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Development of acid-sensitive copolymer micelles for drug delivery*

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Abstract: In recent years, supramolecular micellar assemblies formed from amphiphilic block copolymers have been receiving attention as potential drug carriers. The size of the carriers is ideal for avoiding rapid renal exclusion and reticuloendothelial uptake, and enables them to be targeted to certain tissues such as tumors. One important issue determining the effectiveness of a micellar drug carrier is the ability to control the time over which drug release takes place, or to possibly trigger drug release at a specific location or time. The mildly acidic pH encountered in tumor and inflammatory tissues as well as in the endosomal and lysosomal compartments of cells has inspired the development of micellar carriers capable of releasing their drug load in response to small changes in pH. One approach to the development of these systems has been to incorporate "titratable" groups such as amines and carboxylic acids into the copolymer backbone, thus altering the solubility of the polymer upon protonation and disrupting micelle formation. Another approach has been to incorporate acid-degradable linkages into the copolymer, either for direct attachment of the drug, or to cause a structural change of such magnitude that micellar integrity is lost and the drug is released.

INTRODUCTION

Many biologically active molecules are limited in their therapeutic value by properties such as poor solubility, limited bioavailability, and rapid elimination [1]. In addition, while the beneficial effects of many drugs arise through their interactions with specific tissues, their exposure to other cell types frequently leads to undesirable side effects and toxicity [2]. In recent decades, there has been increased awareness of the need to develop of drug delivery systems to improve the properties of therapeutic compounds, increase their effectiveness, and reduce their harmful side effects.

Micelles formed in aqueous solution by the supramolecular assembly of amphiphilic block copolymers have been receiving increased attention as potential drug carriers (Fig. 1) [3–5]. These nanocontainers are capable of solubilizing hydrophobic drugs in their core and offer many attractive characteristics. The size of copolymer micelles, typically between 20 and 100 nm, is effective not only in avoiding rapid renal exclusion, but is also small enough to avoid uptake by the reticuloendothelial system [6]. This prolongs the circulation of the micellar carrier and the encapsulated drug, but since micelles are composed of individual polymer chains that are small enough to be eliminated via renal filtration, the eventual disintegration of the micelle will allow the polymer to be excreted. This is important since long-term build-up of polymer in the body could lead to toxicity. The size of micelles and their prolonged circulation also facilitates their passive accumulation at pathological sites such as tumors, where the vasculature has increased permeability [7]. Furthermore, polymeric micelles are gen-

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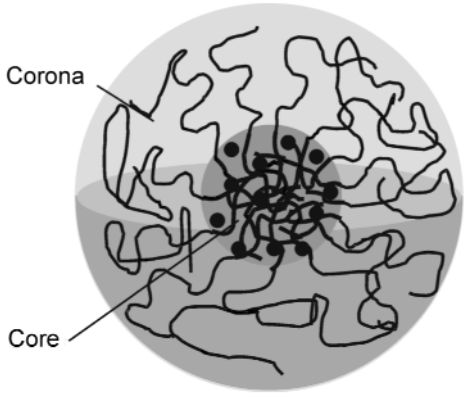

Fig. 1 Sketch of a copolymer micelle with drug loaded in the core.

erally much more stable than surfactant micelles, exhibiting lower critical micelle concentrations (CMCs), slower rates of dissociation, and longer retention of loaded drugs [8].

One important issue determining the effectiveness of a micellar drug carrier is the ability to control the time over which drug release takes place. Fast release may lead to premature loss of drug, causing systemic side effects and negating concentration of the drug at the target site, while slow release may reduce the efficacy of the drug at the site of action and increase drug resistance in cells. This challenge has motivated the development of new micellar systems that are designed to release their drug load in a controlled manner, upon arrival at the target site. In recent years, micelles that are responsive to their environment or to an external stimulus have been designed. For example, drug release has been modulated by temperature from thermo-responsive polymeric micelles [9,10], though this mode of action has remained largely impractical in the normal physiological environment. Ultrasound has been reported to trigger drug release from Pluoronic micelles [11,12].

Change in acidity is a particularly useful environmental stimulus to exploit in the development of drug carriers owing to the numerous pH gradients that exist in both normal and pathophysiological states. For example, it is well documented that the extracellular pH of tumors is slightly more acidic than normal tissues, with a mean pH of 7.0 in comparison with 7.4 for the blood and normal tissues [13–15]. In addition, it is proposed that micelles are taken up by cells via an endocytosis process [16,17]. While the endocytic pathway begins near the physiological pH of 7.4, it drops to a lower pH (5.5–6.0) in endosomes and approaches pH 5.0 in lysosomes [18]. Therefore, polymeric micelles that are responsive to these pH gradients can be designed to release their payload selectively in tumor tissue or within tumor cells. Current approaches toward the development of such systems generally involve either incorporation of "titratable" groups into the copolymer, or linkages that degrade under acidic conditions. The present work briefly reviews these approaches, with special focus on those systems that are sensitive within the range of physiologically accessible pHs and are therefore most relevant to drug delivery applications.

pH-SENSITIVE MICELLES BASED ON "TITRATABLE" GROUPS

Micelles formed from block copolymers containing weak acid or base functions

In recent years, interest in the development of pH-sensitive micelles has been for both scientific and pharmaceutical interest. Several groups have investigated the pH-sensitive micellization of copolymers having weak bases. When the weak bases (generally, amines) are unprotonated, the block of the copolymer that they comprise has a relatively hydrophobic character, and aggregation is promoted. Upon protonation, charges are introduced, thus imparting water solubility to the block and triggering disintegration

of the micelle into unimers as shown in Fig. 2. For example, a poly(2-vinylpyridine)-*block*-poly(ethylene oxide)(P2VP-*b*-PEO) copolymer undergoes spontaneous and reversible micellization in aqueous solutions as the pH is increased from acidic to neutral or basic [19]. P2VP has also been incorporated into triblock copolymers that form micelles having pH sensitivity [20,21]. Armes and coworkers have prepared a series of block copolymers having tertiary amine groups. Some examples are poly[2-(dimethylamino)ethyl methacrylate]-*block*-poly[2-(diethylamino)-ethyl methacrylate] (DMAEMA-*b*-DEAEMA) [22], DMAEMA-*block*-poly[2-(*N*-morpholino)ethyl methacrylate](DMAEMA-*b*-MEMA) [23,24], PEO-DMAEMA [25], and PEO-*b*-DMAEMA-*b*-DEAEMA [26]. These copolymers exhibit pH-dependent micellization owing to their tertiary amine groups, with the behavior of each block copolymer depending on the hydrophobicity and pK_a of the specific amine block involved. The transitional pH is typically in the range of 6–7 for these systems.

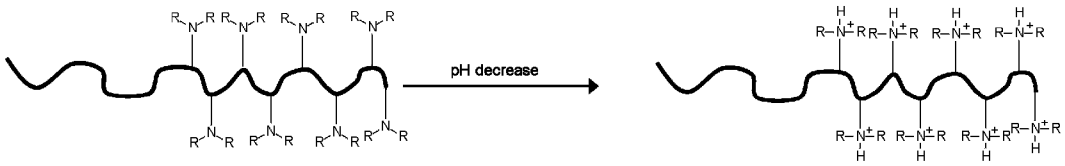

Fig. 2 Change in solubility upon protonation of a copolymer having weakly basic amines in one block.

In contrast, block copolymers having weakly acidic groups such as carboxylic acids tend to aggregate more strongly at acidic pH, where the carboxylic acids are uncharged and more hydrophobic (Fig. 3). In these systems, disruption of micelle formation occurs at neutral or basic pH when the carboxylic acids become ionized. This can be problematic for biological applications, since it is usually desirable to have stable micelles during circulation in the blood at pH 7.4. For example, poly[sodium 2-(acrylamido)-2-methylpropanesulfonate-*block*-poly(sodium 6-acrylamidohexanoate)] and poly(sodium 4-styrenesulfonate)-*block*-poly(sodium 4-vinylbenzoate) form micelles at acidic pHs less than 5, but exist as unimers at and near neutral pH [27,28]. Hydroxyethylcellulose-*graft*-poly(acrylic acid) (HEC-*g*-PAA) forms micellar aggregates at pH less than 4 [29]. Degradable amphiphilic block copolymers of malic acid and malic acid esters, where the pH-sensitive block forms the outer shell of the micelle, were also prepared [30]. As the pH was increased from 2.5 to 7.8, an increase in electrostatic repulsions between the charged carboxylate groups led to a destabilization in the micellar structure, and aggregation was observed. Polybutadiene-*block*-poly(L-glutamic acid) formed vesicles with a size determined by the pH [31]. The size change was attributed to a change in solution conformation of the polypeptide block upon protonation of the carboxylic acid groups. Zwitterionic diblock copolymers such as poly(4-vinylbenzoic acid-*block*-DMAEMA) have also been prepared [32]. This copolymer undergoes self-assembly in aqueous solution to form either micelles or reverse micelles, depending on pH. Precipitation is observed near the isoelectric point of 7.2, but transparent micellar solutions are observed below pH 6 and above pH 9.

Fig. 3 Change in solubility of a copolymer having weakly acidic carboxylic acids in one block.

Researchers attempting to exploit pH-sensitive micellar systems for drug delivery applications have generally focused on systems having transitions in the physiologically accessible pH ranges. For example, Bae and coworkers have prepared pH-sensitive micelles from poly(L-histidine)-*block*-PEO

[PEO-*b*-P(His)] (Fig. 4a) [33]. The micelles were prepared at pH 8, but were destabilized below pH 7.4 as evidenced by light transmittance measurements, light scattering, and fluorescent probe techniques. The triggering pH could be adjusted within the range of 7.2–6.6 by incorporation of different amounts of poly(L-lactic acid)-*block*-PEO (PLLA-*b*-PEO) [34]. Release of the anticancer drug doxorubicin (Dox) could be modulated using pH, and it was found that the toxicity of the Dox-loaded mixed micelles to tumor cells in vitro was strongly dependent on pH with toxicity comparable to free doxorubicin near the triggering pH. Bae and coworkers have also prepared triblock copolymers such as PLLA-*b*-PEO-*b*-polysulfadimethoxine (Fig. 4b) that are pH sensitive owing to the weakly acidic nature of the sulfonamide groups [35]. Micelles formed from these copolymers have a phase transition around pH 7.0, and are expected to be useful as anticancer drug carriers since tumor pH is known to be close to 7.0. Leroux and coworkers have prepared micelles from random copolymers of methacrylic acid (as the pH-sensitive moiety), octadecylacrylate, and *N*-isopropylacrylamide having a phase transition pH of 5.7 (Fig. 4c) [36,37]. The micelles were loaded with the photosensitizer aluminum chloride phthalocyanine and were found to exhibit increased cytotoxicity in vitro relative to the control Cremophor EL surfactant formulation currently used for its in vivo administration. Amphiphilic star polymers were recently prepared using a four-armed multifunctional initiator for sequential polymerization of ethyl methacrylate and *t*-butyl methacrylate, then poly(ethylene glycol)methacrylate, followed by hydrolysis of the *t*-butyl esters [38]. This system was designed as a drug delivery system for oral administration of hydrophobic drugs. Accordingly, as shown in Fig. 5, at the acidic pH of the stomach the carboxylic acids are protonated, while passage of the system into the neutral environment in the intestines should

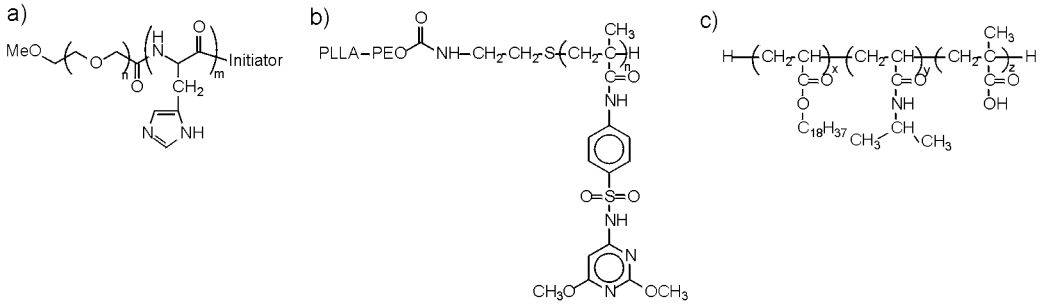

Fig. 4 (a) Poly(L-histidine)-*block*-poly(ethylene oxide); (b) poly(L-lactic acid)-*block*-poly(ethylene oxide)-*block*-polysulfadimethoxine; (c) random copolymer of *N*-isopropylacrylamide, methacrylic acid, and octadecylacrylate.

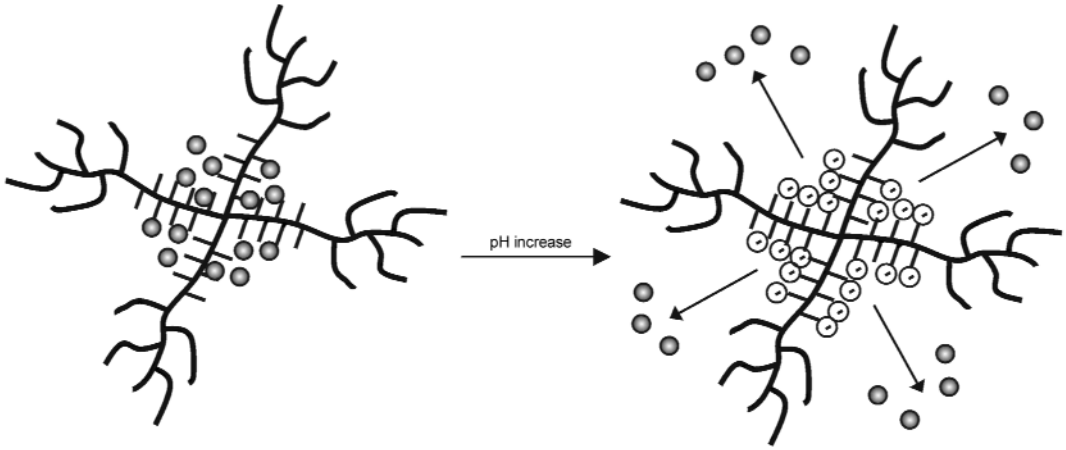

Fig. 5 Schematic for drug release from star polymer of ethyl methacrylate, methacrylic acid, and poly(ethylene glycol)methacrylate (adapted from ref. [38]).

lead to deprotonation of carboxylic acids. This increase in polarity of the core micellar environment was shown to trigger enhanced release of the model hydrophobic drug progesterone.

pH-Sensitive interpolyelectrolyte complexes

Interpolyelectrolyte complexes are formed through the electrostatic interaction of two oppositely charged block copolymers as shown in Fig. 6. These systems have the potential to be pH-sensitive when weakly acidic or basic groups make up the charged blocks as described above. For example, Kataoka and coworkers have demonstrated that stable and considerably monodisperse micelles could be formed by combining PEO-*block*-poly(L-lysine) [PEO-*b*-P(Lys)] and PEO-*block*-poly(aspartic acid) [PEO-*b*-P(Asp)] [39]. It was also found that molecular recognition based on length occurred such that pairs formed from copolymers with the same block lengths of polycations and polyanions, even from mixtures with different block lengths [40]. Kabanov and Eisenberg have shown that micelles formed from PEO-*b*-poly(sodium methacrylate) (PEO-*b*-PMANa), and poly(*N*-ethyl-4-vinylpyridinium bromide) (PEVP) were sensitive to both pH and salt concentration [41].

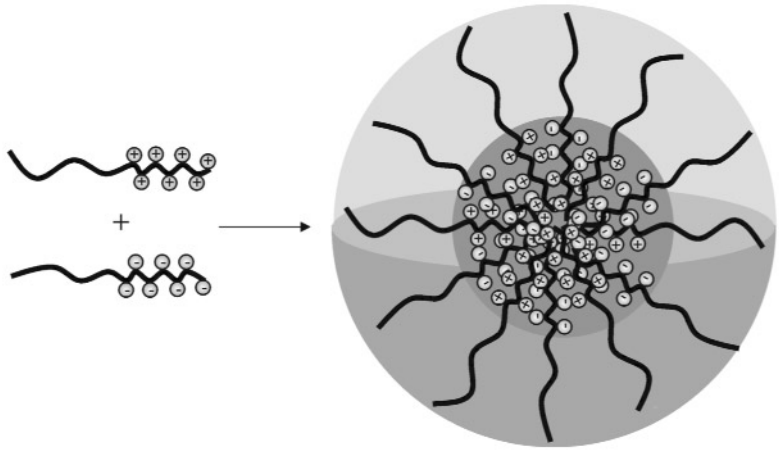

Fig. 6 Formation of an interpolyelectrolyte complex.

Copolymers having cationic blocks have been used for complex formation with polyanionic biomolecules such as DNA and oligonucleotides to form micellar structures. For example, PEO-*block*-polyspermine was complexed with antisense oligonucleotides and the complex was found to enhance the sequence-specific inhibition of gene expression relative to the free oligonucleotide [42]. PEO-*b*-P(Lys) has also been used to prepare complexes with oligonucleotides and DNA [43,44]. Complex formation was found to stabilize the DNA both structurally, and with respect to enzymatic degradation. In addition, a drug delivery system for a zinc porphyrin, a potential photosensitizer for photodynamic therapy, has been prepared. This system consists of a complex between PEO-*b*-P(Asp) and the dendronized porphyrin having 32 primary amine groups on the periphery. Stable micelles were formed from pH 7.4 to 6.2, with a less stable state below pH 6.2. This suggests the potential for triggered release of the photosensitizer at mildly acidic pH [45].

pH-Sensitive polymer-liposome systems

While pH-sensitive liposomes based on mildly acidic or basic lipids have been known for some time, the interest in incorporating polymers into liposomal preparations arose from their ability to improve liposome stability, their ease of association to the liposome surface, as well as polymer properties such

as low immunogenicity, straightforward large-scale synthesis, and structural versatility [46–49]. In addition, it has been found that incorporation of pH-sensitive polymers into liposomal systems can be used to prepare pH-sensitive liposomes.

Polypeptides such as P(Lys) and P(His) are neutral polymers at high pH, but become positively charged with a decrease in pH, dependent on the pK_a. These were the first synthetic polymers found to display pH-dependent fusogenic properties [50–54]. When charged, these polymers can interact with negatively charged membranes, perturbing the lipid packing and promoting aggregation and fusion of the liposomes [55]. Poly(amidoamine)s also show pH-dependent membrane destabilization, proposed to be at least partly due to a distinct conformational change from a hydrophobic coiled structure at neutral pH to a relaxed hydrophilic structure at acidic pH [56,57]. Although the membrane-destabilizing properties of these polymers have inspired their use for the cytoplasmic delivery of genetic material, these polymers have not yet been used for the preparation of pH-sensitive liposomes [58]. Oku and coworkers have found that hydrophobically modified poly(ethyleneimine) (PEI) could be incorporated in the bilayer of phosphatidylcholine (PC) vesicles and induced fusion with negatively charged PC/phosphatidylserine (PS) vesicles only below pH 7 [59]. However, the vesicles were not found to release their contents at acidic pH.

Weak acid polyelectrolytes typically bearing carboxylic acid groups have been more extensively investigated for the preparation of pH-sensitive liposomes. Tirrell has shown that poly(2-ethylacrylic acid) (PEAA) could bind to multilamellar vesicles including liposomes in a pH-dependent manner, and induce the release of probe molecules [60–62]. It is believed that at acidic pH as the carboxylic acid groups become protonated, and as the polymer becomes more hydrophobic and surface adsorption increases [63]. Succinylated poly(glycidol)s bearing long alkyl chains have been incorporated into PC vesicles and were found to trigger release of encapsulated calcein at pH 5.5 [64]. Recently, it has been shown that liposomes with temperature-responsive properties can be prepared by coating liposomes with copolymers of *N*-isopropylacrylamide (NIPAM) having long alkyl chains [65–67]. By incorporating a "titratable" comonomer such as methacrylic acid, Leroux and Drummond have shown that the lower critical solution temperature can be increased to above 37 °C and the coated liposomes are rendered pH-sensitive [68]. Probes such as 8-hydroxypyrene-1,3,6-trisulfonic acid (HPTS) and the Dox have been released from the copolymer-modified liposomes selectively at pHs between 5 and 6 [69,70]. A similar approach has been taken by Winnik and coworkers using the carboxylic acid of glycine as the pH-sensitive group and calcein as a probe [71,72]. A schematic representation of a possible mechanism for pH-triggered release from these polymer-liposome complexes is shown in Fig. 7.

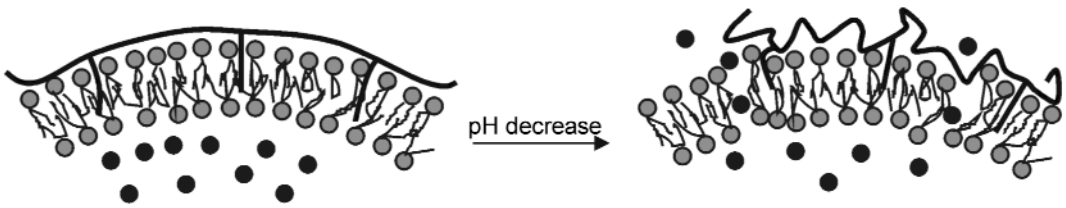

Fig. 7 Schematic representation of a possible mechanism for pH-triggered release by copolymers of NIPAM (adapted from ref. [73]).

ACID-SENSITIVE MICELLES CONTAINING ACID-DEGRADABLE LINKAGES

An alternative approach to "titratable" groups for the preparation of pH-sensitive micelles is to employ an acid-cleavable linkage. This linkage can be used either to directly attach the drug to the copolymer, or to alter the structure of the polymer sufficiently that drug release is triggered upon hydrolysis of the linker. Park and coworkers have prepared PEO-*b*-PLLA with Dox conjugated to the terminal group of the PLLA block by an acid-sensitive *cis*-aconityl or hydrazone linkage as shown in Figs. 8a and 8b,

respectively [74]. Both of these copolymers formed micelles that released free Dox much faster at pH 5 than at pH 7, although release was still quite slow and somewhat incomplete even at acidic pH, likely because hydrolysis released Dox into the hydrophobic core of the micelle, from which diffusion was slow. Despite this setback, the micelle with the hydrazone linkage to Dox displayed higher toxicity than free Dox in vitro. This is likely because the micelles are more readily taken up by cells via an endocytosis process, than is the case for free Dox which enters cells by passive diffusion. In addition, the mechanism of endocytosis may help circumvent the multidrug-resistance effect that occurs for the free drug.

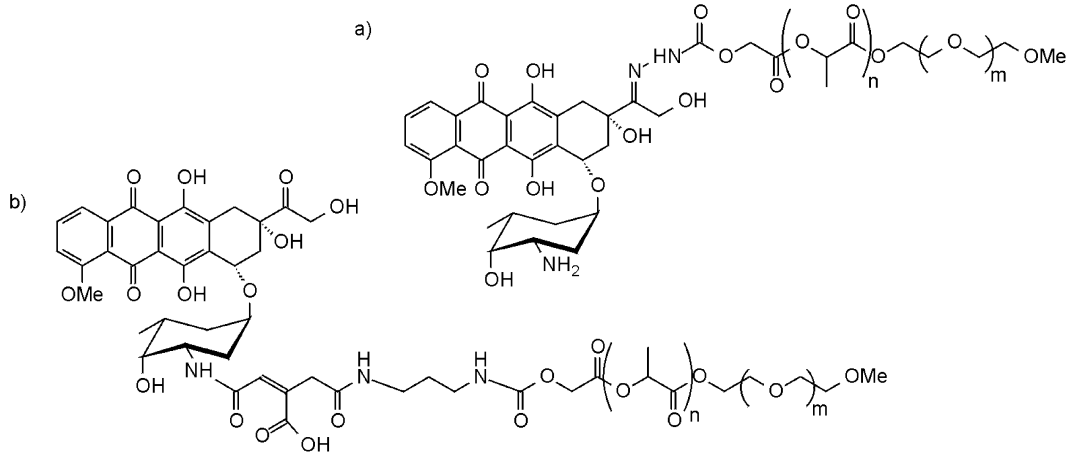

Fig. 8 Doxorubicin conjugated to the terminal end of poly(ethylene oxide)-*block*-poly(L-lactic acid) by (a) a hydrazone and (b) a *cis*-aconityl linkage.

Kataoka and coworkers have recently attached Dox via hydrazone linkage to the aspartic acid units of a PEO-*b*-P(Asp) (Fig. 9a) [75]. The drug coupling procedure resulted in about 68 % of the aspartic acid units being modified with Dox and the resulting block copolymer formed micelles that released free Dox in a time- and pH-dependent manner as the pH was decreased from 7.4 to 3.0. Confocal microscopy was used to show that while free Dox was localized in the nuclei after 24 h of incubation, the micelles containing Dox appeared to be localized in endocytic vesicles. In vitro tests

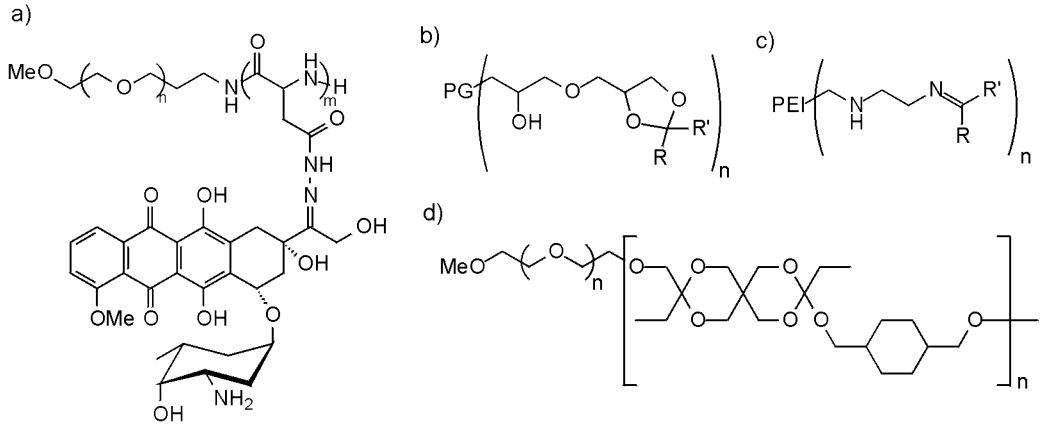

Fig. 9 (a) Poly(ethylene oxide)-*block*-poly(aspartic acid) with doxorubicin attached via hydrazone linkage; (b) hyperbranched polyglycerol (PG) with end groups functionalized using cyclic acetals; (c) hyperbranched polyethyleneimine with end groups functionalized using imines (R = long alkyl chain or hydrogen); (d) poly(ethylene oxide)-*block*-poly(ortho ester).

showed that the toxicity of the micellar system approached that of free Dox, indicating that active Dox must be released from the micelles, likely due to hydrolysis of the hydrazone linkages in the acidic endocytic vesicles.

Employing acid-sensitive linkages in the copolymer itself is an alternative approach, which offers the advantage of being a general system that can be applied to different drugs without changing the linkage. In addition, regulatory approval may prove easier since no chemical modification of the drug is required. As a model system, Haag and coworkers prepared pH-responsive unimolecular micelles based on hyperbranched polyglycerol and PEI with hydrophobic chains attached by either cyclic acetals or imine linkages, respectively (Figs. 9b and 9c) [76]. These systems were soluble in organic solvents, and it was possible to encapsulate polar dyes such as bromophenol blue, congo red, and methyl orange in their cores. The polyglycerol-acetal system was stable at pH >7, but released its contents at pH <3, while the imine based system was less stable at neutral pH, but released its contents at the more practical mildly acidic pH of 6.

Heller and coworkers have introduced a somewhat different approach to pH-sensitive micelles where a pH-sensitive poly(ortho ester) comprises the hydrophobic block of a copolymer with PEO (Fig. 9d) [77,78]. Poly(ortho ester)s are well known to degrade at mildly acidic pH, thus at pH 5.5 the micelles rapidly lose their structure and release the entrapped drug. The micelles have a very low CMC and sizes in the desired range of 50–80 nm. In addition, it has been possible to entrap the anticancer drug taxol at a loading of 40 wt%. Therefore, these pH-sensitive micelles are very promising as delivery systems and are said to be under active development.

Gillies and Fréchet have recently reported an approach based on the attachment of hydrophobic groups to the core-forming block of a copolymer via an acid-sensitive linkage [79]. Cyclic benzylidene acetals were investigated as the acid-sensitive linkages, because they possess several favorable characteristics. First, they contain a hydrophobic aromatic ring that will contribute to micelle formation. In addition, a cyclic benzylidene acetal can mask the polarity of a copolymer-bound 1,3 diol, thus affording a significant solubility change in the copolymer upon hydrolysis. The rate of hydrolysis of benzylidene acetals is generally proportional to the hydronium ion concentration and is expected to increase 250-fold as the pH is changed from 7.4 to 5.0 [80]. Since cyclic acetals generally hydrolyze quite slowly, electron-donating methoxy groups were introduced in the *ortho* and *para* positions to obtain rapid hydrolysis at pH 5.0.

Trimethoxybenzylidene acetals were introduced to approximately 30 % of the carboxylic acid groups of a PEO-*b*-P(Asp). The resulting copolymer shown in Fig. 10a was found to form micelles with

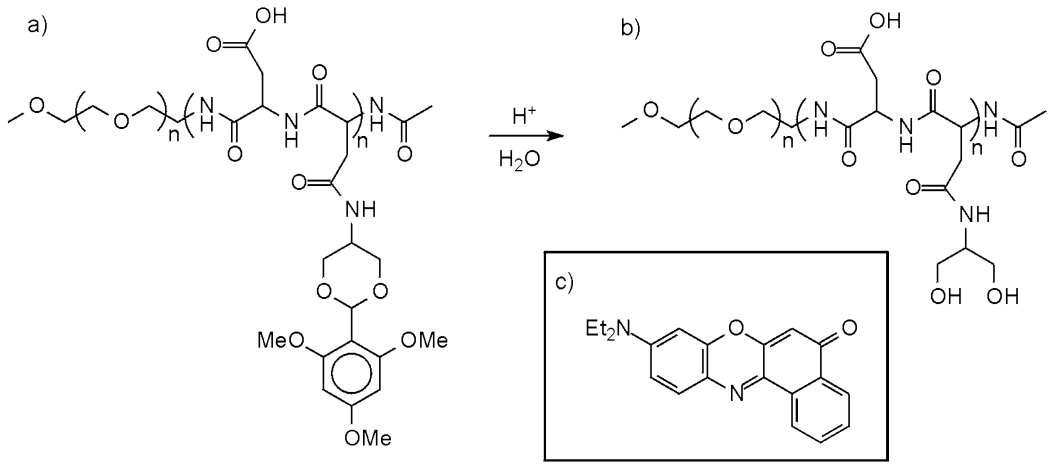

Fig. 10 (a) Poly(ethylene oxide)-*block*-poly(aspartic acid) functionalized with pendant acetal groups before hydrolysis; (b) after hydrolysis; (c) Nile Red.

a CMC of 0.34 mg/ml and a size of 90 nm. At mildly acidic pH, hydrolysis of the acetals is expected to occur, generating hydroxyl groups (Fig. 10b). This solubility change is expected to disrupt the micellar assembly, triggering release of the contents of the micelle.

The hydrolysis of the rate of the acetal moieties in the micelles was measured at 37 °C at pH 5.0 and pH 7.4. Appearance of 2,4,6-trimethoxybenzaldehyde was detected by its absorbance at 292 nm. As shown in Fig. 11a, the hydrolysis was found to be rapid at pH 5.0, with a half-life of 1 h, while at pH 7.4 the half-life was several days. The hydrolysis rate was not affected by incorporation of the acetal groups in the micelle as demonstrated by comparison with a low-molecular-weight model compound.

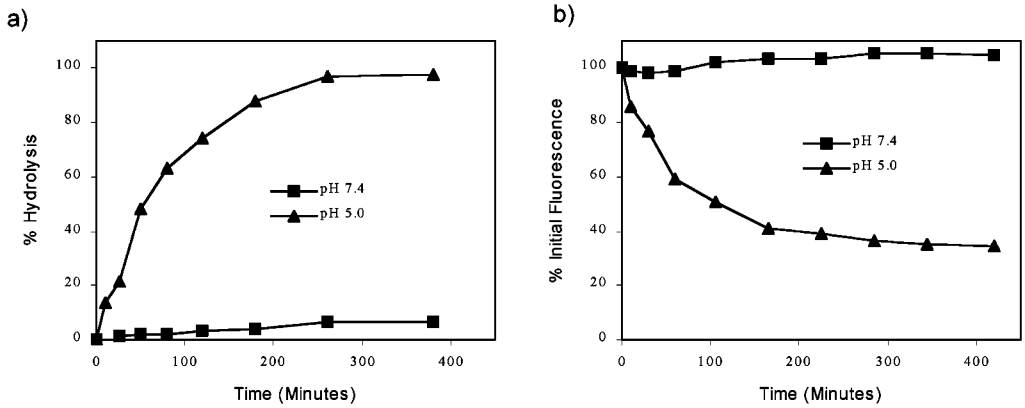

Fig. 11 pH-dependent (a) hydrolysis of acetals in copolymer 14a; (b) Nile Red fluorescence in micelles of copolymer 14a.

As a model system for investigating pH-dependent drug release, the release of micelle-incorporated Nile Red was studied as a function of time at pH 7.4 and pH 5.0 at 37 ºC. Nile Red, shown in Fig. 10c, is a hydrophobic dye that is known to have very low fluorescence in aqueous solutions, but substantially increased fluorescence in hydrophobic environments such as membranes or micelles [81,82]. As shown in Fig. 11b, the fluorescence of Nile Red decreased at pH 5.0 over a timescale similar to that of the acetal hydrolysis, while the fluorescence of the sample at pH 7.4 remained essentially constant over this time period. These observations are consistent with release of Nile Red from the micelle, and the time dependence of these changes strongly suggests that they are indeed due to hydrolysis of the acetals, and not only to a titration effect such as protonation of the residual carboxylic acids of the poly(aspartic acid) block.

Current efforts are directed toward investigation of different copolymer backbones and improving the coupling between the acetal and the copolymer, thus avoiding the presence of residual "titratable" groups along the copolymer backbone after introduction of the acetal. This should lower the CMC and decrease the size of the micelles by removing ionic repulsions at the core of the micelle. In addition, this should provide further insight into the mechanism of micelle disruption. It was found recently that dendrimers having peripheral hydroxyls or amines provide good backbones for the attachment of the hydrophobic acetal groups and that PEO-dendrimer hybrids having the acetals on the dendrimer periphery form pH-sensitive micelles in water (Fig. 12). In addition, in these systems it has proven possible to tune the hydrolysis rate of the acetal by adjusting the structure of the dendrimer and the linkage to the acetal. These systems are also easily tuned by changing the length of the linear PEO or the dendrimer generation (number of branching points) to adjust the number of acetal groups, thus controlling the hydrophilic/hydrophobic balance as well as the copolymer architecture. Therefore, the concept of introducing hydrophobic groups using an acid-sensitive linkage so far seems to be a potentially fruitful and highly versatile approach.

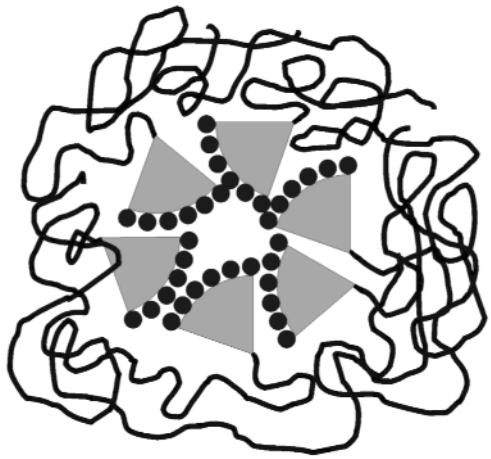

Fig. 12 Micelle formed from PEO-dendrimer hybrids having hydrophobic acetals on the periphery.

CONCLUDING REMARKS

The design and preparation of pH-sensitive micelles is a new and exciting field of research that seeks to exploit the attractive properties of polymeric micelles to improve the selective delivery of therapeutic molecules using physiological triggers. One of the major challenges has been the relatively narrow pH range in which the micellar carrier must both retain the drug over prolonged periods and then release it relatively rapidly. This challenge has been met by many different approaches including incorporation of titratable groups into the copolymer backbone such that the solubility of the polymer is altered by protonation or deprotonation events, and by incorporation of pH-sensitive linkages that are designed to undergo hydrolysis under distinct physiological conditions to either directly release drug, or alter the polymer structure to disrupt or break apart the micelles. In vitro studies undertaken with these systems have provided positive results, thus encouraging the further development of this field. It is likely that in the next decade the current approaches will be further developed and new approaches will be introduced, making pH-sensitive micelles an important part of the drug delivery field.

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