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Double hydrophilic block copolymers self-assemblies in biomedical applications

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Abstract

Double-hydrophilic block copolymers (DHBCs), consisting of at least two different water-soluble blocks, are an alternative to the classical amphiphilic block copolymers and have gained increasing attention in the field of biomedical applications. Although the chemical nature of the two blocks can be diverse, most classical DHBCs consist of a bioeliminable non-ionic block to promote solubilization in water, like poly(ethylene glycol), and a second block that is more generally a pH-responsive block capable of interacting with another ionic polymer or substrate. This second block is generally non-degradable and the presence of side chain functional groups raises the question of its fate and toxicity, which is a limitation in the frame of biomedical applications. In this review, following a first part dedicated to recent examples of non-degradable DHBCs, we focus on the DHBCs that combine a biocompatible and bioeliminable non-ionic block with a degradable functional block including polysaccharides, polypeptides, polyesters and other miscellaneous polymers. Their use to design efficient drug delivery systems for various biomedical applications through stimuli-dependent self-assembly is discussed.

Keywords: Double-hydrophilic block copolymers; pH-responsive polymers; drug delivery systems; polyion complex micelles; degradable copolymers.

Abbreviations: AA, alginic acid; AEM; 2-aminoethyl methacrylate; Alb, albumin; APEG, acryloyl end-capped PEG; API, active pharmaceutical ingredient; ATRA, all-trans retinoic acid; ATRP, atom transfer radical polymerization; BIC, block ionomer complex; γ CAB ϵ CL, γ -(carbamic acid benzylester)- ϵ -caprolactone; CAC, critical association concentration; CL, caprolactone; ClCL, chloro ϵ -caprolactone; CLSM, confocal laser scanning microscope; CMC, critical micelle concentration; CMD, carboxymethyldextran; COSamf, chitooligosaccharides with 5-anhydro-D-mannofuranose at their reducing end; CytC, cytochrome C; Dex, dextran; DG, diammonium glycyrrhizinate; DHBCs, double-hydrophilic block copolymers; DHCs, double hydrophilic copolymers; DIM, diminazene diacetate; DL, drug loading; DLS, dynamic light scattering; DOTA, 1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid; DOX, doxorubicin; DPcZn, dendrimer phthalocyanine zinc; DPP, diphenyl phosphate; DS, degree of substitution; DTT, dithiothreitol; EE, encapsulation efficiency; FA, folate ; FACS, flow cytometry analysis; Gd and Gd³⁺, gadolinium and gadolinium ion; GlcN, (1 \rightarrow 4)-linked units of 2-amino-2-deoxy- β -D-glucopyranose; HA, hyaluronic acid; LA, D,L-lactic acid; Lac-PEG, lactose-conjugated PEG; MA, methacrylic acid; MAL, L-malic acid; MH, minocycline hydrochloride; mPEG, methoxy-poly(ethylene glycol); MRI, magnetic resonance imaging; MSA, mercaptosuccinic acid; MSC, mesenchymal stem cell; MTT, methotrexate; MTX, mitoxantrone; NAEP, 2-(N-acryloyloxy) ethylpyrrolidone; NMP, N-(2-methacryloyloxyethyl) pyrrolidone; NPs, nanoparticles; NVPI, N-vinylphthalimide; PAA, poly(acrylic acid); PAE-API, poly(β -aminoester)-1-(3-aminopropyl)imidazole; PAEP, poly((2-(2-aminoethoxy)ethoxy) phosphazene); PAMPS, poly(2-acrylamido-2-methyl-1-propanesulfonic acid); PAMPSNa, poly(sodium 2-acrylamido-2-methylpropanesulfonate); PAPTAC, poly(3-acrylamidopropyltrimethylammonium chloride); PAsp, poly(L-aspartic acid); PAsp(DET), poly(L-aspartic acid) bearing a N-(2-aminoethyl)-2-aminoethyl group; PATMC, poly(5-allyloxytrimethylethylenecarbonate); PBYP COOH , poly(phosphotriester)s bearing pendent carboxylic acids; PCCL, poly(6-acetoxyl- ϵ -caprolactone); PCEtOx, poly(2-carboxyethyl-2-oxazoline); PCL, poly(ϵ -caprolactone); P(CL-co-DCL), poly(ϵ -caprolactone-co- γ -dimethyl maleamic acid- ϵ -caprolactone); PDEAEMA (poly(2-(diethylamino)ethyl methacrylate); PDMAEMA, poly(2-(dimethylamino)ethyl methacrylate); PDT, photodynamic therapy; PEC, polyelectrolyte complexes; PEG, poly(ethylene glycol); PEGMA, polyethylene glycol methacrylate; PEI, poly(ethyleneimine); PEO, poly(ethylene oxide); PEtOx, poly(2-ethyl-2-oxazoline); PGlu, poly(L-glutamic acid); PIC, polyion complex; PiPrOx, poly(2-isopropyl-2-

oxazoline); PLL, polylysine; PMA, poly(methacrylic acid); PMMA, poly(methyl methacrylate); P2MVP, poly(N-methyl-2-vinyl pyridinium iodide); PNiPAM, poly(N-isopropylacrylamide); PPDO, poly(phosphodiester)s with a negatively charged oxygen atom; PPIL, poly(4-N-piperilactone); PPLG, poly(γ -propargyl-L-glutamate); PSar, poly(sarcosine); PSMA, poly(styrene-alt-maleic anhydride); Pul, pullulan; PVA, poly(vinyl alcohol); PVAc, poly(vinyl acetate); PVAm, Poly(vinyl amine); PVCL, Poly(N-vinylcaprolactam); PVP, Poly(vinyl pyrrolidone); RAFT, reversible addition-fragmentation chain transfer; Rh, hydrodynamic radius; ROP, ring opening polymerization; ROS, reactive oxygen species; sCT, salmon calcitonin; siRNA, small interfering ribonucleic acid; SLS, static light scattering; TEM, transmission electron microscopy; TMC, trimethyl chitosan.

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1. Introduction

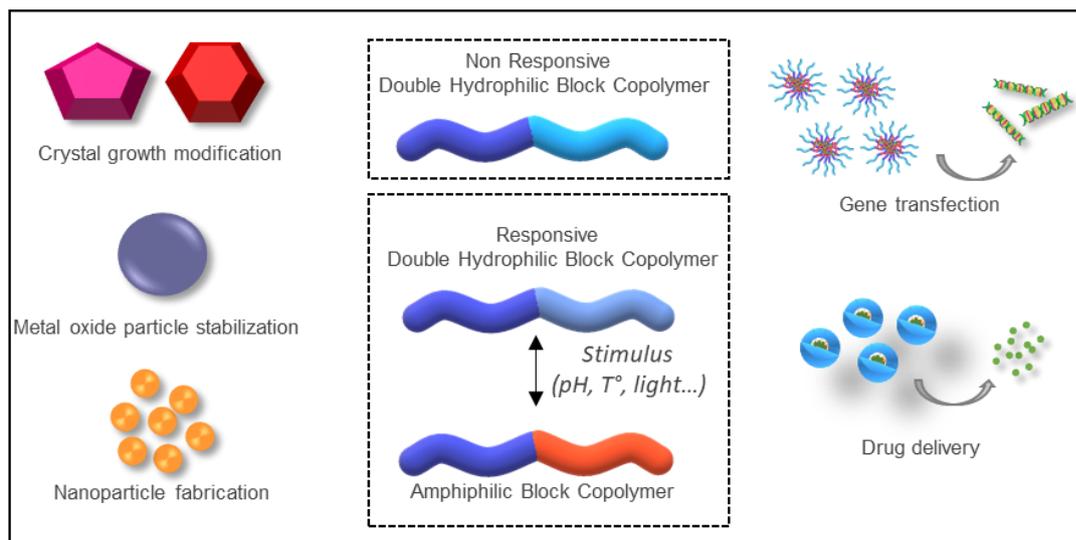
Block copolymers are a category of polymers in which the macromolecule consists of two or more distinct blocks of homopolymer. Block copolymers with two, three, and more blocks are called diblock, triblock, and multiblock copolymers respectively. Block copolymers can be further classified according to their architectural arrangement. For example, linear or star shaped block copolymers can be synthesized. The number of polymer types in block

copolymers may be equal to or lower than the number of blocks. For example, triblock copolymers may be constituted of three polymers (ABC triblock copolymers) or two polymers (ABA triblock copolymers).

The nature of the constituting blocks (hydrophilic or hydrophobic) gives rise to a further categorization within block copolymers. Amphiphilic block copolymers, which consist of at least one hydrophilic and one hydrophobic block, may self-assemble in water to form micelles or aggregates with a hydrophobic core surrounded by a hydrophilic shell. Micelles prepared from amphiphilic block copolymers have been studied primarily as controlled drug delivery systems as their hydrophobic core may serve as a depot for the solubilization of clinically relevant doses of hydrophobic drugs, as reviewed in several excellent publications [1–3].

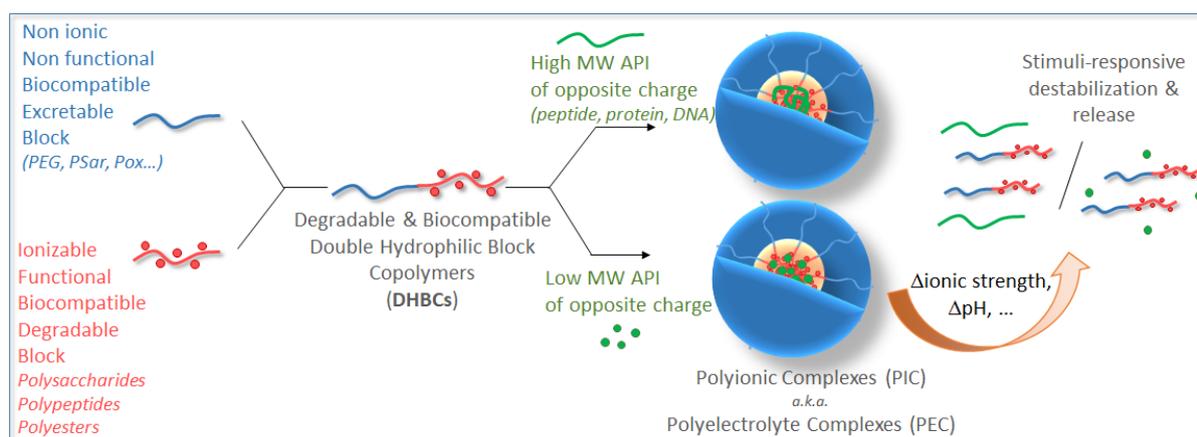
In the last two decades, double-hydrophilic block copolymers (DHBCs), consisting of two or more water-soluble blocks of different chemical nature, have gained increasing attention. In many DHBCs one block (often based on poly(ethylene glycol) (PEG) or poly(ethylene oxide) (PEO)) only promotes solubilization in water, whereas the other block is responsive to an external stimulus or capable of interacting with another polymer or substrate. In aqueous solution under normal conditions, the two hydrophilic blocks are well solvated and DHBCs behave like water-soluble polymers with no amphiphilic characteristics. However, a variation of temperature, ionic strength or pH, as well as a complexation reaction may change the hydrophilicity of one of the homopolymer blocks into hydrophobicity, thereby introducing amphiphilicity, which in turn may lead to the formation of functional structures such as micelles.

Although the first synthesis of a DHBC dates back to 1972 [4], only during the last two decades the potential of DHBCs has been fully recognized for various applications. These include for example crystal growth modification, metal oxide particle stabilization, nanoparticle fabrication, controlled drug delivery and gene transfection (Scheme 1).



Scheme 1. Different type of double hydrophilic block copolymers and their applications.

Despite the increasing interest for this unique class of polymers, recent reviews dedicated to DHBCs are scarce. A comprehensive review concerning DHBCs dates back as far as 2001 [5]. Since then, a limited number of reviews have been published relating to the self-assembly processes of DHBCs, including recent examples of polyion complex (PIC) [6] and block ionomer complex (BIC) micelles [7] (Scheme 2), as well as a recent review dedicated to the self-assembly of completely water-soluble DHBCs in aqueous medium without application of external stimuli but at high concentration [8].



Scheme 2. Formation of Polyionic Complexes (PIC) (also known as Polyelectrolyte Complexes (PEC)) based on degradable double hydrophilic block copolymers.

The present review differs from these previous contributions as we aim at reporting and summarizing recent studies dedicated to the synthesis and the study of the behaviour of DHBCs in solution for biomedical applications. After discussing some recent examples of non-degradable DHBCs, and because biodegradability is in our view of first importance for biomedical polymers, we will subsequently categorize the various degradable DHBCs according to the chemical nature of the responsive or interacting polymer block of the DHBC. We will successively discuss DHBCs which contain polysaccharides, polypeptides, polyesters and other miscellaneous degradable blocks as functional blocks (Table 1). All these polymers are considered biodegradable and bioresorbable as they degrade to non-toxic products in vivo which can be excreted by the kidneys. Despite the existence of other alternatives (poly(2-oxazoline), poly(vinyl pyrrolidone) etc...), they are mostly combined with PEG as a solubilizing block due to its long track record in pharmaceutical formulation, its excretability via the renal pathway up to a molecular weight (MW) of approximately 30 kg/mol [9], and its medium to long-term biodegradability [10,11]. Finally, in a last part we will discuss the main biomedical applications taking advantage of these DHBCs and we will emphasize the remaining challenges and future perspectives associated with this class of copolymers.

2. Non-degradable DHBCs

Many of the non-degradable DHBCs reported in literature are based on a PEG block associated with a poly(acrylic acid) (PAA) or a poly(methacrylic acid) (PMA) block for the anionic ones, or poly(dimethylaminoethyl methacrylate) (PDMAEMA), and poly(ethylenimine) (PEI) for the cationic ones (Table 1). This type of copolymer offers the advantages of a straightforward synthesis as well as pH-responsiveness. Apart from PEG, also poly(vinyl pyrrolidone) (PVP), poly(vinyl caprolactam) (PVCL), poly(vinyl alcohol) (PVOH), poly(vinyl amine) (PVAm) and poly(oxazoline) (POx) have been used as water-soluble block in combination with different

responsive blocks. In the following paragraph examples of such non-degradable DHBCS proposed in various recent biomedical applications are discussed. A summary of the type of self-assemblies obtained with these DHBCs and their characteristics is provided in Table 2.

Shin *et al.* reported on giant polymer vesicles (diameter 5-8 μm) which formed via self-assembly of double-hydrophilic PEG-PAA diblock copolymers at pH 2. This was attributed to protonation of the carboxylic groups on the PAA block (pKa of 4.5), thereby providing hydrogen bonding with itself and the PEG block resulting in aggregation of the polymer chains, thus providing a mechanism for phase segregation of the complex of otherwise soluble polymers. In contrast, no vesicle formation was observed at pH 9. To demonstrate that the polymer vesicles enable the triggered delivery of cargo molecules, Alexa Fluor 488 was incorporated by rehydrating the polymer film with a solution of the hydrophilic fluorophore at pH 2. Upon increase of the pH above 4.5, the encapsulated fluorophore was rapidly and completely released within 30 min due to dissociation of the vesicles [12].

Frangville *et al.* reported on nanoparticles of PEG-PAA crosslinked by gadolinium (Gd^{3+}) ions and their use as magnetic resonance imaging (MRI) contrast agents. The formation of the hybrid inorganic/polymeric nanoparticles (20 nm in diameter) was ascribed to association between Gd^{3+} ions and negatively charged acrylic acid monomer units in the core, which is surrounded by a PEG corona. The integrity of the NPs was maintained even after significant dilution and over a large range of pH and ionic strength values. This was explained by the entanglement of PAA blocks, resulting in additional crosslinks that stabilize the nanoparticle. In vivo experiments showed that the Gd^{3+} / PEG-PAA nanoparticles provide both a higher intensity and a more persistent enhancement of vascular MRI signals, at one-third of the total Gd concentration of the commercially available contrast agent GdDOTA [13].

Raisin *et al.* reported the design of tripartite PIC micelles as original non-viral polymeric vectors suited for mesenchymal stem cell transfection with small interfering ribonucleic acid

(siRNA) using the non-degradable DHBC PEO-*b*-PMA. The formation of PIC micelles is based on the association of the DHBC with a cationic homopolymer (polylysine (PLL) or poly(ethyleneimine) (PEI)), and the siRNA can be associated with the core of micelles by complexation with the cationic polymer. The micelle formulations were designed to exhibit pH-triggered disassembly in an acidic pH range found in endosomes. The tripartite micelles were non-cytotoxic and exhibited an ability to deliver a siRNA targeting Runx2 and to induce efficient gene silencing in murine MSC [14].

In another example, Ramasamy *et al.* investigated the interaction of the cationic drugs doxorubicin (DOX) and mitoxantrone (MTX) with the anionic DHBC PEO-*b*-PAA. The primary amino group of DOX and two secondary amino groups of MTX were responsible for electrostatic interactions with the ionized carboxyl group (pKa ~ 5) of PEO-*b*-PAA. The drug loading (DL) was 45 wt% for MTX and 70 wt% for DOX. A faster release of the two drugs was observed at pH 5 (typical of the environment of cancer cells) compared to the release at physiological pH (7.4). DOX PIC micelles showed a higher cellular uptake than MTX PIC micelles. Both complexes induced apoptosis of cancer cells and suppressed tumour growth to the same level as the respective free drugs, but they exhibited a prolonged blood circulation [15].

Poly(vinyl pyrrolidone) (PVP) is another example of non-ionic water-soluble block that has been used to prepare non-degradable DHBCs. PVP is largely used in pharmaceutical formulations due to its cytocompatibility and strong binding capacity via dipole interactions with drugs [16]. The next paragraph gathers the few recent examples of PVP analogues used to prepare DHBCs with a ionisable block. Gao *et al.* reported on a series of PVP-based DHBCs prepared by RAFT polymerization for drug delivery applications through the formation of PIC micelles with different drugs including coenzyme A and folic acid. This series of DHBCs embedded PVP-*b*-poly(styrene-*alt*-maleic anhydride) (PVP-*b*-PSMA), PVP-*b*-poly(2-

(dimethylamino)ethyl methacrylate) (PVP-*b*-PDMAEMA) and PVP-*b*-poly(2-acrylamido-2-methyl-1-propanesulfonic acid) (PVP-*b*-PAMPS) [17–19]. Another example consists of PVP-*b*-PMA also obtained by RAFT polymerization and used to control calcium carbonate (CaCO₃) morphologies with target applications in biomimetic mineralization [20]. More recently, Destarac et al. reported on another series of PVP-based DHBCs obtained by redox initiated aqueous RAFT/MADIX polymerization. This approach resulted in the synthesis of PVP-*b*-PAA, PVP-*b*-poly(sodium 2-acrylamido-2-methylpropanesulfonate) (PVP-*b*-PAMPSNa), and PVP-*b*-poly(3-acrylamidopropyltrimethylammonium chloride) (PVP-*b*-PAPTAC) foreseen for use in drug delivery and biological inorganic phase templating [21]. Another recent example of potential interest for the design of drug vectors includes a vinyl pyrrolidone analogue, namely 2-(N-acryloyloxy)ethylpyrrolidone (NAEP). NAEP was copolymerized by reversible addition-fragmentation chain transfer (RAFT) polymerization with 2-(diethylamino)ethyl methacrylate (DEAEMA) to yield PNAEP-*b*-PDEAEMA DHBCs that can lead to the formation of pH-responsive micelles [22]. In the same family of analogue, N-(2-methacryloyloxyethyl) pyrrolidone (NMP) was copolymerized by RAFT polymerization with MA to prepare a family of well-controlled DHBCs able to stabilize and control the size of gold nanoparticles, that are largely used in biomedical applications [23], in a pH-dependent manner [24].

Poly(N-vinylcaprolactam) (PVCL) is also used for biomedical applications as a non-ionic water-soluble polymer. It is for example present in the BASF excipient Soluplus® that is proposed to formulate insoluble drugs. As such recent examples of PVCL-based DHBCs have been reported. Liang *et al.* prepared well-defined DHBCs of PVCL and PVP via RAFT polymerization [25]. PVCL is a thermoresponsive polymer with superior biocompatibility in comparison with PNiPAM [26]. The LCST could be lowered by increasing the PVCL segment length and raised by increasing the hydrophilic PVP segment length. Below the LCST the copolymers dissolved completely in aqueous solution, whereas they formed spherical micellar

or vesicular morphologies above the LCST. The size of the self-assembled structures could be controlled via the molar ratio of the PVCL and PVP segments. The PVCL-*b*-PVP copolymers showed non-toxic towards cancer cells. The group of Jerome reported on the cobalt-mediated radical polymerization of VCL initiated from a poly(vinyl acetate) (PVAc) based macroinitiator to yield PVAc-*b*-PVCL block copolymers [27]. These amphiphilic copolymers were subsequently hydrolysed to give poly(vinyl alcohol)-*b*-PVCL (PVA-*b*-PVCL) DHBCs. These polymers became amphiphilic when heated above their LCST in aqueous solution (36-42 °C, depending on their composition) due to dehydration of the PVCL sequence, leading to self-assembled spherical aggregates with a collapsed PVCL core and a soluble PVOH shell. In a follow-up paper the authors used the PVA-*b*-PVCL DHBCs to prepare nanogels by crosslinking the PVA corona above the LCST with a redox-responsive crosslinking agent [28]. The nanogels loaded with the hydrophobic model drug Nile red (NR) had sizes ranging from 50 to 100 nm and were able to release NR in the presence of the reducing agent dithiothreitol (DTT) thanks to cleavage of the disulphide bonds in the crosslinker (Figure 1a and 1b). In addition, the nanogels proved to be cytocompatible towards L929 fibroblasts and to be efficiently uptaken by MEL-5 cancer cells (Figure 1c), which show the potential of the PVA-*b*-PVCL nanogel system for intracellular drug release.

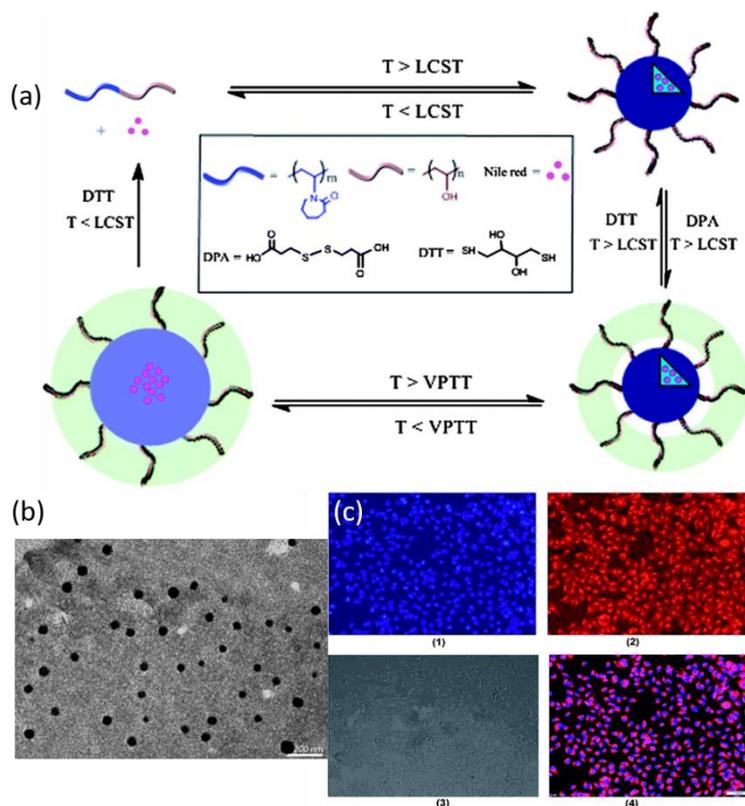


Figure 1. (a) Schematic illustration of the preparation, dissociation of PVA-*b*-PVCL crosslinked nanogels, loading of Nile red (NR) and redox-triggered release behaviors (LCST: lower critical solution temperature, VPTT: volume phase transition temperature) ; (b) TEM images of the crosslinked nanogels (scale bar 200 nm) ; (c) Fluorescence microscopy images of the treated MEL-5 cells after 24 h incubation with the NR-loaded nanogel : (1) nuclei stained with DAPI (blue), (2) fluorescence pattern of NR (red), (3) contrast field pattern, (4) merged images of (1), (2) and (3) (scale bar: 100 nm) (adapted with permission from [28]).

This last example illustrates the use of PVCL in DHBCs, but also of PVA that is another example of non-ionic water-soluble polymer. Cobalt-mediated radical polymerization was also used for the preparation of PVA-*b*-PVP DHBCs [29] as well as pH-responsive PVA-*b*-PAA DHBCs [30]. The latter self-assembled into aggregates below pH 3 due to the protonation of the PAA carboxylic acid groups, which decreases PAA solubility and enables hydrogen bonding between PAA and PVA blocks. Moreover, PVA-*b*-PAA formed PIC micelles with poly(N-methyl-2-vinyl pyridinium iodide)-*b*-poly(ethylene oxide) (P2MVP-*b*-PEO) DHBCs at pH 8 through electrostatic interactions between the core-forming P2MVP and PAA blocks [31]. In the same family of vinyl polymers, Maki *et al.* prepared DHBCs having poly(vinyl amine)

(PVAm) blocks via sequential RAFT polymerization of N-vinylphthalimide (NVPI) and NiPAM, followed by deprotection of the PNVPI block [32]. Aqueous solutions of amphiphilic PNVPI-*b*-PNiPAM block copolymers and PVAm-*b*-PNiPAM DHBCs exhibited a LCST type phase transition between 25-32 °C and 53-75 °C, respectively, in accordance with the higher hydrophilicity of the PVAm-*b*-PNiPAM DHBCs. Although micelle formation was hypothesized for the block copolymers above the LCST, no characterization of these micelles was provided.

Poly(2-oxazoline)s (POx) are an important class of polymers that focuses a strong interest as they have been proposed as alternative to PEG in biomedical applications thanks to their tunable physicochemical properties, good water solubility and excellent biocompatibility [33]. As debates regarding their degradability are still ongoing [34], we chose in this review to present most POx-based DHBCs in this part dedicated to non-degradable DHBCs. Examples of POx DHBCs containing a second block of well-recognized degradability can be found in the corresponding subparts. Zschoche et al. prepared a series of di- and triblock copolymers with temperature-sensitive poly(2-isopropyl-2-oxazoline) (PiPrOx) blocks and pH-sensitive poly(2-carboxyethyl-2-oxazoline) (PCEtOx) blocks [35]. The temperature- and pH-induced reversible phase transition converted these DHBCs into amphiphilic polymers allowing for self-assembly in aqueous solution. The temperature and pH values of the transitions as well as the nature of the formed aggregates (micelles or vesicles) could be controlled via the size and arrangement of the individual blocks. Other examples of POx-containing DHBCs include PEG-*b*-PEtOx star block copolymers [36], PEG-*b*-poly(2-methyl-2-oxazoline) diblock copolymers [37] and PEtOx-*b*-PVP diblock copolymers [38]. In these cases, no application was foreseen and studies aimed at studying the self-aggregation of these DHBCs that was observed without the

application of an external stimulus, suggesting that even in DHBCs subtle differences in hydrophilicity among the constituting blocks are sufficient to drive polymer aggregation.

As shown in this part, polyethers (PEG, PEO), vinyl polymers (PVP, PVCL, PVA...) and poly(2-oxazoline)s (PEtOx) are the non-ionic blocks classically used in non-degradable DHBCs. More exotic blocks like polydehydroalanine to yield PEG- or poly(acrylic acid)-*b*-polydehydroalanine DHBCs [39,40], synthetic glycopolymers in PEG-*b*-poly(mannose) [41], or poly((4-diethylamino)-(E)-stilbene)-*alt*-maleic acid)-*b*-poly(acryloyl morpholine) have been reported [42]. However, they remain largely unexploited in comparison to these classical families. In addition, and although the DHBCs discussed in this part represent promising systems that can potentially address several challenges in the biomedical domain, their non-degradability remains an issue as the polymers may accumulate in the body or, if elimination is possible, in the environment [43]. Therefore, in the remainder of this review we focus on degradable DHBCs containing polysaccharides, polypeptides or polyesters as functional blocks associated with biocompatible non-ionic and bioeliminable blocks, mainly PEG and PEtOx, whereas other non-ionic blocks raising concerns of potential toxicity, like PNIPAM, are not considered.

Table 2: Characteristics of the self-assemblies obtained with non-degradable double hydrophilic block copolymers.

DHBC	Self-assemblies			Stability ^a	Ref.
	Type	Preparation	Characteristics ^a		
PEO- <i>b</i> -PAA	Giant vesicles	gel-assisted rehydration method at pH 2.3	$D_h \sim 5-8.0 \mu\text{m}$ / ξ potential ~ -11 mV (at pH 2.4)	ass. < pH 4.5 < disass.	[12]
PEO- <i>b</i> -PAA	Gd ³⁺ -loaded PIC micelles	addition of Gd ³⁺ solution to PEO- <i>b</i> -PAA 0.1 wt% solution (R = 1)	$D_h \sim 33$ nm / ξ potential ~ 0 mV	disass. < pH 4. stable for DHBC concentrations of 0.1-10 ⁻⁴ wt% stable at 1M NaCl.	[13]
PEO- <i>b</i> -PMA	SiRNA-loaded PIC micelles	(PLL/SiRNA) solution added to PEO- <i>b</i> -PMA solution (R = 1)	$D_h \sim 37-53$ nm / ξ potential ~ 3.5 to 7.9 mV (in water) and $D_h \sim 101$ nm (in PBS) / EE $\sim 90\%$	disass. < pH 6.	[14]
PEO- <i>b</i> -PAA	DOX- or MTX-loaded PIC micelles	mixing of drug and polymer aqueous solutions for 24h (D/P = 0.25 or 0.5)	$D_h \sim 100$ nm / ξ potential ~ -5 to -35 mV (at pH 7) / EE $\sim 90\%$ / DL $\sim 45\%$ (MTX) $\sim 70\%$	drug release at pH 5.	[15]
PVP- <i>b</i> -PSMA & PVP- <i>b</i> -PDMAEMA	Co A-loaded PIC micelles	mixing of PVP- <i>b</i> -PSMA and PVP- <i>b</i> -PDMAEMA / Co A solutions, pH neutralization, dialysis	$D_h \sim 100-130$ nm / EE $\sim 60\%$ / DL $\sim 10-29\%$	drug release at pH 2 < pH 9 < pH 7.4.	[17]
PVP- <i>b</i> -PAMPS & PVP- <i>b</i> -PDMAEMA	FA-loaded PIC micelles	mixing of PVP- <i>b</i> -PAMPS/FA and PVP- <i>b</i> -PDMAEMA solutions, pH neutralization, dialysis	$D_h \sim 170$ nm / EE $\sim 85\%$ / DL $\sim 21\%$	drug release at pH 9 \geq pH 7.4.	[19]
PVP- <i>b</i> -PMA	templating of CaCO ₃	injection of Na ₂ CO ₃ in PVP- <i>b</i> -PMA solution, pH adjustment at 10, addition of CaCl ₂	rhombohedral or multilayered CaCO ₃ calcite microcrystals	not applicable.	[20]

PNAEP- <i>b</i> -PDMAEMA	pH-responsive micelles	direct dissolution at acidic pH followed by self-assembly upon pH increase	$D_h \sim 50-100$ nm	disass. \leq pH 3 ; ass. \geq pH 10. [22]
PNMP- <i>b</i> -PMA	micelles for Au NP templating	direct dissolution in water	$D_h \sim 100$ nm (pH 4) $D_h \sim 200$ nm (pH 2)	ass. $<$ pH 5.3. micellization temperatures $\sim 50-70^\circ\text{C}$. [24]
PVCL- <i>b</i> -PVP	thermo-responsive micelles	direct dissolution in water	$D_h \sim 100-260$ nm	ass. $T > 42-46^\circ\text{C}$. [25]
PVA- <i>b</i> -PVCL	thermo-responsive and reducible nanogels	direct dissolution in water and crosslinking with DPA	$D_h \sim 280-460$ nm (DLS) $D_h \sim 45-110$ nm (TEM)	ass. $T > 36-42^\circ\text{C}$. nanogels stable upon dilution disass. with 10 mM DTT after 24h. [28]
PVA- <i>b</i> -PAA	pH-responsive micelles and PIC micelles	direct dissolution in water (micelles), mixing with P2MVP- <i>b</i> -PEO aqueous solutions (PIC micelles)	$D_h \sim 200$ nm (micelles) $D_h \sim 30-40$ nm (PIC micelles)	ass. pH $<$ 3 (micelles). [30,31]
PCEtOx- <i>b</i> -PiPrOx	pH- & thermo-responsive micelles & vesicles	direct dissolution in water (0.5 g/L)	$D_h \sim 50-230$ nm	ass. $3.5 \leq \text{pH} \leq 5.2$ at 60°C . ass. $T \geq 60^\circ\text{C}$. [35]
PAA- <i>b</i> -PDHA	loose nanoaggregates	direct dissolution in water	$D_h \sim 220$ nm / ξ potential ~ -5 mV (pH $<$ 4)	ass. pH $<$ 4. [39]

Abbreviations : ass. assembly ; Co A coenzyme A ; disass. disassembly ; DOX doxorubicin ; DPA 3,3'-Dithiodipropionic acid ; DTT dithiothreitol ; FA folic acid ; MTX mitoxantrone ; PAA poly(acrylic acid) ; PAMPS poly(2-acrylamido-2-methyl-1-propanesulfonic acid) ; PAPTAC poly(3- acrylamidopropyltrimethylammonium chloride) ; PDHA poly(dehydroalanine) ; PDEAEMA (poly(2-(diethylamino)ethyl methacrylate) ; PDMAEMA poly(2-(dimethylamino)ethyl methacrylate) ; PEO poly(ethylene oxide) ; (PEO)₈ 8 arms star-shaped poly(ethylene oxide) ; PMA poly(methacrylic acid) ; P2MVP poly(N-methyl-2-vinyl pyridinium iodide) ; PNAEP poly(2-(N-acryloyloxy)ethylpyrrolidone) ; PNMP poly(N-(2-methacryloyloxethyl) pyrrolidone) ; PSMA poly(styrene-alt-maleic anhydride) ; PVP poly(vinyl pyrrolidone) ; R charge ratio between positive and negative charges.

^a ranges of provided values correspond to characteristics obtained as a function of the blocks' length of the used DHBCs and/or ratios of compounds. If not specified D_h corresponds to the hydrodynamic diameter measured by DLS.

3. Polysaccharide-based degradable DHBCs

Polysaccharides are a broad class of naturally derived polymers (i.e. obtained from plants, animals or algae) that consist of monosaccharide units bound together by glycosidic linkages [44]. Polysaccharides display a linear or branched architecture and contain various functional groups such as carboxylic acid, amino and hydroxyl groups. These moieties are responsible for the hydrophilicity of many polysaccharides and offer numerous opportunities for chemical derivatization [45]. The molecular weight of polysaccharides may vary significantly (between hundreds and millions of Daltons), which further adds to the diversity of this polymer class [46]. In addition, owing to their native presence within the body, most polysaccharides have a very low toxicity and demonstrate good biocompatibility [47–50], while being enzymatically degradable down to their monomer or oligomer building blocks [51]. This unique combination of features explains their use as micellar systems for drug delivery which has been extensively reviewed [52,53]. Noteworthy, due to the chemical structure of polysaccharides most of their double hydrophilic copolymers (DHCs) have graft topologies rather than block topologies. Recent examples of such DHCs include chitosan-*g*-PEG [54–56], dextran grafted with poly((polyethylene glycol) methacrylate-co-aminoethyl methacrylate) [57] and alginic acid grafted with mPEG [58] that were all used to formulate drug loaded micelles. However, such graft copolymers, despite being double hydrophilic structures that offer interesting self-assembly behaviors are beyond the scope of this review that focuses on DHBCs and will not be further discussed. Examples of polysaccharide-based DHBCs where polysaccharides are used as functional and stimuli-responsive blocks (Table 1). A summary of the type of self-assemblies obtained with these copolymers and their characteristics is provided in Table 3.

Chitosan is a polysaccharide that has been widely used in DHCs but more scarcely in DHBCs. Although chitosan is insoluble in water at physiological pH and other common solvents because

of its strong intra-molecular hydrogen bonding, its copolymerization with PEG or other hydrophilic polymers is known to disrupt the intra-molecular hydrogen bonding of chitosan, thus allowing its water solubilization. For this reason, and despite the fact that chitosan cannot be considered as a well solvated block on its own, the following structures can be considered as double hydrophilic structures. Ganji *et al.* reported on the synthesis of a chitosan-PEG DHBC (Figure 2 1) to be used as a thermosensitive gel. In a first step, the glycosidic bonds of the chitosan main chain were degraded with potassium persulfate to produce oligo-chitosan with a terminal carbonyl group at one scission end and a free radical at the other scission end. Then, the chain end with the free radical was reacted with acryloyl end-capped PEG (APEG, $M_w = 2.10^3 \text{ g}\cdot\text{mol}^{-1}$) giving rise to a diblock-like copolymer structure. The molar ratio of PEG/chitosan within the copolymer could be varied between 0.06 and 0.1 depending on the potassium persulfate concentration used to degrade chitosan. However, the MW of the chitosan segment was not really controlled, and it was also not clearly stated whether one or several acryloyl PEG chains can grow from the chitosan-end radical which may lead to chitosan-*b*-PEG or chitosan-*b*-P(APEG). A gelation time of ca. 10 min was observed at body temperature for concentrations as low as 2 w/v %, which confirmed that these chitosan-PEG DHBC could be used for biomedical application [59].

To yield similar DHBCs, Moussa *et al.* used fully N-deacetylated chitoooligosaccharides with a 5-anhydro-D-mannofuranose at their reducing end (COSamf) to prepare various COS-based building blocks. COSamf with an average number of 22 repeating units of (1→4)-linked units of 2-amino-2-deoxy-β-D-glucopyranose (GlcN) were functionalized via reductive amination. In particular 4-(propargyloxy)aniline and adipic dihydrazide were selected to prepare alkyne or hydrazide functional COSamf and prepare 2 different COS-*b*-PEG diblock copolymers based on chain ends reactions. The first method used copper(I)-catalyzed azide alkyne cycloaddition (CuAAC) reaction between the alkyne-terminated COS block and a commercial mPEG-azide

($M_n=2000\text{g/mol}$) to yield COS-*b*-PEG with $M_n=6520\text{ g/mol}$ and $\bar{D}=1.26$ (Figure 2 2). To get rid of the copper catalyst that may contaminate this first DHBC, the second strategy was based on the hydrazide condensation reaction between the hydrazide terminated COS block and a commercial mPEG-NHS ester ($M_n=2000\text{g/mol}$) to yield COS-*b*-PEG with $M_n=6330\text{ g/mol}$ and $\bar{D}=1.15$ (Figure 2 3). These COS-*b*-PEG DHBCs are foreseen to serve as cationic nanocarriers for the delivery of drugs [60].

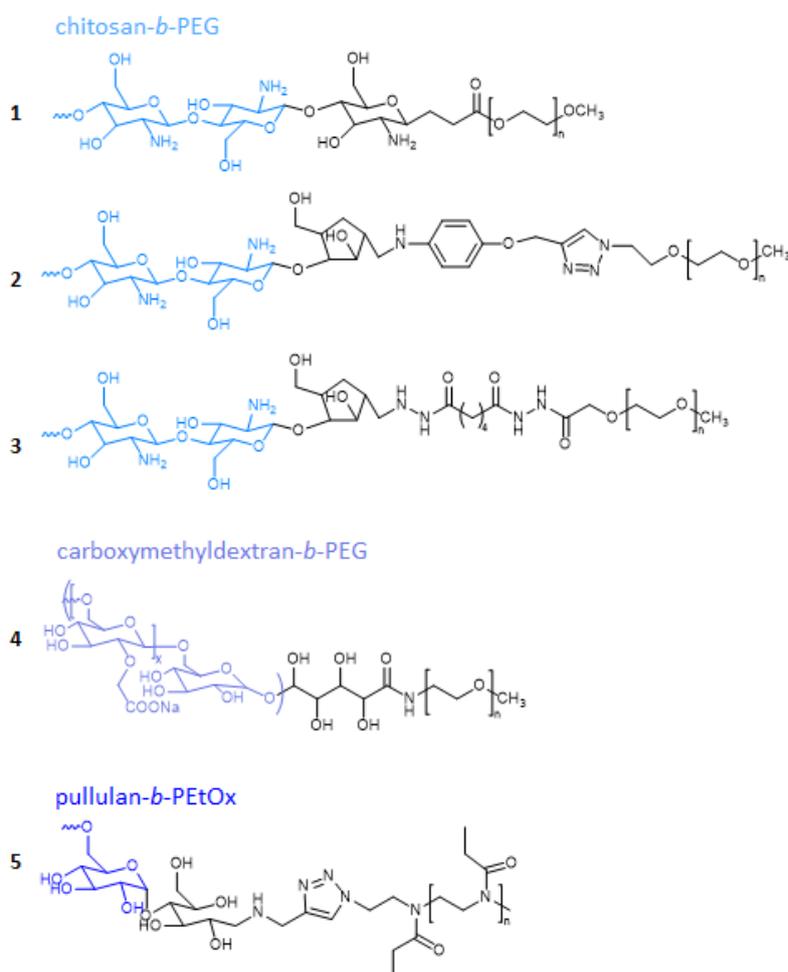


Figure 2. Examples of degradable hydrophilic block copolymers based on polysaccharides.

Dextran is another example of a polysaccharide found in DHBCs. Winnik *et al.* synthesized various block copolymers of dextran ($M_w = 8.3\text{k}$ or 14.7k) and PEG-NH₂ ($M_w = 3\text{k}$ or 7k) via the specific oxidation of the dextran terminal aldehyde group and the covalent linkage of PEG-NH₂ via a lactone aminolysis reaction. Conversion of the neutral diblock copolymers into

polyanions was achieved by carboxymethylation of the dextran block via side chain modification of dextran with chloroacetic acid, leading to carboxymethyl-dextran-PEG (CMD-*b*-PEG) block copolymers (Figure 2 4). The properties of CMD-*b*-PEG in aqueous solutions were analyzed by static and dynamic light scattering (DLS) showing a pH sensitive assembly [61]. In follow up studies, the authors evaluated the ability of (CMD)-*b*-PEG to encapsulate different drugs. The micellization was evaluated as a function of i) the ionic charge density or degree of substitution (DS) of the dextran block with carboxymethyl moieties and ii) the molar ratio of positive charges provided by the drug to negative charges provided by CMD-*b*-PEG. PIC micelles were formed with the cationic and water soluble diminazene diacetate (DIM), an API used as antiparasitic agent, and CMD-*b*-PEG with various DS. Micelles with a charge ratio positive/negative = 2 had a DL ranging from 40 to 65 wt% with a hydrodynamic radius (*R_h*) ranging from 36 to 50 nm, depending on the MW and the DS of the CMD-*b*-PEG. The critical association concentration (CAC) was in the order of 15–50 mg/L for DIM/CMD-*b*-PEG with DS > 60 %, and 100 mg/L for DIM/CMD-*b*-PEG with DS~30 %. Finally, PIC micelles with high DS and charge ratio = 2 allowed for a prolonged release of DIM in vitro compared with a solution of free drug [62]. In another application by the same group, the CMD-*b*-PEG was used to form PIC micelles with minocycline hydrochloride (MH), a semisynthetic tetracycline antibiotic with promising neuroprotective properties for the treatment of neuroinflammatory diseases. PIC micelles with *R_h* of 100 nm and 50 wt% loading of MH were obtained. The MH loaded PIC micelles showed a sustained release of drug from the micelle at physiological pH, thereby allowing to decrease inflammation in the murine microglia (N9) [63]. Lastly, CMD-*b*-PEG copolymers hydrophobized by n-dodecyl groups were used to encapsulate two aminoglycosides: paromomycin and neomycin at DL up to 50 wt%. PIC micelles were stable under physiological conditions (pH 7.4, 150 mM NaCl) in contrast with micelles formed by the unmodified CMD-*b*-PEG and exhibited reduced sizes (around 50 nm) compared to the

non-hydrophobized CMD-*b*-PEG (sizes in the range 75 to 100 nm). The minimal inhibitory concentration of the aminoglycosides encapsulated in PIC micelles was not altered as indicated by their ability to kill *E.Coli* in culture [64].

Brosnan *et al.* synthesized dextran and pullulan based DHBCs, namely dextran-*b*-poly(ethylene oxide) (Dex-*b*-PEO), pullulan-*b*-PEO (Pul-*b*-PEO), and dextran-*b*-poly(sarcosine) (Dex-*b*-PSar). The synthesis route involved reaction of one polysaccharide terminus, existing as an aldehyde group in the equilibrium state, with a hydroxy amine end group of mono-functionalized PEO or PSar, yielding a hydrolytically stable oxime bond between both polymer blocks. Each block had a MW of ca. 20 k yielding DHBCs with MW of ca. 40 k. A direct dissolution at low concentrations (0.1, 0.5, and 1.0 wt%) led after 7 days to the formation of self-assembled polymer vesicles with *Rh* in the range of 250 nm to 700 nm for the lowest and the highest concentrations, respectively. At higher concentrations, above 10 wt%, self-assembly yielded giant polymer vesicles, referred as “aquanelles” with sizes between 2 and 20 μm (Figure 3). These aquanelles’ solutions were stable over 7 days and due to their water permeability, the authors envisioned that they could be well suited for use as artificial cells [65].

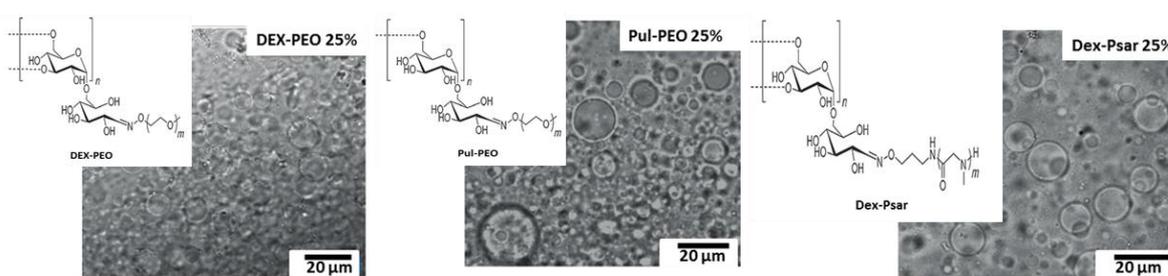


Figure 3. Optical microscopy images of the giant double hydrophilic polymer vesicles referred to as “aquanelles” prepared from Dex-*b*-PEO, Pul-*b*-PEO and Dex-*b*-PSar at 25 wt% (adapted with permission from [65]).

The self-assembly of pullulan-*b*-poly(2-ethyl-2-oxazoline) (Pull-*b*-PEtOx) was studied by Willersin *et al.* The DHBC (Figure 2 5) was synthesized from a pullulan-alkyne (8–38 kg mol⁻¹) and a biocompatible azido-PEtOx (22 kg mol⁻¹) by a CuAAC conjugation. The MW of the Pull block was varied to study its impact on the self-assembly behavior. Sizes between 300 and 500 nm were measured by DLS and static light scattering (SLS) in dilute aqueous solution (0.1–1.0 wt%) with an optimum ratio of 0.4/0.6 (Pull/PEtOx) for the assembly of copolymers in water. Larger particulate structures with sizes around 1 to 2 μm were observed by optical microscopy at a higher concentration (20 wt%) [66].

Table 3: Characteristics of the self-assemblies obtained with polysaccharide-based degradable double hydrophilic block copolymers.

DHBC	Self-assemblies			Stability / Degradation ^a	Ref.
	Type	Preparation	Characteristics ^a		
PEG- <i>b</i> -chitosan	Thermosensitive gel	dissolution of DHBC in PBS at 2 or 3 wt%	gelation time 6-11 min	sol < 35-40°C < gel.	[59]
	DIM-loaded PIC micelles	DIM : mixing of DHBC and DIM Tris buffer solutions (pH 5.3, R = 2),	DIM : CAC 0.014-0.095 g/L / D _h ~70-100 nm / ξ potential~ -3.4 mV / DL~40-65%	dissa. \geq 0.2 M NaCl. stable from pH 4 to 1.1 25% size increase over 2- month storage in Tris (pH 5.3).	
PEG- <i>b</i> -carboxymethyl-dextran	MH-loaded PIC micelles	MH : mixing of DHBC and MH/CaCl ₂ Tris-buffer solutions (pH 7.4, R = 1)	MH : D _h ~200 nm (MH) / DL~50%	stability over 1-month storage in Tris (pH 7.4).	[61–63]
	PAR-loaded PIC micelles NEO-loaded PIC micelles	mixing of DHBC and PAR or NEO PBS solutions (pH 7.4, R = 2.5)	PAR : CAC 0.125 g/L / D _h ~100 nm / DL~50% NEO : CAC 0.06 g/L / D _h ~80-120 nm / DL~50%	PAR : stable at 100 mM NaCl. NEO : stable at 200 mM NaCl and stability > 3 months at 150mM NaCl and pH 7.4.	
PEO- <i>b</i> -dextran PEO- <i>b</i> -pullulan PSar- <i>b</i> -dextran	giant vesicles	direct dissolution in water	R _h ~250 to 700 nm for 0.1 to 1.0 wt% / R _h ~2 to 20 μ m for 10 to 25 wt%	stability > 7 days.	[65]
PEtOx- <i>b</i> -pullulan	Nanoparticles	direct dissolution in water	D _h ~320-500 nm (minor population with D _h ~10-15 nm)	stability for pH 5 to 9 and 2M NaCl.	[66]

Abbreviations : ass. assembly ; disass. disassembly ; DIM diminazene diaceturate ; MH minocycline hydrochloride ; NEO neomycin ; PAR paromomycin ; PEG poly(ethylene glycol) ; mPEG monomethoxy-poly(ethylene glycol) ; PSar poly(sarcosine) ; R charge ratio between positive and negative charges.

^a ranges of provided values correspond to characteristics obtained as a function of the blocks' length of the used DHBCs and/or ratios of compounds. If not specified sizes correspond to the hydrodynamic diameters or radii measured by DLS.

4. Polypeptide-based degradable DHBCs

Polypeptides have an inherent biocompatibility (with the exception of high concentrations and polycationic polypeptides with a high MW) [67] and possess a simple polymeric structure. They can form secondary structure motifs that can mimic protein behavior and introduce additional intermolecular forces such as hydrogen bonding [68,69]. The incorporation of polypeptide sequences, such as pH-responsive poly(L-lysine) (PLL) and poly(glutamic acid) (PGlu), into DHBCs can endow them with additional structural versatility, tunable spatial arrangement of chain segments within self-assembled nanostructures, enhanced biocompatibility and broader applications in the field of biomedicines.

In this part we will discuss recent examples of DHBCs containing polypeptides as the functional block (Table 1). A summary of the type of self-assemblies obtained with these DHBCs and their characteristics is provided in Table 4.

Wu et al. reported on the synthesis of double hydrophilic PEtOx-*b*-PSar copolymers via a one-pot two-step approach (Figure 4). PEtOx–ammonium phosphate was first obtained by polymerization of 2-ethyl-2-oxazoline in the presence of the mild brönsted acid diphenyl phosphate (DPP), and was further used as macroinitiator for the ROP of sarcosine N-carboxyanhydride (Sar-NCA) [70].

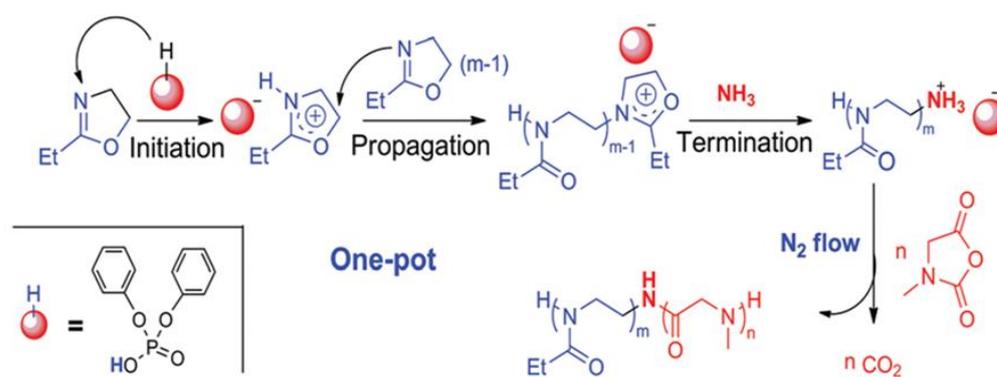


Figure 1. One-pot synthesis of PEtOx-*b*-PSar diblock copolymers [70].

Salmanpour *et al.* synthesized poly(2-ethyl-2-oxazoline)-*b*-poly(benzyl-L-glutamate) (PEtOX-*b*-PbGlu) via cationic ROP of 2-ethyl-2-oxazoline, subsequent amine functionalization of PEtOX using 1-Boc-piperazine and finally N-carboxyanhydride polymerization of benzyl-L-glutamate [71]. PEtOX-*b*-poly(L-glutamic acid) (PEtOX-*b*-PGlu) DHBCs were obtained after removal of the protecting benzyl groups via hydrolysis. In contrast with PEtOX-*b*-PbGlu copolymers, which formed micelles in aqueous solution, PEtOX-*b*-PGlu was freely soluble in water as demonstrated with DLS. Chemical conjugation of the chemotherapeutic agent SN38 to the carboxylic acid groups of the PGlu block via carbodiimide mediated esterification resulted in PEtOX-*b*-PGlu-SN38 conjugates [72]. Thanks to the hydrophobicity of SN38 these polymer-drug conjugates self-assembled in aqueous solution into spherical particles of 90 nm. In vitro experiments with colorectal carcinoma cells demonstrated a higher cellular uptake and a higher cytotoxicity for polymer-conjugated SN38 than for free drug. However, the non-specificity of the hydrolysis reaction may result in premature drug release and side effects.

Also, two sets of double hydrophilic block copolymers with PEG and either poly(L-aspartic acid) (PAsp) or poly(L-glutamic acid) (PGlu) were successfully synthesized by Kasparova *et al.* via ring opening polymerization of their respective protected N-carboxyanhydride monomers using α -methoxy- ω -amino[poly(ethylene glycol)] (PEG-NH₂) as macroinitiator

[73]. The resulting DHBCs were applied in the crystallization of CaCO_3 and BaSO_4 . All DHBCs with a minimum of 10 amino acids were shown to be effective in modifying crystal growth and promoting the formation of different crystal superstructures up to concentrations of 0.05 g/l, such as well-defined ball-shaped, extension and dumbbell particles between 2 and 10 μm in size. CaCO_3 particles with prolonged stability of at least one year were obtained via an aggregation of metastable vaterite nanoparticles.

Kataoka's group developed a method based on charge-conversional PIC micelles, for the efficient delivery of protein into cytoplasm by a cationic DHBC composed of PEG and a cationic segment based on PAsp bearing a N-(2-aminoethyl)-2-aminoethyl group (PAsp(DET)) (Figure 5, middle row), that acts as a buffering moiety inducing endosomal escape with minimal cytotoxicity. This DHBC was associated with protein derivatives. They selected equine heart cytochrome c (CytC; $M_w=12384$ Da), an essential protein in the electron transfer of the mitochondria, as a model protein. CytC was modified with citraconic anhydride or cis-aconitic anhydride to increase the charge density and form anionic CytC derivatives, namely CytC–Cit and CytC–Aco (Figure 5, top row). DLS measurements showed the PIC micelles to have a unimodal size distribution with diameters of about 50 nm and PDI values of about 0.05, also at physiological salt concentration (150 mM NaCl). Spherical PIC micelles were formed at a N/C (amine/carboxylate) ratio of 2. Over 50 % of CytC–Cit was released from the PIC micelles within 4 hours at pH 5.5, whereas only 10 % was released after 8 hours at pH 7.4. Experiments with CytC–Aco showed similar release profiles but with a slower release. The intracellular distribution of the CytC derivatives after incubation for 24 h with HuH-7 hepatocyte-derived carcinoma cells was investigated (Figure 5, bottom row). The charge-conversional PIC micelles containing CytC–Aco or CytC–Cit showed an efficient release of CytC. It was assumed that the

polymer released from the PIC micelles could come into direct contact with the endosomal membrane to induce the efficient escape of the CytC into the cytoplasm [74].

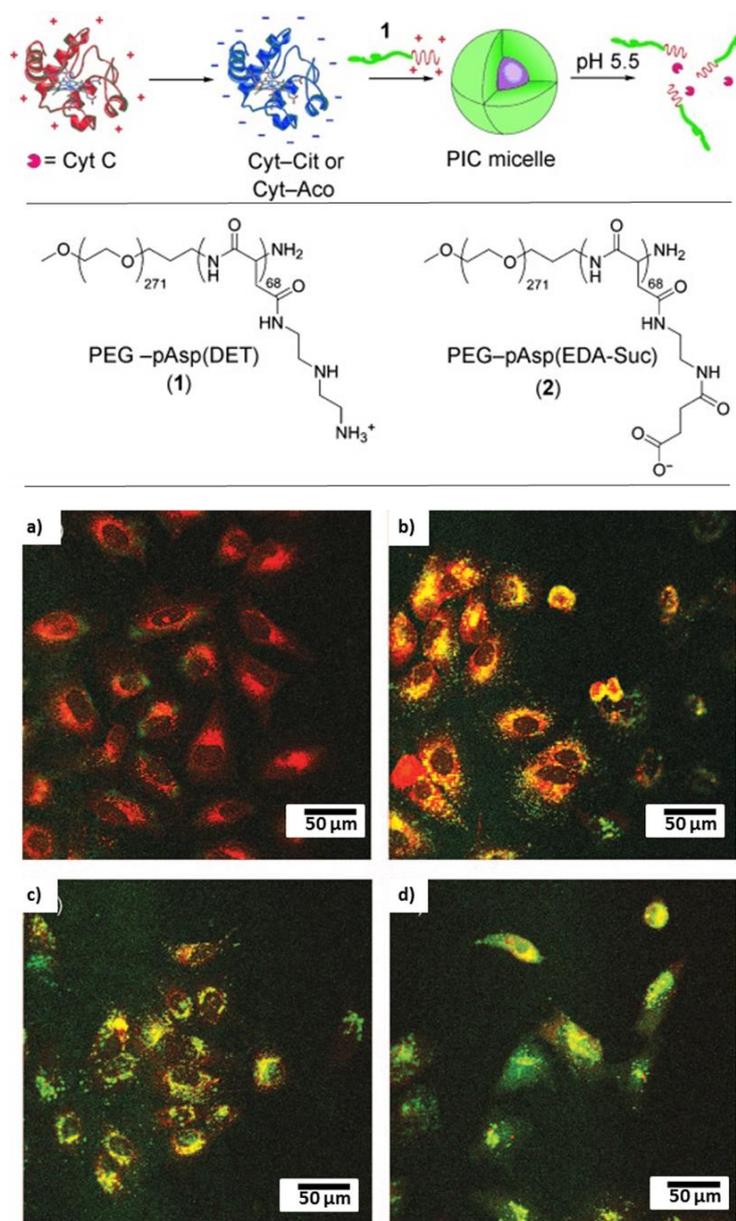


Figure 2. Top row: schematic representation showing the preparation of charge-conversional PIC micelles containing CytC derivatives and PEG-pAsp(DET). Middle row: chemical structures of PEG-pAsp(DET) and of PEG-pAsp(EDA-Suc). Bottom row: CLSM images of HuH-7 delivered by a) free native CytC control, b) succinyl CytC PIC non-charge conversional anionic derivative controls, c) Cyt-Aco PIC micelles, and d) Cyt-Cit PIC micelles after 24 h transfection. Each CytC derivative was labeled with Alexa Fluor 488 (green). The late endosome and lysosome were stained with Lyso-Tracker Red (red). CytC in the endosome was detected as yellow prior release and as green after release (adapted with permission from [74]).

The same group synthesized a similar DHBC PEG-SS-P(Asp(DET)) containing a biocleavable disulfide. The cationic DHBC was complexed with plasmid DNA (pDNA) yielding polyplex micelles with a size around 80 nm, which are stabilized by the hydrophilic PEG blocks. In contrast, aggregation was rapidly observed upon addition of 10 mM dithiothreitol (DTT) as a consequence of the disulfide reduction and PEG cleavage from the micelles. The gene transfection efficiency of the PEG-SS-P(Asp(DET)) micelles was higher than the one of PEG-P(Asp(DET)) micelles as a result of a much more effective endosomal escape thanks to the detachment of the PEG in the endosome [75].

Li et al. synthesized a mPEG-*b*-PGlu derivative bearing mercaptosuccinic acid (MSA) methoxypoly(ethylene glycol)-*b*-poly(γ -propargyl-L-glutamate-*g*-mercaptosuccinic acid) (mPEG-*b*-(PPLG-*g*-MSA)) (Figure 6 1) by combining the ROP of a clickable propargyl-Glu-NCA and the subsequent thiol-yne photoaddition of MSA. The self-assembly of the anionic polymer and cationic DOX.HCl in aqueous medium led to the formation of pH-responsive polymersomes with a size of 20 nm and 99 wt% EE. CLSM (Confocal Laser scanning microscope) and FACS (Flow cytometry analysis) studies confirmed that the FITC-labeled drug delivery polymersomes were taken up by A549 cells via endocytosis. Biodistribution studies in nude mice bearing A549 tumors showed less uptake of the polymersomes by the liver and kidneys compared to the free DOX.HCl, as well as a stronger fluorescence in the tumor (Figure 6 2). These results indicate that polymersomes loaded with DOX.HCl are able to modify the biodistribution of the drug and thereby reduce its systemic toxicity [76].

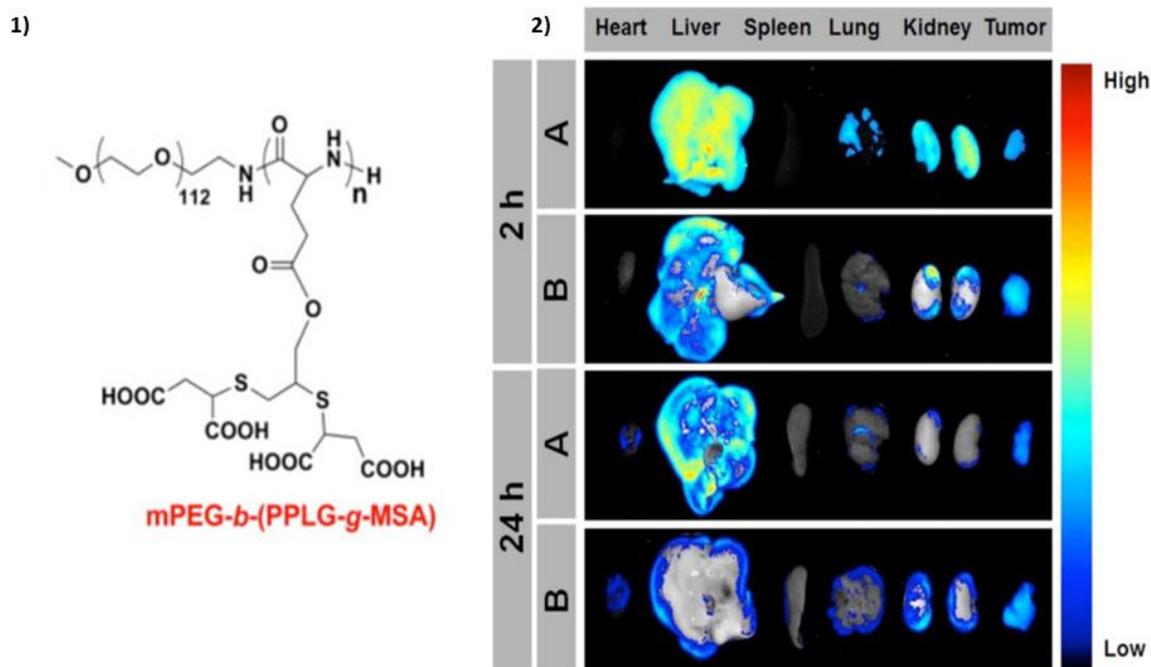


Figure 3. 1) Structure of mPEG-*b*-(PPLG-*g*-MSA) 2) Ex vivo DOX.HCl fluorescence images showing the drug bio-distribution of A) free DOX.HCl and B) mPEG-*b*-(PPLG-*g*-MSA)-DOX HCl polymersomes in nude mice bearing A549 tumors at 2 and 24 h post-injection (adapted with permission from [76]).

Several studies report on the preparation of polypeptide-based PIC micelles for cancer treatment via photodynamic therapy, using either PEG-*b*-PA_{sp} or PEG-*b*-PLL as DHBCs, depending on the nature of the photosensitizers [77,78]. Photodynamic therapy (PDT) involves systemic administration of porphyrin or phthalocyanine-based photosensitizers (PSs), followed by local photoirradiation of solid tumors with a specific wavelength light. As an example, PIC micelles formed via the electrostatic interactions between anionic dendrimer phthalocyanine zinc (DPcZn) and PEG-*b*-PLL (DPcZn/*m*) were prepared for use as an effective photosensitizer for photodynamic therapy at 650 nm. DPcZn/*m* PIC micelles had a size around 50 nm, which is suitable for intravenous administration. DPcZn and DPcZn/*m* exhibited an effective uptake of dissolved oxygen to generate reactive oxygen species ROS under light irradiation with an increase in photocytotoxicity as a function of irradiation time. However, after 60 minutes, DPcZn/*m* exhibited almost 100 times higher photocytotoxicity than free DPcZn, along with a

4 times higher cellular uptake for DPcZn/m due to the charge neutralization of the DPcZn by the micelles [79].

PEG-*b*-PLL DHBCs have also been proposed to yield MRI macromolecular contrast agents. Yokoyama *et al.* substituted ca. 50% of the lysine moieties of PEG₁₁₈-*b*-PLL₃₄ with DOTA mono(N-hydroxysuccinimide ester) before complexation with Gd³⁺ ions. The resulting DHBC with 7 DOTA/Gd moieties formed micelles with a size of 43 nm and larger aggregates of 225 nm with a zeta potential of – 9.55 mV. The Gd-loaded micelles were able to circulate for 48h in colon 26-bearing CDF1 female mice blood stream with a significant accumulation in tumor tissues. This result demonstrates the applicability of this DHBC as a diagnostic tool, which was confirmed by a subsequent study using them for magnetic resonance lymphography [80,81].

In another application, PIC micelles were formed between the cationic antimicrobial peptide MSI-78 and the anionic PEG-*b*-PGlu DHBC to develop antimicrobial agents. The mean diameters of the spherical PIC micelles decreased with an increase in the length of the negatively charged PGlu block with sizes ranging from 80 to 120 nm, as observed by TEM. A sustained release of FITC-labeled MSI-78 was obtained from the PIC micelles for more than 40 hours. Importantly, these PIC micelles greatly decreased the hemolytic toxicity of MSI-78 to human red blood cells, without influencing its antimicrobial activity as shown with maintained MIC (minimum inhibitory concentration) values against Gram-negative *E. coli* and Gram-positive *B. subtilis* and *S. aureus* [82].

Table 4: Characteristics of the self-assemblies obtained with polypeptide-based degradable double hydrophilic block copolymers.

DHBC	Self-assemblies			Stability / Degradation ^a	Ref.
	Type	Preparation	Characteristics ^a		
PEtOx- <i>b</i> -PSar			not evaluated		[70]
PEtOx- <i>b</i> -PGlu	SN38-conjugated micelles	direct dissolution in water, 3 days incubation	17 wt% SN38 conjugated CMC = 0.02 mg/mL / $D_h \sim 91$ nm / ξ potential ~ 2.6 mV	not evaluated.	[71,72]
PEG- <i>b</i> -PGlu and PEG- <i>b</i> -PAsp	templating of CaCO ₃ and BaSO ₄	Double-Jet method (DJM) : slow injection of (Na ₂ CO ₃ and CaCl ₂) or (Na ₂ SO ₄ and BaCl ₂) solutions in DHBC solution	CaCO ₃ : 15–20 nm vaterite particles at 0.05 g/L < DHBC ≤ 1 g/L BaSO ₄ : 2-3 μ m particles at 0.5 g/L < DHBC ≤ 5 g/L	vaterite metastable form maintained over 1 year.	[73]
PEG- <i>b</i> -PAspED	CytC-loaded PIC micelles	Mixing of CytC and DHBC water solutions (R= 2), addition of acetate or phosphate buffer to reach 150 mM NaCl	$D_h \sim 50$ nm	50% CytC release at pH 5.5 vs. 10% at pH 7.4 after 4h.	[74]
PEG- <i>b</i> -PAspED	pDNA-loaded polyplexes	Mixing pDNA and DHBC 10 mM Tris-buffer solutions (pH 7.4) Dropwise addition of DOX.HCl solution in DHBC aqueous solution	$D_h \sim 80-90$ nm and ξ potential ~ 4 mV for N/P ratios >2 $D_h \sim 34$ nm (DLS) 20 nm (TEM) / ξ potential ~ 40 mV / EE $\sim 99\%$ / DL $\sim 16,5\%$	reducible DHBC containing disulfide, loss of PEG block in the presence of 10mM DTT.	[75]
mPEG- <i>b</i> -PPLG-MSA	DOX-loaded polymersomes	at pH 7.4. Overnight stirring, 24h dialysis vs. water		63% DOX release at pH 5.5 vs. 27% at pH 7.4 after 60h.	[76]
PEG- <i>b</i> -PLL	DPcZn-loaded PIC micelles	Mixing of DHBC and DPcZn aqueous NaH ₂ PO ₄ solutions (R =1, pH 7.3)	$D_h \sim 50$ nm	stability of PIC micelles in 10mM PBS in the presence of 10% FBS.	[79]

PEG- <i>b</i> -PLL	Gd ³⁺ -loaded micelles	Dissolution of DHBC in 150 mM NaCl solution.	D _h ~43 & 225 nm / ξ potential~-9.55 mV	stable in the presence of vacant DOTA groups leading to DOTA-DOTA interactions	[81]
PEG- <i>b</i> -PGlu	AMP-loaded PIC micelles	Mixing of DHBC and AMP aqueous solutions (1:1 molar ratio), dialysis 6h vs. water	D _h ~196-277 nm / ξ potential~-16 to -39 mV / EE~ 75-88% / DL~19-23%	80% release at pH 7.4 after 72h.	[82]

Abbreviations : AMP antimicrobial peptide ; ass. assembly ; CytC cytochrom C ; disass. disassembly ; DTT dithiothreitol ; DOX doxorubicin ; DPcZn dendrimer phthalocyanine zinc ; FBS fetal bovine serum ; Gd³⁺ gadolinium ion ; PAsp poly(aspartic acid) ; PAspED poly(aspartic acid(ethylene diamine)) ; pDNA plasmid DNA ; PEtOx, poly(2-ethyl-2-oxazoline) ; PGlu poly(L-glutamic acid) ; PLL poly(lysine) ; PPLG-MSA poly(γ -propargyl-L-glutamate-g-mercaptop succinic acid) ; PSar poly(sarcosine) ; R charge ratio between positive and negative charges ; SN38 7-Ethyl-10-hydroxy camptothecin.

^a ranges of provided values correspond to characteristics obtained as a function of the blocks' length of the used DHBCs and/or ratios of compounds. If not specified sizes correspond to the hydrodynamic diameters or radii measured by DLS.

5. Polyester-based degradable DHBCs and others miscellaneous synthetic blocks

Polyesters are polymers that can be hydrolyzed in accordance with the thermodynamic reversibility of the esterification reaction. This feature, associated with a recognized biocompatibility for some of them, *e.g.* those derived from lactic acid (LA), glycolic acid (GA) or ϵ -caprolactone (CL), explain their wide success in many biomedical applications such as implantable devices, drug delivery systems or scaffolds for tissue engineering. However, polyesters are known for their intrinsic hydrophobicity [83]. The lack of structural diversity of polyesters appears as an important limitation in terms of functionality and physico-chemical properties. As a consequence, various methodologies to introduce functional groups onto polyester backbones have been reported [84–88], for example to poly(ϵ -caprolactone) (PCL) functionalized with hydrophilic groups such as hydroxyl [89], carboxyl [90,91], or amino groups [92]. DHBCs containing such functional polyesters as functional block will be discussed in this part (Table 1). A summary of the type of self-assemblies obtained with these DHBCs and their characteristics is provided in Table 5.

Liu *et al.* synthesized a family of aminated DHBCs in 5 steps via ROP of γ -(carbamic acid benzylester)- ϵ -caprolactone (γ CAB ϵ CL) in bulk using mPEG as macro-initiator to yield, after deprotection, mPEG-*b*-PACL that can be candidates for pH-sensitive drug delivery, especially for anionic hydrophilic drugs or genes (Figure 7 1). The authors studied the solution properties of the various DHBCs as a function of pH and PACL block length and highlighted by DLS the presence of unimers or aggregates with sizes ranging from 50 to 250 nm depending on the parameters previously cited [93].

By copolymerization of caprolactone (CL) and CL bearing reactive groups like chloro ϵ -caprolactone (ClCL) that can be derivatized after polymerization into an azido-PCL,

Charoongchit *et al.* obtained a clickable triblock $(P(\alpha\text{-N}_3\text{-CL-}co\text{-CL})_2\text{-PEG}$ copolymer that was reacted with propargyltrimethyl ammonium iodide to yield the cationic $(P(\alpha\text{-TMA-CL-}co\text{-CL})_2\text{-PEG}$ bearing trimethyl ammonium (TMA) side groups (Figure 7 2). The surface charge of $(P(\alpha\text{-TMA-CL-}co\text{-CL})_2\text{-PEG}$ particles was positive due to the grafted cationic ligand present on the surface of the particles. The authors compared the particle size for different contents of cationic ligand but with constant PCL chain length, and found that the particle size increased with increasing the mol% of cationic ligand.[94] These cationic copolymers showed a capability to entrap enoxaparin, a low molecular weight heparin used in the treatment of deep vein thrombosis and pulmonary embolism [95], with 87% EE and 8% DL [94].

Gao *et al.* reported on the synthesis of PEG-*b*-poly(β -aminoester)-1-(3-aminopropyl)imidazole (PEG-PAE-API) by a Michael-type step polymerization between monoacrylated PEG, 1,6-hexanediol diacrylate, 4,4'-trimethylene dipiperidine and 1-(3-Aminopropyl) imidazole. This copolymer (Figure 8, top) was used to encapsulate the model protein albumin (Alb). The Alb-loaded micelles, with sizes in the range of 50 to 70 nm, showed a charge conversion from neutral to positive when pH values were changed from 7.8 to 6.2, which is compatible with pH changes observed in cancerous tissue or ischemic tissues. The ability of this PEG-PAE-API to deliver protein *in vivo* in acidic tissues was assessed in a rat model of cerebral ischemia. Following intravenous injection with Cy5.5-Alb-loaded micelles a gradual increase in fluorescence signals of the brain ischemic area was observed (Figure 8, bottom), indicating that protein/PEG-PAE-API could be effective for targeting acidic environments and diagnostic imaging [96].

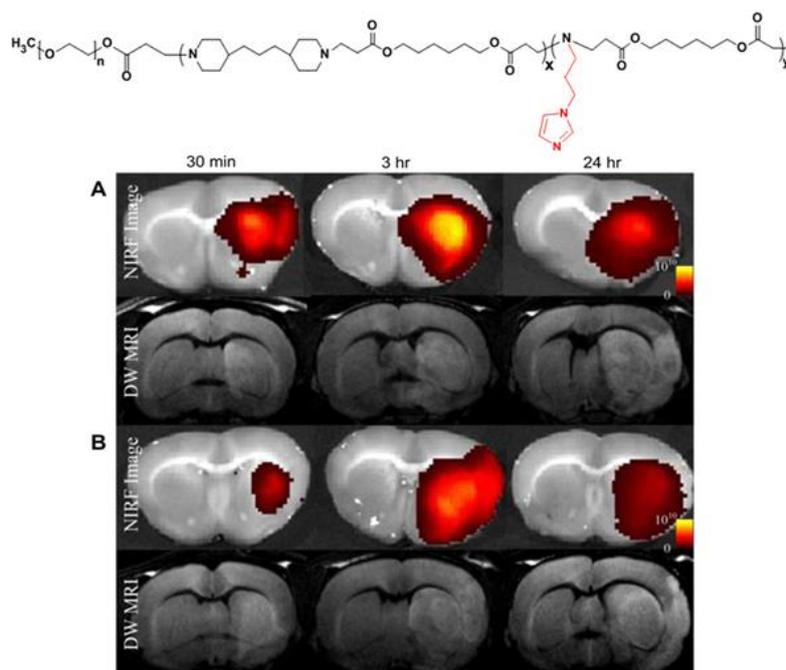


Figure 5. In vivo diffusion-weighted MRI (DW-MRI), near-infrared fluorescence (NIRF) images and signal quantification. In the coronal cross-sectional NIRF images of rat brains of group A (rats injected with PEG-PAE-API-albumin-Cy5.5) (A) and group B (rats injected with albumin-Cy5.5) (B), albumin-Cy5.5 accumulation is clearly visible in the ischemic area of the

right hemisphere, which can be identified as hyperintense lesion on the DW-MRI, in comparison to the left hemisphere.(adapted with permission from [96]).

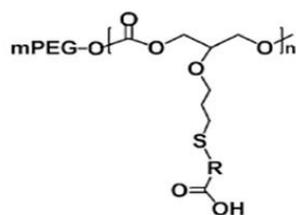
Mahmud *et al.* synthesized in 5 steps a family of PEO-*b*-poly(α -carboxyl- ϵ -caprolactone) DHBCs via ring opening polymerization of α -benzyl carboxylate- ϵ -caprolactone and CL with methoxy-PEO as an initiator, followed by catalytic debenzilation of the protected copolymer (Figure 7 3) [90]. The copolymer with 40% of carboxylated CL units assembled to spherical micelles with a CMC of 1.2×10^{-2} mM and average diameters of 25 nm. According to the authors, this system could be used as delivery systems for the chemical conjugation, optimized solubilization, and controlled delivery of therapeutic agents.

Deng *et al.* synthesized methoxy poly(ethyleneglycol)-*b*-poly(ϵ -caprolactone-co- γ -dimethyl maleamic acid- ϵ -caprolactone) (mPEG-*b*-P(CL-co-DCL) in 3 steps, having a polyester moiety carrying different amounts of acid-labile β -carboxylic amides (Figure 7 4) [97].

The copolymer formed stable micelles in water with diameters of 100 to 150 nm and with critical micellar concentrations (CMCs) of 3.2–6.3 $\mu\text{g/mL}$. The DL and EE of this copolymer for DOX were 3-4 times higher than those of amphiphilic copolymer mPEG-*b*-PCL micelles. The mPEG-*b*-P(CL-co-DCL) polymer micelles are negatively charged and stable in neutral solution, but, because of the hydrolysis of the β -carboxylic amides in acidic conditions (pH=6.0) the polymer becomes positively charged (Figure 7 4'). This negative to positive charge reversal triggered by the variation of the pH led to a very fast drug release under acidic conditions, and also improved the cellular uptake by electrostatic absorptive endocytosis. Also, the hydrolysis of the acidic group in the polyester upon pH decrease from 7.4 to 5.4 led to a faster release in acidic environment (already mentioned). The mPEG-*b*-P(CL-co-DCL) micelles showed a very low cytotoxicity up to a concentration of 1 mg/mL.

Zhuo *et al.* used a thiol-ene “click” reaction between pendent carbon-carbon double bonds of mPEG-*b*-poly(5-allyloxytrimethylethylenecarbonate) (mPEG-*b*-PATMC) and various thiol-bearing molecules to prepare four different acid modified copolymers mPEG-*b*-PATMC-*g*-SRCOOH (R = CH₂, CH₂CH₂, (CH₂)₁₀ and CH(COOH)CH₂), denoted as P1, P2, P3 and P4, Figure 9 1) [98]. The micelles mean diameters determined by DLS for all the copolymers were below 130 nm, and by TEM the authors showed that the copolymer micelles were dispersed in spherical shape with average diameters from 25 to 35 nm (Figure 9 2). The negatively charged copolymers were used for the encapsulation of the positively charged drug DOX via synergistic hydrophobic and electrostatic interactions. The DL of the acid-modified copolymer micelles were all higher than 10 % and the EE were higher than 60 %. The DOX-loaded copolymer micelles showed a pH-dependent release behavior. After 50 hours, the release was limited to 20% at pH 7.4 against ca. 70% at pH 5.0. Overall, the copolymer P3 with the 11-mercaptoundecanoic moieties was the best candidate for DOX formulation as it showed a lower CMC value, smaller particle size, good stability and blood compatibility, as well as higher drug loading capacity. Moreover, cellular investigations revealed an efficient cancer cellular uptake and potent cytotoxic activity of DOX-loaded micelles based on P3 copolymer, probably due to a suitable hydrophobicity and charge density [98].

1)



R= (P1) -CH₂- ; (P2) -CH₂CH₂- ; (P3) -(CH₂)₁₀- ; (P4) -CH(COOH)CH₂-

2)

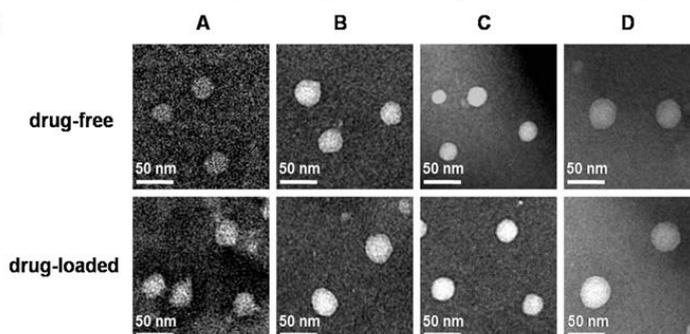


Figure 6. 1) Structure of 4 different acid modified copolymers mPEG-*b*-PATMC-*g*-SRCOOH. 2) Transmission electron microscope images of drug-free and drug-loaded polymeric micelles based on (A) P1, (B) P2, (C) P3 and (D) P4. (adapted with permission from [98]).

Zhang et al. prepared PEG-*b*-poly(lactic acid-*co*-malic acid) (Figure 7 5) copolymers via polycondensation between D,L-lactic acid (LA), L-malic acid (MAL), and monomethyl polyethyleneglycol using stannous chloride (SnCl₂) as the catalyst [99]. The copolymer was used to encapsulate the DOX via electrostatic interactions between the carboxyl side group of MAL units and the amino groups of DOX. The DL was 18.2% with good stability in aqueous solution, and TEM images showed spherical nanoparticles in a size range of 110-140 nm. The cumulative DOX release increased under acidic conditions because of the protonation of the acidic group in the MAL. This effect was however limited as after 60 hours, 80% of the DOX was released at pH 7.4, against 94% at pH 5.8.

Zhang et al. prepared a pH-responsive amphoteric block copolymer poly(6-acetoxyl- ϵ -caprolactone)-*b*-poly(4-N-piperilactone) (PCCL-*b*-PPIL) (Figure 7 6) by bulk ring-opening polymerization of 4-N-benzyl formate-piperilactone in the presence of the hydroxyl-terminated

poly(6-(p-methylbenzyl acetate)- ϵ -caprolactone) as macroinitiator, followed by removal of the protecting groups. The PCCL-*b*-PPIL copolymer contains carboxyl groups and secondary amine groups in each segment, leading to protonation of the secondary amine groups of the PPIL segments at pH < 5.7 and the formation of PCCL-core micelles. At pH > 6.8 the carboxyl groups of the PCCL segments were deprotonated and PPIL-core aggregates were formed. Noteworthy, the morphology of the self-assemblies changed from spherical at pH 5 with a diameter of 65 nm to worm-like micelle upon pH increase to 8. Thanks to the carboxyl and the secondary amine groups, fluorescent molecules were attached to the copolymer to form stimuli-responsive fluorescent materials [100].

Our group recently reported on a straightforward, 3-step synthetic strategy for the preparation of DHBCs with PCL blocks containing carboxylic acid, amine or hydroxyl functional moieties [101]. PEG-*b*-PCL copolymers were prepared via ROP of CL employing mPEG as macroinitiator, followed by post-polymerization functionalization of the PCL blocks with pendant alkyne groups using an anionic modification technique [102,103]. Reaction of the alkyne groups with mercaptosuccinic acid, 4,5-diamino-6-hydroxy-2-mercaptopyrimidine or 1-thioglycerol via thiol-yne photoaddition resulted in PEG-*b*-PCL copolymers with carboxylic acid, amine or hydroxyl functionalized PCL blocks, respectively (Figure 10a). For PEG_{1.9k}-*b*-PCL_{1.1k}(OH)₅₂ (containing 52% of hydroxyl groups with respect to CL units) no pH dependency was expected and therefore its aqueous solution behavior was only tested at pH 7.4. This copolymer self-assembled into micelles above the CMC of 1 mg/mL with a diameter of 225 nm. PEG_{1.9k}-*b*-PCL_{1.3k}(COOH)₅₅ and PEG_{1.8k}-*b*-PCL_{1.4k}(NH₂)₅₈ (containing 55% of carboxylic acid and 58% of amine groups with respect to CL units, respectively) self-assembled into pH-responsive micelles with sizes ranging from ~190 nm (in case of ionized DHBCs) to ~130 nm (in case of non-ionized DHBCs). PEG_{1.9k}-*b*-PCL_{1.3k}(COOH)₅₅ formed stable PIC micelles with DOX at pH 7.4. When the pH was decreased to 5.0, the PIC micelles disassembled due to the

loss of electrostatic interactions between DOX and the carboxylic groups on the DHBC, leading to the release of the drug. The DOX loaded PIC micelles were highly cytotoxic towards MCF-7 cancer cells (Figure 10b), demonstrating the potential of this type of degradable DHBC for the intracellular delivery of electrostatically charged, hydrophobic drugs.

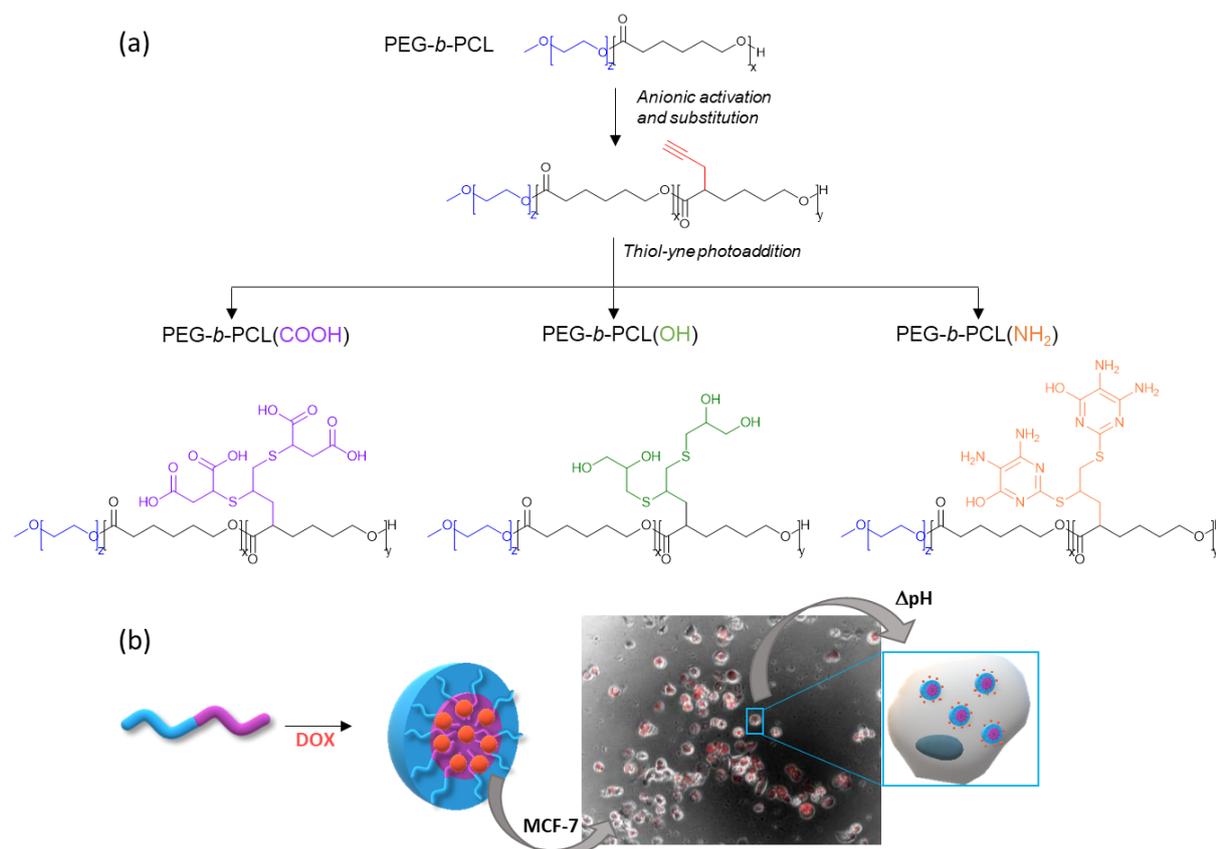


Figure 7. (a) Synthesis of carboxylic acid, amine or hydroxyl functionalized DHBCs from mPEG-*b*-PCL in 2 steps and (b) illustration of mPEG-*b*-PCL(COOH)/DOX PIC micelles formation and their internalization in MCF-7 cancer cells (adapted with permission from [101]).

Zeynep et al. synthesized DHBCs composed of a PEO block and a polyphosphoester block by a combination of organocatalyzed ring opening polymerization, thiol–yne click chemistry and protection/deprotection methods. They prepared poly(phosphotriester)s bearing pendent carboxylic acids (PEO-*b*-PBYP₃COOH) with an affinity for calcium by ROP of butynyl phospholane, as well as poly(phosphodiester)s with a negatively charged oxygen atom on each repeating monomer unit (PEO-*b*-PPDO⁻) by ROP of allyl phospholane (Figure 7 7 and 7’). The

authors exploited this family of DHBCs to formulate CaCO_3 particles, that can be used for encapsulation, and showed an efficient decrease of particle sizes by a factor of 6 while preventing their aggregation compared to formulations with hyaluronic acid (HA) [104]. In a follow up work, the authors reported on lysozyme-loaded CaCO_3 particles prepared via a supercritical CO_2 process, where CO_2 serves as a source of carbonate ions, using either PEO-*b*-PBYP COOH or HA as templating agent. With PEO-*b*-PBYP COOH a twice higher loading of active lysozyme was obtained in the particles compared to HA. Furthermore, a smaller size and a deeper encapsulation of lysozyme in the particle core was observed as well as a more efficient incorporation of the protein (Figure 11) [105].

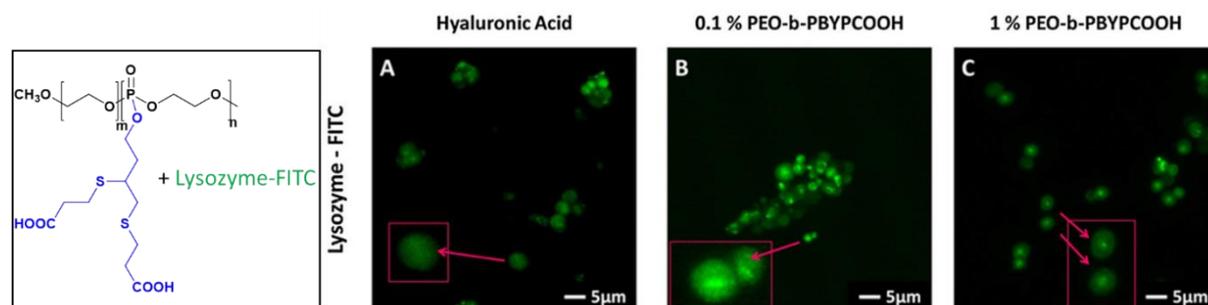


Figure 8. Confocal images of CaCO_3 particles with lysozyme-FITC in the presence of (A) 0,1 % HA; (B) 0.1 % PEO-*b*-PBYP COOH and (C) 1 % PEO-*b*-PBYP COOH (adapted with permission from [105]).

In another study, poly(2-(2-aminoethoxy)ethoxy)phosphazene (PAEP) was coupled to folate-PEG-COOH or mPEG-COOH using a DCC/NHS activation (Figure 7 8). DNA was condensed by the resulting cationic PEG-PAEP DHBCs at various N/P ratios to form PEG-PAEP/DNA polyplexes that were compared with PAEP/DNA polyplexes. It was shown that the pegylation of the PAEP decreased the cytotoxicity toward Hela cells and improved the transfection efficiency [106].

Table 5: Characteristics of the self-assemblies obtained with polyester-based degradable double hydrophilic block copolymers.

DHBC	Self-assemblies			Stability / Degradation ^a	Ref.
	Type	Preparation	Characteristics ^a		
mPEG- <i>b</i> -P(γ -NH ₂ -CL)	pH-responsive micelles	direct dissolution in water precipitation method: addition of EXP water	D _h ~50-250 nm / ξ potential~-10 to 30 mV	diass. \leq pH 5 \leq ass.	[93]
PEG- <i>b</i> -(P(TMACL- <i>co</i> -CL)) ₂	EXP-loaded PIC micelles	solution in DMF solution of DHBC (with 20% TMA), dialysis 24h vs. water	D _h ~290 \pm 80 nm / ξ potential~4 mV / EE~ 87% / DL~8%	not evaluated for EXP-loaded micelles.	[94]
PEG- <i>b</i> -PAE-API	Alb-loaded PIC micelles	solution of Alb and DHBC (1:10) in PBS at pH 6 followed by pH neutralization at pH 7.4	D _h ~50-70 nm / ξ potential~0 mV / EE~ 87% / DL~8%	~30% size decrease when pH \geq 6.9. ~20% size increase from water to NaCl 150 mM.	[96]
PEO- <i>b</i> -P(α -COOH-CL- <i>co</i> -CL)	micelles	precipitation method: addition of acetone solution of DHBC in water followed by solvent evaporation	D _h ~20-38 nm with second population (ca. 50%) with D _h ~260-370 nm / CMC ~44 \times 10 ⁻² μ M-1.2 \times 10 ⁻² mM	not evaluated.	[90]
mPEG- <i>b</i> -P(CL- <i>co</i> -DCL)	DOX-loaded PIC micelles	precipitation method: DMF solution of DHBC and DOX added in water followed by dialysis vs. PBS	Unloaded micelles : CMC ~3-6 μ g/mL / D _h ~115-150 nm / ξ potential~-14 mV (pH 7.4) DOX-loaded micelles : D _h ~110-136 nm / ξ potential~-7 mV (pH 7.4) / EE~ 58-84% / DL~9-13%	hydrolysis of acid-labile β -carboxylic amides at pH 6. 90% DOX release at pH 5.5 after 6h vs. ~10% at pH 7.4 after 24h.	[97]
mPEG- <i>b</i> -PATMC- <i>g</i> -SRCOOH	DOX-loaded PIC micelles	dialysis method : DMF solution of DOX.HCl, Et ₃ N, DHBC dialysed vs. water 24h	. Unloaded micelles : CMC ~3-78 mg/mL / D _h ~73-125 nm / ξ potential~-10 to -17 mV . DOX-loaded micelles : D _h ~83-142 nm / ξ potential~-4 to -	35-65% DOX release at pH 5 vs. ~20% at pH 7.4 after 48h. 33% to 66% increase of DOX release with 0.1 and 0.5M	[98]

mPEG- <i>b</i> -P(LA- <i>co</i> -MAL)	DOX-loaded PIC micelles	addition of DOX.HCl, Et ₃ N water solution in DHBC water solution, dialysis vs. water 24h	8 mV / EE~ 60-91% / DL~10-16% D _h ~110-140 nm (TEM) / CAC 0.8 g/L / DL~18%	NaCl compared to no NaCl, respectively. 94 % DOX release at pH 5.8 vs. ~80% at pH 7.4 after 70h. 90 % DOX release at 0.9% NaCl vs. ~55% at 0.3% NaCl after 70h.	[99]
PCCL- <i>b</i> -PPIL	zwitterionic pH-responsive micelles	direct dissolution of DHBC in water	. pH < 5 spherical micelles with D _h ~100 nm / ξ potential~10 to 60 mV . pH > 8 worm-like micelles with D _h ~200 nm / ξ potential~-10 to -40 mV	pH < 5.7 micelles with PCCL core and cationic PPIL corona. pH > 6.8 micelles with PPIL core and anionic PCCL corona. precipitation for 5.7 < pH < 6.8.	[100]
mPEG- <i>b</i> -P(CL- <i>co</i> -CL(COOH) ₂)	DOX-loaded PIC micelles	DMF solution of DOX.HCl, Et ₃ N, DHBC added in water, dialysis vs. water and PBS 24h	. Unloaded micelles : CMC ~ 0.9-1.7 mg/mL / D _h ~190-220 nm / ξ potential~-30 mV (pH 7.4) . DOX-loaded micelles : EE~30-69% / DL~4.5-10%	75 % DOX release at pH 5.0 after 8h vs. ~25% at pH 7.4 after 24h.	[101]
PEO- <i>b</i> -PBYP-COOH	Lys-loaded CaCO ₃ particles	mixing of a CaCl ₂ DHBC and Lys in glycine buffer, and injection in scCO ₂	vaterite particles D _h ~2 μm / ξ potential~-1.5 to -10 mV	degradation in water after 24h. plateau of Lys release at ~60% after 90 min.	[104, 105]
FA-PEG- <i>b</i> -PAEP	pDNA-loaded polyplexes	vortexing of DHBC and pDNA solutions (R = 15-25)	D _h ~88-110 nm / ξ potential~7.5 mV	not evaluated.	[106]

Abbreviations : Alb Albumin ; DOX doxorubicin ; EXP enoxaparin ; FA folate ; Lys Lysozime ; PAE-API poly(β-aminoester)-1-(3-aminopropyl)imidazole ; PAEP poly(2-(2-aminoethoxy)ethoxy)phosphazene ; PATMC, poly(5-allyloxytrimethylethylenecarbonate) ; PCCL-*b*-PPIL poly(6-acetoxyl-ε-caprolactone)-*b*-poly(4-N-piperilactone) ; P(CL-*co*-CL(COOH)₂) poly(ε-caprolactone-*co*-α-ε-caprolactone) ; P(CL-*co*-DCL) poly(ε-caprolactone-*co*-γ-dimethyl maleamidic acid-ε-caprolactone) ; P(α-COOHCL-*co*-CL) Poly(α-carboxyl-ε-caprolactone-*co*-ε-caprolactone) ; Poly(α-carboxyl-ε-caprolactone) ; P(γ-NH₂-CL) Poly(γ-amino-ε-caprolactone) ; P(TMACL-*co*-CL) Poly(trimethyl ammonium ε-caprolactone-*co*-ε-caprolactone) ; R charge ratio between positive and negative charges ; scCO₂ supercritical CO₂.

^a ranges of provided values correspond to characteristics obtained as a function of the blocks' length of the used DHBCs and/or ratios of compounds. If not specified sizes correspond to the hydrodynamic diameters or radii measured by DLS.

6. DHBCs for biomedical applications: unique advantages, current challenges and future perspectives

As illustrated in the course of this review, DHBCs are well-suited for biomedical use. This resides in their wide applicability in aqueous systems explained by some key advantages compared to classical amphiphilic systems. A first advantage is the chemical flexibility offered by the functional block that can be synthesized in such a way that it becomes amphiphilic in response to a stimulus. Temperature and pH have been largely exemplified in this work and are the two main parameters considered for triggering the change of macromolecular conformation in water, especially for drug delivery. However, ionic strength and the nature of the counter ions can also be considered, for example in the case of zwitterionic DHBCs [107] or of DHBCs exhibiting self-assembly upon addition of metal ions to form supramolecular hydrogels [108]. Another physical trigger rising more and more focus in drug delivery is light. Classically, light is used to activate dimerization, cleavage or conformational transition of light-sensitive constituents of the macromolecular structure which allows disassembly or swelling of the drug delivery system leading to release of their payload [109]. This approach has rarely been reported for DHBCs with only few examples of soluble coumarin-functional DBHCs being turned into insoluble nanogels with a controllable degree of swelling thanks to a coumarin-based reversible UV-crosslinking [110,111]. Light-trigger could also be used to generate DHBCs as exemplified by Wu *et al.* who took advantage of the photocleavable nitrobenzyl moiety [112]. Upon UV irradiation, a block containing 5-(2-(dimethylamino)ethoxy)-2-nitrobenzyl acrylate units was turned into a hydrophilic water-soluble block bearing hydrophilic acrylic acid groups, which led to the formation of a DHBC. This last example illustrates the potential of light to generate DHBCs *in situ*. Of notice, no examples of light-responsive degradable DHBCs are described to date which constitutes to our opinion an unexplored opportunity. However, further development of light-responsive DHBCs for biomedical applications raises the critical question of the

cytocompatibility of the photosensitive groups and the byproducts generated upon their photoactivation.

Coming to the biomedical applications, like their amphiphilic counterparts DHBCs can be found in a large array of applications including medical imaging [13,113], sensing of external microenvironment [112,114], theranostics [115] and most of all in drug and gene delivery as largely exemplified in this review with numerous examples of PIC micelles. A second key advantage of DHBCs explain their success in this domain. Many relevant substrates, including drugs and biomolecules, are hydrophilic (although not necessarily water soluble), contain groups allowing electrostatic interactions and are present in aqueous environments. To address such substrates, the soundest molecular design of a surfactant is that of a DHBC as they can accommodate higher loadings and guaranty their load retention compared to more conventional nano-carriers (such as core-shell micelles from amphiphilic copolymers) by forming PIC micelles thanks to electrostatic interactions between the polymer and the cargo molecule. In addition, the stimulus-responsiveness of the functional block allows for on-demand drug or gene release within short times and with high efficiency. Of notice, this stimuli-responsiveness being also responsible for the transition of the functional block from a hydrophilic to a hydrophobic block, it allows DHBCs to accommodate hydrophobic cargos as well. This feature makes them highly versatile as most active pharmaceutical ingredients can therefore be encapsulated in DHBCs with proper optimization of the formulations.

Despite the enormous progress that has been achieved in the field, several challenges remain. These will be discussed below together with perspectives how they could be addressed in future research. A first challenge concerns the lack of homogeneity in the conditions used to prepare the DHBC self-assemblies. Although their natures are diverse and do not allow a single

procedure, it is to note that DHBCs of the same class are not always formulated the same way (dissolution, co-solvation etc...) which makes the comparison difficult among them. This is in particular true when it comes to drug loading of self-assemblies. For a same drug, various ways of incorporation can generally be found (see Tables 2 to 5), and therefore, the resulting drug loadings cannot be only ascribed to the chemical structure of the DHBCs. This largely hinders the comparison of performances between the DHBCs which on the long term slows down the iterative process of design improvements that can lead to a real breakthrough in drug formulation and delivery.

Secondly, and in relation with the first point, the complex behavior of DHBCs in solution should be pointed out and correlated with their functional behavior. Although this complex behavior is the cornerstone of their applicability as stimuli-responsive systems, most DHBCs are only studied and used by polymer groups with a main interest in the structure design and applications. On the other hand, in the case of DHBC studies by physico-chemists, the dimension of application is generally not considered, with for example in depth studies of solution behaviors of the DHBCs under various conditions, but no study of the corresponding drug-loaded systems (*e.g.* spatial repartition of drug, drug-polymer interactions etc...) under physiologically relevant conditions (*e.g.* presence of proteins). It is therefore our opinion that one of the levers for the future developments of DHBCs resides in the combined approaches of both communities.

Thirdly, most of the DHBC systems have only been tested *in vitro*. This is clearly demonstrated with the works cited in this review, where less than 10% of the structures reported have been tested *in vivo* [13,15,58,72,76,77,81,96]. To facilitate clinical translation, the therapeutic effects should be studied *in vivo* together with their toxicity, long-term biodegradability and accumulation. This is especially important for DHBCs carrying cationic moieties since concerns have been raised regarding the *in vitro* and *in vivo* toxicity of such polymers [116].

Fourthly, the preparation of functional synthetic polymer blocks may be challenging, which cripples reproducibility. It also suffers from low yields due to multi-step reactions, which makes translation into the clinic less feasible. In this regard the use of polysaccharides, which inherently contain pending functional groups that can be easily modified, is particularly attractive for the conception of DHBCs. However, the control of their molecular weight and the difficulty to generate only diblock copolymers and not graft copolymers limits their practical use as illustrated by the low number of polysaccharides-based DHBCs. It is therefore our belief that the development of scale up friendly chemical strategies allowing for a rapid preparation of functional synthetic polymer blocks is one challenge to be tackled by the polymer community. Of notice, post-chemical modifications of selected polymer structures to generate macromolecular diversity appears to be highly attractive, especially with the use of “click chemistry” methodologies, that allow a rapid fine tuning of the polymers’ structures [76,94,98,101,104].

Fifthly, concerns exist regarding the immunogenicity of PEG [117,118], which is present in many DHBCs as the water-soluble non-ionic block. These concerns justify the consideration of alternatives for use in DHBCs. Other non-ionic water-soluble blocks such as POx and PVP have been explored, but further research is warranted in terms of *e.g.* toxicity to investigate whether they can realistically serve as an alternative for PEG. In this regard, in view of the non-degradability of PEG, POx and PVP, a degradable, non-ionic and water-soluble block would represent a significant step forward in the field of biomedical DHBCs.

Lastly, although most polymers reported in this review are considered as degradable due to expected degradability of the backbones of the functional blocks (polypeptides, polyesters etc...), the degradation of these DHBCs is not investigated, even though biodegradability is a key property for polymers in biomedical applications. Despite degradation being highly likely, this degradation may be strongly impacted by the chemical modifications carried out on these

backbones and the influence of the selected functional groups on the degradation behavior of the DHBCs should be systematically investigated. Future research should therefore provide insight in the mass loss over time of DHBCs as well as the chemical structure and the toxicity of the degradation products both *in vitro* and *in vivo*.

7. Concluding remarks

Double hydrophilic block copolymers are a class of copolymers with numerous advantages in the frame of drug delivery systems design and drug formulation, including stimuli-responsive self-assembly behavior or improved loadings through electrostatic interactions. In particular, DHBCs exhibiting a functional degradable block chosen among polysaccharides, polypeptides or polyesters can further improve these systems by providing fully bio-eliminable structures. Such degradable DHBCs have demonstrated their potential for various biomedical applications ranging from pH triggered release of high amounts of drugs and proteins, to efficient delivery of nucleic acids or self-assembly driven templating of inorganic cargos. However, a number of limitations are clearly still present, including the absence of homogeneity in self-assembly testing, missing *in vivo* data, laborious synthesis of functional polymer blocks and lack of data concerning degradation of DHBCs. Nevertheless, the authors believe that DHBCs will play an increasingly important role in biomedical applications in the years to come thanks to their wide applicability in biological systems, the possibility of stimulus-responsiveness and their high cargo loading. Overall, this review highlights both the potential of degradable DHBCs for advanced drug delivery systems and the opportunities that are still open to further improve these copolymers towards applications at an industrial and clinical level.

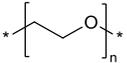
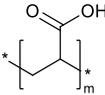
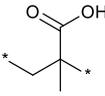
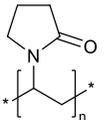
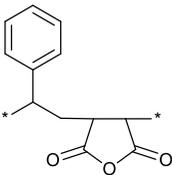
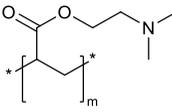
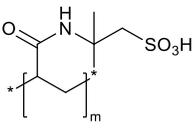
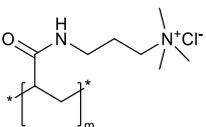
Declaration of Competing Interest

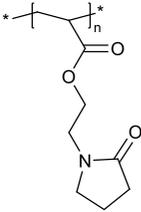
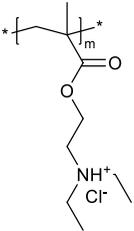
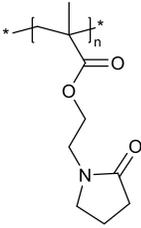
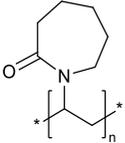
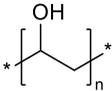
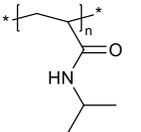
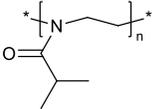
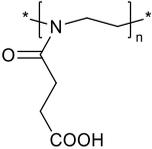
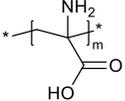
We declare that there is no conflict of interest related to this article.

Acknowledgements

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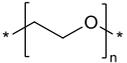
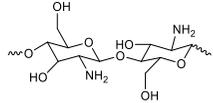
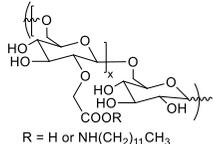
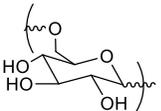
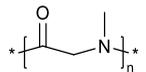
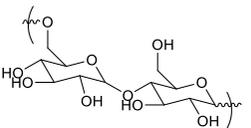
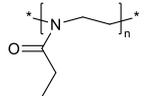
Table 1 (non-degradable DHBCs): chemical structure of double hydrophilic block copolymers and their biomedical applications (chemical structures are only provided once for each block).

Solubilizing blocks	Stimuli-responsive blocks	Applications	Ref.
PEO 	PAA 	Self-assembly into giant vesicles	[12]
PEO	PAA	Association with gadolinium (Gd ³⁺) for imaging	[13]
PEO	PMA 	Transfection of mesenchymal stem cells using SiRNA	[14]
PEO	PAA	Encapsulation of DOX or MTX	[15]
PVP 	PSMA 	Encapsulation of Co A	[17]
PVP	PDMAEMA 	Formation of PIC-micelles with anionic PVP-DHBCs	[17]
PVP	PAMPS 	Encapsulation of FA / inorganic phase templating	[19,21]
PVP	PMA	templating of CaCO ₃	[20]
PVP	PAPTAC 	inorganic phase templating	[21]

PNAEP		PDEAEMA		pH-responsive micelles	[22]
PNMP		PMA		Templating of Au NP	[24]
PVCL		PVP		Thermosensitive micelles	[25]
PVCL		PVA		Thermosensitive and redox-sensitive micelles	[27,28]
PVA		PAA		pH-responsive micelles and PIC micelles	[30,31]
PNiPAM		PVAm		Thermoresponsive micelles	[32]
PiPrOx		PCEtOx		Thermosensitive and pH-sensitive micelles	[35]
PDHA		PAA			[39]

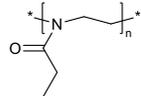
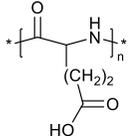
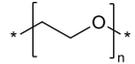
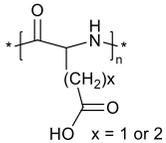
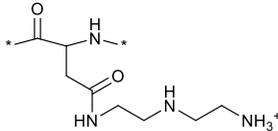
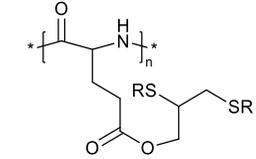
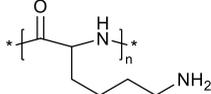
Abbreviations : Co A coenzyme A ; DOX doxorubicin ; MTX mitoxantrone ; PAA poly(acrylic acid) ; PAMPS poly(2-acrylamido-2-methyl-1-propanesulfonic acid) ; PAPTAC poly(3- acrylamidopropyltrimethylammonium chloride) ; PCeTOx poly(2-carboxyethyl-2-oxazoline) ; PDEAEMA (poly(2-(diethylamino)ethyl methacrylate) ; PDHA poly(dehydroalanine) ; PDMAEMA poly(2-(dimethylamino)ethyl methacrylate); PEG poly(ethylene glycol) ; mPEG monomethoxy-poly(ethylene glycol) ; PEO poly(ethylene oxide); PEtOx Poly(2-ethyl-2-oxazoline) ; PHPMA poly(N-(2-hydroxypropyl) methacrylamide) ; PiPrOx poly(2-isopropyl-2-oxazoline) ; PMA poly(methacrylic acid) ; PNAEP poly(2-(N-acryloyloxy)ethylpyrrolidone) ; PNIPAM poly(N-isopropyl acrylamide) ; PNMP poly(N-(2-methacryloyloxyethyl) pyrrolidone) ; PSMA poly(styrene-alt-maleic anhydride) ; PVA poly(vinyl alcohol) ; PVAm poly(vinyl amine) ; PVCL, Poly(N-vinylcaprolactam) ; PVP poly(vinyl pyrrolidone).

Table 1 (continued polysaccharide-based degradable DHBCs): Classification and chemical structure of double hydrophilic block copolymers and their biomedical applications (chemical structures are only provided once for each block).

Solubilizing blocks	Stimuli-responsive blocks	Applications	Ref.
PEG 	Chitosan 	Thermosensitive gel	[59]
mPEG	Oligochitosan	Foreseen for drug delivery applications	[60]
PEG	Carboxymethyl-dextran  R = H or NH(CH2)11CH3	Encapsulation of diminazene diacetate, minocycline hydrochloride, paromomycin and neomycin	[61–64]
Dextran 	PSAr 	Formation of giant vesicles to be used as model cells and organelles	[65]
Dextran	PEO	Formation of giant vesicles to be used as model cells and organelles	[65]
Pullulan 	PEO	Formation of giant vesicles to be used as model cells and organelles	[65]
Pullulan	PEtOx 	-	[66]

Abbreviations : PEG poly(ethylene glycol) ; mPEG monomethoxy-poly(ethylene glycol).

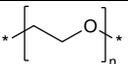
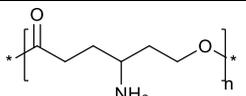
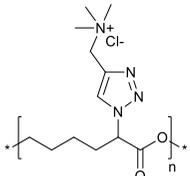
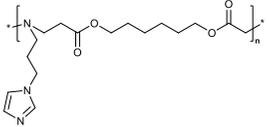
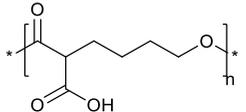
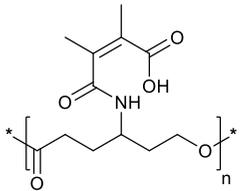
Table1 (continued polypeptide-based degradable DHBCs): degradable double hydrophilic block copolymers and their biomedical applications (chemical structures are only provided once for each block).

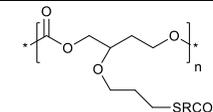
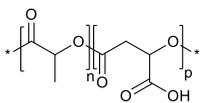
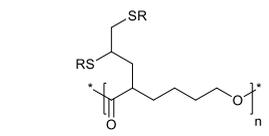
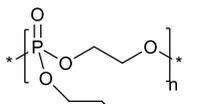
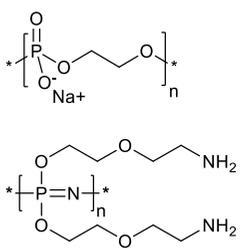
Non-ionic blocks	Ionic blocks	Applications	Ref.	
PEtOx		PSAr	-	[70]
PEtOx		PGlu	SN38-conjugate with antitumoral activity	[71,72]
PEG		PGlu and PAsp	Crystallization of CaCO ₃ and BaSO ₄	[73]
PEG		PAspED	Encapsulation of equine heart CytC	[74]
PEG		PAspED	Transfection of plasmid DNA	[75]
mPEG		PPLG-MSA	Encapsulation of DOX	[76]
PEG		PLL	Encapsulation of phthalocyanine zinc for photodynamic therapy	[79]

PEG	PGlu	Encapsulation of antibacterial peptide MSI-78	[82]
PEG	PLL	DOTA/Gd ³⁺ conjugate for MR diagnostic	[80,8 1]

Abbreviations : CytC cytochrome C ; DNA deoxyribonucleic acid ; DOTA 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid ; DOX doxorubicin ; Gd³⁺ gadolinium ion ; PAsp poly(aspartic acid) ; PAspED poly(aspartic acid(ethylene diamine)) ; PEtOx, poly(2-ethyl-2-oxazoline) ; PGlu poly(L-glutamic acid) ; PLL poly(lysine) ; PPLG-MSA poly(γ -propargyl-L-glutamate-g-mercaptoposuccinic acid) ; PSar poly(sarcosine) ; SN38 7-Ethyl-10-hydroxy camptothecin.

Table1 (continued polyester-based degradable DHBCs): degradable double hydrophilic block copolymers and their biomedical applications (chemical structures are only provided once for each block).

Solubilizing blocks	Stimuli-responsive blocks	Applications	Ref.
mPEG	 P(γ -NH ₂ -CL)	 -	[93]
PEG	P(α -TMA-CL- <i>co</i> -CL)	 Encapsulation of enoxaparin	[94]
Monoacrylated-PEG	PAE-API	 Encapsulation of albumin	[96]
PEO	P(α -COOHCL- <i>co</i> -CL)	 -	[90]
mPEG	P(CL- <i>co</i> -DCL)	 Encapsulation of DOX	[97]

mPEG	PATMC- <i>g</i> -SRCOOH	 $R = \text{CH}_2, (\text{CH}_2)_2, (\text{CH}_2)_{10}, \text{CH}(\text{COOH})\text{CH}_2$	Encapsulation of DOX	[98]
PEG	P(LA- <i>co</i> -MAL)		Encapsulation of DOX	[99]
mPEG	P(CL- <i>co</i> -CL(COOH) ₂) and P(CL- <i>co</i> -CL(NH ₂) ₂)	 $R = \text{CH}(\text{COOH})\text{CH}_2\text{COOH}$ or $(\text{C}_4\text{H}_4\text{N}_2)\text{-4,5-(NH}_2\text{)-6-OH}$	Encapsulation of DOX	[101]
PEO	PBYP ⁺ COOH and PPDO ⁻	 $R = \text{CH}_2\text{CH}_2\text{COOH}$	Templating agent lysozyme-loaded CaCO ₃ particles	[80, 81]
FA-PEG	PAEP		Transfection of plasmid DNA	[106]

Abbreviations : DNA deoxyribonucleic acid ; FA folate ; PAE-API poly(β -aminoester)-1-(3-aminopropyl)imidazole ; PAEP poly(2-(2-aminoethoxy)ethoxy)phosphazene ; PATMC, poly(5-allyloxytrimethylethylenecarbonate) ; PBYP⁺COOH poly(phosphotriester) ; PCCL-*b*-PPIL poly(6-acetoxyl- ϵ -caprolactone)-*b*-poly(4-N-piperilactone) ; P(CL-*co*-CL(COOH)₂) poly(ϵ -caprolactone-*co*- α -propane-2,3-dimercaptosuccinic acid- ϵ -caprolactone) ; P(CL-*co*-CL(NH₂)₂) poly(ϵ -caprolactone-*co*- α -propane-2,3-dimercapto-4,5-diamino-6-hydroxy-pyrimidine- ϵ -caprolactone) ; P(CL-*co*-DCL) poly(ϵ -caprolactone-*co*- γ -dimethyl maleamidic acid- ϵ -caprolactone) ; P(α -COOHCL-*co*-CL) Poly(α -carboxyl- ϵ -caprolactone-*co*- ϵ -caprolactone) ; P(LA-*co*-MAL) Poly(lactic acid-*co*-malic acid) ; P(γ -NH₂-CL) Poly(γ -amino- ϵ -caprolactone) ; PPDO⁻ anionic poly(phosphodiester) ; P(α -TMA-CL-*co*-CL) Poly(trimethyl ammonium ϵ -caprolactone-*co*- ϵ -caprolactone) ;

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