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Interaction of liposomes with silica nanocapsules: from lipid bilayer coating to multi-liposomal composites

Tatiana V. Panova,*[a] Andrey V. Sybachin,[a] Yongliang Zhao[b] and Xiaomin Zhu[c,d]

[a] *Department of Chemistry, M. V. Lomonosov Moscow State University, 119991 Moscow, Russian Federation. E-mail: tvk@genebee.msu.ru*
[b] *Shanghai Dilato Materials Co., Ltd, 200433 Shanghai, P. R. China*
[c] *DWI – Leibniz-Institute for Interactive Materials, 52074 Aachen, Germany*
[d] *Institute for Technical and Macromolecular Chemistry, RWTH Aachen University, 52056 Aachen, Germany*

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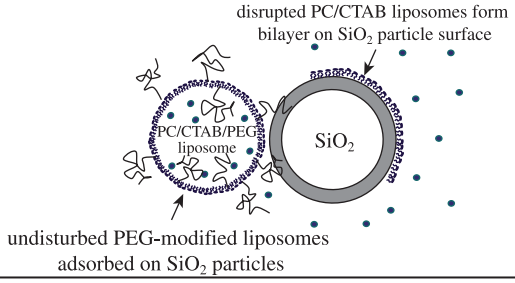
A new delivery system has been developed that combines liposomes and silica nanocapsules. It was found that in the presence of cationic unilamellar liposomes, silica nanocapsules are instantly coated with a lipid bilayer. In contrast, cationic stealth liposomes modified with polyethylene glycol can adsorb on the surface of silica to form multi-liposomal composites in which the liposomes remain intact.



Keywords: liposomes, silica nanocapsules, multi-liposomal composite, lipid bilayer, liposomal container.

Liposomes are promising candidates for biomedical applications as nanocarriers of various bioactive substances.[1–4] Despite the advantages of conventional liposomes, such as great diversity, excellent biocompatibility, ease of preparation and the possibility of encapsulating both hydrophilic and hydrophobic substances, cationic vesicles have a high affinity for negatively charged components of biological media. This interaction leads to the aggregation of cationic liposomes or their unwanted adsorption on non-target objects. This fact limits their use as components of drugs administered intravenously; however, cationic liposomes can be used in nasal formulations or in the field of dental surgery.[5,6] Thus, despite the tremendous progress in liposome research in recent years, challenges remain regarding, *e.g.*, the stability of liposomal preparations and the formation of multi-liposomal conjugates for combination therapy.[7]

Being mechanically strong, chemically inert, non-toxic and biocompatible, silica-based materials have a high potential for biomedical applications.[8] Amorphous silica is 'generally recognized as safe' by the US Food and Drug Administration, evidenced by its everyday use in food additives and vitamin supplements. Furthermore, it has been demonstrated to be biodegradable.[9] The silica nanocapsules (SNs) less than 100 nm in diameter used in this work are gaining increasing attention. This size range offers significant advantages such as reduced reticuloendothelial system uptake, enhanced extravasation into targeted sites, increased cellular uptake and easier penetration through a barrier.[10] Many methods have been developed to synthesize materials of this kind, including hard-core templating,[11] soft templating using microemulsions,[12,13] vesicles,[14] micelles or polymer aggregates[15] and selective etching.[16] SNs have been functionalized with fluorescent dyes or loaded with drugs or inorganic nanoparticles as functional cargos for various biomedical applications.[17] In this work, an attempt was made to use the synergistic effects of liposomes and SNs to create advanced functional delivery systems through the formation of complexes in which silica particles act as adsorption centers for liposomes. Such systems could synergistically combine the advantages of liposomes with those of mesoporous SNs.

First, we estimated the surface charge of SNs.† The charge density of the particles in Tris–HCl buffer (pH 7.4) was measured by titration with the oppositely charged polycation, polyhistidine hydrochloride (PHis).[18] The dependence of the electrophoretic mobility (EPM) of SNs on the PHis concentration is shown in Figure 1.† It is seen that the addition of PHis gradually neutralizes the negative charge of the silica surface. Complete neutralization of the surface charge of SNs was achieved at a PHis concentration (expressed in histidine monomeric units) of 4×10^{-4} mol dm^{-3}; based on these data, the total available negative charge of SNs was 1×10^{-4} mol mg^{-1}. The high surface charge of SNs makes them a promising candidate for the adsorption of cationic liposomes.

The interaction of cationic liposomes with SNs was studied by measuring the change in EPM of SNs dispersion upon the

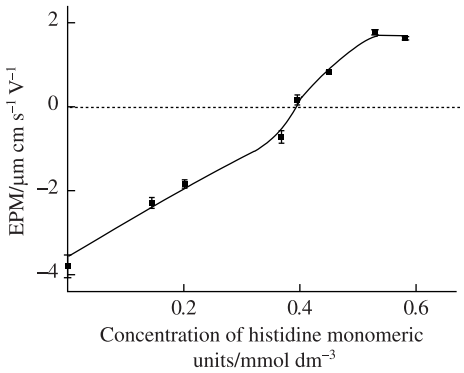


Figure 1 The dependence of EPM of SNs (4 mg cm^{-3}) on the concentration of the PHis polycation.

† For details, see Online Supplementary Materials.

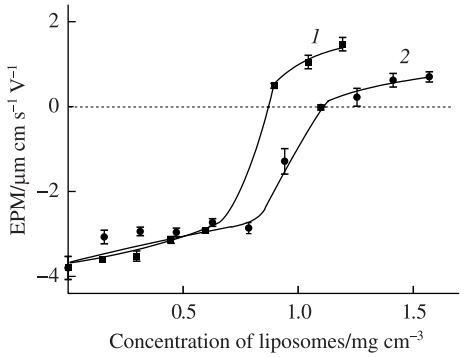


Figure 2 The change in EPM of SNs dispersion upon the addition of (*1*) PC/CTAB and (*2*) PC/CTAB/PEG liposomes. Concentration of SNs was 5 mg cm^{-3}.

addition of liposomes. The cationic liposomes were additionally loaded with NaCl, which made it possible to determine the integrity of the liposomal membrane straightforwardly. When a defect is formed, small ions leak from the inner cavity of liposomes, increasing the conductivity of the solution.[19] A suspension of cationic unilamellar liposomes, characterized by a mean diameter of 80 nm and filled with 1 M NaCl, was prepared by sonication from electrically neutral phosphatidylcholine (PC) and a cationic surfactant, cetyltrimethylammonium bromide (CTAB), the molar fraction of which was 0.1, and added to the aqueous dispersion of SNs.† The EPM value of the SNs dispersion increases to zero upon addition of PC/CTAB liposomes (Figure 2, curve *1*), and the ratio of charged groups of SNs and PC/CTAB liposomes at the neutralization point is about 0.5, which means that only CTAB molecules on the outer leaflet of the liposome are involved in the interaction. A further increase in the concentration of liposomes in the system leads to recharged particles, and after SNs saturation with adsorbed liposomes, the EPM reaches a plateau.

The adsorption of liposomes on rigid flat surfaces or surfaces with large curvature is often accompanied by deformation and disruption of the vesicles.[20] The integrity of the PC/CTAB liposomes in contact with SNs was monitored by measuring conductivity.† The dependence of the relative conductivity ($\Omega - \Omega_0$), where Ω and Ω_0 are the conductivity of the system with and without liposomes, respectively, on the concentration of added liposomes is shown in Figure 3. It can be assumed that increasing the liposome concentration in suspension provides a linear growth of conductivity due to increasing the concentration of charged particles (Figure 3, curve *1*). The addition of liposomes to the SNs suspension results in a much sharper increase in conductivity than that caused only by an increase in the concentration of liposomes (Figure 3, curve *2*). This increase demonstrates that the added vesicles rupture after adsorption on the SNs surface while the payload (NaCl) is released

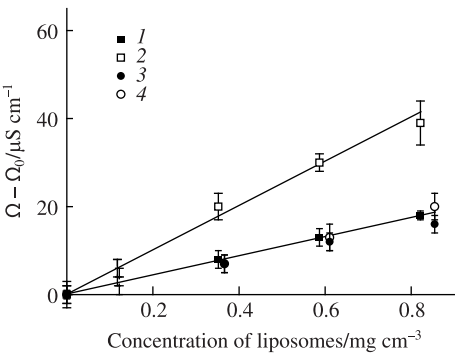


Figure 3 The dependence of relative conductivity of the system on the concentration of added liposomes: (*1*) PC/CTAB liposomes, (*2*) SNs with PC/CTAB liposomes, (*3*) PC/CTAB/PEG liposomes and (*4*) SNs with PC/CTAB/PEG liposomes. SNs concentration was 5 mg cm^{-3}.

from their internal cavities. To detect the presence of intact liposomes in the suspension, we completely disrupted all the vesicles by adding the nonionic surfactant Triton X-100 to the final mixture (0.8 mg cm^{-3}), which led to a slight increase in conductivity from 39 ± 5 to 54 ± 5 μS cm^{-1}. At the same time, for a suspension of individual vesicles, the addition of Triton X-100 leads to a much more significant jump in conductivity from 18 ± 1 to 55 ± 3 μS cm^{-1}. These results indicate that most of the PC/CTAB liposomes were already destroyed after contact with SNs. Additional particle size measurements in the system show the formation of aggregates several microns in size, most likely due to the association of SNs neutralized with cationic surfactants.† Thus, the adsorption of cationic liposomes on the SNs surface due to electrostatic attraction leads to the disruption of liposomes. As previously observed for such systems,[21] the interaction of the head groups of lipid molecules is strong enough to ensure the rupture of liposomes with the formation of a bilayer on the silica surface.

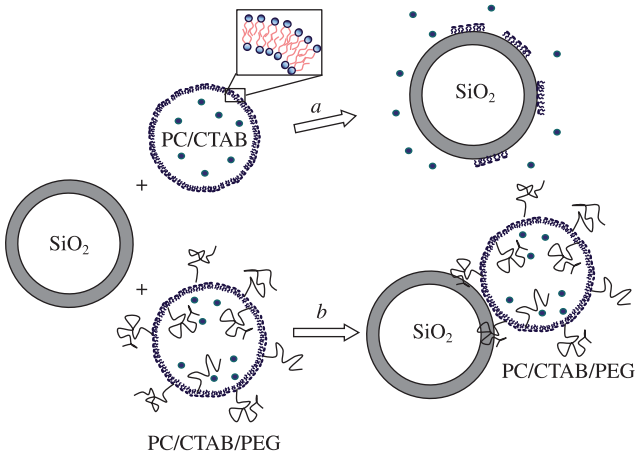
One possible approach to overcoming vesicle fusion and fission is to 'soften' the liposome–surface interaction. It has previously been demonstrated that liposomes can successfully adsorb without rupture on soft surfaces such as polymer micelles or cross-linked microgels.[22,23] We decided to modify liposomes with polyethylene glycol (PEG) chains to obtain so-called stealth liposomes. PEG acts as a 'softening spacer' between the liposomal membrane and the rigid SN surface. This approach is commonly used to mask the surface of particles by preventing adsorption and any unwanted contact with oppositely charged species.[24]

To test this assumption, we prepared a suspension of PEGylated cationic unilamellar liposomes filled with 1 M NaCl from PC, CTAB and Brij 58 (10 mol% CTAB and 20 mol% Brij 58)† and added it to an aqueous dispersion of SNs. The change in the EPM value of the mixture with an increase in the concentration of PC/CTAB/PEG liposomes is shown in Figure 2, curve *2*. The curve profile is similar to that obtained for non-PEGylated PC/CTAB liposomes, indicating the electrostatic nature of the interaction. Thus, incorporating PEG fragments into the liposomal membrane does not change the mechanism of their interaction with SNs.

The integrity of the stealth liposomes in the presence of SNs was investigated by measuring conductivity.[25] When comparing the dependence of conductivity on the concentration of liposomes with and without SNs (Figure 3, curves *3* and *4*), no differences are observed. It should be noted that the relative conductivity for all systems does not change after two hours of observation, which indicates that PEGylated liposomes retain their integrity on the SN surface. The addition of Triton X-100 to the suspension leads to the complete disruption of liposomes and a significant increase in conductivity up to a maximum value of 57 ± 5 μS cm^{-1} for both suspensions. Hence, PEGylation of cationic liposomes allows their electrostatic adsorption on the SN surface without disrupting the integrity of the liposomal membrane. Further confirmation of this was obtained by measuring the hydrodynamic size of SN aggregates with PC/CTAB/PEG liposomes, and the mean hydrodynamic diameter was found to be 240 nm with a PDI of 0.22. This size can be attributed to the sum of the mean diameter of one SN and two mean diameters of PC/CTAB/PEG liposomes.

Based on the experimental data presented above, the following scheme of interaction of cationic liposomes with SNs was proposed (Scheme 1). After adsorption on SNs, binary PC/CTAB liposomes undergo fusion and form a supported lipid bilayer on the SN surface (see Scheme 1, pathway *a*). In contrast, modification of the liposome surface with flexible PEG chains allows the formation of multi-liposomal SN composites in which liposomes retain their integrity (see Scheme 1, pathway *b*).

In conclusion, it was shown that cationic liposomes are instantly disrupted upon contact with the surface of silica nanocapsules,



Scheme 1 The interaction of SN with (*a*) PC/CTAB liposomes and (*b*) PC/CTAB liposomes modified with PEG.

forming a bilayer lipid coating. At the same time, stealth cationic liposomes can be adsorbed on a silica nanocapsule, leading to the formation of sufficiently stable multi-liposomal composites, promising for creating nanocontainers with high potential in biomedical applications.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2021.11.020.

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