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Payload release by liposome burst: Thermal collapse of microgels induces satellite destruction

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Abstract

We present a smart liposome carrier system for stimulated release, consisting of cationic, thermo-responsive microgels. At low temperature, the swollen microgels adsorb about 200 anionic liposomes, 50 nm in diameter, per microgel. When heated from 39 °C to 41 °C, the microgel–liposome complex particles collapse from approx. 370 nm down to approx. 270 nm. Upon the thermo-induced collapse, the adsorbed liposome satellite layer is squeezed until the initially spherical liposomes explode and release their payload (antitumor drug doxorubicin) into the surrounding. This burst release mechanism, taking place over a narrow temperature range, is newly reported and of possible biomedical importance.

Key words: Liposomes; Nanocontainer; Microgel; Controlled release; Complexes; Thermo-sensitivity

Among the wide variety of medicinal containers for encapsulation and release of drugs, spherical bilayer vesicles composed of lipid molecules (liposomes) are of particular interest. Due to their unique structure, liposomes are able to entrap both hydrophilic and hydrophobic guests: the former dissolve within the internal water pool, whereas the latter enter the lipid bilayer.[1–3] Being constructed from native and synthetic lipids, liposomes are biocompatible nano-sized objects; modification of liposomes by low-toxic polysaccharides or poly(ethylene oxide)s allows an enhanced circulation time and bioavailability of the drugs.[1–5]

Recently, a method has been described of anionic liposome adsorption on the surface of colloidal particles covered by grafted polycationic chains ("spherical polycationic brushes") that maintained liposome integrity.[6] This allows the concentration of dozens of liposomes within a rather small volume while adsorbed liposomes can be loaded by various compounds with a controllable ratio.[7] The use of liposomes with embedded pH-sensitive amphiphiles leads to multi-liposomal constructs releasing their content upon acidification.[8] However incorporation of synthetic amphiphiles into the liposomal membrane can influence the liposome toxicity and the stability of their complexes with colloidal adsorbents. Therefore, it would be alluring to have stimulus-sensitive multi-liposomal constructs without artificial amphiphiles.

In the present communication we describe electrostatic adsorption of conventional anionic liposomes on the surface of thermo-sensitive microgel (μG) particles. We demonstrate herein that immobilized liposomes retain their encapsulating power at lower temperature and quickly release their cargo, an antitumor antibiotic doxorubicin (Dox), at higher temperature. These findings make a multi-liposomal container promising for passive targeting" due to selective penetration of 200-400 nm particles in the capillaries of tumors and other inflammation areas.[9,10] The

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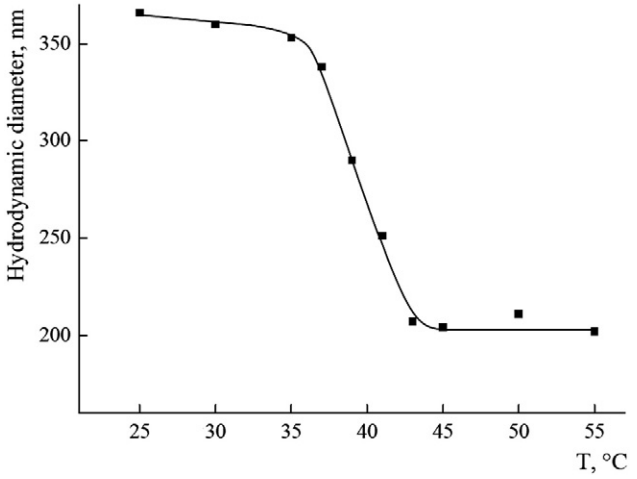

Figure 1. Temperature-dependent hydrodynamic diameter of microgel conc. 0.072 mg/mL; 0.01 M Tris buffer with pH 7.

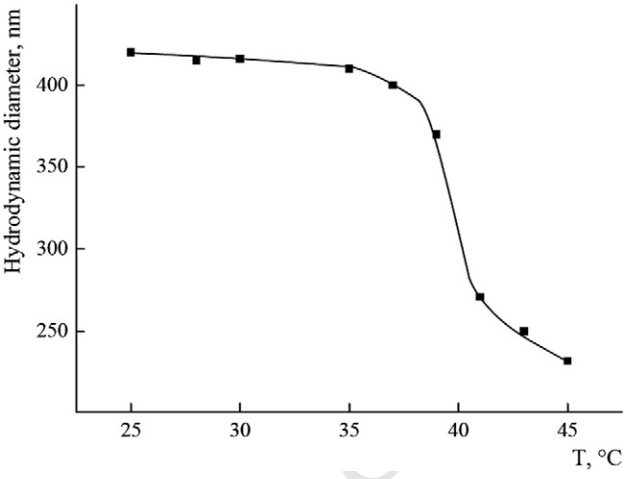

Figure 3. Temperature-dependent hydrodynamic diameter of μG/liposome complexes. EL/PS^{1-} (7:3) liposomes; Microgel conc. 0.072 mg/mL; 0.01 M Tris buffer with pH 7.

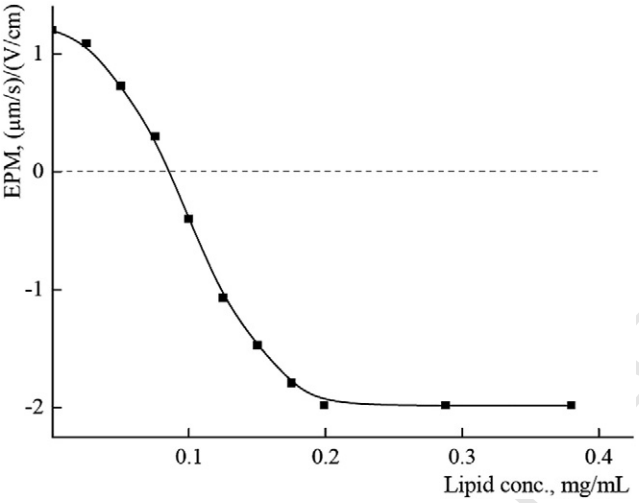

Figure 2. EPM of μG/liposome complexes vs. concentration of added EL/PS^{1-} (7:3) liposomes. Microgel conc. 0.072 mg/mL; 0.01 M Tris buffer with pH 7; 25 °C.

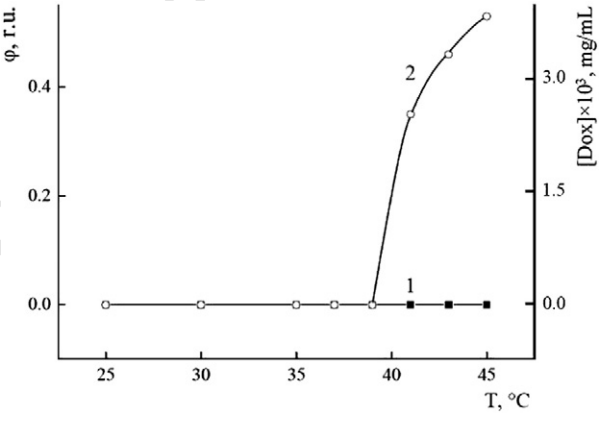

Figure 4. Temperature-dependent relative fluorescence of encapsulated Dox (left) and concentration of released Dox (right). Free EL/PS1-(7:3) liposomes (1) and μG/liposome complex (2). Microgel conc. 0.072 mg/mL; lipid conc. 0.25 mg/mL; encapsulated Dox conc. 0.00725 mg/mL; 0.01 M Tris buffer with pH 7.

total negative charge of the container renders it compatible with other components of biological liquids.

The μG particles were synthesized by precipitation polymerization of a mixture composed of dimethylaminopropyl methacrylamide (DMAPMA, 10 mol% or 14 wt%), *N*-isopropylacrylamide (NIPAM, 85 mol%) and a cross-linking agent, *N*,*N*-methylenebisacrylamide (5 mol%) as described previously.[9] The dried microgel sample was swollen in double-distilled water for 3 days at 25 °C. According to dynamic light scattering (DLS), the solution contained μG particles with the mean hydrodynamic diameter of 365 nm and a narrow distribution by size.

Owing to NIPAM groups, the μG particles show thermo-sensitive properties: they are in the swollen state at lower temperature and collapsed at higher temperature; a hydrodynamic diameter of μG particles measured by DLS at different temperatures is presented in Figure 1[11,12] with standard deviations for this and other plots shown in Supplementary S1. The figure reflects a progressive decrease of the diameter in an entire temperature range from 25 to 55 °C (curve 1) with a sharper drop in diameter in between 32 and 43 °C with a mean of 39 °C, which can be referred to as a volume phase transition temperature (Tc) from the swollen to the collapsed state. By specifying different ratios between charged groups and NIPAM groups in water-soluble copolymers and copolymer gels, Tc can be varied within wide limits.

A μG solution in a pH 7 buffer was mixed with a suspension of unilamellar anionic liposomes (ca. 50 nm in diameter) prepared conventionally by sonication and composed of zwitter-ionic egg lecithin (EL) and anionic phosphatidylserine (PS^{1-}) in a molar ratio of 7:3 (see the preparation details in Supplementary S2).

Binding of anionic EL/PS^{1-} liposomes to cationic μG was accompanied by a mutual neutralization of their charges that was