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Immobilization of Superoxide Dismutase Enzyme on Layered Double Hydroxide Nanoparticles

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Abstract : Antioxidant enzymes are the most efficient defense systems against reactive oxygen species, which cause severe damage in living organisms and industrial products. However, their supplementation is problematic due to their high sensitivity to the environmental conditions. Immobilization on carrier nanoparticles is a promising research direction towards the improvement of their functional and colloidal stability. In that way, their applications in biomedical treatments and manufacturing processes in the food, textile and cosmetic industry can be extended. The main goal of the present research was to prepare and formulate antioxidant bionanocomposites composed of superoxide dismutase (SOD) enzyme, anionic clay (layered double hydroxide, LDH) nanoparticle and heparin (HEP) polyelectrolyte. To characterize the structure and the colloidal stability of the obtained compounds in suspension and solid state, electrophoresis, dynamic light scattering, transmission electron microscopy, spectrophotometry, thermogravimetry, X-ray diffraction, infrared and fluorescence spectroscopy were used as experimental techniques. LDH-SOD composite was synthesized by enzyme immobilization on the clay particles via electrostatic and hydrophobic interactions, which resulted in a strong adsorption of the SOD on the LDH surface, i.e., no enzyme leakage was observed once the material was suspended in aqueous solutions. However, the LDH-SOD showed only limited resistance against salt-induced aggregation and large irregularly shaped clusters formed during short term interval even at lower ionic strengths. Since sufficiently high colloidal stability is a key requirement in most of the applications mentioned above, the nanocomposite was coated with HEP polyelectrolyte to develop highly stable suspensions of primary LDH-SOD-HEP particles. HEP is a natural anticoagulant with one of the highest negative line charge density among the known macromolecules. The experimental results indicated that it strongly adsorbed on the oppositely charged LDH-SOD surface leading to charge inversion and to the formation of negatively charged LDH-SOD-HEP. The obtained hybrid materials formed stable suspension even under extreme conditions, where classical colloid chemistry theories predict rapid aggregation of the particles and unstable suspensions. Such a stabilization effect originated from electrostatic repulsion between the particles of the same sign of charge as well as from steric repulsion due to the osmotic pressure raised during the overlap of the polyelectrolyte chains adsorbed on the surface. In addition, the SOD enzyme kept its structural and functional integrity during the immobilization and coating processes and hence, the LDH-SOD-HEP bionanocomposite possessed excellent activity in decomposition of superoxide radical anions, as revealed in biochemical test reactions. In conclusion, due to the improved colloidal stability and the good efficiency in scavenging superoxide radical ions, the developed enzymatic system is a promising antioxidant candidate for biomedical or other manufacturing processes, wherever the aim is to decompose reactive oxygen species in suspensions.

Keywords : clay, enzyme, polyelectrolyte, formulation
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