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Preparation and characterization of siliceous material using liposomes as template

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Material synthesis using unilamellar liposomes with a high sol–gel temperature transition phase as a template leads to a new silica material.

Due to potential applications of templated inorganic materials (catalysis, controlled release) their synthesis has been the subject of numerous publications.[1,2] Biomimetic silica-based materials can be prepared using surfactants,[3,4] polymers[5] and phospholipids[6] using a technique based on the hydrolysis and condensation of a neutral silicon alkoxide precursor (TEOS).[7,8] A variety of self-assembled organic molecules such as lyotropic lamellar phases and microemulsions[9] is used as a template. The aim of this study is to prepare silica microparticles using unilamellar liposomes as template. Liposomes which are phospholipid vesicles are used as biodegradable and biocompatible drug carriers in clinical and cosmetic practices. Moreover, progress in the field of new materials synthesis seems to be promising for pharmaceutical applications. The use of zwitterionic phospholipids (non toxic) as templates requires a modified approach since the liposome structure is sensitive to low pH and high ionic strength and organic solvents are not allowed for pharmaceutical applications. The silica growth is directed by the receptiveness of the quaternary ammonium surface of the phospholipid to the silica.[10]. The present article aimed to study (i) the morphological characterization of the new material and (ii) the behavior of the liposome phospholipids. The morphological characterization, which is expected to reveal the presence of the silica coating, is provided by different methods such as electron microscopy (TEM and SEM), FTIR measurements and nitrogen adsorption. The presence of the entrapped materials (phospholipids) is studied by FTIR.

The synthesis process follows a two step pathway. Firstly, the liposomes preparation and secondly the synthesis of the material. For the liposomes preparation, a suitable amount of L-α-dipalmitoylphosphatidylcholine (sol–gel phase transition temperature = 41 °C; kindly provided by Lipoïd) is dissolved in chloroform. After complete removal of the chloroform (at 40 °C under reduced pressure), phosphate buffer solution (PBS, 150 mM pH 7.4) is added in order to obtain a 10 mg ml^{-1} lipids suspension. The size of the liposomes is reduced above 41 °C using an extruder (Lipex Biomembranes Inc.) with polycarbonate membranes (mean size diameter: 450, 220 and 100 nm, Nucleopore). Liposome size measurements are performed by quasi-elastic light scattering at a 90° angle (SEMATECH, SM 633/RTG, France) using monomodal analysis. The mean size is determined to be 105 nm (polydispersity: 0.2519). An enzymatic method (PAP 150, Biomerieux) is used to determine the phospholipid concentration in the liposome preparation: 9.5 mg ml^{-1}.

For the material synthesis the inorganic precursor, tetraethylorthosilicate (TEOS, Sigma Aldrich), is added to the liposomal suspension (TEOS/DPPC molar ratio 8 : 1) at room temperature and stirred overnight. Sodium fluoride (NaF, Sigma Aldrich): 4% mole TEOS is then added and the reaction medium stirred at room temperature (48 h). The preparation is centrifuged at 10000 rpm and DPPC concentration is determined in the supernatant (0.1 mg ml^{-1}). The characterization is carried out on the as-synthesized material (sample A). In a second step the material is washed (sample B) with a solution of isopropanol/acetic acid. The last step consists in the characterization of the calcined material (sample C) after heating 24 h at 500 °C. The morphological characterization was performed on the three samples by conventional transmission electron microscopy (JEOL 1200 EXII) and scanning electron microscopy (Hitachi S 4500) on a dried sample (40 °C). The specific surface area was assessed by nitrogen adsorption measurements according to the Brunauer–Emmett–Teller standard method (conditions for the outgassing treatment before nitrogen adsorption measurements: 35 °C, 12 h, 10^{-2} Torr). Measurements were carried out at 77K using an adsorption analyzer ASAP 2010 from Micromeritrics. The IR spectra (Bruker, Equinox 55 with SPECAC diffuse reflectance cell) are collected at room temperature.

SEM micrographs (Fig. 1a) show microparticles with a diameter of one to several microns consisting in aggregated nanoparticles; most of all these nanoparticles have a spherical shape. TEM results (Fig. 1b) are consistent with the SEM observations and confirm the presence of aggregated hollow nanocapsules with a mean diameter close to the unilamellar liposomes one (105 nm). The silica wall of these nanocapsules is thin enough to be partially transparent to the electron beam. The wall thickness is estimated to be 6 to 9 nm from TEM micrographs. The material morphology is the same whatever the treatment used after the synthesis (unwashed or washed materials). After calcination, the vesicles burst and silica vesicles fragments are observed (SEM and TEM micrographs, not shown).

The phospholipid bilayer rigidity is ensured by using a phospholipid of high phase transition temperature. Hubert[9] has already demonstrated that the use of lipids which have a high phase transition temperature leads to spherical morphologies. DPPC exhibits a high sol–gel phase transition temperature (41°C) and we have observed that the resulting nanoparticles are spherical in shape. Moreover, it seems that aggregation occurs during the synthesis and generates microparticles which consist of aggregated nanocapsules with an average diameter of 112 nm. The nanocapsule size is consistent with the liposome size distribution (105 nm). As we use a zwitterionic phospholipid, the addition of TEOS may induce an aggregation of the liposomes explaining such a morphology. We cannot exclude a possible reorganization of the phospholipid and/or the effect of

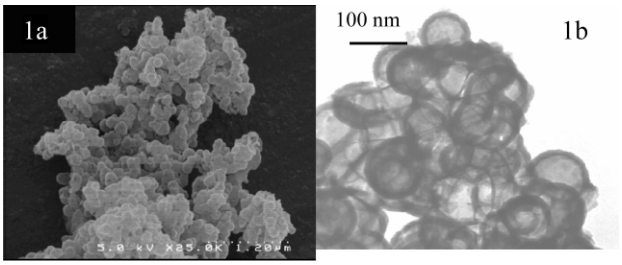

Fig. 1 (a) SEM micrograph and (b): TEM micrograph of aggregated nanoparticles.

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the mechanical stresses exerted by the condensed silica network[9] on the synthesized material morphology.

The synthesis conditions and especially the silicon alkoxide/phospholipid ratio (8 : 1) ensure a homogeneous deposit of the silica on the vesicles surfaces which are completely covered. Nitrogen adsorption isotherms (Fig. 2) show that the as-synthesized and washed materials have neither micro nor mesoporosity (type III isotherm). The surface increased from 55 $m^2\ g^{-1}$ to 90 $m^2\ g^{-1}$ after washing with the solvent. Calcination results in the rupture of the nanoparticles and as a consequence, in the surface increase to 110 $m^2\ g^{-1}$.

FT-IR spectra (Fig. 3) collected on the as-synthesized and on the washed material, display vibrational bands characteristic of the C=O, CH and CH_2 groups which demonstrate that the mild processing conditions allow the quantitative entrapment of preformed liposome in the samples. Moreover, no band was observed corresponding to free silanol groups, indicating that the phospholipids interact with the silica surface. Calcination at a high temperature (500 °C) results in the disappearance of these characteristic vibrational bands.

In conclusion, a new silica material is described using liposomes as template. The use of unilamellar liposomes with a narrow size distribution leads to a well-defined internal size. We are currently investigating the phase transition behavior of the entrapped phospholipid by fluorescence depolarization in order to explain the phospholipids interaction with the silica wall. The synthesis of these microparticles can be adapted to the encapsulation of molecules (that can be entrapped in conventional liposomes) in order to improve their solubility or their stability with the advantage of an increase of the liposome stability for oral administration.

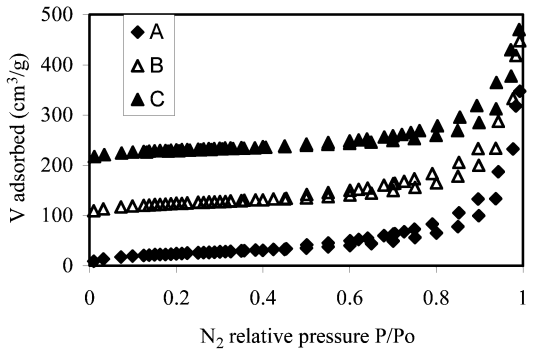

Fig. 2 Nitrogen adsorption and desorption isotherms of unwashed (A) , isopropanol/acetic acid washed (B) and calcined samples (C) (the second and third isotherms (B and C) have been offset on the vertical scale respectively by 100 and 200 $cm^3\ g^{-1}$).

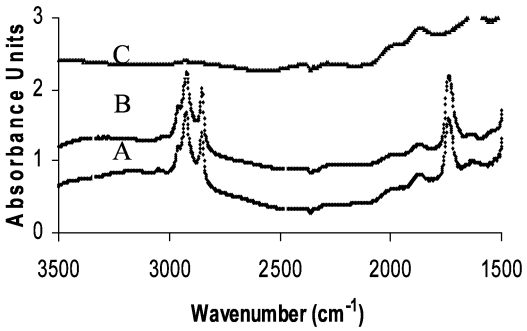

Fig. 3 FT-IR spectra of as-synthesized (A), isopropanol/acetic acid washed (B) and calcined samples (C).

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