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Synthetic asymmetric lipid-like organosilanes for liposomal nanohybrid cerasomes toward potential medical applications

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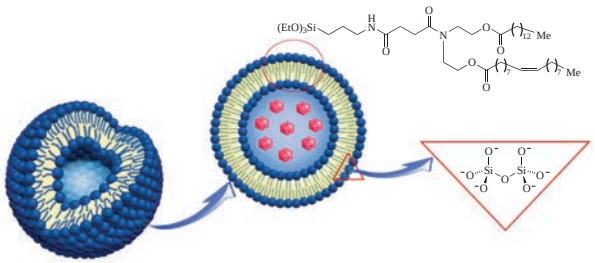
The design and synthesis of lipidic organoalkoxysilanes with different higher fatty acids as hydrophobic acyl chains for the construction of bilayer vesicles called 'cerasomes' were accomplished and their properties, including hydrodynamic diameter, zeta potential and encapsulation efficiency of water-soluble doxorubicin, were determined. Experiments have shown that an asymmetric hydrophobic block makes it possible to obtain cerasomes with acceptable size and stability. At the same time, the drug loading is quite high.



Nowadays, a controlled drug delivery technology represents one of the frontier areas of modern medicine and pharmaceutics due to the great capability to regulate the drug release thus improving the therapeutic efficacy and reducing side effects.[1–4] Liposomes, the well-known class of biocompatible and non-cytotoxic biomimetic materials,[5–7] can minimize the drug uptake by normal cells and enhance the drug accumulation in tumor cells.[8] Nevertheless, the primary drawback hampering the application of liposomes is the general lack of stability under physiological conditions, which often leads to a burst release of the encapsulated drugs causing undesirable side reactions.[9,10]

To solve the problems related to stability, cerasomes were proposed.[11] They are drug delivery systems based on cerasome-forming lipids (CFL), which comprise silicon-containing head groups, hydrophobic alkyl tails, and linkers. They have attracted attention due to the capability of forming bilayer vesicles in aqueous medium, which are coated with a polymeric siloxane network.[12]

Cerasomes are usually produced using proamphiphilic organoalkoxysilanes[13] in a combination of self-assembly and sol–gel processes, so they are organic-inorganic hybrids with precisely designed nanostructures.[14] One of the vesicle characteristics is the ability to encapsulate various types of drugs, including both hydrophilic and hydrophobic molecules (Figure 1). It has been demonstrated that blank cerasomes and cerasomes loaded with doxorubicin exhibited a remarkably high stability towards surfactant solubilization, long-term storage, and other factors that are prone to destabilize conventional liposomes.[15,16] In addition, the nontoxic silica surface protects the inner lipid bilayer and can easily undergo the bioconjugation with silane-coupling compounds.

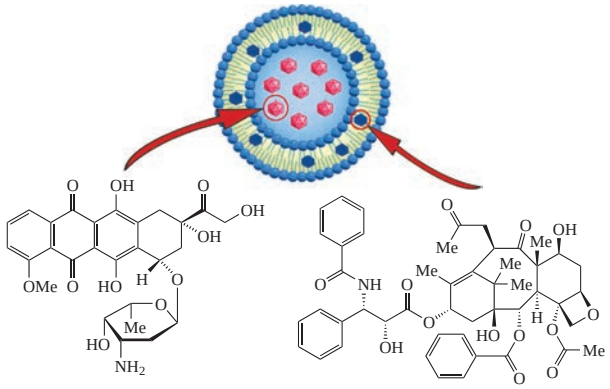
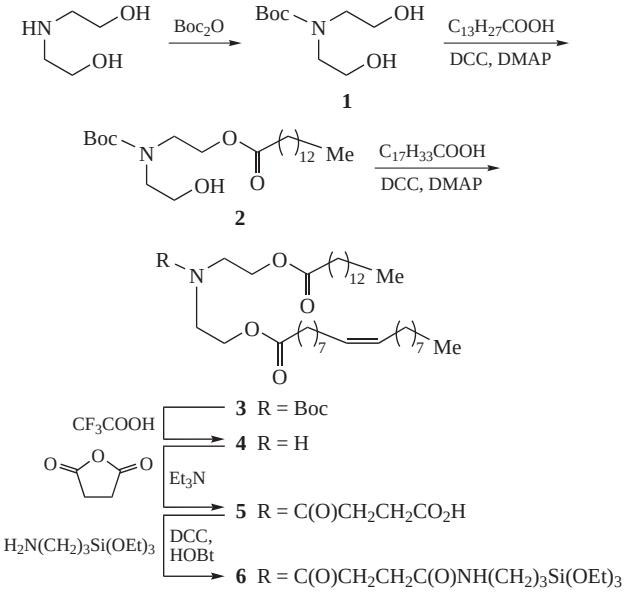


Figure 1 Schematic diagram for encapsulation of drug molecules of various types by cerasome.

This work was aimed at the design and synthesis of new hybrid lipid bearing asymmetric hydrophobic tails (Scheme 1), and investigation of physico-chemical properties of aqueous dispersions of this compound.

The synthesis of the target product was carried out according to the known procedures.[17,18] Briefly, diethanolamine was treated with di-*tert*-butyl pyrocarbonate to give precursor **1**, which was used to prepare compounds **2** and **3**. The product **4** of the deprotection of amino group by trifluoroacetic acid was treated



Scheme 1

with succinic anhydride in the presence of Et_3N to yield compound **5**. The reaction of the latter with 1-hydroxybenzotriazole (HOBt) in the presence of DCC, followed by the addition of (3-aminopropyl)triethoxysilane afforded final product **6**. The detailed synthesis will be reported elsewhere. The structure of obtained compounds was confirmed by NMR spectroscopy and mass spectrometry.†

The thin film of compound **6** was hydrated with 5% acetic acid solution to prepare cerasomes.‡ It should be noted that a spontaneous vesicle formation and polymerization of siloxane groups occurred simultaneously with the formation of polymer network on the surface of the bilayer.

The efficiency of drug encapsulation into the cerasomes was evaluated using the antitumor antibiotic doxorubicin as the example. The doxorubicin loading was carried out during the hydration of thin film. Removal of the drug that was not incorporated into the vesicles was performed by column chromatography (gel filtration) and dialysis.§

Experimental results acquired by dynamic light scattering (DLS) revealed that the mean hydrodynamic diameter of blank cerasomes and cerasomes loaded with DOX were 80±0.4 and 113±0.1 nm with a polydispersity index of 0.271 and 0.276, respectively (Figure 2). This might be caused by extra electrostatic interactions between the positively charged DOX and negatively charged silanol groups, which did not form Si–O–Si bonds during the preparation of DOX-loaded cerasomes.

The zeta potential is an important parameter to be investigated if nanoparticles are intended for applications as the drug delivery carriers.[19] The zeta potential of blank cerasomes was evaluated relative to that of drug-loaded cerasomes. The blank cerasomes were positively charged, so the zeta potential was 38.49±0.03 mV. This might be due to the polymerization of siloxane network on the cerasome surface in 5% acetic acid used in the experiments. Nevertheless, a significantly increased zeta-potential value (79.99±0.06 mV) was observed for the DOX-loaded cerasomes. Such a result was caused by the protonatable amino residues of doxorubicin fraction that was adsorbed on the outer surface of cerasomes, which thus provided the increased charge (Figure 3).

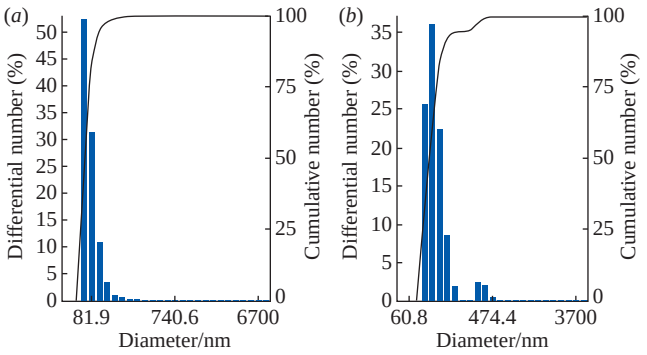


Figure 2 Hydrodynamic diameters of (*a*) blank and (*b*) DOX-loaded cerasomes.

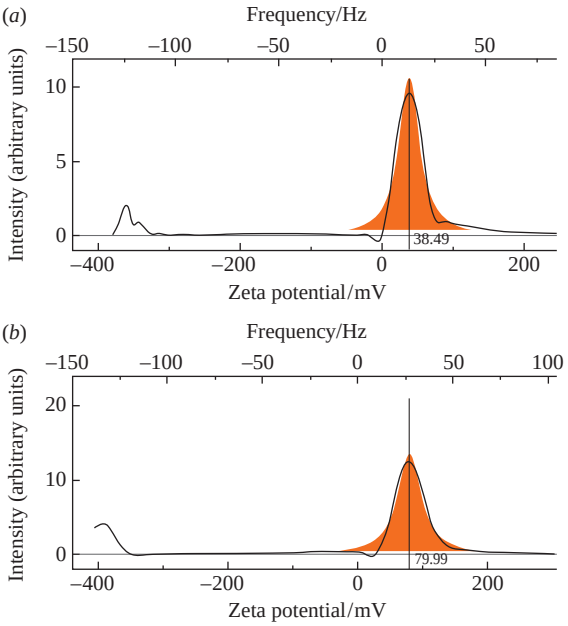


Figure 3 The zeta potentials of (*a*) blank and (*b*) DOX-loaded cerasomes.

To estimate the potential carrier capacity, the entrapment efficiencies for the hydrophilic drug DOX were determined using spectrophotometry data. Once the free drug was removed, a change in the optical density of transmitted light was recorded every hour. The moment when the change was stopped was considered as the completion of doxorubicin excess removal. DOX was entrapped into the aqueous compartment of cerasomes with encapsulation efficiencies of 92±0.04%. The efficiency was calculated according to the known method.[20] Two ways to remove the drug excess provided similar results within a small error. This indicates a high accuracy of the method for calculating the encapsulated amount of substance. We assume that the structure containing the asymmetric acyl tails in its hydrophobic core can provide the vesicle with a compact bilayer, which promotes good results of DOX entrapment efficiency.

To evaluate the morphological stability of dispersions, the change in optical density was measured. The results revealed that the density of dispersion was not significantly changed during storage for one month. This indicates a high stability provided by the formation of polysiloxane network aggregate surface, which prevents the adhesion and fusion of particles. Furthermore, the stability of the cerasomes was evaluated in surfactant dissolution experiments. The optical density change of vesicles consisting of conventional 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC) liposomes drastically increased upon the addition of Tween 80 surfactant (~40 μmol), which indicated the disintegration of vesicles. The amount of solubilized substance increased with the surfactant concentration in the solution in the region of existence of the spherical vesicles and dramatically increased during the formation of lamellar micelles, thus reflecting the rearrangement of vesicles in the solution. The solubilization leads to swelling of vesicles and, accordingly, to a growth of their size. By contrast, the optical density change of cerasomes based on CFL **6** demonstrated almost no change at all, even in the presence of Tween 80 in the higher amount (80 μmol) (Figure 4).

In conclusion, the hybrid lipid containing asymmetric hydrophobic tails of myristic and oleic acids was proposed, successfully

† *Compound* **6**. ^{1}H NMR, δ: 0.66 (m, 2H, CH_2Si), 1.68 (m, 2H, CH_2CH_2Si), 3.20 (q, 2H, $CH_2CH_2CH_2Si$), 3.85 (q, 6H, OCH_2Me) and hydrocarbon tails 0.88 (t, 6H, Me), 1.29 (s, 36H, CH_2), 2.04 (dd, 4H, $CH_2CH{=}CHCH_2$), 5.33 (d, 2H, HC=CH). MS (MALDI), *m*/*z*: 882.54 $[M]^+$.

‡ *Preparation of cerasomes*. Chloroform (5 ml) was added to a sample of CFL **6** (10 mg), and the mixture was stirred for 1 h. The solvent was evaporated to form a thin film, which was dried for 3 h. The film was hydrated in 5% acetic acid. The obtained dispersion was sonicated in a sonicator bath at 45 °C for 50 min.

§ Doxorubicin (400 μg DOX per 2 mg of CFL) was dissolved in acetic acid (5 ml, 5%). This mixture was added to a thin film of CFL and sonicated in a sonicator bath. To clear the cerasome dispersions from the DOX that was not incorporated into the vesicles by gel filtration, the dispersion (1 ml) was loaded onto a NAP-25 chromatographic column (GE Healthcare) and eluted with a buffer solution (pH 7.8). For dialysis, the cerasome dispersions were placed in dialysis bags (MFPI, USA) with a pore size of 6–8 kDa and incubated for 48 h. The purification process was monitored by spectrophotometry.

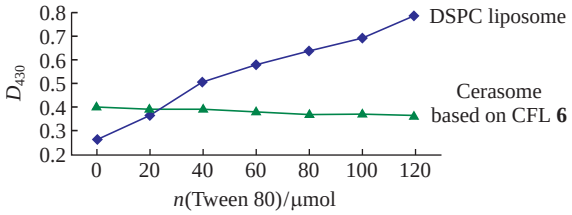


Figure 4 Effect of the addition of Tween 80 on the optical density change of blank cerasomes based on CFL **6** and DSPC liposomes.

synthesized and used for the design of cerasomes *via* the self-assembly and sol-gel processes. The properties of cerasomes such as size, zeta-potential, entrapment efficacy of drug, and stability of particles, were estimated. The oleic acid usage can reduce the overall cytotoxicity of drug delivery system, thus getting closer to the green chemistry concept. The vesicles possess the smaller size than that of known analogues, which is undoubtedly their advantage promoting a better penetration into the cells. In addition, the high zeta-potential values prevent premature aggregation of dispersions. The high resistance towards aggregation was also confirmed experimentally using the surfactant. Therefore, the obtained new material with improved characteristics makes a contribution to the relevant field of chemistry.

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