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Mixed micelles self-assembled from block copolymers for drug delivery

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ABSTRACT

Mixed micelles self-assembled from two or more dissimilar block copolymers provide a direct and convenient approach to improve physical stability and enhance drug loading capacities of conventional polymeric micelles for drug delivery. The versatility of this approach also allows for the concomitant integration of multiple functionalities into a single system — a feat that is synthetically challenging to accomplish with micelles formed from a single co-polymer. Through the careful selection and blending of structurally and/or functionally diverse block copolymers, a population of novel and multi-functional micelles bearing desirable attributes of each constituent copolymer can be easily fabricated without the need for elaborate synthetic schemes. As such, this review is focused on the various strategies used to form and stabilize mixed micelles for drug delivery and the methodologies employed to ascertain the establishment of mixed micelle formation. *In vivo* evidence demonstrating the effectiveness of mixed micelles will be presented. Lastly, future perspectives for the development of mixed micelle systems for drug delivery will also be discussed.

1. Introduction

Biodegradable polymeric micelles self-assembled from block copolymers have emerged as promising vehicles for drug delivery, with four systems having been clinically examined with encouraging results [1–4]. The synthetic versatilities and ease of incorporating functionalities are highly attractive attributes of this class of materials. Conventional systems most frequently utilize amphiphilic block copolymers, which spontaneously self-assemble in aqueous milieus to form core-shell type micelles at copolymer concentrations above the critical micelle concentration (CMC) in a thermodynamically driven process [5,6]. The core-shell type model may also be derived from ionic interactions between block copolymers with opposing charges. Key features of this system include the capacity to solubilize hydrophobic drugs in the core of polymeric micelles and a hydrophilic shell for the stabilization and protection of therapeutic cargos present in the core from the external medium.

Besides the ability of polymeric micelles to function as a depot for hydrophobic drugs, nano-sized drug-loaded particles also have distinct advantages *in vivo* compared to the free drug. Firstly, polymeric micelles, typically in the size range of 20–200 nm, are sufficiently large to avoid premature elimination via glomerular filtration in the kidneys, but are small enough to enter blood vessels and to capitalize on the enhanced permeation and retention (EPR) effect for passive accumulation in the target tumor tissues (Fig. 1) [7]. On the cellular level, the small size of drug-loaded micelles not only facilitates uptake, but also provides an alternative route of internalization via the endosomal pathway, which is speculated to be a major course for nanoparticles to circumvent drug reflux mechanisms associated with multi-drug resistance (MDR) [8]. Poly(ethylene oxide) (PEO) is the most frequently employed structural motif for the hydrophilic component of block copolymers due to its high aqueous solubility and non-toxicity [1,9]. Through its well-established ability to form an aqueous shell around the hydrophobic drug-loaded core, it provides steric stabilization and charge shielding capabilities to prevent non-specific adsorption of serum proteins and/or complement and antibodies, a process commonly known as opsonization, that could otherwise predispose foreign particles to rapid clearance by macrophages and/or endothelial cells of the reticuloendothelial system (RES) in the liver, spleen and bone marrow. The benefit of altered pharmacokinetic properties arising from the encapsulation of drugs in polymeric micelles is two-fold; firstly, by preventing premature elimination and clearance, an appreciable increase in the blood circulation time of the drug is achieved. Secondly, together with the more precise delivery of drugs to target tissues, the dose administered can be reduced; hence circumventing non-specific organ toxicities (e.g. cardiotoxicity induced by doxorubicin) associated with the high systemic exposure to cytotoxic agents.

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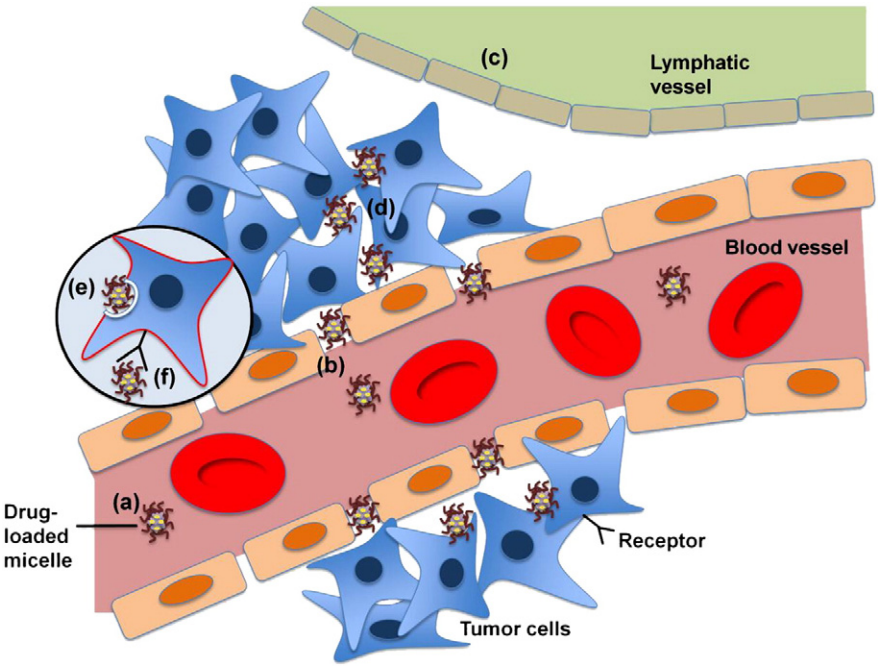

Fig. 1. Passive accumulation of drug-loaded copolymer micelles in tumor tissues via the EPR effect [3,113] (a) Block copolymer micelles effectively evade innate clearance mechanisms, resulting in prolonged blood circulation time; (b) nano-sized micelles, typically around 20–200 nm diameter, efficiently extravasate through the leaky tumor vaculature, where the endothelial gap junctions vary between 400–600 nm; (c) impaired lymphatic drainage occur in tumor tissues; (d) a high interstitial concentration of drug-loaded micelles is thus retained in the tumor; (e) non-specific or (f) specific receptor-mediated internalization of drug-loaded micelles is effected.

To successfully exploit polymeric micelles for drug delivery, several important parameters, including micelle stability, drug loading capacity, size, size distribution and incorporation of functionalities, have to be carefully considered and optimized in each application. The stability of the block copolymer-derived micelles is imperative to its ultimate utility as a drug delivery vehicle and is governed by thermodynamic and kinetic principles [1–4]. For a micelle to be thermodynamically stable, the copolymer concentration should be above its CMC, defined as the concentration at which the individual copolymer chains associate to form micelles at a given temperature. This poses a significant challenge following the systemic administration of drug-loaded polymeric micelles due to the 'sink condition' or infinite dilution encountered in the blood stream. As such, it is desirable to select a copolymer with a lower CMC as the premature dissociation of copolymer micelles potentially culminates in the deleterious liberation and precipitation of the hydrophobic and often cytotoxic payloads. The CMC of block copolymers is influenced by the hydrophilic–lipophilic balance (HLB) of the block copolymer [3]. Generally, an increase in the hydrophobic block length of a copolymer results in a lower CMC if the hydrophilic segment is kept constant. The kinetic stability of copolymer micelles comes into the picture when the concentration of the copolymer falls below the CMC. Unlike micelles formed from low molecular weight surfactant molecules, the disassembly of copolymer-based micelles at a concentration below CMC occurs at a relatively slower rate and is dependent upon the physical state of the core (glass transition temperature T_g above or below 37 °C), the interactions between the hydrophobic blocks, the length (or molecular weight) of the hydrophobic block and the proportion of the hydrophilic to hydrophobic blocks [1,4].

The drug loading capacity is primarily dependent on the degree of miscibility between the drug and polymer among other factors [1,6]. The degree of compatibility between the drug and polymer can be predicted by the Flory-Huggins interaction parameter [1–3,6]; expressed as $\chi_{drug\text{-}polymer} = (\delta_{drug} - \delta_{polymer})^2 \ (V_{drug}/RT)$, where $\chi_{drug\text{-}polymer}$ represents the interaction parameter between the drug and core-forming polymer, δ_{drug} and $\delta_{polymer}$ are the Hildebrand–Scatchard solubility parameters of the drug and polymer respectively, V_{drug} is the molar volume of the drug and lastly, R and T correspond to the ideal gas constant and temperature. A smaller value of $\chi_{drug\text{-}polymer}$ signifies a greater degree of drug-polymer compatibility. Thus, the drug loading capacity of micelles can be enhanced by carefully selecting block copolymers with hydrophobic segments that are compatible and can be tuned to elicit favorable interactions with drug molecules in the core.

The size and its distribution is another important property of polymeric micelles. The size of a polymeric micelle is dependent on several factors including copolymer molecular weight, relative proportion or length of hydrophobic and hydrophilic blocks, drug loading level and micelle aggregation number [6]. Wide size distributions, which often arise from the self-aggregation of individual micelles in solution, are a common problem of many copolymer micellar systems [1]. A broad size distribution is undesirable for drug delivery applications as varied pharmacokinetic properties for different size populations can be expected. For instance, particles less than 200 nm tend to accumulate in the liver, where 100–200 nm sinusoidal fenestrations exist [10]. Aggregates greater than 7 μm, on the other hand, have the tendency to be physically entrapped in the lungs [11]. Approaches to improve micellar stability, such as through the incorporation of the steric stabilizing PEO, can help to prevent self-aggregation of drug-loaded micelles, which is driven by the hydrophobic-hydrophobic type of interaction between the cores of individual micelles. The incorporation of various functionalities such as a targeting ligand to seek out overexpressed surface receptors on diseased cells coupled with pH- or temperature-responsive drug release in polymeric micelles provides a more efficient and controlled therapy [4,12,13]. However, the concomitant incorporation of multiple functionalities in a single copolymer micellar system is technically challenging to achieve.

Polymeric micelles formed from single copolymers are often lacking in one or more departments primarily due to limitations in the number of building blocks. The combination of two or more dissimilar block copolymers (e.g. AB + CB [14] or AB + AC [15] or AB + CD [16]) to form mixed micelles, on the other hand, is a practical and efficient approach to address many of the aforementioned issues without the need for complicated synthetic schemes. Shim et al. has proposed a model for the comicellization of diblock copolymers in solution, which was described to be dependent on the relative concentrations of the two species of

diblock copolymers [17]. In addition, in an ideal case, the CMC of a mixed micelles system can be mathematically derived from the CMC values and molar fractions of its constituents [18]. Mixed micelles fabricated from chemically diverse amphiphilic block copolymers have been employed in various situations. Some of the advantages that mixed micelles possess over their individual constituents include significant improvements in thermodynamic (by lowering CMC) [19] and kinetic (through enhancing the hydrophobic interactions, stereocomplexation, H-bonding, ionic interaction or chemical cross-linking between the core-forming blocks) stability [14], improved drug loading [20,21], exquisite size control to prevent precipitation [22] and ease of incorporating multiple functionalities (e.g. for stimuli-responsive drug release and cellular targeting) [23]. Thus, the focus of this review will be on the use of mixed micelles for drug delivery applications.

In the following sections, we will describe in detail mixed micelles formed from different block copolymers and delve into the core interactions involved in mixed micelles formation and stabilization, specifically hydrophobic interaction, stereocomplexation, hydrogen bonding, ionic interaction and chemical cross-linking as illustrated in Fig. 2, with emphasis on their roles in drug delivery applications through the use of specific examples. As the formation of mixed micelles is vital to the success of the system, various methods employed to verify mixed micelles formation will be evaluated. *In vivo* evidence demonstrating the effectiveness of the mixed micelles concept in enhancing overall therapeutic efficacies and toxicity profiles will also be presented. Lastly, we will conclude by outlining the future perspectives for the development of mixed micelle systems for drug delivery.

2. Formation of mixed micelles (classified through the nature of interactions) and their use in drug delivery

Mixed micelles self-assembled from different block copolymers interacting through weak hydrophobic interactions, and interactions via molecular recognition such as stereocomplexation, H-bonding, ionic interactions as well as chemical cross-linking in the core are expected to behave differently. As such, in this section, we will discuss mixed micelles formed from block copolymers through the abovementioned interactions and their applications in drug delivery, and provide an insight on how the interactions can be characterized.

2.1. Hydrophobic interactions

By far the most frequently studied type of noncovalent interactions in mixed micellar systems are hydrophobic interactions between different block copolymers. Table 1 gives an overview of the mixed micellar systems that rely on hydrophobic interactions for micellization, and are used as drug delivery vehicles. In this context, we limit our discussion to the assembly of two different synthetic block copolymers that form mixed micelles in aqueous solution through hydrophobic interactions between the hydrophobic blocks of the copolymers. Incorporation of various other substances such as vitamin E [24], modified phospholipids [25–27], D-α-tocopheryl polyethylene glycol succinate (TPGS) [28,29] and graft copolymers [30–34] with block copolymers was also reported in the literature but this will not be examined here.

Block copolymers of hydrophilic poly(ethylene oxide) (PEO) and hydrophobic poly(propylene oxide) (PPO), commercialized as Pluronic polymers, are the most researched materials used to form mixed micelles systems for drug delivery [20,22,26–28,35–39] as they are readily available and biocompatible with low toxicity [40]. However, the limitations lie in their inability to encapsulate large amounts of hydrophobic drug and in their high critical micelle concentrations (CMC) which lead to low stability of micelles and dissociation when diluted by the blood upon intravenous injection [41]. Mixing Pluronic polymers with a more hydrophobic copolymer can greatly improve the stability of the resulting micelles and thus bioavailability of the

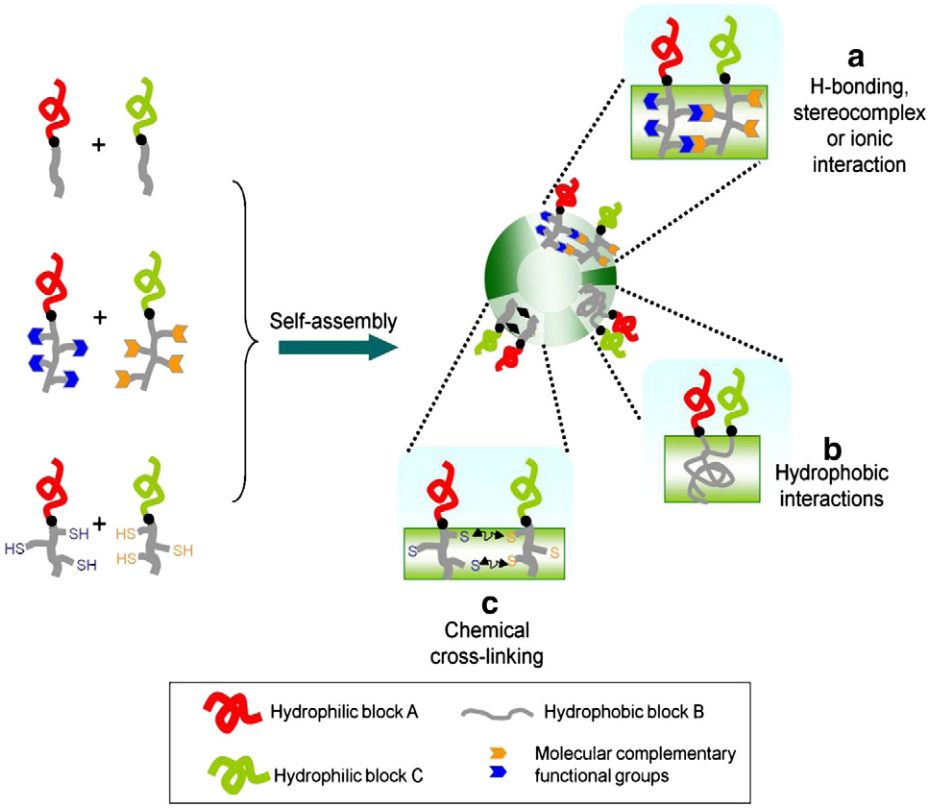

Fig. 2. Schematic presentation of the formation of mixed micelles through various core interactions. (a) Hydrogen bonding, stereocomplexation or ionic interaction; (b) Hydrophobic interactions; and (c) Chemical cross-linking (e.g. disulfide bond).

Table 1
Overview of mixed micelles made from synthetic amphiphilic block copolymers as drug delivery agents.

Polymer system	Block copolymer 1	Block copolymer 2	Drug incorporated	System significance of mixing	Ref.
Pluronics	Pluronic L61 Pluronic L62 Pluronic L85	mPEG-*b*-poly(lactide)	Docetaxel	To enhance bioavailability and to enhance intracellular uptake of docetaxel relative to mPEG-*b*-poly(lactide) micelles	[39]
	Pluronic L61	Pluronic F127	DOX	To stabilize Pluronic L61 micelles by preventing aggregation and separation of liquid phase	[22]
	Pluronic F127 modified with sulfate groups	Poly(propylene sulfide)-*b*-PEO	Sirolimus	To stabilize Pluronic F127 micelles by lowering CMC	[36]
	Pluronic P105	Pluronic L101	PTX	To improve solubility and blood circulation retention of PTX	[20]
	Pluronic P105 modified with folate	Pluronic L101 modified with folate		To overcome MDR in MCF-7/ADR cells	[37]
Polyesters	mPEG-*b*-poly(D, L-lactide)	mPEG-*b*-P(NnPAAm-co-VIm)	DOX	To improve micellar stability of mPEG-*b*-P(NnPAAm-co-VIm) and to prevent dispersion of mPEG-*b*-poly(D, L-lactide) in dilute conditions	[14]
	PEG-*b*-PLA	Poly(lactide)-*b*-PNIPAM	Ibuprofen	To form PEG channels upon collapse of PNIPAM shell at high temperatures	[15]
	PLA-*b*-PEG-*b*-PLA	Folate-PEG-*b*-PLA	BBSKE	Folate-PEG-*b*-PLA contains folate for targeting to MCF-7 breast cancer cells	[51]
	AP-*b*-PEG-*b*-PLA	mPEG-*b*-poly(β-amino ester)	DOX	To form pH-sensitive mixed micelles with tumor-specific AP peptide	[56]
	DOX-*b*-PLGA-*b*-mPEG	PLGA-*b*-PEG-*b*-Folate	DOX	To increase loading of DOX inside micelles and to target cancer cells with folate moiety	[23]
Polypeptide	poly(L-glutamic acid)-*b*-PPO-*b*-poly(L-glutamic acid)	PEG-*b*-PPO	DOX	To form PEG channels upon collapse of PLGA shell at acidic pH	[46]
	Poly(L-histidine)-*b*-PEG	PLLA-*b*-PEG-*b*-Poly(L-histidine) -TAT	DOX	To form pH-sensitive mixed micelles with non-specific cell penetrating peptide moiety	[55]
	Poly(L-histidine-co-phenylalanine)-*b*-PEG	PLLA-*b*-PEG-Folate	DOX	To form pH-sensitive mixed micelles to target MDR cancer cells	[52,53]
	Poly(L-histidine)-*b*-PEG	PLLA-*b*-PEG PLLA-*b*-PEG-Folate	DOX		[47,48,114,115]

AP, CRKRLDRN peptide; BBSKE, 1,2-[bis(1,2-benzisoselenazolone-3(2 H)-ketone)] ethane; DOX, doxorubicin; MDR, multidrug resistance; mPEG, methoxy poly(ethylene glycol); NnPAAm-co-Vim, N-n-propylacrylamide-co-vinylimidazole, PEG, poly(ethylene glycol); PEO, poly(ethylene oxide); PLA, poly(lactide); PLLA, poly(L-lactic acid); PLGA, poly(D,L-lactic-co-glycolic acid); PNIPAM, poly(N-isopropylacryamide); PPO, poly(propylene oxide); PTX, paclitaxel; TAT, transactivator of transcription.

encapsulated drugs. For example, Mu and coworkers mixed Pluronic copolymers L61, L62 and P85 each with methoxy poly(ethylene glycol)-*b*-poly(lactide) (mPEG-*b*-PLA) that exhibited lower CMC at different ratios [39]. All of the mixed micelles were in nanosize ranging from 19.6 to 28.5 nm, whereas pure Pluronic polymers formed large aggregates of ~1000 nm. Anti-cancer drug docetaxel was encapsulated in these mixed micelles and the mixed micelle formulation was found to be more effective in both *in vitro* and *in vivo* systems, and had higher *in vivo* bioavailability than the commercial formulation, Taxotere®. In another instance, Pluronic F127 was mixed with poly(propylene sulfide)-*block*-poly(ethylene oxide) (PPS-*b*-PEO) containing PPS that is more hydrophobic than PPO in F127. Thus, the mixed micelles possessed a CMC value intermediate between that of pure PPS-*b*-PEO micelles (very low CMC) and that of pure Pluronic micelles (high CMC) [36]. PPS block is known to undergo a degradation mechanism whereby PPS is oxidized to hydrophilic polypropylene sulfoxide under physiological conditions which is later cleared *in vivo* through renal filtration [42]. Furthermore, the molecular weight (MW) of the PPS-*b*-PEO polymer is less than 40 kDa which is the MW cutoff for elimination by renal filtration [43].

Mixed micelles have also been formed by two Pluronic copolymers with different hydrophobicity/hydrophilicity ratios to improve the properties of the micelles for drug delivery. For example, Pluronic F127 with a longer hydrophilic block and higher molecular weight stabilized the self-assembly of Pluronic L61 with a relatively longer hydrophobic block, counteracting the precipitation of Pluronic L61 in water [22]. This mixed micelle formulation loaded with doxorubicin (SPC1049C) was approved for clinical trials and was delivered to patients through intravenous infusion [44]. To increase the drug loading of paclitaxel instead, Pluronic L101 was mixed with Pluronic 105 at a small percentage to expand the micellar core of Pluronic 105 so that more paclitaxel molecules can be encapsulated [20]. This is because Pluronic L101 formed lamellar aggregates in water due to its long hydrophobic PPO blocks and thus addition of Pluronic L101 made Pluronic 105 micelles less compact to allow space for loading of more paclitaxel molecules.

In addition to improving CMC, size, *in vivo* bioavailability and drug loading capacity, the mixed micelle approach has also been employed to modulate the lower critical solution temperature (LCST) of micelles. Methoxy poly(ethylene glycol)-*block*-poly(N-n-propylacrylamide-co-vinylimidazole) (mPEG-*b*-P(NnPAAm-co-VIm)) is a temperature-sensitive copolymer and has a LCST of 31 °C [14]. At temperatures higher than its LCST, the polymer forms micelles. However, at temperatures below the LCST, the micelles are dissociated as P(NnPAAm-co-VIm) becomes hydrophilic. Therefore, when a drug is encapsulated with the copolymer, its storage at room temperature below the LCST may cause premature drug release. By mixing the copolymer with methoxy poly-(ethylene glycol)-*block*-poly(D,L-lactide) (mPEG-*b*-PLA) of low CMC, the mobility of the temperature-sensitive copolymer was limited due to the interactions with PLA block, thus lowering the LCST. Therefore, stable mixed micelles were able to form at room temperature and in dilute solutions.

While it is common for two different block copolymers with the same hydrophilic block but different hydrophobic blocks (AB + AC) to form mixed micelles to improve on stability, diblock copolymers of the same hydrophobic block but different hydrophilic blocks (AB + CB) can form mixed micelles with the shell having dual functions [15,45,46]. Shi's group reported mixed micelles formed from poly(*tert*-butylacrylate)-*block*-poly(N-isopropylacrylamide) ($PtBA_{45}$-*b*-$PNIPAM_{91}$) and P*t*BA-*block*-poly(4-vinylpyridine)($PtBA_{60}$-*b*-$P4VP_{80}$), having P*t*BA core surrounded by a mixed P4VP/PNIPAM shell [45]. With an increase in temperature, PNIPAM became increasingly insoluble and thus PNIPAM chains in the shell shrank and aggregated due to its increasing hydrophobicity, leaving the P4VP chains stretched out. Similarly, an increase in pH would deprotonate P4VP blocks, making them insoluble and so, PNIPAM chains stretched out in the shell with P4VP chains collapsed. The collapse of the corresponding chains upon exposure to

the stimuli would affect the release rate of the enclosed drug molecules. The same group later reported mixed micelles from PLA-*b*-PNIPAM and PEG-*b*-PLA and used these mixed micelles to deliver ibuprofen. The release of the drug from PEG chain channels was studied when PNIPAM chains shrank at 37 °C [15]. The release rate of ibuprofen from the PEG channels was found to be influenced by the ratio of PNIPAM and PEG chains in the shell, and the addition of NaCl limited the release of ibuprofen with the condensation of PEG channels. Lin et al. further showed that collapse of temperature- or pH-sensitive blocks in the corona slowed the release of drug from the micellar core [46].

Nanosized polymeric micelles with anticancer drugs in their cores can accumulate in solid tumors due to the EPR effect as described in Fig. 1. To further limit the effects of the polymeric micelles on tumor cells only, active targeting of the micelles via biological ligands have been employed. Ligands whose receptors are exclusively found or overexpressed on tumor cells are normally conjugated onto the surface of polymeric micelles, the uptake of the surface-functionalized micelles by the cells depends on the density of the ligands. The mixed micelle approach provides the possibility of varying the surface density of the ligands. For example, folate receptor is overexpressed on the surface of many types of cancer cells. Folate has been introduced onto the surface of mixed micelle systems to target cancer cells [20,23,47–53]. Park's team reported mixed micelles made from doxorubicin-*block*-poly(D,L-lactic-co-glycolic acid)-*block*-PEG (DOX-*b*-PLGA-*b*-PEG) and PLGA-*b*-PEG-Folate, and used them to encapsulate DOX [23]. The cytotoxicity of DOX-loaded mixed micelles with folate was greater than that of DOX-*b*-PLGA-*b*-PEG micelles without folate for KB cells. In addition, the content of DOX was higher in tumors formed from implantation of KB cells in nude mice when intravenously delivered by the folate-functionalized mixed micelles. Bae's team mixed poly(L-lactic acid)-*block*-PEG-folate with poly(L-histidine)-*b*-PEG-folate [47] or poly(L-histidine)-*b*-PEG [48] or poly(L-histidine-co-L-phenylalanine)-*b*-PEG (Poly(His-co-Phe)-*b*-PEG) [52,53] while encapsulating DOX. All mixed micelle systems were destabilized under acidic environments such as at the tumor tissues and endosomes/lysosomes due to the pH-sensitivity of histidine, thus releasing DOX. All systems effectively killed DOX-resistant ovarian cancer cells in both *in vitro* and *in vivo*. Pluronic polymers were also modified with folate to increase the uptake of drug-encapsulated mixed micelles in drug-resistant cell lines such as MCF-7/ADR (Fig. 3) [20]. When ligand-functionalized amphiphilic linear-dendritic block copolymers are used in micellar delivery vehicles, mixed micelles can also serve as tools for manipulating the presentation of ligand on a micelle surface by generation of 'patchy micelles' where ligand is presented in predetermined clusters and densities; it was found that presenting folate at optimal cluster sizes of 3 to 4 folate significantly enhanced extracellular uptake both *in vitro* and in mouse tumor models compared to smaller or larger cluster arrangements [54]. Other than folate, peptides [55,56] and chemical functional groups [36] can be introduced to mixed micelles systems. For the latter, Pluronic F127 was modified to expose sulfate groups on the shell of the mixed micelles when it was blended with PPS_{20}-*b*-PEO_{44} for attachment to collagen in the extracellular matrix [36].

From the above examples, the mixed micelles are an exciting approach that provides a wide range of opportunities to improve micelle stability and design micelle systems with multiple functions. To verify the formation of mixed micelles, a number of methods have been reported, many of which are not specific to the nature of interactions in the mixed micelles and can be applied to almost all types of mixed micelles. In the mixed micelles made from Pluronic F127 and PPS_{20}-*b*-PEO_{44} micelles, analytical ultracentrifugation (AUC) analysis was used in tandem with ^{1}H-NMR analysis to prove the formation of the mixed micelles [36]. A single peak in the size exclusion chromatograph (SEC) would indicate that the micelles formed are completely mixed if the individual polymers form micelles with different sizes. Similarly, one dynamic light scattering (DLS) peak also suggests narrow diameter distribution with low PDI, indicating that only one population of mixed micelles is formed [20,39,49]. Two-dimensional proton nuclear overhauser effect (2D NOE) plots of individual polymers and mixed micelles can signify the formation of mixed micelles from the cross peaks present in the spectrum due to physically close protons from chemically different polymers [15,25]. On the other hand, transmission electron microscopy (TEM) is a practical technique to visualize the morphology of micelles. Mixed micelles were observed to be spherical in shape, just like their one-component counterparts [15,51,56]. The formation of mixed micelles

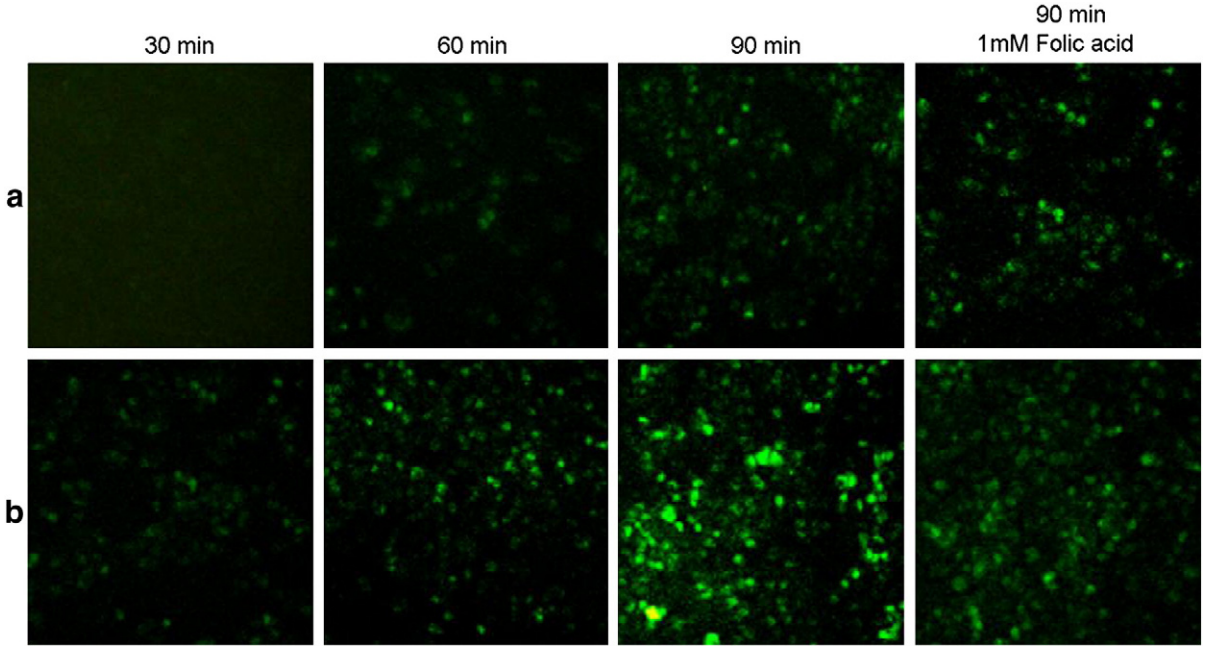

Fig. 3. Uptake of FITC-labeled PL/PTX (Pluronic P105/L101 mixed micellar formulation with paclitaxel) and FOL-PL/PTX micelles at different times and effect of free folic acid on uptake of the two micelles in MCF-7/ADR cell sublines at 90 min: (a) FITC-labeled PL/PTX micelle; (b) FITC-labeled FOL-PL/PTX micelles (Reprinted with permission from Ref. [20], Copyright (2007) Elsevier).

can be evaluated theoretically with regular solution theory [35,57] using the interaction parameter, β. The parameter β is a gauge indicating the degree of interactions between two components and its divergence from ideal solutions. Negative β denotes an attractive interaction between the two components in the mixed micelles and as β gets increasingly negative, the favorable interactions between the two components increase i.e. mixed micelles are easier to form than the micelles from individual polymers. This parameter is dependent on the mixing ratio of the two components. The equations to obtain parameter β can be easily found in references [35,57].

The hydrophobic interactions within the micelles can be studied via isothermal titration calorimetry (ITC) analysis at different temperatures. ITC analysis has not been done on mixed micelles formed from block copolymers as of now but widely used in mixed micelles of block copolymer with surfactants [58]. The main parameter affecting hydrophobic interactions is temperature as rising temperature is known to increase hydrophobic interactions [59]. Thereby, by varying temperature, the micellization of two copolymers driven by hydrophobic interactions would be altered. In the differential enthalpy curves from ITC analysis, the value of enthalpy would increase as the temperature increases thereby confirming hydrophobic interactions as the driving force for micellization of two polymers. Although ITC analysis is useful to confirm hydrophobic interactions, another approach is needed to evaluate the formation of coherent mixed micelles.

2.2. Stereocomplexation

In comparison to the relatively well-studied hydrophobic interactions, the study of stereocomplexation between individual enantiomers having different stereochemistry has been less pervasive. Typical examples of iso- and syndiotactic stereocomplexes include poly(methyl methacrylate), polythiiranes, polyoxiranes, polylactones and polylactides (PLA) [60]. More recently, O'Reilly has reported the synthesis and block polymerization of optically pure N-acryloy-D-leucine methyl ester and N-acryloyl-L-leucine methyl ester, and studied the micelle and mixed micelle formation by a variety of techniques including TEM, DLS, atomic force microscopy (AFM) etc [61]. Among these examples, the PLAs have attracted a great deal of attention because the PLA homo- and block copolymers are biodegradable, produced from renewable resources, and nontoxic to the human body and the environment with applications ranging from medicine to engineering thermoplastics [62–65]. Moreover, since both polylactide and polyethylene oxide are FDA-approved, and block copolymers can be readily dispersed in water, significant effort has been devoted towards the sequestration and delivery of therapeutic cargos using self-assembled polylactide-*b*-polyethylene oxide micelles. Cyclic lactide monomers have two stereocenters, denoted as L- and D-enantiomers, that generate a number of types of polylactides including optically active poly(L-(−)-*S*-lactide) (L-PLA) and poly(D-(+)-*R*-lactide) (D-PLA), racemic poly(DL-lactide) (*rac*-PLA) and meso poly(DL-lactide) (*mes*-PLA). Advances in catalysis and control of the microstructure have allowed the preparation of isotactic, syndiotactic, heterotactic and stereoblock polylactide with varying properties. Since Ikada et al. first reported stereocomplex formation from mixtures of L-PLA and D-PLA in both the melt and solution, numerous studies have been performed on the formation of the stereocomplex and its crystalline structure, morphology, and physical structure [66,67]. For example, Sarasua *et al.* demonstrated that the complex formation stems from H-bonding from specific $CH_3\cdots O$ C and $C_\alpha H\cdots O$ C interactions between both stereoisomers of polylactide from combined study with fourier transform infrared (FTIR) spectroscopy and molecular modeling [68]. Moreover, the stereocomplex offers opportunities in macromolecular engineering as Sun *et al.* observed thermal history-dependent phase transitions from multilamellar vesicle to lamellar to honeycomb morphology in an incompatible blend of enantiomeric PLA block copolymers, which may be attributed to the formation and melting of stereocomplexes [69,70]. Fujiwara and coworkers reported the formation of hydrogels triggered by stereocomplexation of triblock copolymers of PLA-PEO copolymers [71].

Leroux et al. showed that stereocomplex block copolymer micelles obtained from mixtures of poly(ethylene glycol)-*b*-poly(L-lactide) (PEG-PLLA) and poly(ethylene glycol)-*b*-poly(D-lactide) (PEG-PDLA) exhibited enhanced kinetic stability and redispersion properties superior to micelles prepared with isotactic or racemic polymer alone [72]. They showed that mixtures of the freeze-dried copolymer micelles exhibited melting points and x-ray diffraction (XRD) patterns consistent with the formation of the stereocomplex. The stabilized micelles were also less likely to aggregate into larger, less desirable supramolecular structures that are easily cleared by the mononuclear phagocyte system. Similar results were reported by Chen and coworkers in the comparison of PEG-PLLA, PEG-PDLA and their stereocomplexed micelles having CMC values ranging from 0.8 to 4.8 mg/L and sizes ranging from 40 to 120 nm with the stereocomplexed samples having the lowest values [73]. Fukushima and coworkers [74] have extended the scope of possibilities from stereocomplexed PLA-PEO block copolymers by modifying the architecture of the amphiphilic copolymer. Amphiphilic block copolymers comprised of PEG as the hydrophilic component and poly(carboxytrimethylene carbonate) (PMTC) as the hydrophobilc block having latent initiator functionalities for the subsequent ring opening polymerization of stereoregular lactides. Polymerization of either L-lactide or D-lactide from the ROP initiators in the PMTC block generated the lactide grafts in the hydrophobic block. Comb-shaped block copolymers of predictable molecular weights and narrow polydispersities were obtained with up to 8-PLA branches. Mixed micelles having exceptionally low CMCs were obtained with small (25–30 nm) and narrow size distributions. Another example of the role of architecture and stereocomplexation includes a simple and versatile approach to miktoarm co- and terpolymers from carbonate functional oligomers [75]. In this study, the key building block employed is a carboxylic acid functional cyclic carbonate, derived from 2,2-bis(methylol)propionic acid, that was readily coupled to a hydroxyl functional monomethylether poly(ethylene glycol) oligomer [76,77]. Ring-opening of the cyclic carbonate using functional amines generated a carbamate linkage bearing a functional group capable of initiating ring-opening polymerization, together with a primary hydroxyl group for ring-opening polymerization. Two tandem polymerization steps were possible, which adds the second two arms, thus generating the targeted ABC miktoarm terpolymer. The resulting amphiphilic miktoarm terpolymers containing poly (D- and L-lactide) formed polylactide stereocomplexes in the bulk. In aqueous solution, mixtures of the Y-shaped miktoarm copolymers poly(ethylene glycol)-poly(D-lactide)-poly(D-lactide) and poly(ethylene glycol)-poly(L-lactide)-poly(L-lactide), or the miktoarm poly (ethylene glycol)-poly(D-lactide)-poly(L-lactide) form stabilized micelles in the 20 to 30 nm range, with a significantly lower critical micelle concentration (10.0 to 15.8 mg/L) than those derived from conventional stereoregular linear copolymers.

Micelles derived from the PEG-PDLA/PEG-PLLA stereocomplex showed higher drug loading levels, more efficient loading and more controlled release rates than either of the copolymers alone. Leroux and coworkers [72] sequestered the water insoluble paclitaxel (0.5% w/w) into the core of the stereocomplex by hydrophobic interactions using a solvent evaporation technique at an efficiency of 87 to 92%. The incorporation of the cargo at these loading levels had little effect on the size (~40 nm) but the distributions were narrower than micelles from either of the loaded copolymers alone. The kinetic stability was studied in the presence of sodium dodecyl sulfate (SDS), a destabilizing agent, and the loaded micelles derived from the stereocomplex showed significantly enhanced stability relative to the

copolymers alone. Yang et al. [21] showed that the PEG-PDLA/PEG-PLLA stereocomplex showed high paclitaxel-loading levels (3–5%) by both direct dissolution and dialysis with sizes ranging from 107 to 276 nm. The release of paclitaxel from the micelles derived from the stereocomplex was significantly slower than the micelles from the single copolymers. Chen et al. showed that the PEG-PDLA/PEG-PLLA stereocomplex entrapped rifampin with high efficiency and loading levels (7.5–10.6%) and the complex had little effect on the crystallinity of the core by the retention of the high melting point [73]. Nederberg et al. showed that the Y-shaped PEG-PDLA, PEG-PLLA and stereocomplex showed high paclitaxel loading levels (7.9–11.6%), with the sterecomplex having the highest level and smallest size [75]. The release of paclitaxel from the micelles in PBS buffer (simulated physiological conditions) showed no initial burst, but occurred in a sustained manner with near zero-ordered release kinetics over a ten-day period. Tan et al. showed that paclitaxel will self-assemble during the dialysis process under certain conditions and form micrometer-long fiber-like structures [78]. If this process is done in the presence of the PEG-PDLA and PEG-PLLA copolymers, a co-assembly of the paclitaxel fibers with the copolymers stereocomplex will occur. From XRD and thermal analysis measurements the paclitaxel is soluble in the polylactide core while the characteristic XRD peaks of the stereocomplex are preserved. Moreover, the paclitaxel-loaded block copolymer fibers have a PEG-shell, allowing sustained release and preventing protein adsorption under physiological conditions.

The use of mixed micelles formed from mixture of two block copolymers, AB + AC, with distinctly different properties can self-assemble into particles with discrete sites often denoted as "patches." From this strategy, unprecedented and diverse specific particle anisotropy can be achieved [79–81]. Pitera suggested from coarse-grain molecular dynamics simulations that a binary mixture of two different diblock copolymers with a common hydrophobic block but dissimilar hydrophilic blocks self-assembles into a "patchy" spherical micelle in water [82]. Moreover, it was shown that phase separation of the two hydrophilic blocks on the surface of the micelle could occur to generate the patches. However, the majority of polymer mixtures are incompatible or immiscible, although a sufficient difference in the properties between shell-forming blocks is necessary for the mixed micelles to be effective in applications. The formation of stable mixed micelles requires their spontaneous formation due to strong interactions between two blocks constituting either core or shell of micelles.

For example, Kim et al. demonstrated mixed micelle formation based on a stereoselective association between different stereoisomers PEG-PDLA and poly(*N*-isopropylacrylamide)-*b*-poly(L-lactide) (PNIPAAM-PLLA) in water [19]. Differential scanning calorimetry (DSC) and TEM showed stereocomplex formation between enantiomeric PLA blocks that constitutes the core of mixed micelles compensated the repulsion between the shell-forming PEG and PNIPAAM blocks to form narrowly-dispersed nanoscopic spheres with diameters ranging from 30 to 40 nm (Fig. 4). Both transmittance and DSC measurements for aqueous block

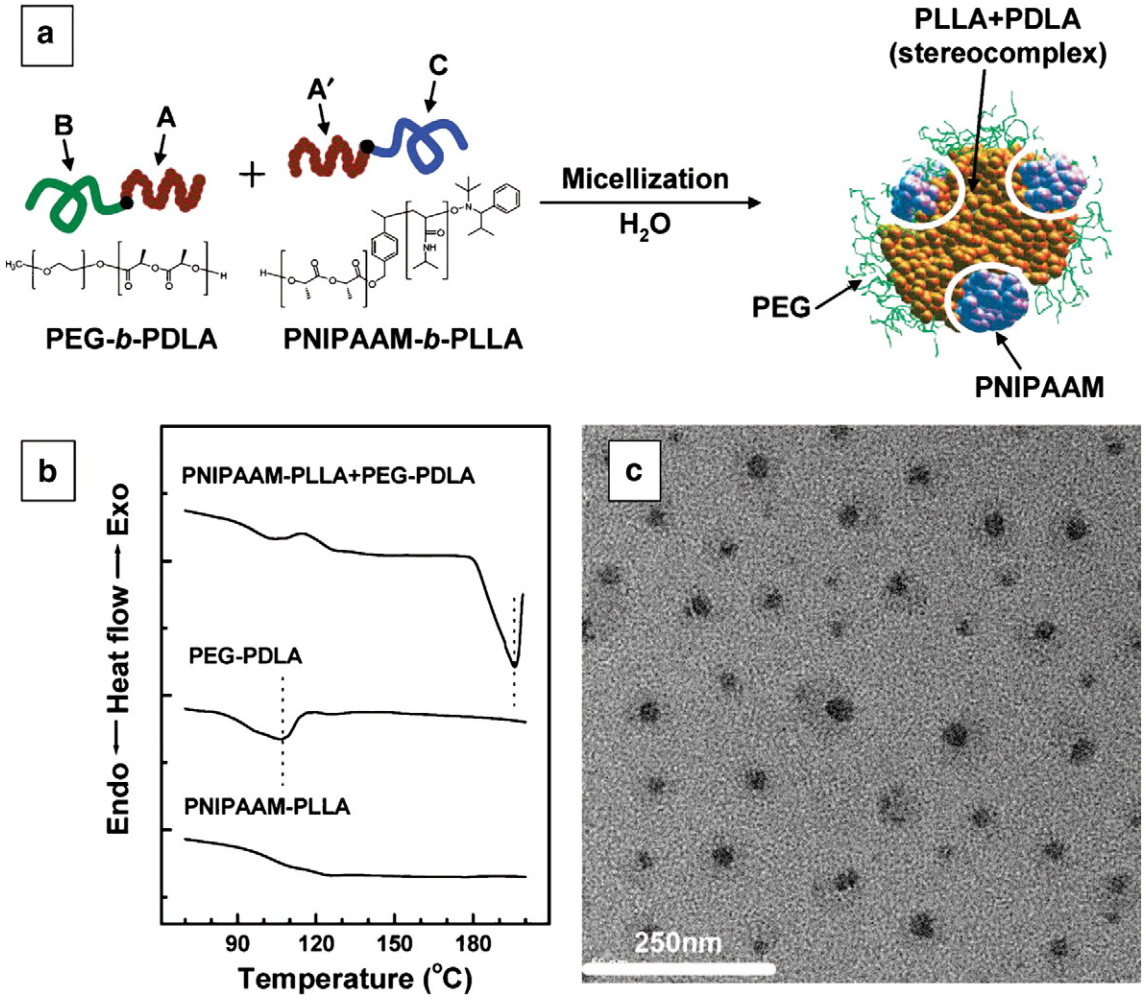

Fig. 4. Mixed micelles formed from dissimilar stereocontrolled block copolymer mixture. (a) The PEG-PDLA + PNIPAAM-PLLA mixed micelles were prepared by dispersing both block copolymers in deionized water. (b) DSC thermograms. (c) TEM image of air-dried micelles shows the stereocomplex mixed micelles with diameters of 20–40 nm. (Reprinted with permission from Ref. [19], Copyright (2009) American Chemical Society).

copolymer solutions showed temperature-responsive behavior of mixed micelles in the aqueous solution with potential use in drug-delivery systems.

The polymer mixtures described above are of polylactide having identical chemical composition but opposite enantiomeric configurations and form homo-stereocomplexes. Domb demonstrated stereo-selective hetero-complexes between two dissimilar polymers, specifically L-peptides and poly(D-PLA) [83–85]. The hetero-complex was detailed with both thermal analysis and AFM measurements. As a means to deliver peptides, no initial burst was observed upon delivery and the controlled release of the peptide was dependent on the detachment of the stereocomplex, facilitated by the polylactide degradation.

2.3. Hydrogen bonding

Like stereocomplexation, hydrogen bonds are short-range interactions that only form if the two interacting groups (i.e. hydrogen bonding donor and receptor) are in close proximity to each other. It is hypothesized that two different amphiphilic copolymers self-assemble into micelles first due to unfavorable interactions of the hydrophobic blocks with water and then hydrogen bonds form when the interacting groups in the hydrophobic blocks of the two copolymers come together, stabilizing the hydrophobic core. Recently, we reported that block copolymer of PEG and urea-functionalized polycarbonate formed micelles with a CMC lower than that of a block copolymer of PEG and polycarbonate without urea groups as the urea groups can form strong hydrogen bonds between themselves, leading to stronger interactions between the hydrophobic polycarbonate blocks [86]. The micelles formed from the block copolymer with urea groups were kinetically more stable, had greater loading capacity for DOX and had a more compact structure with nanosize after DOX loading. Although hydrogen bonding has the potential to drive the formation of mixed micelles, the research on hydrogen-bonding mixed micelles is relatively less pervasive than other types of mixed micelles. The research done so far mostly involves the micellization of block copolymers in solvents other than pure aqueous medium [87–89] or the use of copolymers (in block form or otherwise) with and without mixing with homopolymers in organic solvents [16,90,91]. Copolymers and homopolymers were also mixed in organic solvents to produce vesicles mediated by the hydrogen-bonding [91–94]. These mixed micelle systems were not customized for drug delivery purposes but merely studied to explore their behaviors in different milieus. As of now, there are no published works on mixed micelle systems comprising of two synthetic block copolymers with H-bonding complexation in water. This review aims to inspire researchers alike to fill this void and broaden our understanding of H-bonding within mixed micelles. Henceforward, we will briefly review the characterization of H-bonding between copolymers and/or homopolymers in solvents other than water as well as discuss the various factors influencing H-bonding, which can be applied to the design of future studies on mixed micelles of such nature in aqueous media.

FTIR spectroscopy is commonly applied in polymer systems featuring interpolymer H-bonding. FTIR spectra are useful in indicating hydrogen bonds between specific hydrogen bond acceptors and donors in the different blocks of the copolymers when there is a shift of peak frequency of bands to a lower wavenumber. For example, the hydrogen bonds between polystyrene-*block*-poly(4-vinyl phenol) (PS-*b*-PVPh) and poly(methylmethacrylate)-*block*-poly(4-vinylpyridine) (PMMA-*b*-P4VP) were studied by FTIR in THF and DMF [91,94]. Information extracted from the spectra such as the difference in the shift of the wavenumbers of hydroxyl bands of P4VP after mixing with PS-*b*-PVPh then alluded to the difference in strength of the H-bonding between the PVPh and P4VP blocks in different solvents.

Competitive H-bonding is another approach that can be used to confirm H-bonding as the interactions formed between two different copolymers can be broken with the introduction of a competitive compound that can form stronger interactions with either of the polymers. Gohy et al. added urea in poly(methacrylic acid)-*block*-poly-(ethylene oxide) ($PMAA_{21}$-*b*-PEO_{177}) micelles in aqueous medium, whereby its self-assembly was hypothesized to be driven by the intramolecular H-bonding of PMMA to PEO due to the much longer length of PEG block [95]. Intramolecular complexes formed the core of micelles, surrounded by the shell of uncomplexed PEO segments. Upon addition of urea, $PMAA_{21}$-*b*-PEO_{177} micelles were dissociated as the copolymer formed hydrogen bonds with urea instead, validating the fundamental role of H-bonding in the self-assembly of the copolymer into micelles in water. This competitive H-bonding effect is the reason why abovementioned mixed micelle systems were commonly studied in apolar solvents such as toluene [16,96] or aprotic organic solvents such as THF and DMF in the first place [91,94].

Mixed micelles formed through hydrogen bonding can be tuned systemically with changing several parameters to modulate the H-bonding as it is reversible. Firstly, high temperatures can break hydrogen bonds, dissociating mixed micelles systems. The mixture of poly(styrene)-*block*-poly(4-vinylpyridine) (PS-*b*-P4VP) and poly (acrylic acid) (PAA) in DMF formed micelles having a core of P4VP and PAA complex and a shell of PS. The hydrodynamic radius, R_h, of the mixed micelles was studied with temperature. The radius of the micelles remained consistent until temperatures reached 55 °C whereby the R_h started to decrease [90]. Changing the pH of the milieu by adding acidic and basic water to organic solvents can also disrupt the hydrogen bonds in mixed micelles as the hydrogen bonding acceptors and donors can be protonated or ionized with the change in pH, rendering them unable to form hydrogen bonds with one another. The disruption of the hydrogen bonds between P4VP and PAA, and the addition of water, reversed the core/shell structure of the micelle into a PS core and a P4VP shell. Finally, the solvent used in dissolving the different copolymers plays a very important role in the formation of H-bonding between them. Polar solvents may take part in H-bonding with the copolymers, disrupting the H-bonding complexation of the mixed micelles. On the other hand, dissolving these mixed micelles systems in different solvents will yield different results when temperature and pH are changed due to the difference in degree of solvents' polarity and subsequent competition for hydrogen bonds as shown by Lefèvre et al. [90].

2.4. Ionic interactions

Besides the short-range interactions such as hydrophobic, stereo-complexation and hydrogen bonding interactions, long-range ionic interactions or charge transfer have also been employed to form mixed micelles. In this instance, the driving force of the micellization is the strong electrostatic attraction between a pair of oppositely charged block copolymers. This class of mixed micelles is referred to as polyion complex (PIC) micelles. When the pair of block copolymers is dissolved in water, oppositely charged blocks of the copolymers come together to from aggregates through long-range electrostatic interaction, driving the formation of mixed micelles. Compared to conventional mixed micelles formed through hydrophobic or hydrogen bonding interactions, PIC micelles have the added advantage of encapsulating ionic drugs/compounds such as folic acid [97], coenzyme A [98], oligonucleotides and plasmid DNA [99] and siRNA [100] in the core of the micelles. Electrostatic attraction was employed as the dominant force in the assembly of “patchy” supramolecular particles consisting of urea-adamantyl poly(propylene imine) dendrimer and ureido acetic acid in addition to short-range hydrogen-bonding [101].

While hydrophobic interactions may take a backseat in the micellization of PIC micelles, they still play a role in driving the

formation of mixed micelles under certain conditions. This interplay between the hydrophobic interactions and electrostatic interactions was demonstrated by Cohen Stuart and Schlaad's team with the structural changes of positively charged poly(4-(2-amino hydrochloride-ethylthio) butylene)-*block*-poly(ethylene oxide) ($PAETB_{49}$-*b*-PEO_{212}) and negatively charged poly(4-(2-sodium carboxylate-ethylthio) butylene)-*block*-poly(ethylene oxide) ($PCETB_{47}$-b-PEO_{212}) PIC micelles [102]. When these two block copolymers were fully ionized at intermediate pH values, the mixed micelles formed with PEO shell had a swollen core with gel-like properties as the copolymers interacted through electrostatic attraction. With an increase or decrease in pH, as one copolymer became less ionized, the micellar core became less swollen with water being expelled out and the core was more compact. Hydrophobic interactions then became the dominant driving force in the micellization of the PIC micelles. Thus, the polymer chains in the mixed micelles became immobile and tended to be irresponsive to environmental stimuli. This agrees with the works reported by He's group [103] who studied the unimacromolecule exchange between individual mixed micelles formed from polystyrene-*block*-poly(acrylic acid) (PS_{24}-*b*-PAA_{116}) and polystyrene-*block*-poly(amino propylene-glycol methacrylate) (PS_{51}-*b*-$PAPMA_{140}$) in water. After mixing, there was a strong electrostatic interaction between the shell-forming chains of negatively charged PAA and positively charged PAPMA, forming PIC micelles. These ionic interactions, among many reasons, were thought to encourage the exchange of different unimer chains from different micelles as the interactions are long-range. The swollen cores of the individual and mixed micelles aided in the free exchange of unimer chains. Thus, the structure of the copolymers and how compact the core is, determined by the dominant interactions holding the different components in the mixed micelles, affect the mobility of polymer chains and responsiveness of these PIC micelles.

The ionic interaction within PIC micelles is also reversible and can be influenced by pH, salt concentration and chain length of the charged block. pH affects the degree of ionization of the interacting blocks. As pH increases, the positively charged (basic) block polymer will become less ionized and eventually becomes neutral. Similarly, the negatively charged (acidic) block polymer will become less ionized with protonation as pH decreases. Upon pH changes to PIC micelles system, the increasing neutralization of one block copolymer results in a decrease in the electrostatic attraction between the different block copolymers while hydrophobic interactions become dominant in the micellization as was the case in the $PAETB_{49}$-*b*-PEO_{212} and $PCETB_{47}$-*b*-PEO_{212} PIC micelles mentioned earlier [102]. R_h, of the PIC micelles was studied with pH change and the change in size with different pH values can also prove the existence of electrostatic attraction between the oppositely charged block copolymers in a PIC system. pH-dependent drug release was reported for PIC micelles incorporating charged drugs/compounds in their cores. Luo et al. showed that Coenzyme A (Co A) release from PIC micelles consisting of poly(N-vinylprrolidone)-*block*-poly(styrene-alter-maleic anhydride) (PVP-*b*-PSMA) and PVP-*block*-poly(N,N-dimethylaminoethyl methacrylate) (PVP-*b*-PDMAEMA) was pH-dependent owing to the pH-dependent structural transition of the positively charged PDMAEMA block and its electrostatic interaction with negatively charged Co A and PDMAEMA block [98]. At pH 7.4 (small intestine pH), the electrostatic repulsion between Co A and PSMA block coupled with the expansion of PDMAEMA block resulted in Co A being leaked from the micellar core. Higher pH environments (i.e. 9.18) forced PDMAEMA block to be compacted in a coil formation, hindering the release of Co A while under the acidic pH of 2, strong hydrogen bonds were formed between Co A and PSMA block, trapping Co A in the micellar core (Fig. 5). This system was later modified by incorporating folic acid into PVP-*b*-PDMAEMA and PVP-*b*-poly(2-acrylamido-2-methyl-1-propanesulfonic acid) (PAMPS) PIC micelles [97]. In this instance, folic acid release was improved slightly instead

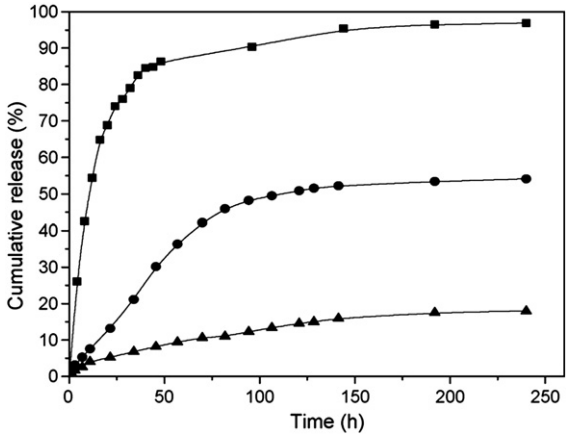

Fig. 5. In vitro release profile of PIC micelles loaded with Co A at pH 2.0 (▲, the gastric pH conditions), pH 7.4 (■, the pH of small intestine) and pH 9.18 (●) at 37 °C. (Reprinted with permission from Ref. [98], Copyright (2009) Elsevier).

at pH 9.18 compared to that at pH 7.4 due to the repulsion of folic acid to negatively charged PAMPS block while PDAEMA was being neutralized. These examples show that by tuning the pH, drug release from PIC micelles can be controlled which is useful when polymeric micelle encapsulating therapeutics are delivered *via* oral administration to the different pH environments in the gastrointestinal tract.

Electrostatic attraction between the oppositely charged block copolymers in PIC micelles can also be challenged with the use of salts. The inter copolymer ionic interactions can break with an increase in concentration of salt ions in the PIC system as block copolymers will form ionic interactions with the salt ions instead. Therefore, stability of PIC micelles is pervious to a critical salt concentration above of which dissociation of PIC micelles occurs. The dissociation of poly(2-vinylpyridine)-*block*-poly(ethylene oxide) ($P2VP_{41}$-*b*-PEO_{204}) and poly(methacrylic acid)-*block*-poly-(ethylene oxide) ($PMAA_{21}$-*b*-PEO_{177}) PIC micelles was tracked with the decrease in fluorescence intensity of pyrene at 363 nm with the increase in NaCl concentration in the aqueous system [95]. Pyrene was used as a fluorescence probe as its intensity decreases when its environment changes from non-polar to polar, the latter of which exists when charged block copolymers exist freely in solution. Since there is a high concentration of salts present in the physiological environment, it is important to evaluate stability of mixed micelles formed through ionic interaction in a simulated physiological environment.

The degree of polymerization (DP) of block copolymers involved is also known to influence their self-assembly into PIC micelles. Harada and Kataoka laid the groundwork by mixing poly(ethylene glycol)-*block*-poly(L-lysine) (PEG-P(Lys)) and poly(ethylene glycol)-*block*-poly(α,β-aspartic acid) (PEG-P(Asp)) copolymers with the same unit ratio of L-lysine and aspartic acid units, resulting in the formation of PIC micelles of 15 nm diameter and very low polydispersity [104]. They expanded the work by mixing two sets of these copolymers with different DP of the poly(amino acid) blocks (18 and 78 repeating units) in aqueous solution. Using gel filtration chromatography to track the micellization of copolymers of different DP, it was found that PIC micelles were only formed when the pair of oppositely charged polymers matched in their block length [105]. Unmatched pairs of block copolymers would just interact to neutralize the charges without self-assembling into micellar structures. The motivation for a pair of block copolymers with same poly(amino acid) chain length to recognize each other is for a uniform arrangement of copolymers where the hydrophilic PEG corona and poly(amino acid) core are regularly delineated. Another motivation is to have equal number of poly(amino acid) residues to neutralize the charges for stable PIC micelles. The question to which of these motivations would be more

dominant in the chain length recognition phenomenon is answered by Gohy and coworkers [95]. Their PIC system of $P2VP_{41}$-*b*-PEO_{204} and $PMAA_{21}$-*b*-PEO_{177} discussed earlier has mismatched hydrophobic block (P2VP and PMMA) chain lengths yet uniform PIC micelles were formed. The reason for the formation of PIC micelles was attributed to the difference in the degree of ionization between the P2VP and PMMA blocks such that an unequal number of the two block residues still results in an equal concentration of negative and positive charges for neutralization to occur. The mixing fraction of the different block copolymers in the PIC micelles can also be tuned to vary the concentration of the charges on the block copolymers.

2.5. Chemical cross-linking

Chemical cross-linking is a versatile and commanding approach to forming mixed micelles. This strategy opens up new possibilities in the choice of polymers used by conferring stability to block copolymers that are traditionally unable to form stable micelles [106,107] as well as suppressing the dissociation of PIC micelles [108]. Chemical cross-linking has also been used to stabilize mixed micelles with varying stoichiometries of surface-presented functional groups [109]. In the following paragraphs, we shall explore the various means of manipulating chemical cross-linking to form stable mixed micelles.

Chemical cross-linking can be used to stabilize the core of polymeric micelles such that incompatible polymers can still form stable aggregates [106,107]. Diblock copolymers of the AB + CB variety can be chemically cross-linked to form core-stabilized micelles [107]. Hui et al. showed that by using 1,4-dibromobutane as a cross-linker for P2VP, polystyrene-*block*-poly(2-vinylpyridine) (PS-*b*-P2VP) and poly(ethylene oxide)-*block*-poly(2-vinylpyridine) (PEO-*b*-P2VP) formed stable mixed-shell micelles in DMF despite the fact that the PEO and PS chains are highly incompatible [107]. After transferred into water, the mixed micelles presented enough PEO molecules in the shell to prevent aggregation [107].

Core stabilization may be utilized to counter the threat to the integrity of the micelles posed by environmental changes such as changes in solvent, temperature and pH [106,108]. This stabilization was achieved by the formation of poly(pentaerythritol tetraacrylate) (poly(PETA)) networks in the micellar core, created using UV-induced free radical polymerization of PETA [106]. The maintenance of the morphology of micelles containing a mixed PEO/poly(2-hydroxyethyl methacrylate) (PHEMA) shell and a poly(propylene oxide) (PPO) core in aqueous solution at different concentrations, under ultrasonic irradiation and in methanol demonstrates the efficiency of this approach [106].

Joralemon et al. showed that shell cross-linked mixed micelles could be prepared from poly(acrylic acid-*b*-methyl acrylate) (PAMA) and mannosylated PAMA [109]. After forming mixed micelles with varying stoichiometric ratios of non-mannosylated and mannosylated PAMA, a condensation-based cross-linking between the acrylic acid groups of the shell-forming polymeric chains via 2,2′-(ethylenedioxy) bis(ethylamine) locks the micellar configuration. These micelles with the surface-presented mannose functional groups have the potential for targeting cell surfaces that express mannose receptors.

Among all the cross-linkers reported in the literature, the biodegradable ones are preferred especially in the design of an injection formulation. Kakizawa et al. established the efficacy of using disulfide bonds to stabilize such micelles. PIC micelles formed between poly(ethylene glycol)-*block*-poly(L-lysine) (PEG-P(Lys)) and poly(α,β-aspartic acid) (P(Asp)) are stabilized by the formation of disulfide bonds between the thiol groups that were conjugated to the lysine block via the primary amines in the lysine residues [108]. Besides improving the stability of the PIC micelle, disulfide bonds provide reversibility to the cross-linking. This means that the disulfide bridges can be destroyed in a reducing environment such as the intracellular environment. This opens up the possibility of the micelle releasing its payload within the cell instead of within the relatively less reducing extracellular fluid [108].

3. *In vivo* applications

Similar to micelles formed from single polymers, mixed micelles serving as drug delivery carriers must be evaluated *in vivo* in terms of efficacy and toxicity. The *in vivo* toxicity of polymeric micelles is known to be dependent upon their biodistribution and biodegradability [2,110]. In cancer therapy, mixed micelles formed through hydrophobic interactions [22,23,26,39,48,55,56] and stereocomplexation [21] have been demonstrated to possess superior *in vivo* drug delivery efficacies over free drugs and single-polymer micelle formulations alike. In most cases, the favorable effects were attributed to the enhanced pharmacokinetic properties conferred by the greater stability of the mixed micelles.

The early work by Alakhov and coworkers clearly underlined the potential of mixed micelles to improve drug delivery for cancer treatment [22]. In this study, the aggregation tendencies of pure Pluronic L61 micelles was overcome by combining L61 with the more hydrophilic L127 in the SPC1049C formulation of DOX. The resultant mixed micelles demonstrated more efficient tumor inhibition in a wide spectrum of *in vivo* mice tumor models compared to free DOX following intravenous administration. This observation was in line with the results obtained from pharmacokinetic analyses in C57BL/6 mice, which revealed prolonged drug residence time and a greater degree of drug accumulation in subcutaneously implanted tumors derived from Lewis lung carcinoma 3LL M-27 cells than free DOX. In a more recent study by Mu et al., the enhanced thermodynamic stability (lowered CMC) of docetaxel-loaded mixed micelles comprising of Pluronic P85 and MPEG-PLA (MPP) resulted in improved bioavailability and extended drug circulation time in comparison to MPP micelles and the commercially available Taxotere® [39]. More importantly, intravenous administration of MPP/P85 mixed micelles resulted in significantly reduced tumor volumes than MPP micelles and Taxotere® at the end of 24 days. Evidence from both studies, among others, therefore support the viewpoint that enhanced dissolution and stability profiles of mixed micelles aids in preserving the physical integrity of drug-loaded micelles, and can lead to avoidance of unwanted toxicity and premature renal loss of free drug from unstable micelles. A corresponding increase in blood circulation time of the drug-loaded mixed micelles thus allows for enhanced accumulation in solid tumors, as evidenced in several *in vivo* imaging studies [48,51,52,56], via the EPR effect.

Together with enhanced passive tumor accumulation, the ease and flexibility conferred by the mixed micelles system to incorporate targeting ligands and/or stimuli (e.g. low tumor pH)-responsive moieties was also found to be useful in improving the selectivity of drug uptake by or drug release to tumor cells; leading to enhanced anti-tumor effects *in vivo* [23,47,48,51–53,55,56]. This effect is expected to minimize non-specific toxicities in healthy tissues/organs. Indeed, in a study by Lee et al., the tumor pH-activated display of the cell penetrating peptide, transactivator of transcription (TAT), in DOX-loaded mixed micelles comprising of poly(L-lactic acid)-*b*-PEG-*b*-poly(L-histidine)-TAT and poly(L-histidine)-*b*-PEG gave rise to significant tumor regression in a variety of xenografted tumors types, which included a drug resistant human ovarian tumor, in nude mice compared with free DOX control micelles [55]. Unlike free DOX and DOX-loaded non-pH dependent TAT presenting control micelles, minimal body weight loss following administration of the test micelles was observed. This study, together with several others, has also provided promising evidence demonstrating the potential of mixed micelle systems in overcoming multi-drug resistance in cancer therapy [22,39,48,52,55].

The biodegradability of the polymers is another important factor that affects the *in vivo* toxicity of mixed micelles. Mixed micelles

formed through reversible non-covalent interactions such as hydrophobic interaction, hydrogen bonding, stereocomplexation and ionic interaction can eventually be dissociated into individual polymer chains and may accumulate in organs/tissues after administration. Therefore, for the use of mixed micelles in parenteral formulations, an important consideration lies in the biodegradability of the constituent copolymers as well as the biocompatibility of their degradation products. It is well-documented that the degradation product of PLA is biocompatible and PLA has since been approved by FDA for use in numerous injectable drug delivery products [111], sutures, orthopaedic screws and medical devices [112]. PEG has also been approved for use in many PEGylated protein formulations such as PEGylated interferon-alpha-2b (Pegintron®), PEGylated interferon-alpha-2a (Pegasys®) and PEGylated L-asparaginase (Oncaspar®) by the FDA. Block copolymers of PLA and PEG, which are the most commonly utilized polymers in mixed micelles formulation, however, are still required to gain FDA's approval prior to their clinical applications. If the polymers used for the formation of mixed micelles in parenteral formulations are not biodegradable, attention has to be taken to ensure that their molecular weights are below 40 kDa so that the unimers may be excreted from the kidney after dissociation of the micelles [43]. In addition, mixed micelles with improved stability, drug loading capacity and multiple functionalities can also be used in topical, nasal and oral drug delivery formulations. In these applications, the biodegradability of the polymers may not be essential if the size of the micelles is sufficiently large to avoid micellar absorption by the skin, gastrointestinal tract and nasal mucous layer.

While not all of the mixed micelle systems introduced in this review are fully biodegradable and biocompatible, innovative concepts governing the formation of mixed micelles and their characterizations are still expected provide researchers with new insights and motivation to design and utilize more functional and biofriendly polymers in the development of optimized mixed micelle systems for *in vivo* drug delivery applications.

4. Conclusion and future perspectives

Mixed micelles as a concept is a stepping stone to improve the use of polymeric micelles as drug delivery vehicles. From the numerous examples highlighted in this review, it is evident that the rational combination of two or more copolymers to form mixed micelles is a direct and convenient approach to improve the physical stability and enhance drug loading capacities of conventional polymeric micelles for drug delivery. The versatility of this approach also allows for the concomitant integration of multiple functionalities into a single system — a feat that is synthetically challenging to accomplish with micelles formed from a single co-polymer. The combination of pH- and temperature-responsive drug release functionalities, for instance, allows drug-loaded mixed micelles to capitalize on various pathologically- or externally-induced microenvironmental changes to release drugs at desired target sites in a highly controlled manner. Furthermore, the mixed micelles system also offers a straightforward approach to optimize surface densities of ligands, which is expected to result in more efficient cellular targeting and internalization of the drug-loaded polymeric micelles.

The nature of interactions between the hydrophobic blocks of the copolymers plays an important role in the formation and behaviors of mixed micelles in their environment. Of the various core interactions outlined, the short-range hydrophobic interactions feature as the major non-covalent driving force for current mixed micelles systems. This is closely followed by the long-ranging polyionic complexation. Chemical cross-linking and other non-covalent interactions, such as stereocomplexation and hydrogen bonding, however, remain less exploited. As the molecular recognition that exists between molecularly complementary functional groups such as urea/acid and amine/acid (via hydrogen bonding) or enantiomeric moieties (e.g. L-PLA and D-PLA via stereocomplexation) offers an excellent opportunity to precisely guide the association of mutually interacting copolymers, more extensive investigations into mixed micelles formation of such nature are warranted.

Another major challenge to researchers lies in the need to achieve a delicate balance between the formation of physiologically stable copolymer micelles and polymeric micelles that can undergo rapid dissociation at the desired sites for drug release and the eventual elimination of the unimers from the body. The continued development of advanced methodologies is imperative to the advancement of mixed micelles as a drug delivery system. Although various methods to verify the formation of a single coherent population of mixed micelles have been reported, a dearth of innovative tools and techniques that can validate and characterize the specific interactions between the copolymers exists. Therefore, the concurrent development of characterization methodologies is crucial for the evaluation and optimization of the mixed micelles formed as a result of the various interactions outlined in this review.

As discussed in the earlier section, current *in vivo* evaluations of efficacy and safety of mixed micelles are limited to those formed through hydrophobic interactions and stereocomplexation. As the various reversible core interactions are expected to affect the stability (thermodynamic and kinetic) and dissolution profiles of drug-loaded mixed micelles, resulting differences in the rate of micellar disintegration, drug release properties and degradation rates (if applicable) are expected to lead to significantly varied pharmacokinetic properties and therapeutic efficacies *in vivo*. Thus, *in vivo* proofs of concept for drug-loaded mixed micelles formed through the other modes of interaction should be given more attention. Additionally, toxicity issues, which are greatly influenced by the biodegradability (and its rate) of copolymers and biocompatibility of their degradation products, are a major concern for the successful application of mixed micelle-based drug delivery systems in the clinics. At present, degradation profiles of mixed micelles formed from stereocomplexation [60] exist as the more well-characterized among the various forms of core interactions detailed in this review. Although several *in vivo* studies utilizing mixed micelles have provided evidence demonstrating comparable or superior toxicity profiles to free drugs, with no added toxicity arising from the mixed micelles carriers [22,39,48,52,55], detailed biodegradation studies are still essential to the regulatory approval of novel mixed micelles formed from the various interactions. This aspect, together with efficacy and pharmacokinetic studies in preclinical evaluations, should therefore be one of the major focuses for researchers working on the various mixed micelle systems for drug delivery applications.

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physical properties of polymeric micelles as well as provide a comprehensive overview of micelle characterization techniques.
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