Electronic supplementary materials *Mendeleev Commun.*, 2023, **33**, 337–339

Liposomes as nanoreactors for copper nanoparticles synthesis

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Reagents. 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine, DPPC (Avanti Polar Lipids Inc, USA); copper (II) sulfate pentahydrate, $CuSO_4 \cdot 5H_2O$ (Reahim, Russia); hydrazine, N_2H_4 (Reahim, Russia).

Preparation of liposomes. Liposomes loaded with $CuSO_4$ solution were prepared by extrusion technique [O. V. Zaborova, *Russ. J. Gen. Chem.*, 2020, **90(4)**, 762] from DPPC. The phase transition temperature of DPPC is 41°C, thus the membrane of obtained liposomes is in solid phase state. Aliquot of lipid solution in ethanol was placed in a round-bottom flask and organic solvent was evaporated under vacuum. Obtained thin lipid film was dispersed in 1 M $CuSO_4$ solution and then was passed 27 times through a 50 nm polycarbonate membrane with use of Avanti® mini-extruder. Obtained suspension of unilamellar liposomes was dialyzed against deionized water for 24 h. Stock suspension of liposomes (20 mg/mL) was stored at 4°C and used within 3 days.

UV-VIS spectrophotometry. The optical density (A) spectra of the samples were recorded using a Helios Alpha spectrophotometer (quartz cell with 1 cm optical path, 300–950 nm) using deionized water as reference. The total volume of the sample was 1 mL.

High angle annular dark field scanning TEM (HAADF-STEM). HAADF-STEM images, energy dispersive X-ray (EDX) spectra and maps were obtained using a probe-corrected Titan Themis Z transmission electron microscope equipped with a Super-X detection system and operated at 200 kV.

Dynamic Light Scattering (DLS). Mean hydrodynamic diameter was determined by dynamic light-scattering at fixed scattering angle (90°) in a thermostatic cell with a NanoBrook Omni particle sizer and zeta potential analyzer (Brookhaven Instruments, USA) at 22 °C. Software provided by the manufacturer was employed to calculate hydrodynamic diameter values.

Nanoparticle Tracking Analysis (NTA). NTA was conducted using a ZetaView® PMX420-QUATT instrument (Particle Metrix GmbH, Germany), while the data were analyzed by ZetaView NTA software. Operating instructions of the manufacturer were followed before calibrating the instrument with a known concentration of 100 nm polystyrene nanoparticles (Applied Microspheres B.V., Netherlands). The standards were suspended in particle-free water, whereas the investigated samples were diluted 1:1000 with Milli-Q water. Particles were counted and size-distributed at one cycle of 11 frames per cycle under a sensitivity of 85 and shutter value of 32.

Calculations. Theoretical size of CuNPs in liposomes was calculated as:

$$d_{CuNP} = \sqrt[3]{\frac{C_{CuSO4} \cdot M_{Cu} \cdot d^3}{\rho_{Cu} \cdot 1000}}, \quad \text{(S1)}$$

where d_{CuNP} – diameter of Cu nanoparticles, nm; C_{CuSO4} – $CuSO_4$ concentration in liposomes, mole/L; M_{Cu} – copper molar mass, g/mole; d – diameter of liposomes, nm; ρ_{Cu} – copper density, mg/mL.