

*Encapsulation of diphtheria anatoxin into ordered mesoporous silica*

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Different concentrations (w/w) of diphtheria anatoxin (D-ANA) were encapsulated into SBA-15 ordered mesoporous silica [1], which is composed by micropores (diameter less than 2 nm), mesopores (mean diameter of 10 nm) and macropores (diameter higher than 50 nm). The encapsulation yield of the antigen diluted in phosphate-buffered saline (PBS) solution inside the silica, after a drying process, was determined by nitrogen adsorption isotherm (NAI), which provided BET surface area and the BJH pore size distribution. These results indicated that for a mass ratio of 5SBA-15:1ANA all mesopores are filled with the antigen, suggesting that it is the optimal concentration of D-ANA into SBA-15. Furthermore, the NAI results revealed that the micropores and mesopores are also filled by PBS. Analyzing the SAXS data, simulated by a theoretical model, which included form and structure factors [2], we concluded that D-ANA is inside the mesopores and that the mass ratio of 5SBA-15:1ANA is excessive, indicating the presence of D-ANA also in the macropore region. Transmission Electron Microscopy (TEM) images further confirmed these latter results. In-situ SAXS measurements of D-ANA release from the silica matrix were performed in mimetic intestine fluid. Dynamic Light Scattering (DLS) obtained from D-ANA in PBS showed that the antigen did not aggregate and hydrodynamic particle sizes between 5 to 10 nm were observed, which is also consistent with the ability of D-ANA to enter the mesopores. X-ray absorption spectroscopy with X-ray microscopy data at the carbon K-edge revealed that the D-ANA is distributed homogeneously in all the silica particles. The thermal stability of D-ANA increased when encapsulated inside SBA-15, as determined by Thermogravimetry (TG). The immunogenic complex D-ANA in the SBA-15 vehicle will be tested in mice in order to check the production of antibodies, regarding the exceptional adjuvant protecting characteristics of SBA-15 [3].

[1] Zhao D et al. (1998). *Science* 279(5350), 548–552.

[2] Sundblom A et al. (2010). *J. Phys. Chem. C* 113, 7706–13.

[3] Carvalho L V et al. (2010). *Vaccine* 28, 7829–36.

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