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Lipid sponge-phase nanoparticles as enzyme carriers - structure and intermolecular interaction controlling the enzyme encapsulation

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Non-lamellar lipid aqueous phases, such as reverse cubic or hexagonal phases, can be used to entrap smaller biomolecules. The curvature of the lipid aqueous interfaces in these phases and hence the size of the aqueous cavities depends on the composition, water content and temperature. Normally the size of the cavities is similar or smaller than biomacromolecules, such as large enzymes. This poses a challenge when lipid phases as matrices for enzymes and other functional biomacromolecules. Here, we will present a lipid system, based on mixtures of acylglycerides and acyldiglycerides, which are able to form highly swollen sponge phases (L3), with aqueous pores up to 13 nm of diameter. The structure and composition of the particles were revealed by combining small angle neutron scattering (SANS), light scattering, cryo-TEM, size exclusion chromatography and Raman spectroscopy. The Raman spectroscopy results for the sponge phases show large similarities in lipid chain conformation and head group interactions as in the lamellar and reverse bicontinuous cubic phase in the same lipid system [3]. This might be expected as all three structures are formed by lipid bilayers, albeit of different curvature. The L3 phase was found to be easy to disperse into sponge-like nanoparticles (L3 NPs) in excess aqueous solution by simply shaking [2]. We investigate encapsulation of two key types of enzymes of different sizes, used in food processing, namely Aspartic protease (34 KDa) and Beta-galactosidase (460 KDa). They are today delivered into the process as solutions with a considerable amount of preservatives and still with limited shelf-life and limited control of the enzyme activity. The SANS results reveal differences in the L3 NPs with and without enzyme that can be interpreted as inclusion of the protein in the liquid crystalline phase. We also performed neutron reflectometry, which verified the enzyme lipid interaction and enzyme penetration in the bilayer. These findings are verified by size exclusion chromatography, Raman spectroscopy and the enzymatic activity of the encapsulated enzyme, which surpasses the storage stability of pure enzymes in solution.

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