering to track the stability of PEG-\(b\)-poly(ortho ester)-\(b\)-PEG micelles following incubation at 37 °C in the presence of bovine serum albumin. Their results showed that the impact of serum proteins on the stability of micelles differs significantly according to the nature and composition of the block copolymer [52]. Likewise, interactions between drug and protein molecules can be liable for rapid dissociation of the drug from the vector. While the binding of proteins to PEG-\(b\)-poly(5-benzyloxy-trimethylene carbonate) micelles was found to be insignificant, the release of the hydrophobic drug ellipticine was accelerated in the presence of bovine serum albumin [53]. It is argued that assessing the partitioning of the drug between the micellar system and the aqueous medium containing protein may allow for a more accurate prediction of the system’s fate in vivo.

Depending on the volume of distribution and rate of removal of the vector from the blood compartment, the polymer concentration may fall below the critical association concentration (CAC) following intravenous administration. In contrast to low molecular
weight surfactants, polymeric micelles exhibit significantly lower CAC values, indicating greater thermodynamic stability. Nevertheless, numerous efforts have been directed at better understanding the dynamics of dissociation to improve the stability and in vivo performance of polymeric micelles. It is seen that the rate at which the micelles tend to dissociate is related to the composition, physical state and cohesion of the micelle core. For instance, it has been shown that micellar stability correlates well with the length of the hydrophobic segment in the amphiphilic copolymer, with higher proportions of hydrophobic polymer conferring greater thermodynamic stability. We have demonstrated, by a steady-state pyrene fluorescence technique, that increasing the PDLLA proportion in PVP-b-PDLLA-b-PVP triblock copolymers from 27 to 55 mol% led to a decrease in CAC values from 19.9 to 5.1 mg/L [26]. Alternatively, Jette et al. used size exclusion chromatography to show that an increase in the length of the core-forming segment could reduce fenofibrate leakage while enhancing the resistance of PEG-b-PCL polymeric micelles to dissociation [54]. Other groups have focused on modifying the properties of the core-forming blocks in an attempt to enhance their hydrophobicity. For instance, Adams et al. developed PEG-b-poly(N-hexyl-L-aspartamide) copolymers containing acyl side chains of varying lengths conjugated to the aspartic acid core segments. A good correlation was found between the level of stearate substitution and the stability of the system, as assessed by the time-dependent hemolytic activity of encapsulated amphotericin B toward bovine erythrocytes [55,56]. Finally, benzyl ester derivatives of PEG-b-PAsp copolymers formed camptothecin-loaded micelles which exhibited greater stability at higher degrees of esterification (decreased CAC and in vitro release rate) [57].

Micelle stability is also strongly related to the physical state of the core-forming polymer, be it amorphous or crystalline. Block copolymer micelles comprised of a hydrophobic block with a glass transition temperature (Tg) exceeding 37 °C are said to have “frozen” cores, i.e. the molecular motions of the chains in the core are constrained, generally accounting for greater kinetic stability upon dilution [45]. Kataoka et al. deployed a pyrene probe to monitor temperature dependence of the CAC of PEG-b-PDLLA copolymers. While the CAC was constant at a temperature range below the Tg, it drastically increased at temperatures above the Tg, suggesting enhanced core fluidity and a decrease in the stability of the micelles [58]. Burt et al., in turn, reported the superior stability of paclitaxel-loaded PEG-b-PDLLA versus PEG-b-P(DLLA-co-CL) micelles, resulting from the reduced fluidity of the PDLLA core (as determined by fluorescence intensity and anisotropy techniques) [25]. This finding correlates with the higher Tg of the PDLLA block. Block copolymer micelles can be made up of a crystalline core. Crystallinity contributes to micelle stability and may confer greater drug retention properties by decreasing the rate of diffusion of the drug from the core. We recently developed an approach to increase the crystallinity and kinetic stability of PEG-b-PLA block copolymer micelles. It has been shown that blending the isotactic stereoisomers PDLA and PLLA in a 1:1 molar ratio leads to the formation of a crystalline triclinic unit cell in which the chains exhibit a 3_1 helical conformation, displaying a melting point 50 °C above that reported for the enantiomeric components [59]. We prepared equimolar mixtures of PEG-b-PDLA and PEG-b-PLLA enantiomeric copolymers which self-assembled into spherical micelles with narrow distributions and mean diameters of 30–50 nm. The formation of stereocomplexes was confirmed by X-ray diffraction analysis and modulated differential scanning calorimetry. A time-dependent stability study by DLS revealed that these stereocomplex micelles exhibited kinetic stability superior to that of isotactic or racemic polymeric micelles. Stability could be improved slightly by increasing the length of the PLA chains in the copolymer (Fig. 3). Enhanced stability of the micellar system was due to strong van der Waals interactions between PLA chains in the stereocomplexes, resulting in a more compact conformation and denser packing of the polymer [30].

Cross-linking of the shell or core of hydrophobic micelles is yet another promising stratagem to improve the stability of micelles and is often evidenced by a decrease or even the absence of a CAC [60]. Shuai et al. performed core cross-linking of paclitaxel-loaded PEG-b-PCL micelles by radical polymerization of double bonds introduced into the
PCL blocks [61]. These micelles exhibited significantly enhanced stability against dilution with aqueous solvents, i.e. no micelle collapse was detected upon dilution (1000 ×), as determined by DLS and gel permeation chromatography. More recently, Kataoaka et al. prepared trypsin-loaded PEG-b-P(Asp) PICM cross-linked with glutaraldehyde via Schiff base formation between protein and polymer molecules in the core [62]. The cross-linked micelles showed remarkable stability against high salt concentrations while preserving the activity of the incorporated protein.

 Likewise, strong cohesive forces between the drug and the polymer core segments can also confer physical stability to the system. Lee et al. demonstrated that PEG-b-PDLA copolymers with additional carboxylic acid groups could establish hydrogen bonds with the incorporated drug papaverine [63]. These interactions resulted in a controlled release profile in vitro wherein 90% of the drug was released within 7 days versus 10 h for micelles without COOH moieties. A drug may otherwise be covalently conjugated to the core-forming polymer through various linkers, which consist mainly of hydrolyzable bonds (including esters [64], amides [65], carbamates [28]) or pH-sensitive bonds (such as β-thiopropionate [66], hydrazones [67] and cis-acetylonyl [68]). Li et al. found that the higher the level of methotrexate ester conjugation, the greater the micelle stability and the slower the drug release from the carrier [64].

 If the above discussion on the effects of dilution also applies to PICM, these nanocarriers are nonetheless particular in that other factors, including ionic strength and pH, affect their stability. The CAC of PICM has been measured by pyrene fluorescence, light scattering as well as turbidimetry studies [8]. CAC values for PICM of poly(isobutylene)-b-PMAA coupled with the cationic poly(N-ethyl-4-vinylpyridinium bromide) were found to be similar to those established for poloxamers [69]. Harada et al. determined the CAC values of PEG-b-PLys carrying antisense ODN to be around 140–170 mg/L [70]. These values are superior to the CAC of most amphiphilic copolymer micelles and suggest that dissociation of PICM upon dilution could seriously compromise their in vivo performance. The stability of PICM is also dictated by the ionic strength, or salt concentration, of the aqueous solvent. The presence of salts in solution can shield electrostatic interactions between core-forming, oppositely charged polycations.
and trigger either micelle dissociation or structural changes. On the other hand, pH will have a bearing on the degree of ionization of polyions and their mutual capacity to participate in coupling reactions. The composition of polyions influences the resistance of PICM to salt and pH variations. Indeed, Dufresne et al. demonstrated that, among several polymers presenting pendant amino groups with different degrees of substitution, polycations with the highest $pK_a$ values formed the most stable PICM towards basification and an increase in ionic strength. In this respect, PEG-$b$-P(AEMA), a copolymer bearing primary amino groups, proved to be promising for the formulation of heparin [11]. In a parallel study, the susceptibility of PICM to dissociate in the presence of salts was found to be inversely related to the length of the core-forming polyions. PICM of PEG-$b$-poly(-nBA-co-MAA) and PLys of various chain lengths (viz. 1800, 8300, and 30,200) were prepared and submitted to escalating NaCl concentrations. Fig. 4 illustrates how PICM of greater stability were formed when the length of the polycation was increased. This phenomenon can be ascribed to cooperative electrostatic forces of greater magnitude for PICM of larger molecular weight.

7. Long circulating properties

In theory, the prolonged circulation time of polymeric micelles allows for their preferential accumulation at certain biological sites characterized by vascular abnormalities (including tumorous and inflamed tissues) through the enhanced permeation and retention (EPR) effect. Tumors exhibit newly formed vessels with poorly aligned endothelial cells and wide fenestrations; according to the tumor model, endothelial pores with sizes varying from 10 to 1000 nm can be found. This phenomenon is typically coupled with defective lymphatic drainage. As a result, tumors present erratic fluid and molecular transport dynamics. To achieve EPR-mediated cancer targeting, the plasma concentration of the drug must remain sufficiently high for a prolonged period of time, ideally over 6 h [71]. However, in practice, little is known of the fate of drug-loaded polymeric micelles after their intravenous administration. Burt et al. demonstrated that, within minutes of intravenous administration, paclitaxel was swiftly released from its carrier while the PEG-$b$-PDLLA copolymer chains were found to accumulate in the kidney and undergo extensive renal excretion [25]. Several factors have been linked to the long-circulation properties of colloidal vectors, among which size distribution and steric hindrance conferred by the presence of a hydrophilic corona are the most critical.

The hydrophilic corona of polymeric micelles can be composed of various types of polymers. PEG, the most commonly used shell-forming polymer, is one of the few synthetic polymers approved by the FDA for internal use. Its biocompatibility and lack of toxicity have largely contributed to its acceptance. When hydrated, PEG forms a dense brush of polymer chains stretching out from the core of the micelle. Owing to its high aqueous solubility, high mobility and large exclusion volume [72], PEG imparts steric stability by minimizing the interfacial free energy of the micellar core and by impeding hydrophobic intermicellar attractions [73]. $^1$H-NMR analysis suggests that PEG chains anchored to a polyester core extend outward into the aqueous environment, displaying flexibility and mobility comparable to PEG molecules dissolved in water [74]. The hydrophilic corona is vital in preventing
opsonin adsorption and subsequent clearance by the mononuclear phagocyte system in the liver and spleen [1]. This is especially true for PICM which might present cores with residual positive charges. Kursa et al. have shown that the neutral nature of the PEG corona could effectively shield the cationic PEI and prevent nonspecific interactions with negatively charged plasma proteins in the biological environment, thereby ensuring prolonged circulation times [75]. Moreover, while PEG chains are usually between 1 and 15 kDa in length [76], some data indicate that the longer the chains and the denser the hydrophilic brush, the greater the resulting “stealth” effect and blood circulation times [72]. Peracchia et al. found that the conformation of PEG anchored to polymeric nanoparticles could influence protein adsorption and complement-mediated phagocytosis [77]. Indeed, PEG attached by both ends to the nanoparticles formed a more compact conformational cloud which showed greater hindrance against blood protein adsorption. Lastly, the PEG chain end can be functionalized to tether ligands not only to actively target certain tissues in the body but also to modify the properties of the corona. Kataoka et al. prepared acetaldehyde-functionalized PEG-b-PDLLA micelles to which an anionic peptidyl ligand (tyrosyl-glutamic acid) was coupled to confer a negative surface charge for enhanced “stealth” properties [78]. Up to 25% of the administered polymeric micelles were still circulating 24 h after intravenous injection, suggesting that the elimination half-life of the carrier resembled that of well-established vectors such as PEGylated liposomes.

8. Cellular internalization

Recently, increasing efforts have focused on gaining insights into the pathway of cellular internalization as well as the subcellular localization of micellar carriers and their payload. Maysinger et al. conducted exhaustive studies to elucidate the mechanisms governing the cellular uptake of block copolymer micelles [79]. As such, confocal laser scanning microscopy (CLSM) data suggested that the incorporation of a hydrophobic molecule into PEG-b-PCL polymeric micelles could significantly decrease its rate of cellular uptake. The latter was determined to be an endocytic process in light of its time, pH, energy, and temperature-dependence [80]. Furthermore, labeling of the block copolymers with a fluorescent probe confirmed that the nanocarriers were internalized as intact micelles, as opposed to free unimer chains [81]. Elsewhere, triple-labeling confocal microscopy in live cells indicated that the micelles were predominantly localized in the cytoplasm and distributed within several cytoplasmic organelles, but not in the nucleus [82]. Similarly, we showed that paclitaxel-loaded PVP-b-PDLLA block copolymer micelles tended to gather within subcellular compartments, mainly lysosomes (Fig. 5C), whereas free paclitaxel diffused throughout the cytoplasm and nucleus (Fig. 5A). Yet, much remains to be elucidated with respect to interactions at the cellular and subcellular levels between a drug-loaded carrier and its target in vivo.

9. Targeting

9.1. Ligand-mediated targeting

In cellular-specific targeting, pilot molecules are installed at the end of the hydrophilic segment so that they may extend outward from the micelle corona and readily encounter and interact with membrane receptors. The main purpose of functionalization of the hydrophilic corona is to modulate the biodistribution of polymeric micelles and induce specific cellular uptake by receptor-mediated endocytosis. Certain types of tissues are known to overexpress specific protein receptors on their surface. The localization of such site-specific receptors has contributed to several advancements in the field of targeted drug delivery. The tethering of a ligand to the outer shell of micelles is most often achieved through the post-modification of a copolymer with bifunctional spacer molecules [83] or via the direct synthesis of heterobifunctional block copolymers [84]. Targeted micelles generally exhibit greater cellular uptake and improved in vitro efficacy than their unmodified counterparts. Table 1 highlights several systems that have emerged in the past few years and demonstrates the potential of polymeric micelles for the active targeting of drugs and other therapeutic compounds.
9.2. Stimuli-responsive polymeric micelles

9.2.1. Biological stimuli

Other targeting approaches rely on the fact that many pathological processes present either a slight increase in temperature or decrease in pH. For instance, contrary to the normal blood pH of 7.4, extracellular pH values in tumorous tissues were determined to be around 6.8–7.0. This is mainly attributed to the higher rate of aerobic and anaerobic glycolysis compared to normal cells [85]. Cellular compartments, such as endosomes and lysosomes, exhibit even lower pH levels of approximately 5–6. pH-sensitive block copolymer micelles capable of dissociating in response to decreased pH levels have been designed to free their incorporated drug molecules upon accumulation at the tumor site and/or entry into the cytoplasm. Recently, PEG-b-PDMAEMA-b-PDEAEMA triblock copolymers were found to self-assemble into micellar structures at physiological pH (7.1–7.3) and efficiently solubilize the hydrophobic compound dipyridamole. Acidification of the aqueous media provoked the protonation of tertiary amine DEAEMA units followed by dissociation of the micellar system, as demonstrated by rapid in vitro release of the drug at pH 3 [86]. The sensitivity of a nanocarrier to changes in environmental conditions may differ with respect to the nature of the block copolymer making up the system. For instance, to tailor the pH at which micelle dissociation and drug release are triggered, Lee et al. prepared mixed micelles of PEG-b-PHis and PEG-b-PLLA where the latter shifted the micelle response to pH values close to the extracellular pH of tumors (7.2–6.6) [85]. More recently, these authors reported the preparation of pH-sensitive mixed micelles which combined biotin-PHis-b-PEG-b-PLLA and PEG-b-PHis. At pH > 7, the PHis tethering the biotin was mostly deionized and hydrophobic, accounting for its attraction to the micellar PLLA core. However, as the pH was slowly decreased, the PHis segments became progressively ionized and extended outward through the PEG brush surrounding the core, thus exposing the biotin moieties for proper ligand–receptor interactions. At pH < 6.5, ionization of PHis in the PEG-b-PHis block copolymer contained in the core induced micelle dissociation and endosome disruption, theoretically
ensuring cargo release into the cytoplasm following ligand-mediated cellular internalization [87]. Alternatively, we showed that incorporation of MAA units in the PNIPAM shell of polymeric micelles induced conformational changes at pH values corresponding to that of the endosome/lysosome [34]. Polymeric micelles loaded with the photosensitizer aluminum chloride phthalocyanine exhibited greater cytotoxicity against EMT-6 mouse mammary cells relative to the control Cremophor EL (CrmEL) formulation [88]. This enhanced activity was reduced in the presence of chloroquine, a weak base impeding the acidification of the endosomal/lysosomal compartments [89], and was ascribed to the capacity of the polymer to destabilize the endosomal membrane upon a pH-triggered conformational change [34]. As mentioned previously, cross-linking of the micelle core is a newly developed strategy aiming at augmenting the physical stability and circulation time of a drug delivery system. Hence, ODN-containing PEG-b-PLyss micelles were core cross-linked through disulfide bonds. Since the intracellular concentration of glutathione, a disulfide-reducing agent, is approximately 300 times higher than that of the extracellular fluid, disulfide-stabilized PICM may one day be applied to the cytoplasmic delivery of genes and related compounds [90]. Finally, intracellular drug delivery could be achieved through acid-sensitive linkers [68,91]. For example, Kataoka worked on developing environment-sensitive polymeric micelles based on PEG-b-PAsp-hydrazone-doxorubicin. In vitro, this system released the drug at a pH-dependent rate, and the optimal hydrazone cleaving pH was found to correspond to that characterizing late endosomes and/or lysosomes (pH 5). Results from in vivo assays on mice bearing C26 tumors demonstrated the enhanced therapeutic effect and reduced toxicity of the micelle-incorporated drug [21].

Environment-responsive polymeric micelles can be a promising approach to the oral delivery of hydrophobic drug molecules. Jones et al. prepared pH-responsive unimolecular polymeric micelles (UPM) composed of hydrophobic EMA and MAA, with hydrophilic PEG-methacrylate units [92]. UPM present a core-shell architecture comparable to that of polymeric micelles, but are inherently resistant to dissociation upon dilution. At acidic pH, the carboxylic acid groups were fully protonated whereas they were more than 40% ionized at pH 7, causing a change in the polarity of the core. This increased polarity promoted the diffusion of progesterone, a

<table>
<thead>
<tr>
<th>Pilot molecule</th>
<th>Polymer</th>
<th>Incorporated molecule</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>PEG-b-PDLLA</td>
<td>N/A</td>
<td>[105]</td>
</tr>
<tr>
<td>Galactose</td>
<td>PEG-b-PDLLA</td>
<td>N/A</td>
<td>[106]</td>
</tr>
<tr>
<td>Galactose</td>
<td>PEG-b-PDLLA</td>
<td>N/A</td>
<td>[84]</td>
</tr>
<tr>
<td>Mannose</td>
<td>PEG-b-P(DMAEMA-co-VP)</td>
<td>Plasmid DNA</td>
<td>[107]</td>
</tr>
<tr>
<td>Mannose</td>
<td>Poly(acrylic acid)-b-poly(methyl acrylate)</td>
<td>N/A</td>
<td>[108]</td>
</tr>
<tr>
<td>Lactose</td>
<td>PEG-b-PDLLA</td>
<td>N/A</td>
<td>[109]</td>
</tr>
<tr>
<td>Lactose</td>
<td>PEG-b-PDLLA</td>
<td>N/A</td>
<td>[9]</td>
</tr>
<tr>
<td>Lactose</td>
<td>PEG-b-PDMAEMA</td>
<td>Plasmid DNA</td>
<td>[10]</td>
</tr>
<tr>
<td>Folate</td>
<td>PEG-b-PCL</td>
<td>Paclitaxel</td>
<td>[110]</td>
</tr>
<tr>
<td>Folate</td>
<td>PEG-b-PLGA</td>
<td>Doxorubicin</td>
<td>[65]</td>
</tr>
<tr>
<td>Folate</td>
<td>PEG-b-PLLA, PEG-b-PHis</td>
<td>Doxorubicin</td>
<td>[85]</td>
</tr>
<tr>
<td>Transferrin</td>
<td>PEG-b-PEI</td>
<td>Plasmid DNA</td>
<td>[75]</td>
</tr>
<tr>
<td>Transferrin</td>
<td>PEG-g-PEI</td>
<td>Phosphorothioate oligonucleotide</td>
<td>[83]</td>
</tr>
<tr>
<td>Epidermal growth factor</td>
<td>PEG-g-PEI</td>
<td>Plasmid DNA</td>
<td>[111]</td>
</tr>
<tr>
<td>Transferrin</td>
<td>Poly(acrylic acid)-b-poly(methyl acrylate)</td>
<td>N/A</td>
<td>[112]</td>
</tr>
<tr>
<td>HIV-1 Tat protein</td>
<td>Poly(acrylic acid)-b-poly(methyl acrylate)</td>
<td>N/A</td>
<td>[113]</td>
</tr>
<tr>
<td>Antigen binding fragment of the OV-TL16 antibody</td>
<td>PEG-g-PEI</td>
<td>Plasmid DNA</td>
<td>[75]</td>
</tr>
</tbody>
</table>
hydrophobic hormone, out of the micellar system. Along these lines, Sant et al. developed pH-sensitive micelles composed of PEG-b-P(AlA-co-MAA), where the hydrophobic AlA were either EA or nBA [35]. Due to the presence of pendant carboxylic groups on the MAA segments in the core, the copolymers self-assembled at pH < 4.7, whereas above this value, the micelles dissociated owing to ionization of the COOH moieties. The pH at which micellization occurred decreased with a reduction in the length of the hydrophobic block. Three poorly water-soluble drugs, namely indomethacin, fenofibrate, and progesterone, were successfully loaded into these micelles. As in the case of UPM, it was possible to trigger drug release in a pH-dependent manner by changing the pH of the release medium from 1.2 to 7.2. Taken together, these data demonstrate the potential of pH-responsive UPM and polymeric micelles to target drugs to the more basic environment of the intestine following oral administration. Fenofibrate, a hypolipidemic agent with dissolution rate-limited oral bioavailability, was chosen to test the in vivo efficacy of pH-sensitive micelles. Oral bioavailability studies revealed 156% and 15% increases versus a fenofibrate coarse suspension and a commercial formulation, respectively. To our knowledge, this study is one of few reporting the enhanced oral bioavailability of a poorly water-soluble drug through incorporation into pH-sensitive polymeric micelles [93].

9.2.2. External stimuli

Other drug delivery systems are designed to release their payload in response to exogenous stimuli. Ultrasounds have been successfully applied to trigger the in vitro and in vivo release of doxorubicin from poloxamer micelles [94–96]. Local heating at solid tumor sites could also be exploited. PNIPAM was incorporated in polymeric micelles to create thermoresponsive systems where the corona precipitated under local hyperthermia and initiated drug release. Chung et al. reported the in vitro “on/off” control over doxorubicin release from PNIPAM-b-poly(butylnmethacrylate) copolymer micelles via heating and cooling cycles [97]. The authors further suggested that this thermosensitivity could be tailored by modifying the composition of the core-forming block [98].

10. Applications in anticancer drug delivery

Recently, PEG-b-PGlu block copolymer micelles carrying cisplatin, an anticancer drug, demonstrated prolonged circulation times and enhanced therapeutic efficacy versus the free drug when administered to tumor-bearing mice. The system combines both sufficient stability to ensure prolonged circulation in the bloodstream and adequate drug release kinetics upon accumulation at the delivery site via the EPR effect [3]. Yet, more often than not, correlation between in vitro and in vivo results constitutes a major challenge to researchers in the field. For many polymeric micelle systems, promising in vitro stability and efficacy data on culture cells do not ultimately translate into long-circulating, efficacious vectors when investigated in animal models.

In spite of these shortcomings, polymeric micelles have nonetheless become promising alternatives to the use of nonionic, low-molecular weight surfactants in terms of reduced toxicity and improved biocompatibility. Taxol®, the commercial form of paclitaxel, is formulated in micelles of CrmEL, a low-molecular weight surfactant known for its side effects (acute hypersensitivity and severe neurotoxicity) [99]. Burt et al. were the first to report the solubilization of high levels of paclitaxel (25 wt.%) by PEG-b-PDLLA micelles [100] and to demonstrate the biocompatibility and lack of toxicity of the carrier in vitro and in vivo [25]. Recently, a novel PEG-b-PDLLA injectable micelle formulation of paclitaxel called Genexol® showed a three-fold increase in the maximum tolerated dose (MTD), while providing much improved antitumor efficacy versus Taxol® in mice [101]. After encouraging results in terms of toxicity and efficacy during the course of Phase I clinical trials, Genexol® is currently undergoing Phase II trials in Korea in patients with advanced breast and non-small cell lung cancers [102]. We previously reported the use of PVP-b-PDLLA block copolymers for the solubilization of paclitaxel. In a first study, in vitro IC\textsubscript{50} values of PVP-b-PDLLA block copolymers were at least two orders of magnitude higher than CrmEL/ethanol, demonstrating the lower toxicity of the polymeric carrier. Both drug-loaded carriers exhibited equal cytotoxicity against murine C26, EMT-6 and human OVCAR-3 cell lines. The MTD
of Taxol® in healthy Balb/C mice was 20 mg/kg, whereas that of paclitaxel-loaded polymeric micelles was not reached even at doses as high as 100 mg/kg. Studies were conducted on C26 tumor-bearing mice with 20 mg/kg Taxol® and 60 mg/kg PVP-b-PDLLA-paclitaxel formulations, whereby the latter demonstrated greater antitumor activity [103].

Similarly, docetaxel, a synthetic analogue of paclitaxel, is available as Taxotere®, a polysorbate 80-based micelle formulation. In vitro, PVP-b-PDLLA was found to be less toxic than polysorbate 80, while drug-loaded polymeric micelles demonstrated similar cytotoxic activity to Taxotere® against murine C26 and EMT-6 cell lines. Data from in vivo studies revealed that the acute toxicity of the docetaxel molecule. Lastly, biodistribution and pharmacokinetic studies showed comparable results for both formulations [104].

11. Conclusion

Polymeric micelles hold promise for the delivery of a large array of chemically diverse therapeutic compounds. Their utility in the field of drug delivery is based on their characteristic self-assembly into core-shell nanostructures in aqueous milieu. In general, hydrophobic or electrostatic interactions are the driving force behind the segregation of the core from the surrounding media. Apart from enhancing the water-solubility of many hydrophobic drugs, polymeric micelles can modify the biodistribution of drugs through either passive or active targeting strategies. Designing vectors with sufficient physical stability to withstand dissociation upon dilution and ensure drug transport within the bloodstream to specific biological sites is of prime importance to achieving successful drug delivery. Issues of in vivo stability and drug retention, or lack thereof, have inevitably become focal points of research conducted on micellar systems.

Acknowledgements

This work was supported financially by the Canada Research Chair Program, the Natural Sciences and Engineering Research Council of Canada, and Labopharm Inc. We thank Elvire Fournier for the preparation of fluorescent micelles for the confocal laser scanning microscopy study.

References


[97] J.E. Chung, M. Yokoyama, T. Aoyagi, Y. Sakurni, T. Okano, Thermo-responsive drug delivery from polymeric micelles constructed using block copolymers of poly(V-isopropyla-


