

Ph.D. Theses

# **Systematic Design of Antioxidant Hybrid Biocatalysts Based on Inorganic Nanozymes and Polystyrene Latex Beads**

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## 1. Introduction

Antioxidant natural enzymes play pivotal protective role in living organisms with unparalleled catalytic potential, specificity, and selectivity due to their complex chemical structures. In addition, antioxidant enzymes are widely used in numerous industrial fields such as in pharmaceutical and food manufacturing processes. Despite the advantages, these biocatalysts possess several inherent flaws that severely limit their applicability. For example, they are costly to extract and purify due to high sensitivity to operational conditions including the pH, temperature, type of solvent, and presence of inhibitors. Upon applying undesignated conditions, the vital structural features of the comprising proteins can undergo severe alternations leading to loss of catalytic activity. These limitations have prompted a strong demand for versatile alternatives with similar or indistinguishable biocatalytic properties.

Beside metal complexes, cyclodextrins, and polymers, numerous nanostructured materials have emerged to exhibit enzyme-like catalytic properties. These so-called nanozymes present promising alternatives due to easy and low-cost production, structural stability, and highly tunable physicochemical properties, which enable their use in wide pH and temperature ranges, where the mimicked enzymes become inactive. Also, these artificial enzymes have diverse structures and compositions such as metallic nanoparticles, metal oxides/chalcogenides, carbon materials, and metal-organic frameworks. These nanostructures were found to mimic numerous enzymes such as superoxide dismutase (SOD), catalase (CAT) and horseradish peroxidase (HRP). These enzymes are of special importance not only within living systems, but also in numerous industrial fields.

Surfaces of nanosized particles, however, possess high Gibb's energy, and when dispersed in a liquid medium, tend to cluster into larger aggregates, which can be maximized under certain experimental conditions such as at isoelectric point (IEP), high ionic strength, and in presence of surfactants or polyelectrolytes. Since the enzymatic action occurs on the surfaces, aggregation of particles can largely limit their catalytic applications, and thus, particle stabilization is crucial for efficient use as enzyme-mimicking biocatalysts. A common stabilization approach is the immobilization of nanozymes onto larger support materials such as graphene, clays, and latex beads, which prevent aggregation, enable separation, and preserve the particles' large surface area. The resulting nanozyme-support composites are foreseen to be versatile antioxidants with excellent enzyme-like catalytic potential and sustained catalytic surface and thus, would be of huge importance especially in industries.

## 2. Objectives

In the present work, we aspire to deliver multi-functional antioxidant composites based on inorganic nanozymes and latex beads. Hence, Prussian blue (PB NPs), manganese oxide (MnO<sub>2</sub> MFs), and cerium oxide (CeO<sub>2</sub> NPs) nanoparticles were prepared as potential antioxidant materials and further formulated by immobilization onto amidine (AL) or sulfate (SL) functionalized polystyrene latex beads.

Initially, the experimental conditions such as pH, ionic strength, and latex concentration were aimed to be optimized to enable effective composite formation. In addition, the surface charge of metal oxide particles was subjected to adjust with adsorption of poly(diallyldimethylammonium chloride) (PDADMAC) polyelectrolyte prior to their heteroaggregation with the relevant latex particles. The colloidal properties were carefully probed during PDADMAC adsorption as well as nanozyme-latex heteroaggregation as it enables the determination of the optimal PDADMAC/nanozyme and latex/nanozyme mass ratios, which play a crucial role in the stability of the formulated composites. Finally, the structure as well as the biocatalytic potential of the composites were thoroughly examined.

## 3. Experimental Methods

Several instrumental techniques were employed throughout the doctoral work. First, the chemical structures of PB NPs, MnO<sub>2</sub> MFs, and CeO<sub>2</sub> NPs were confirmed via X-ray photoelectron spectroscopy (XPS) and X-ray diffraction (XRD) techniques.

Dynamic (DLS) and electrophoretic (ELS) light scattering were used extensively, primarily to obtain the hydrodynamic radii ( $R_h$ ) and zeta potential ( $\zeta$ ) values of the particles, respectively. The latter parameter is a quantity related to the surface charge of the particles. The time-resolved mode of DLS was used to obtain the aggregation rate of particles. The light scattering methods were also used to probe the dependence of  $R_h$  and  $\zeta$  as well as the aggregation rate on the pH and ionic strength applied in the dispersion. Besides, during composite formation, the MnO<sub>2</sub> MFs and CeO<sub>2</sub> NPs often required surface functionalization to adjust their surface charge, and thus, DLS and ELS were not only used to optimize the dose of nanozymes to be immobilized onto the latex particles, but also the dose of PDADMAC polyelectrolyte to be adsorbed on MnO<sub>2</sub> MFs and CeO<sub>2</sub> NPs for surface charge adjustments.

For visualization of individual particles and their respective composites, transmission electron microscopy (TEM), scanning electron microscopy (SEM), and atomic force microscopy (AFM) were used to probe the morphology as well as to confirm the immobilization of the nanozymes onto the relevant latex support. In addition, Raman spectroscopy was employed to confirm the formation of CeO<sub>2</sub> NPs and its successful immobilization onto its designated latex beads.

The UV-Vis spectrophotometry was used to assess enzymatic activities and the antioxidant potential of the bare nanozymes as well as the formulated composites. The same technique was also applied to confirm the successful formation of PB NPs and CeO<sub>2</sub> NPs.

#### **4. Prospective Novel Findings**

**T1: The dependence of aggregation state and surface charge of nanozymes (PB NPs, MnO<sub>2</sub> MFs and CeO<sub>2</sub> NPs) and their relevant latex support on the pH and the ionic strength served as a versatile tool to set the optimal experimental conditions for efficient preparation of the antioxidant latex-nanozyme composites.**

T1.1 The PB NPs, prepared by co-precipitation of ferric (K<sub>3</sub>[Fe(CN)<sub>6</sub>]) and ferrous (FeCl<sub>2</sub>) salts, maintained similar hydrodynamic radii and negative surface charge in acidic, neutral, and alkaline pH regimes. Deprotonated surface moieties such as hydroxyl groups give rise to strong electrostatic repulsion, and hence, remarkable colloidal stability for further processing.

T1.2 The MnO<sub>2</sub> MFs, prepared by reduction of KMnO<sub>4</sub>, underwent heavy aggregation and possessed low surface charge in both acidic and neutral media, whereas in alkaline solutions, MnO<sub>2</sub> MFs developed significant negative surface charge that prevented particle aggregation through electrostatic repulsion due to deprotonated surface moieties. The aggregation state of MnO<sub>2</sub> MFs was largely affected by the salt level, in line with DLVO theory. Heavy aggregation occurred at characteristic critical coagulation concentration (CCC) of 12.2 mM.

T1.3 The CeO<sub>2</sub> NPs, synthesized by oxidation of Ce(III) salt in ethylene glycol, possessed positive and negative surface charge in acidic and alkaline conditions, respectively, with characteristic IEP around neutral pH regime. Significant aggregation of CeO<sub>2</sub> NPs was observed in the presence of salt, in agreement with the DLVO theory.

However, the salt-induced aggregation was pH-dependent and more pronounced in acidic media; the CCC values of CeO<sub>2</sub> NPs at acidic and alkaline conditions were 79.1 mM and 5.1 mM, respectively.

T1.4 Amidine (AL) and sulfate (SL) functionalized polystyrene latex particles exhibited superb colloidal stability. No significant change in particle size or zeta potential was observed within the pH range 3-11. High resistance against salt-induced aggregation was observed, the CCC of SL and AL were 224.5 mM and 100 mM, respectively.

**T2: Polyelectrolyte functionalization of negatively charged metal oxides served to induce charge reversal enabling composite formation with negatively charged SL particles via electrostatic forces. Adsorption of oppositely charged PDADMAC led to charge neutralization and reversal of nanozymes at appropriate doses. Analysis of such trends helped to identify the interparticle forces involved and hence, to predict the colloidal stability.**

T2.1 The MnO<sub>2</sub> MFs maintained considerable negative charge in the alkaline pH regime allowing the surface functionalization by PDADMAC, an oppositely charged strong polyelectrolyte. ELS measurements revealed that at low PDADMAC doses, negatively charged particles are present, while high doses resulted in positively charged particles due to charge reversal. The transition point occurred at the IEP at a dose of 15.5 mg PDADMAC/g MnO<sub>2</sub>, where opposite charges on materials are balanced, resulting in heavy aggregation. The dispersions were stable at doses away from the IEP. The selected PDADMAC dose for further studies was 200 mg PDADMAC/g MnO<sub>2</sub>, at which the PDADMAC-functionalized MnO<sub>2</sub> MFs (PMn) have sufficiently high magnitude of surface charge to resist aggregation.

T2.2 The significantly negative charge of CeO<sub>2</sub> NPs in the alkaline pH regime enabled the functionalization with positively charged PDADMAC. The CeO<sub>2</sub> NPs maintained their negative charge at low PDADMAC doses, while at high doses, charge reversal was observed, resulting in positively charged particles. The IEP occurred around 1 mg PDADMAC/g CeO<sub>2</sub>, giving rise to heavily aggregating particles with zero overall charge. A PDADMAC dose of 40 PDADMAC mg/g CeO<sub>2</sub> was selected for heteroaggregation with latex beads, at which the PDADMAC-functionalized CeO<sub>2</sub> NPs (PCe) possessed high positive charge and formed processable dispersion.

T2.3 The DLVO theory accounts for above trends in charge-aggregation relation. The non-aggregating state and colloidal stability of functionalized metal oxides at high and low PDADMAC doses are a result of strong electrostatic repulsion due to high magnitude of surface charge of the polyelectrolyte-functionalized nanozymes. Around the IEP, electrostatic forces are absent due to balanced opposite charges on the individual hybrid particles and hence, heavy aggregation of the non-charged nanozyme-PDADMAC particles occur under the influence of van der Waals forces, causing destabilization of the colloidal system.

**T3: The antioxidant composites (AL-PB, SPMn, SL-CeO<sub>2</sub>, and SPCe) were prepared by controlled heteroaggregation under the influence of electrostatic forces. The individual hybrid particles were stabilized by electrostatic/steric repulsion away from the systems' IEP, while van der Waals forces dominate in the absence of sufficiently high charge. Nanozyme coverage on the lattices depended on the type of surface functionalities.**

T3.1 The PB NPs and AL particles are oppositely charged at pH 4.0 and thus, the formation of AL-PB composites was achieved via spontaneous heteroaggregation under the influence of electrostatic and van der Waals attraction. At low PB doses, hybrid AL-PB particles maintained positive charge. Around the system IEP (76.6 mg PB/g AL), however, the AL-PB particles have no overall charge and undergo heavy aggregation and phase separation under the influence of van der Waals forces. Higher PB NP doses result in charge reversal and negatively charged AL-PB composites that remained in dispersed state.

T3.2 Since non-functionalized MnO<sub>2</sub> MFs and SL are like-charged at pH 9.0, direct immobilization was not attainable. The positively charged PMn particles were formed with a PDADMAC dose of 200 mg PDADMAC/g MnO<sub>2</sub> (PMn). The charge and aggregation of the SL-PDADMAC-MnO<sub>2</sub> (SPMn) composite was found to be dependent on the dose of PMn particles. Low PMn doses led to negatively charged SPMn particles, while charge neutralization occurred at a dose of 7.1 mg PMn/g SL. Higher doses caused charge reversal and result in positively charged SPMn with strong repulsion between the individual hybrid SPMn particles that gave rise to remarkable colloidal stability. Major interparticle forces were of DLVO origin.

T3.3 At pH 4.0, the CeO<sub>2</sub> NPs and SL are oppositely charged, and thus direct immobilization was achieved through heteroaggregation, while the overall charge on the resulting composite (SL-CeO<sub>2</sub>) depended on the CeO<sub>2</sub> dose. The IEP of the SL-CeO<sub>2</sub> system occurred at 100 mg CeO<sub>2</sub>/g SL, where van der Waals forces triggered significant aggregation of the SL-CeO<sub>2</sub> particles. Lower and higher doses led to formation of negatively and positively charged SL-CeO<sub>2</sub> particles, respectively. The high overall charge gave rise to high colloidal stability via electrostatic repulsion. At pH 9.0, however, both particles are similarly charged and hence, PDADMAC were adsorbed onto CeO<sub>2</sub> NPs to induce positive surface charge allowing spontaneous heteroaggregation with SL particles via DLVO-type and Coulombic forces. Low and high PCE doses resulted in highly charged composites (SPCe), while around the IEP (16 mg PCE/g SL), significant aggregation occurred under the influence of van der Waals forces.

T3.4 The extent of surface coverage of latex with nanozymes was largely affected by the presence of PDADMAC. Less decorated composites (SPMn and SPCe) were observed when PDADMAC-functionalized nanozymes are heteroaggregated with latex. Such difference in coverage is also observed in composites that are based on the same materials, as the case with SL-CeO<sub>2</sub> and SPCe, which exhibited IEPs around 16 mg CeO<sub>2</sub>/g SL and 100 mg PCE/g SL, respectively. Hence, the adsorption of PDADMAC not only resulted in charge reversal, but also modified the latex capacity to accommodate nanozymes on the surface.

**T4: The immobilization and PDADMAC functionalization of nanozymes did not compromise their antioxidant potential or enzyme-like activity, it rather resulted in minimal deterioration (AL-PB), similar (SPMn), or enhanced activity (SPCe and SL-CeO<sub>2</sub>), compared to bare nanozymes. Also, prolonged thermal treatment of nanozymes and their composites had little to no effect on their enzyme-like activity.**

T4.1 Bare PB NPs showed excellent horseradish peroxidase (HRP) and superoxide dismutase (SOD) activities that scavenge H<sub>2</sub>O<sub>2</sub> and superoxide radical anions, respectively. Upon immobilization of PB NPs onto AL particles, the AL-PB composite showed similar SOD-like and HRP-like activities with only minimal deterioration in the activity compared to the original PB NPs.

T4.2 Bare MnO<sub>2</sub> MFs were found to exhibit catalase (CAT)- and SOD-like activities. The CAT enzyme catalyzes the breakdown of H<sub>2</sub>O<sub>2</sub> into water and O<sub>2</sub>. Results showed that the PDADMAC functionalization and the subsequent immobilization of MnO<sub>2</sub> MFs on SL had no effect on both activities. Also, prolonged thermal treatment up to 75 °C did not affect the SOD activity, along with minimal loss in yield for both bare and immobilized MnO<sub>2</sub> MFs alike. Considering that natural SOD and CAT permanently lost catalytic activities at elevated temperatures, such heat tolerance of the enzymatic function of the SPMn composite is remarkable.

T4.3 Antioxidant CeO<sub>2</sub> NPs showed excellent HRP-like activity and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical reduction potential. Compared to bare CeO<sub>2</sub> NPs, the SPCe and SL-CeO<sub>2</sub> composites showed better HRP-like activity. On the other hand, the DPPH (a model organic radical to assess antioxidant ability) reduction potential of CeO<sub>2</sub> NPs in SPCe was enhanced, while SL-CeO<sub>2</sub> showed similar activity as bare CeO<sub>2</sub> NPs. Although less covered, the SPCe exhibited better enzyme-like activity than the fully covered SL-CeO<sub>2</sub>, which emphasizes the substantial effect of the aggregation status of nanozymes on their biocatalytic activity as well as their enhanced activity when nanozymes are present in composite form. The reduced coverage was attributed to the presence of PDADMAC on the nanozyme's surface.

## 5. Potential Applications

Within living organisms, antioxidant enzymes help to combat oxidative stress that is believed to be linked to numerous adverse health conditions such as aging, asthma, diabetes, male infertility, AIDS, autism as well as neurodegenerative diseases such as Alzheimer's and Parkinson's disease. In addition, enzymes are also widely used in numerous fields such as sensing, food manufacturing, environmental remediation, and cosmetics. Thus, the formulated composites are rendered potential alternatives that are expected to find use in such applications.

The formulated hybrids also present model composites that have economic edge. The formulation of latex-nanozyme composites is straightforward, economical, and rarely requires extreme conditions or sophisticated equipment. Therefore, the laboratory formulation of such composites can be easily scaled up to industrial production, where the composites can be recycled and regenerated. Also, the preservation of catalytic activity after high-temperature thermal treatment is highly beneficial in applications that take place at high temperatures such



as in rubber industry and thermal biosensors. Also, the structure of the composites was preserved during solvent evaporation due to strong electrostatic binding between the components and the resulting high surface area gave rise to excellent antioxidant potential. The structural integrity is vital in such potential applications as effective drug delivery of therapeutic nanoparticles, packing materials, biomarker detection, and sensing. In addition, the high colloidal stability, owing the high magnitude of overall charge, was maintained at various experimental conditions. The stable dispersions of antioxidant composites have promising use in numerous applications including pharmaceuticals, food/beverage industries, paints, and wound healing. Most importantly, the formulation of composites may enable their use in various biomedical applications such as Alzheimer's treatment, tumor-specific therapy, cancer therapy, and radiotherapy of malignant tumors.

Lastly, the systematic control over colloidal stability by altering the pH, ionic strength as well as doses provides a model approach for controlled manipulation of colloidal stability. Such initiation of aggregation of highly stable particles followed by phase separation is crucial in many applications such as water treatment and environmental remediation. In addition, the controlled aggregation of latex particles shows promising potential in the contemporary issue of the risks of increased introduction of micro and nanoplastics in air and natural water sources, where long-term exposure of such particles is believed to be linked to chronic health problems such as pneumonia and bronchitis.

## 6. Scientific Activities

MTMT ID: 10067930

### Research/Review Articles – related to dissertation

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1. **Nizar B. Alsharif**, Gergely F. Samu, Szilárd Sáringér, Adél Szerlauth, Dóra Takács, Viktoria Hornok, Imre Dékány, Istvan Szilagyí  
*Antioxidant colloids via heteroaggregation of cerium oxide nanoparticles and latex beads*  
COLLOIDS AND SURFACES B: BIOINTERFACES, 216 (2022) 112531  
SJR indicator: **Q1** **Impact factor**<sub>2021</sub>: 5.999  
**Independent citations**: 2
2. **Nizar B. Alsharif**, Szabolcs Muráth, Bojana Katana, Istvan Szilagyí  
*Composite materials based on heteroaggregated particles: Fundamentals and applications*  
ADVANCES IN COLLOID AND INTERFACE SCIENCE, 294 (2021) 102456  
SJR indicator: **D1** **Impact factor**<sub>2021</sub>: 15.190  
**Independent citations**: 7

3. **Nizar B. Alsharif**, Katalin Bere, Szilárd Sáringer, Gergely F. Samu, Dóra Takács, Viktória Hornok, Istvan Szilagyí  
*Design of hybrid biocatalysts by controlled heteroaggregation of manganese oxide and sulfate latex particles to combat reactive oxygen species*  
 JOURNAL OF MATERIALS CHEMISTRY B, 9 (2021) 4929-4940  
 SJR indicator: **Q1** **Impact factor**<sub>2021</sub>: 7.571  
**Independent citations: 1**
4. **Nizar B. Alsharif**, Gergely F. Samu, Szilárd Sáringer, Szabolcs Muráth, Istvan Szilagyí  
*A colloid approach to decorate latex particles with Prussian blue nanozymes*  
 JOURNAL OF MOLECULAR LIQUIDS, 309 (2020) 113066  
 SJR indicator: **Q1** **Impact factor**<sub>2020</sub>: 6.165  
**Independent citations: 10**

### Research Articles – unrelated to dissertation

1. Marko Pavlovic, Szabolcs Muráth, Xénia Katona, **Nizar B. Alsharif**, Paul Rouster, József Maléth, Istvan Szilagyí  
*Nanocomposite-based dual enzyme system for broad-spectrum scavenging of reactive oxygen species*  
 SCIENTIFIC REPORTS, 11 (2021) 4321  
 SJR indicator: **D1** **Impact factor**<sub>2021</sub>: 4.997  
**Independent citations: 10**
2. Szabolcs Muráth, **Nizar B. Alsharif**, Szilárd Sáringer, Bojana Katana, Zoltán Somosi, Istvan Szilagyí  
*Antioxidant materials based on 2D nanostructures: A review on recent progresses*  
 CRYSTALS, 10 (2020) 148  
 SJR indicator: **Q2** **Impact factor**<sub>2020</sub>: 2.589  
**Independent citations: 17**
3. Bojana Katana, Dóra Takács, Felix D. Bobbink, Paul J. Dyson, **Nizar B. Alsharif**, Matija Tomšic, Istvan Szilagyí  
*Masking specific effects of ionic liquid constituents at the solid–liquid interface by surface functionalization*  
 PHYSICAL CHEMISTRY CHEMICAL PHYSICS, 22 (2020) 24764-24770  
 SJR indicator: **Q1** **Impact factor**<sub>2020</sub>: 3.676  
**Independent citations: 3**

### Summary

Total publications:	<b>7</b>	Of which related to Dissertation:	<b>4</b>
Total independent citations:	<b>50</b>	Of which related to Dissertation:	<b>20</b>
Total impact factor:	<b>46.187</b>	Of which related to Dissertation:	<b>34.925</b>

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**Conference Participation – related to dissertation**

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1. **N. Alsharif**, K. Bere, G. F. Samu, I. Szilagyí (poster presentation/**virtual**)  
*Design of biocatalyst by immobilization of manganese oxide nanozymes onto sulfate latex support*  
Royal Society of Chemistry, 2022 #RSCPoster Twitter Conference (1-2 Mar 2022)
2. **N. Alsharif**, K. Bere, G. F. Samu, I. Szilagyí (oral presentation/**virtual**)  
*Hybrid biocatalysts based on manganese oxide and sulfate latex particles*  
33<sup>rd</sup> Australian Colloid & Surface Science Student Conference (31 Jan - 2 Feb 2022)
3. **N. Alsharif**, G. F. Samu, I. Szilagyí (poster presentation/**virtual**)  
*Decorating amidine latex particles with Prussian blue nanozymes*  
Geneva Colloids 2021 (8-9 Apr 2021)
4. **N. Alsharif**, G. F. Samu, I. Szilagyí (oral presentation/**virtual**)  
*The colloidal stability and enzymatic activity of Prussian blue nanoparticles immobilized onto polystyrene latex particles*  
XLIII. Kémiai Előadói Napok, (27-29 Oct 2020)

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**Conference Participation – unrelated to dissertation**

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1. **N. Alsharif**, T. Szabó, I. Szilagyí, E. Illés (poster presentation/**on-site**)  
*Heterocoagulation- and heteronucleation-synthesized iron oxide/graphene oxide nanocomposites for high-performance magnetic hyperthermia*  
33<sup>rd</sup> Conference of the European Colloid & Interface Society (ECIS), Leuven, Belgium (8-13 Sep 2019)