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Title: Synthesis of Molecularly Imprinted Polymers – catalytic activity of obtained materials

Since their inception in 1931, there has been an increasing interest in the application of molecularly imprinted polymers (MIPs) to a diverse range of disciplines. MIPs can be prepared in various shapes and sizes, starting from block monoliths, spherical microdroplets, ending on nanohydrogels, which makes them attractive for vast range of applications. With high selectivity and specificity, these polymers can withstand harsh chemical environments, such as extremes of pH, temperatures or high concentrations of organic solvents. MIPs have successfully been created and developed against all major target classes including peptides, proteins and other macromolecular structures, as well as smaller chemical entities such as drugs, their metabolites, pollutants, explosives, etc. This same molecular recognition capacity also enables MIPs to function as catalysts, or enzyme analogues. Conceptually inspired by this possibility, the work presented in this thesis was focused on exploiting the technical possibilities offered by MIP systems which possess either catalytic, a combination of catalytic and molecular recognition and simple molecular recognition properties. These were based, respectively, on using MIPs as artificial enzymes, by introducing additional molecular recognition capabilities into natural enzymes and by imprinting natural enzymes. For this, three types of imprinted materials were prepared, the first possessing intrinsic catalytic activity, the second by bioimprinting using an enzyme as monomer and the third by using an enzyme as the template for polymer synthesis.

The first approach consisted in the preparation and assessment of the properties of molecularly imprinted polymers with catalytic centres that mimic the active sites of metalloenzymes. The MIPs synthesis was based on suspension polymerization of functional monomers (4-vinylpyridine and acrylonitrile) with trimethylolpropane trimethacrylate as a cross-linker in the presence of transition metal ions and 4-methoxybenzyl alcohol as a template. Four metal ions were chosen for imprinting from among the microelements that are the most commonly found the native enzymes: Cu^{2+}, Co^{2+}, Mn^{2+}, and Zn^{2+}. To prepare catalysts, the required loading of metal ions was obtained by a sorption process. The imprinted catalysts with Cu^{2+}, Co^{2+} and Zn^{2+} were successfully used for hydroquinone oxidation in the presence of hydrogen peroxide. The Mn^{2+}-imprinted catalyst showed no

activity due to the insufficient metal loading. Cu^{2+}-MIP showed the highest efficiency. In the case of Cu- and Co-MIP catalysts, their activity was additionally increased by the use of surface imprinting technique. The MIPs systems were additionally characterised by swelling in water and 0.001 M HCl solution, nitrogen content, porosity, sorption and catalytic properties. The stability of the polymers produced with Cu^{2+}, Co^{2+} and Zn^{2+} ions was examined by their properties after 10 and 18 months of synthesis.

Subsequently, the second approach exploited here was inspired by MIPs application to immunoassay and bioimprinting processes. For this cross-linked protein nanoparticles were developed using horseradish peroxidase (HRP) to be used as a single recognition and signalling agent in ELISA-like immunoassays. Using a solid phase synthetic approach, the nanoparticles were prepared from HRP as a monomer and glutaraldehyde as a crosslinker fulfilling the dual-purpose role of both achieving the template-derived cavities, and increasing the operational and thermal stability of the crosslinked enzyme nanoparticles. As a template two antibiotics were used: vancomycin and ampicillin. The enzymatic constitution of the nanoparticles is responsible for their signalling capability, whilst their recognition properties are due to the imprinting process. For the development of the ELISA-like assay, a competitive format was selected where the target molecule was immobilised onto the wells of microplates by amine coupling. The molecular imprinted crosslinked HRP was then used as a replacement for both antibodies and conjugate during the assay. In addition, competitive binding and cross-reactivity assays for crosslinked HRP in the presence of a second antibiotic were performed. The results show high affinity and selectivity of the imprinted HRP nanoparticles towards the template used during the imprinting procedure. Additionally, crosslinked HRP nanoparticles possessed increased storage and thermal stability as compared to native HRP, assessed enzymatically, by circular dichroism and storage assays.

The final approach consisted in combining the molecular imprinting technique for enzyme recognition, creating MIPs chaperone particles with affinity for HRP. These were prepared in order to determine if they can be used to stabilise or increase enzyme activity and lifetime. Nanoparticles were prepared by solid phase imprinting using water soluble monomers and immobilised HRP as template. Polymerisation was initiated by ammonium persulphate and tetramethylethylenediamine as a catalyst system in aqueous medium in the presence of glass beads with immobilised HRP enzyme. For the synthesis, N-isopropylacrylamide, N-tertbutylacrylamide and acrylic acid were used as the functional monomers and N, N'-methylenebisacrylamide as the crosslinker. The size of the produced nanoparticles was

determined by the dynamic light scattering method and their concentration using a spectrophotometer. Subsequently, aggregation studies were performed over 5 days, which demonstrated that nanoparticle aggregates increase their size from 142.6 nm at first measurement to approximately 240 nm, and then gradually decreased over the next 5 days to achieve the size of 174.70 nm. As a final application for chaperone MIPs, the effect of the presence of nanoparticles on the HRP catalytic activity at different temperatures (at 4 ^{o}C, 18 ^{o}C, 37 ^{o}C and 58 ^{o}C) was investigated over 72 h. The results indeed confirm that chaperone MIPs can increase HRP stability during storage for 72 h at 4, 18 and 37 °C. The nanoMIPs did not influence the thermal stability of the HRP at 58 °C