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## Neutron scattering study of lipid sponge-phase nanoparticles as enzyme carriers for food processing

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Immobilization of enzymes into different support materials has been widely used as means to control their activity and stability. This has mainly been done in context of bioanalytical, preparative or biomedical purposes. In the present study we investigate two key types of enzymes used in food processing, namely Aspartic protease (34 KDa) and Beta-galactosidase (460 KDa), which are used in processing of dairy products. Mostly these proteins are 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. Here we have used lipid liquid crystalline phases as enzyme carriers, based on their established capability for drug delivery, protein encapsulation or crystallization. They can form a wide range of self-assembled structures with aqueous cavities of nano-scale dimensions. Reverse cubic or hexagonal lipid aqueous phase can be used to entrap smaller biomolecules yet it is still challenging to encapsulate bioactive macromolecules, such as proteins. Here, we will present a novel lipid system able to form highly swollen sponge phases (L3), with aqueous pores up to 13 nm of diameter. We will show that this structure is preserved even if in excess aqueous solution, where they form sponge-like nanoparticles (L3 NPs) in which the two enzymes are included. The structure and composition of the particles was revealed by combined measurements using small angle neutron scattering (SANS), light scattering, cryo-TEM, size exclusion chromatography and Raman spectroscopy. The SANS results reveal differences in the L3 NPs with and without enzyme. To reveal the nature of the interaction between the enzymes and the lipid matrix, we further studied the adsorption of both proteins on the lipid layers formed by the L3 NPs. These data reveal partial penetration of the enzymes in the lipid bilayers. The results of this study will be discussed in terms of the ability of these nanoparticles to encapsulate and release of the proteins in the lipid matrix.

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