Polymersomes

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Light-Switchable Vesicles from Liquid-Crystalline Homopolymer–Surfactant Complexes**

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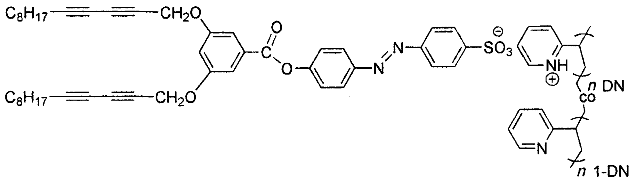
Vesicles are supramolecular assemblies of natural or synthetic amphiphilic molecules that enclose a volume with a thin membrane. In living cells low-molecular-weight phospholipids form vesicles (often termed liposomes) that play a central role in compartmentalization functions, nutrient transportation, and DNA protection.[1] Vesicles attract growing interest because of their various applications ranging from cosmetics to drug delivery.[2] Polymer vesicles, or polymersomes, formed by high-molecular-weight compounds, are significantly more stable to lysis by surfactants than the ones formed from low-molecular-weight amphiphiles, while preserving low immunogenicity.[3] Polymersomes are generally fabricated from amphiphilic linear block copolymers,[4] hyperbranched polymers,[5] and dendrimers,[6] or by polymerization of preformed low-molecular-weight vesicles.[7]

Complexation of polymer chains by low-molecular-weight amphiphiles through noncovalent interactions is a common tool in the design of nanostructured macromolecular materials.[8] Most frequently, lamellar phases are formed, where layers of ligands and polymer backbones alternate. It is logical to assume that the complexes are capable to form vesicles such as lipid–DNA complexes, which self-assemble in a multilamellar structure ($L_{\alpha}C$) consisting of alternating DNA and lipid layers.[9] However, in the case of synthetic polymers the vesicle formation was reported so far only for the complexes of block ionomers with oppositely charged surfactants.[10] The stoichiometric homopolymer complexes are generally not soluble in water, whereas the nonstoichiometric complexes are known to form "mixed micelles" consisting of clusters of hydrophobic surfactant chains surrounded by polar polyelectrolyte backbones.[11] There is, yet, a unique and versatile approach for the synthesis of supramolecular complexes by neutralization of a polybase with amphiphilic acid molecules.[12] It allows for a facile control of the degree of neutralization (DN), that is, the degree of substitution. The main question to be addressed here is whether this type of system is prone to spontaneous curvature of the smectic layers to eventually form vesicles. The possibility of vesicle formation from polybase–amphiphilic acid molecules can pave the way to fabrication of a new type of polymersomes.

In this work, we prepared complexes of poly(2-vinylpyridine) (P2VP) with an amphiphilic sulfonic acid, 4′-[3,5-di(trideca-2,4-diynyloxyl)]azobenzene-4-sulfonic acid (Scheme 1), and studied their self-assembly in bulk and in water suspensions. The presence of an azo group can render the system photoswitchable,[13] whereas the diacetylene groups can be used to further stabilize the self-assembled structure by UV-polymerization.[7a,b,14]



Scheme 1. Chemical structure of complexes of P2VP and 4′-[3,5-di(trideca-2,4-diynyloxyl)]azobenzene-4-sulfonic acid; DN = degree of neutralization.

According to small-angle X-ray scattering (SAXS) data, the supramolecular complexes corresponding to different DN values, namely 25, 50, and 100 %, exhibit lamellar structures. This is concluded from the set of characteristic h00 reflections visible in the medium-angle range (see Figure S1 in the Supporting Information). The layer thickness calculated using several orders of the 100 reflection equals 6.02 nm and is largely independent from the DN for the values above 25 %.

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Importantly, for a DN value of 25 % the 100 reflection is shifted towards low angles ($d = 7.78$ nm) and noticeably broadened. This fact can be attributed to the increased concentration of structural defects in this system containing an excess of P2VP chains. Indeed, the structure of this complex marks a transition from amorphous P2VP to a liquid-crystalline (LC) polymer complex, and is at the verge of the LC phase formation. The length of the sulfonic acid molecule in the assumption of the fully stretched alkyl chain conformation is about 3.6 nm, so the smectic layer can be viewed as a bilayer of the ligand with partially interdigitating alkyl tails and the head groups linked to the P2VP backbones.

Different ways of vesicle preparation have been tried. For example, we were not able to directly disperse the complexes into water through thin-film hydration. However, upon injection of tetrahydrofuran (THF) solutions of the complexes with DN values of 25 and 50 % into a large amount of water, stable turbid dispersions were obtained. The complex with a DN of 100 % seems to be too hydrophobic to be dispersed in water in this way. For the complex with a DN of 25 % the dynamic light scattering (DLS) measurements showed a bimodal distribution of dispersed particles with two median diameters of 2.1 μm and 42 nm (Figure S2). Using optical microscopy the formation of spherical particles with an average diameter of (4.5 ± 1.4) μm was observed (Figure S3). At the same time, cryo-field emission scanning electron microscopy (FESEM) micrographs confirmed the formation of microsized vesicles with a thin membrane (Figure 1). The membrane thickness was estimated to be less than 20 nm, so it possibly comprises one or just a few smectic layers.

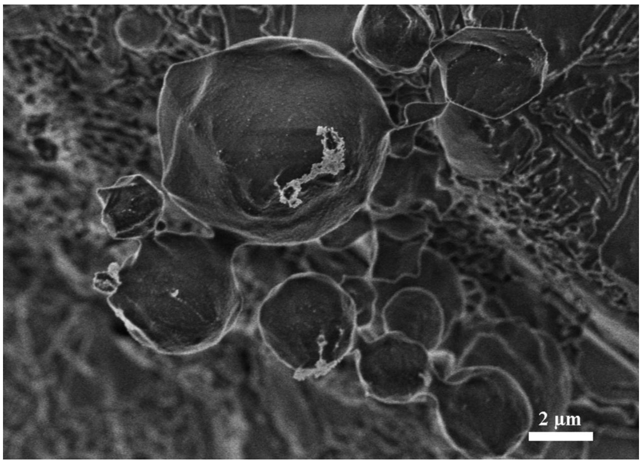

Figure 1. Cryo-FESEM micrographs of microsized vesicles formed by the complex with a DN value of 25 %.

The nanoparticles with the size below 500 nm were visualized by cryo-TEM. Figure 2 shows the internal structure of these particles which consist of concentric alternating layers; therefore, they can be assigned to onionlike multilamellar vesicles. The characteristic spacing of 6.7 nm was derived from the one-dimensional power spectral density function computed from the micrographs (see the inset in Figure 2).

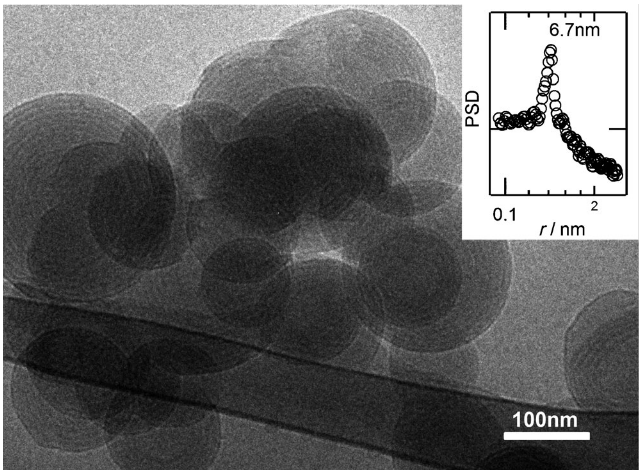

Figure 2. A typical cryo-TEM micrograph of the multilamellar vesicles formed by the complex with a DN value of 25 % together with the corresponding 1D power spectral density function (PSD, inset). The r stands for the reciprocal distance in nm^{-1}.

The vesicle structure was also addressed by SAXS which revealed a strong interference maximum at 6.55 nm (Figure 3 a). This value is in good agreement with the d spacing in the cryo-TEM micrographs. In addition, it is rather close to the smectic spacing of the complex in the bulk (see Figure S1).

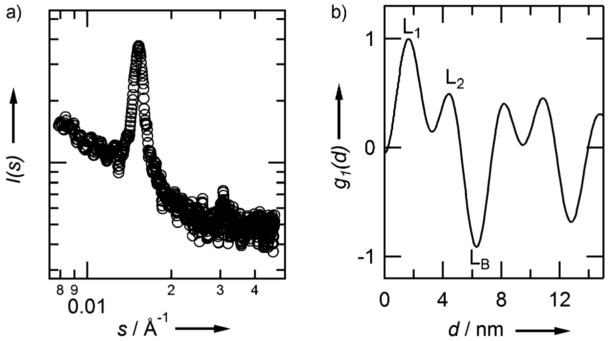

Figure 3. a) SAXS curve measured for water suspension of vesicles formed by the complex with DN = 25 % and b) the corresponding interface distribution function $g_1(d)$. The distances L_1 and L_2 indicated on the right panel stand for the thickness of the two constitutive sublayers of the smectic layer with a total thickness L_B.

The only minor difference between the SAXS patterns of the complex in water dispersions and in the bulk is that the vesicles exhibit a faint second order of the main interference maximum whereas this peak is not observed for the bulk sample. This difference can be accounted for by slight variations in the relative thickness of the constitutive sublayers of the smectic layer because of a limited swelling of the P2VP-rich phase in water.

A more detailed analysis of the vesicle microstructure was performed using a 1D interface distribution function (IDF)[15] (Figure 3 b). The IDF of the vesicles exhibits a shape typical of a two-phase lamellar system. The first minimum at 6.45 nm corresponds to the nearest-neighbor distance of the layered stack, being virtually identical to the value obtained from cryo-TEM. The maximum at 4.63 nm can be attributed to the most probable thickness of the sublayer containing the P2VP backbones and complexed sulfonic acid groups, while the maximum at 1.81 nm corresponds to the adjacent alkyl-chain layer. The sharp first two positive peaks indicate that the sublayers have a relatively narrow distribution of thickness.

Based on the SAXS data, a packing model for the complex of a DN value of 25 % in the vesicles has been proposed (Figure 4). The amphiphilic sulfonic acid molecules form bilayers and the P2VP backbones are sandwiched between them. The shell of the microsized vesicles most probably consists of only one or a few such bilayers. According to cryo-FESEM measurements, the ligand molecules alone do not form particles in water. The complex corresponding to a DN value of 50 % can be dispersed in water and particles of about 200 nm in size were formed as shown by DLS and cryo-FESEM, however, no formation of vesicles was evidenced. Therefore, it seems that the vesicles can only be formed at low DN values such as 25 %. This fact can be explained as follows. On the one hand, the excess of P2VP favors the formation of chain entanglements and thereby improves the mechanical properties of the vesicles. Importantly, the polymer chains here are oriented mainly parallel to the vesicle wall. This orientation makes the system more stable than conventional polymersomes where the backbones are perpendicular to the layers.[3b] Indeed, one can see in Figure 2 that the system tolerates a high curvature of the walls: the radius of the layer can be as small as 50 nm. On the other hand, at low DN values the sulfonic acid molecules have a high degree of freedom and are able to slide along the P2VP chains to adapt to the local curvature and environment. This adjustment mechanism would become impeded at higher DN values. The minimum DN value at which the vesicles are formed likely reflects the transition point from an amorphous P2VP-rich system to a LC polymer complex. It is also important to mention that although P2VP is hydrophilic, it is insoluble in water at neutral pH. Therefore, no dissociation of the complex is expected under these conditions. Finally because of a large amount of free binding sites, this system offers a possibility to incorporate different functional molecules into the vesicle structure.

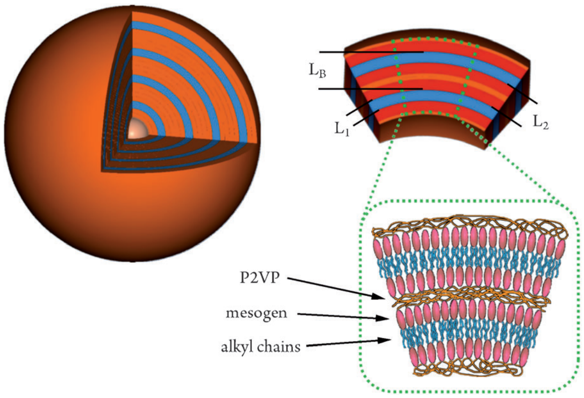

Figure 4. Proposed molecular model of the vesicles formed by the complex with a DN value of 25 %.

Since the vesicles contain photosensitive azo groups, it was instructive to follow the impact of the *trans–cis* photoisomerization on the vesicle structure. To this end, the vesicles were irradiated by UV light (254 nm), and were simultaneously studied by UV/Vis spectroscopy (Figure 5, top panel). The intensity of the absorption band at 350 nm corresponding to the π–π* transition of the azo group gradually decreased with exposure time, indicating a *trans–cis* transition. After 90 min of irradiation, the complex was precipitated (see the inset in Figure 5, top panel).

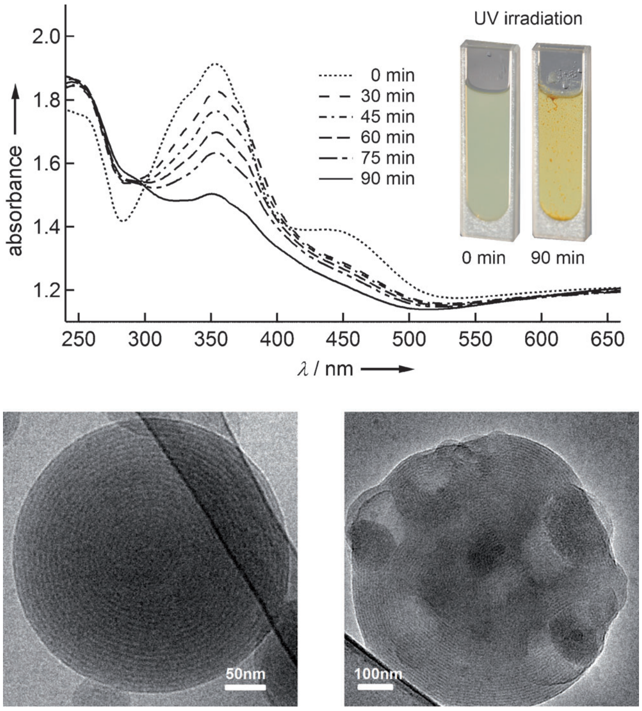

Figure 5. Top panel: UV/Vis spectra of the complex with a DN value of 25 % in water upon UV irradiation. The concentration of the complex is 0.1 mg mL^{-1}. Bottom panel: Cryo-TEM micrographs of the multilayer vesicles before (left) and after (right) UV irradiation for 45 min.

The impact of UV light on the morphology of the vesicles was also examined by cryo-TEM (Figure 5, bottom). Upon UV irradiation, we can observe the appearance of deformed vesicles with locally disrupted regions. Importantly, the smectic layering in these regions has completely disappeared. We used grazing-incidence SAXS (GISAXS) to investigate the structure of the thin films prepared by a drop-casting method from suspensions of vesicle before and after UV irradiation (Figure S4). It can be seen that the smectic order in the complexes vanishes after 90 min of UV exposure. Meanwhile, the disappearance of the vesicle particles is evidenced by DLS measurements. The *cis* isomer has a bent shape with a low anisometry, which can perturb the LC order in the vesicles.[16] Therefore, it can be concluded that the collapse of the vesicles results from the UV-induced isomerization. During this process the vesicles lose their fine microphaseseparated layered structure and transform to isotropic droplets that are no more stabilized by the outer hydrophilic shell of P2VP. It is noteworthy that, during UV irradiation no absorption bands in the range of 500 to 600 nm were detected, indicating that no polymerization of the diacetylene groups has occurred. This is possibly due to the progressive disruption of the smectic phase during UV irradiation.

In conclusion, we demonstrate for the first time that a LC complex of a polybase and amphiphilic sulfonic acid with a DN value of 25 % forms unilamellar microsized as well as onionlike multilamellar nanosized vesicles. The structure of the complex in the vesicles was found to be similar to that in the bulk, where the polymer backbones are sandwiched between the bilayers formed by the ligand molecules. In contrast to conventional polymer vesicles, the polymer chains in this system are mainly parallel to the vesicle surface, contributing to their mechanical stability. A large amount of remaining free binding sites makes it possible to incorporate different functional molecules into the vesicles. Furthermore, a collapse of the vesicles can be induced by UV irradiation because of the *trans–cis* transition of the azo groups, which leads to the isotropization of the layered structure. This feature could make this system promising for controlled delivery applications.

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[1] “Structure and Dynamics of Membranes—from Cells to Vesicles”: R. Lipowsky, E. Sackmann, *Handbook of Biological Physics*, *Vol. 1*, Amsterdam, Elsevier, **1995**.

[2] a) D. D. Lasic, D. Papahadjopoulos, *Medical applications of liposomes*, Elsevier Science, Dordrecht, **1998**; b) V. P. Torchilin, V. Weissig, *Liposomes: A practical approach*, Oxford University Press, Oxford, **2003**.

[3] a) M. Antonietti, S. Förster, *Adv.Mater.* **2003**, *15*, 1323 – 1333; b) D. E. Discher, A. Eisenberg, *Science* **2002**, *297*, 967 – 973; c) P. Tanner, P. Baumann, R. Enea, O. Onaca, C. Palivan, W. Meier, *Acc. Chem. Res.* **2011**, *44*, 1039 – 1049.

[4] a) K. Kita-Tokarczyk, J. Grumelard, T. Haefele, W. Meier, *Polymer* **2005**, *46*, 3540 – 3563; b) K. B. Aubrecht, R. B. Grubbs, *J. Polym. Sci. Part A* **2005**, *43*, 5156 – 5167.

[5] Y. Zhou, W. Huang, J. Liu, X. Zhu, D. Yan, *Adv. Mater.* **2010**, *22*, 4567 – 4590.

[6] a) V. Percec, D. A. Wilson, P. Leowanawat, C. J. Wilson, A. D. Hughes, M. S. Kaucher, D. A. Hammer, D. H. Levine, A. J. Kim, F. S. Bates, *Science* **2010**, *328*, 1009 – 1014; b) J. Van Hest, D. Delnoye, M. Baars, M. Van Genderen, E. Meijer, *Science* **1995**, *268*, 1592 – 1595.

[7] a) B. Hupfer, H. Ringsdorf, H. Schupp, *Chem. Phys. Lipids* **1983**, *33*, 355 – 374; b) H. Ringsdorf, B. Schlarb, J. Venzmer, *Angew. Chem.* **1988**, *100*, 117 – 162; *Angew. Chem. Int. Ed. Engl.* **1988**, *27*, 113 – 158; c) S. Liu, D. F. O’Brien, *J. Am. Chem. Soc.* **2002**, *124*, 6037 – 6042.

[8] a) C. F. J. Faul, M. Antonietti, *Adv. Mater.* **2003**, *15*, 673 – 683; b) O. Ikkala, G. ten Brinke, *Science* **2002**, *295*, 2407 – 2409; c) W. J. MacKnight, E. A. Ponomarenko, D. A. Tirrell, *Acc. Chem. Res.* **1998**, *31*, 781 – 788; d) C. K. Ober, G. Wegner, *Adv. Mater.* **1997**, *9*, 17 – 31.

[9] C. R. Safinya, *Curr. Opin. Struct. Biol.* **2001**, *11*, 440 – 448.

[10] A. V. Kabanov, T. K. Bronich, V. A. Kabanov, K. Yu, A. Eisenberg, *J. Am. Chem. Soc.* **1998**, *120*, 9941 – 9942.

[11] E. Dickinson, E. Goddard, K. Ananthapadmanabhan, *Interactions of Surfactants with Polymers and Proteins*, CRC, Boca Raton, **1993**, p. 295.

[12] a) O. Ikkala, J. Ruokolainen, G. Ten Brinke, M. Torkkeli, R. Serimaa, *Macromolecules* **1995**, *28*, 7088 – 7094; b) X. Zhu, U. Beginn, M. Möller, R. I. Gearba, D. V. Anokhin, D. A. Ivanov, *J. Am. Chem. Soc.* **2006**, *128*, 16928 – 16937.

[13] a) E. Mabrouk, D. Cuvelier, F. Brochard-Wyart, P. Nassoy, M. H. Li, *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 7294; b) P. Han, N. Ma, H. Ren, H. Xu, Z. Li, Z. Wang, X. Zhang, *Langmuir* **2010**, *26*, 14414 – 14418.

[14] a) G. Wegner, *Z. Naturforsch. B* **1969**, *24*, 824 – 832; b) D. H. Charych, J. O. Nagy, W. Spevak, M. D. Bednarski, *Science* **1993**, *261*, 585 – 588; c) W. Spevak, J. O. Nagy, D. H. Charych, *Adv. Mater.* **1995**, *7*, 85 – 89.

[15] a) W. Ruland, *Colloid Polym. Sci.* **1977**, *255*, 417 – 427; b) D. A. Ivanov, G. Bar, M. Dosie‘re, M. H. J. Koch, *Macromolecules* **2008**, *41*, 9224 – 9233; c) M. Rosenthal, D. V. Anokhin, M. Defaux, G. Portale, D. A. Ivanov, *J. Appl. Crystallogr.* **2011**, *44*, 805 – 811.

[16] A. Natansohn, P. Rochon, *Chem. Rev.* **2002**, *102*, 4139 – 4176.