

Formation of responsive enzyme-loaded gelatin microgels using water-in-water emulsions

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The research work has focused on the preparation and characterization of microgel particles, obtained by cross-linking in the disperse phase of water-in-water (W/W) emulsions, with the final aim of studying the microgels as carriers for the delivery of the enzyme β -galactosidase. The system was composed of water, gelatin and maltodextrin, because of its excellent biocompatibility and the easy formation of W/W emulsions in this system, due to mutual immiscibility between gelatin and maltodextrin. Stable gelatin-in-maltodextrin emulsions have been prepared by dispersing the gelatin aqueous phase into the maltodextrin aqueous phase. For proper understanding of this biopolymer mixture, the influence of pH, temperature and chaotropic/cosmotropic salts on the phase behaviour was investigated.

Genipin, a biocompatible cross-linker, has then been added to cross-link the gelatin-based aqueous droplets, and thus obtaining stable microgel dispersions of 10-20 μm (Fig.1a), which remained stable over a period of at least 1 month. The microgels have been purified and freeze dried, obtaining leaf-like structured macroporous particles (Fig. 1b). The influence of cross-linker, polymer, NaCl concentration and pH on microgel properties have been tested. High genipin concentrations (10 mM) increase bond formation between polymer chains, leading to rigid particles with low swelling ratios. Larger microgel swelling ratios have been observed at $\text{pH} < 4$, because gelatin proteinaceous chains become electrostatically charged at those pH values, leading to repulsion between adjacent chains and producing expansion of the microgel network. Dissolution of the microgel particles was observed after 1 day at pH 2, probably because of partial hydrolysis of polymer chains at such low pH. NaCl and polymer concentration did not affect microgel size.

Enzyme activity within gelatin macrogel cross-linked with genipin has been evaluated, as preliminary tests before encapsulation of the enzyme inside the microgels. While gelatin concentration did not have an effect on enzyme activity, increasing genipin concentrations decreased enzyme activity. After 2 weeks at 4°C, gels crosslinked with 1 mM Genipin retained 30 % of activity; whereas the gels crosslinked with 10 mM Genipin only had 10 % of activity, in comparison to the non-crosslinked samples. The enzyme did however not lose activity in presence of previously crosslinked microgels. The efficiency of enzyme encapsulation into microgels, and their release and stability, are subject to current study.

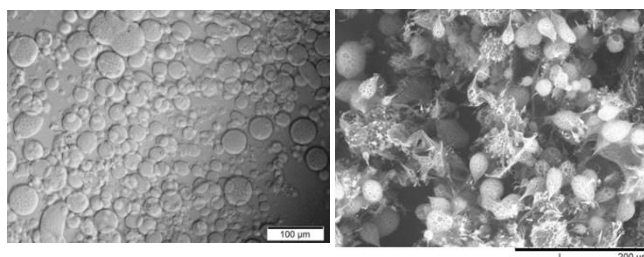


Fig. 1. Microscopic image (a) of gelatin microgel dispersion and scanning electron microscope image after freeze-drying the sample (b)

Acknowledgements Financial support from the People Programme (Marie Curie Actions) of the European Union's Seventh Framework Programme FP7/2007-2013/ under REA grant agreement n°606713 (BIBAFODDS project).