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Bioinspired Polymer Vesicles Based on Hydrophilically Modified Polybutadienes

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Bioinspired polymers are raising more and more attention as advanced materials for key applications in materials science or biomedicine. Especially interesting are the so-called “biohybrids” or “molecular chimeras”,[1] which are polymers being made of biological (peptide, sugar, or nucleic acid) and synthetic parts. Polymers with main-chain biological segments can nowadays be synthesized by design in high quality[2−4]—but often in low quantity. Polymers with pendent biofunctionalities, on the other hand, are easily available through chemical modification, basically without limitation in quantity. The most promising strategies involve “click chemistry”, i.e., Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition of azides onto alkyne side chain polymers,[5] or free-radical addition of mercaptans onto an alkene side chain polymers.[6,7]

The latter approach enables one to generate a platform of “hydrophilically modified polybutadienes” from readily available starting materials. Such hydrophilically modified polybutadienes are random copolymers being, as can be recognized by the chemical structure in Figure 1, closely related to the polymeric amphiphiles introduced by Ringsdorf et al.[8] (also compare to amphiphilic homopolymers,[9] polysoaps, and hydrophobically modified polymers[10]). As such, they should be useable for the production of functional polymer colloids by design, especially for that of bioinspired polymer vesicles or membranes—a field so far being reserved to block copolymers.[11,12]

Four polymeric amphiphiles and biohybrids were produced by free-radical additions of ω-functional mercaptans onto a 1,2-polybutadiene with a number-average of 40 repeat units (see the list in Table 1). Details of synthetic procedures and analytics can be found elsewhere[13] and in the Supporting Information.

The weak polyelectrolytes **1**−**3** could be dispersed in water (polymer concentration ∼0.1 wt %) at room temperature in a certain range of pH, as monitored by eye (clear solution) and by measurement of the light scattering intensity at an angle of 90° (Figure 2a). Flocculation or precipitation of polymer was recognized by a steep increase of turbidity and scattering intensity. The polyanion **1** readily dispersed in neutral to basic media when acid residues were in carboxylate form. The polymer started to precipitate when the pH was decreased (using 0.1 N HCl) to a value of 6.0 or lower. The polymer redispersed upon addition of 0.1 N NaOH. The scattering intensity was the same as at the beginning of the titration cycle, indicating that the process was fully reversible. Accordant observations were made for the polycation **2**, which was disperable in neutral to acidic media (→ ammonium form) and started to precipitate sharply at pH 8.2. The poly(amino acid) **3** dispersed in very acidic and in basic media; precipitation occurred around the isoelectric point (which for methionine is at pH 5.74) between pH 2.3 and ∼9.

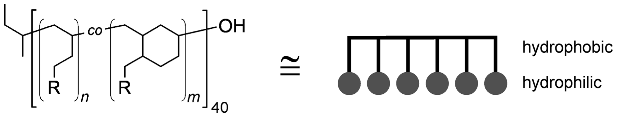
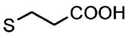
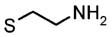
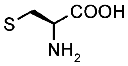
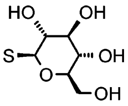


Figure 1. General chemical structure of the hydrophilically modified 1,2-polybutadienes prepared (R: see Table 1) and idealized sketch of a polymeric amphiphile.

Table 1. List of Hydrophilically Modified Polybutadienes (See Chemical Structure in Figure 1)

Sample	R	f[a]	n[b]	m[b]	w_{hydro}[c]
1		0.91	0.82	0.09	0.27
2		0.76	0.52	0.24	0.11
3		0.71	0.40	0.30	0.31
4		0.55[d]	0.10	0.45	0.55

[a] Degree of functionalization, determined by elemental analysis.[13] [b] Fractions of comonomer units: $n = 2f - 1$, $m = (1 - n)/2$. [c] Weight fraction of hydrophilic groups. [d] Sample contains traces of double bonds (^{1}H NMR).

Evidently, **1**−**3** responded to changes in pH as would be expected for weak polyelectrolytes. The peculiarity is that the copolymer chains form aggregates rather than dissolve on a molecular level. Dynamic light scattering analyses (DLS, scattering angle 90°) of 0.3 wt % solutions of **1** and **3** in 0.1 N NaOH and of **2** and **3** in 0.1 N HCl showed the presence of aggregates with apparent hydrodynamic radii of $R_h^{app} \sim 170$ nm (**1** and **2**) and $R_h^{app} \sim 130/120$ nm (**3**, low/high pH) (Figure 2b). Aggregates with $R_h^{app} \sim 130$ nm were also observed for the nonionic glucose-modified sample **4**, directly dissolved in water at a concentration of 0.1 wt % (Figure 2c).

Small-angle X-ray scattering (SAXS) indicated the presence of vesicles in 5 wt % solutions of **1**−**3** (Figure 3a). (Samples were directly dissolved in water, and then the pH of the solution was adjusted to either pH 7 (**1** and **2**) or pH 11 (**3**).) The asymptotes of scattering curves at low scattering vectors ($s = 2/\lambda \sin \Theta$) obey the characteristic $I(s) \propto s^{-2}$ (I: scattering intensity). An additional local maximum arising at $s \sim 0.13$ nm^{-1} suggests that the membrane has a multilamellar structure with a lamellar spacing of about 7 nm (Figure 3c). Evidently, the layers formed by the polymeric amphiphiles **1**−**3** are about as thick as a bilayered membrane of a liposome (∼5 nm). Note that the weight fractions of hydrophilic units of **1**−**3** ($w_{hydro} = 0.11-0.31$, Table 1) are smaller than or similar to that of, for instance, 1-palmityl-2-oleyl-phosphatidylcholine ($w_{hydro} = 0.24$) occurring in the membranes of higher organisms.[14]

The nonionic sample **4** instead self-assembled into unilamellar vesicles (Figure 3c), as suggested by SAXS (10 wt % solution) and transmission electron microscopy (TEM) (see Figure 3a,b). SAXS data could be reasonably analyzed on the basis of a model

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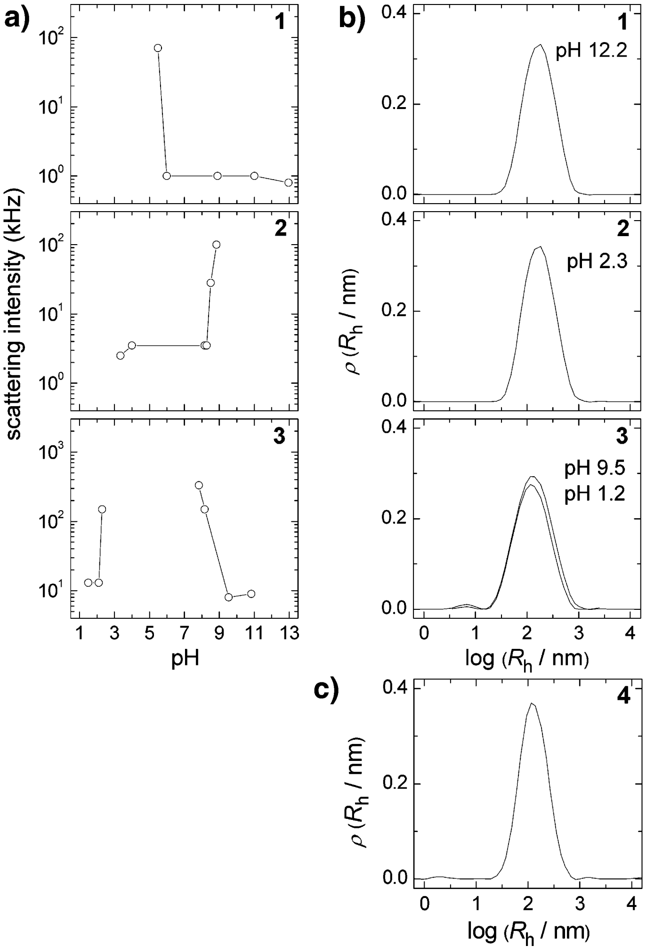

Figure 2. (a) Results of light scattering analysis of dilute aqueous solution of **1**−**3** (top to bottom) in dependence of pH; lines are to guide the eye. (b) Size distributions of aggregates (DLS) in 0.3 wt % aqueous solutions of **1**−**3** at given pH. (c) Size distribution of aggregates (DLS) in 0.1 wt % aqueous solution of **4**.

of vesicles having a thin membrane of constant density, as described by Förster and Burger[15] (see Supporting Information); the thickness of the membrane is <4 nm. According to static light scattering (SLS) and DLS (measurements were done with four samples containing 0.025−0.1 wt % polymer at scattering angles between 12° and 145°), the radius of gyration is $R_{g,0} = 310 \pm 10$ nm and $R_{h,0} = 265 \pm 20$ nm, respectively. The value of the characteristic ratio $R_{g,0}/R_{h,0}$ is close to unity, as expected for vesicles.[16] It is noteworthy that the discrepancy between values of R_h^{app} and $R_{h,0}$ is due to an angular dependency of DLS measurements (see Supporting Information), owing to effects of polydispersity and softness of particles. The mass coverage of the vesicle shell or the thickness of the vesicle membrane could not be determined by SLS because the mass content of vesicles was considerably lower than the total mass concentration of polymer (see the signal of unimers at $\log(R_h/\mathrm{nm}) \sim 0.3$ in Figure 2c and consider the intensity weighting in scattering experiments).

On the basis of SAXS results, the membrane formed by **4** is as thin as that of a liposome and is significantly thinner than that of a block copolymer vesicle (>10 nm).[11] Interestingly, vesicles are formed despite of the high value of $w_{hydro} = 0.55$ (Table 1); phospholipids are usually less hydrophilic, and polybutadiene-*block*-poly(ethylene oxide)s of such composition would have assembled into spherical or cylindrical micelles.[17] Owing to the "comb-shaped" molecular structure of **4** (and also **1**−**3**), polymer backbones must not be oriented orthogonally but parallel to the interface (see the illustration in Figure 3c). Whether the membrane has a bilayered or an interdigitated structure, however, cannot be judged.

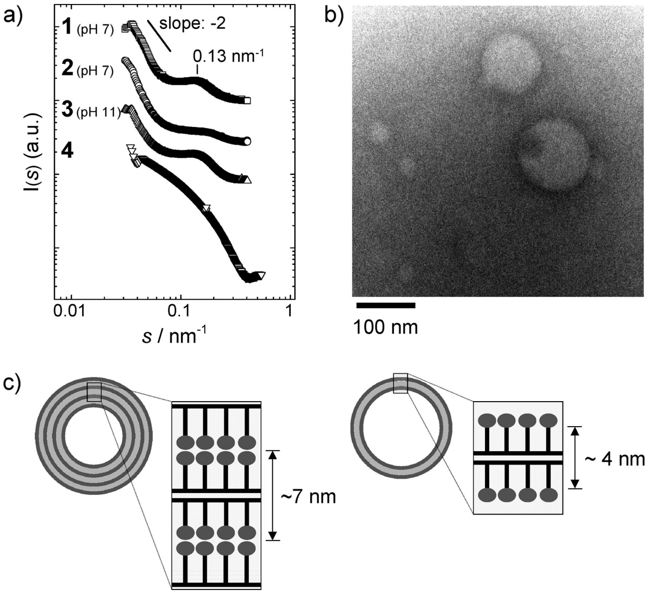

Figure 3. (a) SAXS curves measured for solutions of **1**−**3** (5 wt %) and **4** (10 wt %) in water. (b) TEM image of vesicles as formed in a 0.5 wt % solution of **4** in water (specimen stained in OsO_4 vapor). (c) Schematic illustration of the structure of multilamellar vesicles **1**−**3** (left) and unilamellar vesicles **4** (right) with a bilayered membrane (interdigitated structures not shown).

In summary, we applied free-radical addition of mercaptans to produce a series of hydrophilically modified polybutadienes, including stimulus-responsive materials and biohybrids, which can be considered as polymeric amphiphiles. As such, they can self-assemble into unilamellar or multilamellar vesicles through direct dissolution in water like lipids do.[8] Because of the similarity to liposomes, the biohybrid systems in particular could readily be used as model membranes to study biological recognition processes or for biomedical applications (drug carriers).[3,12]

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Supporting Information Available: Descriptions of experimental procedures and of analytical methods and instrumentation as well as extended discussion of scattering data. This material is available free of charge via the Internet at http://pubs.acs.org.

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