

THESES OF DOCTORAL (Ph.D.) DISSERTATION

# **Halloysite Nanotube Colloids: From Fundamentals to Applications as Antioxidant Agents**

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## **I. Introduction**

Halloysite clay nanotubes (HNTs) are naturally occurring layered aluminosilicates ( $\text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4 \cdot n\text{H}_2\text{O}$ ) with hollow tubular structure. The size of HNTs varies between 40–70 nm in outer diameter and 10–20 nm in inner diameter, and their length is typically span between 500–1500 nm. The outer surface is negatively charged due to the presence of deprotonated silanol groups, while the inner lumen is consisted of aluminol groups, thereby it becomes positively charged at intermediate pH. This dual-charge nature combined with biocompatibility and wide-range accessibility makes HNTs attractive for a variety of applications, such as important building blocks in composites used in biomedical, environmental, and catalytic processes.

Since HNT particles are dispersed in liquid media containing dissolved electrolytes in many applications, a thorough understanding of their colloidal behaviour is necessary before using them in such media. In addition to aqueous environment, novel solvents like ionic liquids (ILs) became important components in systems containing HNT materials. There is a scientific consensus that once nanoparticle dispersions containing simple electrolytes or ILs are involved in the application procedures, the colloidal stability of the samples is a crucial issue.

Nanoparticles have recently become popular candidates as supports for biocatalytic compounds. For instance, antioxidant enzymes, such as superoxide dismutase (SOD), show the greatest potential in preventing and regulating the negative effects of reactive oxygen species (ROS), but their supplementation is complicated due to high sensitivity to environmental conditions. This issue might be solved by immobilization on solid supports. Apart from enzymes, embedment of molecular and artificial antioxidants in nanostructures also attracts widespread contemporary interest. The role of the solid carrier is to stabilize the structure during the supplementation process and deliver the bioactive agents to the desired place in living organisms or in industrial samples.

Owing to their advantageous properties discussed above, HNTs are promising candidates as solid supports in immobilization of antioxidants or other molecules of biological interest. However, in many HNT applications, surface functionalization with polymeric compounds is required, since the general observations were that such a functionalization can enhance the adsorption of the guest molecules along with optimization of the colloidal stability of the nanotubes.

## **II. Objectives**

The main objective of my PhD research was the comprehensive investigation of HNT colloids from both fundamental (dispersion stability study in the presence of charged species) and applied (development of antioxidant composites) aspects. This goal was achieved through the following sub-objectives.

First, quantitative assessment of the colloidal stability of HNT particles in the presence of various monovalent (NaCl, KCl, and CsCl) and multivalent ( $\text{MgCl}_2$  and  $\text{LaCl}_3$ ) inorganic electrolytes in aqueous dispersions.

Second, the clarification of the role of the structure of ionic liquid (IL) constituents in their assembly at the solid/liquid interface in HNT dispersions and in the development of interparticle forces.

Third, tuning colloidal stability of HNT particles with natural and synthetic polyelectrolytes via surface functionalization.

Fourth, to develop stable antioxidant colloids composed of HNT, polyelectrolytes, and bioactive substances such as enzymes and enzyme mimicking metal complexes.

These goals have been achieved through experimental approaches using various microscopy, spectroscopy, and scattering methods, while certain results were compared to data obtained by model calculations using existing theories.

## **III. Experimental**

Commercially available HNTs were used, while its very limited dispersibility in aqueous dispersions was improved with an alkaline treatment to increase the amount of silanol groups on the external surface. Alkali-treated halloysite nanotubes (denoted as h-HNTs thereafter) were used in all investigations. Other chemicals were at least analytical grade and used without further purification.

Electrophoretic (ELS) and dynamic light scattering (DLS) were used to study the charging and aggregation properties of h-HNT in aqueous dispersions. Zeta potentials were calculated by appropriate models, while the tendencies for aggregation were expressed as stability ratios

determined in time-resolved DLS experiments. These techniques were also used to follow the polyelectrolyte adsorption and its effect on the colloidal stability.

Two types of polyelectrolytes were applied for surface functionalization of h-HNTs. First, a natural one, protamine sulfate (PSP), was used, and second, a synthetic polyimidazolium-based polymer (IP-2). The latter polymer was obtained in cooperation with Prof. Paul J. Dyson (EPF Lausanne, Switzerland). Functionalized h-HNT particles were obtained through adsorption of the polyelectrolytes on the oppositely charged surface.

Commercially available ILs were characterized by measuring the viscosity and refractive index of the IL solutions. These data were used for the analysis of the light scattering measurements.

Structural characterization of the particles and composites in solid state was performed by Raman and/or FT-IR spectroscopy. Raman spectra were recorded to reveal the structural features of the functionalized h-HNT particles, and the FT-IR technique was used to detect enzyme adsorption.

Electron paramagnetic resonance (EPR) spectroscopy experiments were conducted to explore the structural changes in the coordination geometry around the copper(II) ions upon immobilization. The measurements were performed under the supervision of Dr. Nóra V. May at the EPR laboratory at the Centre for Structural Science (Research Centre for Natural Sciences, Hungarian Academy of Sciences) in Budapest, as part of a collaboration.

Morphological studies of the bare and functionalized particles were performed by scanning (SEM) and transmission electron microscopy (TEM).

h-HNT particles were used as solid supports in immobilization processes of enzymes or enzyme-like components. The antioxidant activity of immobilized and native SOD enzyme or SOD-mimic was tested using the biochemical Fridovich assay. The evaluation of the test reaction was based on colour change, which was followed by UV-Vis spectrophotometry.

#### **IV. New scientific results**

**T1. The direct Hofmeister series of monovalent cations in the presence of negatively charged hydrophobic surfaces was established for the h-HNT particles based on surface charge and aggregation properties.**

T1.1. Different extent of cation adsorption was confirmed by recording electrophoretic mobilities, whose magnitude decreased with the salt concentration, and they remained negative in the entire regime investigated. A significant increase in the magnitude of the mobilities was observed in the  $\text{Cs}^+ < \text{K}^+ < \text{Na}^+$  order indicating ion specific interactions with the surface of the h-HNT particles.

T1.2. The affinity of the inorganic cations to the h-HNT surface influenced their destabilizing power leading to an increase in the critical coagulation concentration (CCC) of h-HNT dispersions in the  $\text{Cs}^+ < \text{K}^+ < \text{Na}^+$  order. This trend, together with the one obtained with electrophoretic mobilities, agrees with the direct Hofmeister series for negatively charged hydrophobic surfaces.

T1.3. The CCCs determined in the experiments were compared to calculated values using the DLVO (Derjaguin, Landau, Verwey and Overbeek) theory and a good agreement was found. This fact shed light on that the aggregation mechanism is controlled by the interplay between the repulsive electrical double layer and attractive van der Waals forces, while ion specific adsorption is responsible for the variation of the CCCs in the presence of different cations.

**T2. Multivalent counterions destabilized the h-HNT dispersions at lower CCC values than the monovalent ones and the results were explained within the Schulze-Hardy rule.**

T2.1. Significant deviations in the electrophoretic mobility values with the salt ( $\text{KCl}$ ,  $\text{MgCl}_2$  and  $\text{LaCl}_3$ ) concentrations confirmed that the affinity of the metal ions to the surface increases with the valence. For  $\text{La}^{3+}$  counterion, charge neutralization and subsequent reversal occurred at appropriate concentrations.

T2.2. The sequence in the CCC values, followed the  $\text{K}^+ > \text{Mg}^{2+} > \text{La}^{3+}$  order. This tendency is caused by the different extent of the ion adsorption and agrees qualitatively with the Schulze-Hardy rule. The quantitative agreement can be derived from the DLVO theory only for surfaces of unrealistically high charges.

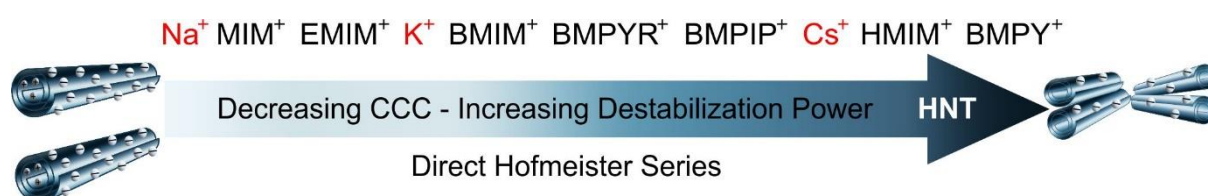
T2.3. Comparison of experimental and calculated CCC data revealed that the particle aggregation mechanism in each system is governed by double layer and van der Waals forces indicating homogeneous charge distribution on the h-HNT surface irrespectively of specific adsorption of the cations, which, in turn, influences the location of CCC data.

**T3. The Hofmeister series has been extended with IL cations of different structures (BMIM<sup>+</sup>, BMPYR<sup>+</sup>, BMPIP<sup>+</sup>, BMPY<sup>+</sup>) or alkyl chain lengths (MIM<sup>+</sup>, BMIM<sup>+</sup>, EMIM<sup>+</sup>, HMIM<sup>+</sup>).**

T3.1. It was unambiguously confirmed that both the magnitudes of electrophoretic mobilities at the same IL concentration and the CCCs decreased in the BMIM<sup>+</sup> > BMPYR<sup>+</sup> > BMPIP<sup>+</sup> > BMPY<sup>+</sup> order due to the different affinity of the cations to the h-HNT surface, with the strongest adsorption for BMPY<sup>+</sup>.

T3.2. It was found that increasing the alkyl chain length of the imidazolium cations led to an increased affinity of the counterions to the surface and subsequently, such charge compensation caused weakening of the electrical double layer repulsion in the MIM<sup>+</sup> > EMIM<sup>+</sup> > BMIM<sup>+</sup> > HMIM<sup>+</sup> order. While the particle aggregation mechanism could be predicted by the DLVO theory irrespectively of the type of ILs, the order of the obtained CCC values followed the above trend confirming similar charge-aggregation relation as for inorganic salts. Such a tendency originates from the hydrophobic nature of the counterions, which became more pronounced with increasing the alkyl chain length on the imidazolium ring.

T3.3. Based on results from electrophoretic and aggregation studies performed on negatively charged h-HNT particles, the direct Hofmeister series for the inorganic and IL cations was established as follows: Na<sup>+</sup> > MIM<sup>+</sup> > EMIM<sup>+</sup> > K<sup>+</sup> > BMIM<sup>+</sup> > BMPYR<sup>+</sup> > BMPIP<sup>+</sup> > Cs<sup>+</sup> > HMIM<sup>+</sup> > BMPY<sup>+</sup>. This order predicts the colloidal destabilization power of the cations, as illustrated in Figure 1.



**Figure 1.** Schematic representation of the direct Hofmeister series established for the negatively charged h-HNTs for inorganic (red) and IL (black) cations.

**T4. Positively charged protamine sulfate (PSP) and polyimidazolium (IP-2) polyelectrolytes have been shown to adsorb strongly onto oppositely charged h-HNT particles. The effect of the polyelectrolyte concentration on the stability regimes was unambiguously determined.**

T4.1. The negative electrophoretic mobility values, measured at low polyelectrolyte doses reflect that the surface charge of h-HNT was compensated only to a small extent by the adsorbed PSP or IP-2. Such an adsorption process is clearly indicated by an increase in the mobilities upon increasing the PSP or IP-2 concentration leading to charge neutralization at intermediate doses. The adsorption process continued after the charge neutralization point, and charge reversal of the h-HNT particles occurred. At high doses, the surface became saturated with adsorbed PSP or IP-2 layer, and hence, the electrophoretic mobilities reached a plateau.

T4.2. The h-HNT particles were stable at low and high polyelectrolyte doses, as indicated by very high or not even measurable stability ratios. In the intermediate regime, however, the stability ratios formed a U-shape curve with a minimum reached at PSP or IP-2 doses corresponding to the charge neutralization points. The stability ratios were unity within the experimental error at this minimum, indicating rapid particle aggregation and unstable suspensions. These findings are in qualitative agreement with the prediction of DLVO theory, since attractive van der Waals forces destabilize the samples upon charge neutralization, while sufficiently charged particles are stable due to repulsion between the electrical double layers.

T4.3. Dispersion characteristics of h-HNT-PSP and h-HNT-IP-2 (prepared at polyelectrolyte doses, where the saturated layer is formed) are very similar. Coating of the h-HNT particles by these polyelectrolytes resulted in a remarkable colloidal stability. The IP-2 polymer showed a surprising behaviour, as its ability to mask the ion specific effects was discovered. Accordingly, the colloidal stability and corresponding CCC values of h-HNT-IP-2 particles depended only on the ionic strength applied in the dispersions and were irrespective of the type of ions present. In other words, the IP-2 coating resulted in the formation of indifferent surfaces, which are insensitive to ion specific effects in contrast to bare h-HNT systems.

**T5. A highly active antioxidant material consisting of h-HNT, PSP, and SOD enzyme was developed by self-assembly of the biomacromolecules on the nanoparticulate support.**

T5.1. SOD was adsorbed on the h-HNT-PSP carrier via electrostatic and hydrogen bonding interactions leading to the formation of the h-HNT-PSP-SOD bionanocomposite. Remarkable structural and colloidal stability was confirmed by spectroscopic, scattering, and microscopic techniques.

T5.2. The superoxide radical anions scavenging activity was higher for h-HNT-PSP-SOD system than for native SOD indicating that the SOD enzyme kept its functional integrity upon immobilization, while the overall positive charge of the composite gave rise to substrate attraction and to higher activity.

**T6. The influence of h-HNT, h-HNT-PSP and h-HNT-IP-2 particles on the SOD-like activity of an enzyme mimicking metal complex was assessed. It was found that surface functionalization plays an important role in complex adsorption on the particles.**

T6.1. Adsorption of Cu(Cit) complex was the strongest on h-HNT-PSP due to enhanced electrostatic and hydrogen bonding interactions between the complex and the PSP chains on the surface. The Cu(Cit) adopted a dimeric structure both in solution and upon immobilization.

T6.2. The presence of Cu(Cit) complex did not affect the colloidal stability of the bare h-HNT dispersions and no particle aggregation occurred in the samples within the entire concentration range investigated. However, the size data for the h-HNT-PSP and h-HNT-IP-2 increased significantly with increasing complex concentration indicating aggregation of these particles. Such a process occurred at lower complex concentration for h-HNT-PSP in line with the mobility data. Adsorption of Cu(Cit) on the oppositely charged surface led to charge neutralization. Under this experimental condition, the repulsive interparticle forces originating from the overlap of the electrical double layers vanish and thus, attractive dispersion forces predominate giving rise to particle aggregation.

T6.3. Higher superoxide radical anion scavenging activities were obtained in the presence of polyelectrolyte-coated particles than for the Cu(Cit) in solution due to possible structural changes upon surface-complex interaction and to beneficial interferences between the radicals and the oppositely charged particles.



## **V. Applications**

During the PhD research, the basic colloidal features of h-HNT particles as well as their applicability in antioxidant systems were comprehensively studied. Since this topic has not been systematically explored before, the reported data and conclusions are unique, and the findings are expected to attract attention in the research communities in academia or in more applied disciplines.

Given the growing interest in anisotropic nanoparticles including HNT materials suspended in electrolyte or IL solutions, the present results provide important quantitative information on the interfacial properties and subsequently, on the aggregation tendencies of the particles. It is believed that these results give valuable new insights into the development of processable h-HNT dispersions in electrolyte solutions or in their mixtures either for biomedical or industrial uses.

Besides, h-HNT was proven to be a suitable carrier for the immobilization of SOD enzyme. The obtained h-HNT-PSP-SOD composite dispersion can be considered as a highly active antioxidant with the advantages of the heterogenization process, such as easier separation from the reaction mixture. Functionalization of the surface of h-HNTs with polyelectrolytes is beneficial for both colloidal and structural stabilization. These findings make the obtained hybrid material a promising antioxidant candidate in medical processes such as antioxidant treatment of inflammatory bowel diseases.

To overcome the limitations of natural or immobilized enzymes, such as their high sensitivity to environmental conditions and loss of enzyme activity at a higher temperature, the dispersions containing both HNT and an enzyme-mimicking metal complex can be considered since the desired salt and heat tolerances can be achieved by the optimization of the experimental conditions. To obtain such antioxidant additives is particularly important in manufacturing processes in the textile and fuel industry, where heat resistivity is a key requirement.

Summarily, the findings should attract considerable attention among scientist dealing with basic research or product development, since despite of the large-scale availability and advantageous properties of HNT, similar systematic studies, combining materials and colloid chemistry approaches, were rarely reported in the literature so far.

## VI. Scientific publications

MTMT ID: 10066292

### Papers related to the theses

1. **Bojana Katana**, Gábor Varga, Nóra V. May, and István Szilágyi  
*Superoxide dismutase mimicking nanocomposites based on immobilization of metal complexes on nanotubular carriers*  
 JOURNAL OF MOLECULAR STRUCTURE 1256 (2022) 132492.  
 DOI: 10.1016/j.molstruc.2022.132492  
 Independent citations: 1  
 SJR indicator: Q2  
 IF<sub>2021</sub>: 3.841
  
2. **Bojana Katana**, Dóra Takács, Adél Szerlauth, Szilárd Sáringer, Gábor Varga, Andrej Jamnik, Felix D. Bobbink, Paul J. Dyson, and István Szilágyi  
*Aggregation of Halloysite Nanotubes in the Presence of Multivalent Ions and Ionic Liquids*  
 LANGMUIR 37 (2021) 11869–11879.  
 DOI: 10.1021/acs.langmuir.1c01949  
 Independent citations: 3  
 SJR indicator: Q1  
 IF<sub>2021</sub>: 4.331
  
3. **Bojana Katana**, Dóra Takács, Edit Csapó, Tamás Szabó, Andrej Jamnik, and István Szilágyi  
*Ion Specific Effects on the Stability of Halloysite Nanotube Colloids - Inorganic Salts versus Ionic Liquids*  
 JOURNAL OF PHYSICAL CHEMISTRY B 124 (2020) 9757–9765.  
 DOI: 10.1021/acs.jpcc.0c07885  
 Independent citations: 13  
 SJR indicator: Q1  
 IF<sub>2020</sub>: 2.991
  
4. **Bojana Katana**, Paul Rouster, Gábor Varga, Szabolcs Muráth, Karine Glinel, Alain M. Jonas, and István Szilágyi  
*Self-Assembly of Protamine Biomacromolecule on Halloysite Nanotubes for Immobilization of Superoxide Dismutase Enzyme*  
 ACS APPLIED BIO MATERIALS 3 (2020) 522–530.  
 DOI: 10.1021/acsabm.9b00953  
 Independent citations: 17  
 SJR indicator: Q1  
 IF<sub>2020</sub>: N/A

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Papers not related to the theses

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1. Dóra Takács, Tamás Péter, Zsófia Vargáné Árok, **Bojana Katana**, Snežana Papović, Slobodan Gadzuric, Milan Vraneš, and István Szilágyi  
*Structure–Stability Relationship in Aqueous Colloids of Latex Particles and Gemini Surfactants*  
 JOURNAL OF PHYSICAL CHEMISTRY B 126 (2022) 9095-9104.  
 DOI: 10.1021/acs.jpcc.2c06259 SJR indicator: Q1  
 Independent citations: - IF<sub>2021</sub>: 3.466
  
2. Dóra Takács, **Bojana Katana**, Adél Szerlauth, Dániel Sebők, Matija Tomšič, and István Szilágyi  
*Influence of adsorption of ionic liquid constituents on the stability of layered double hydroxide colloids*  
 SOFT MATTER 17 (2021) 9116-9124.  
 DOI: 10.1039/D1SM01074C SJR indicator: Q1  
 Independent citations: 2 IF<sub>2021</sub>: 4.046
  
3. Nizar B. Alsharif, Szabolcs Muráth, **Bojana Katana**, and István Szilágyi  
*Composite materials based on heteroaggregated particles: Fundamentals and applications*  
 ADVANCES IN COLLOID AND INTERFACE SCIENCE 294 (2021) 102456.  
 DOI: 10.1016/j.cis.2021.102456 SJR indicator: D1  
 Independent citations: 8 IF<sub>2021</sub>: 15.190
  
4. **Bojana Katana**, Dóra Takács, Felix D. Bobbink, Paul J. Dyson, Nizar B. Alsharif, Matija Tomšič, and István Szilágyi (shared first authorship)  
*Masking specific effects of ionic liquid constituents at the solid–liquid interface by surface functionalization*  
 PHYSICAL CHEMISTRY CHEMICAL PHYSICS 22 (2020) 24764–24770.  
 DOI: 10.1039/D0CP02805C SJR indicator: Q1  
 Independent citations: 4 IF<sub>2020</sub>: 3.676
  
5. Szabolcs Muráth, Nizar B. Alsharif, Szilárd Sáringer, **Bojana Katana**, Zoltán Somosi, István Szilágyi  
*Antioxidant Materials Based on 2D Nanostructures: A Review on Recent Progresses*  
 CRYSTALS 10 (2020) 148.  
 DOI: 10.3390/cryst10030148 SJR indicator: Q2  
 Independent citations: 17 IF<sub>2020</sub>: 2.589

6. Snežana Papović, Milan Vraneš, Aleksandar Tot, István Szilágyi, **Bojana Katana**, Khalaf Alenezi, and Slobodan Gadžurić

*Physicochemical Investigations of a Binary Mixture Containing Ionic Liquid 1-Butyl-1-methylpyrrolidinium Bis(trifluoromethylsulfonyl)imide and Diethyl Carbonate*

JOURNAL OF CHEMICAL & ENGINEERING DATA 65 (2020) 68–80.

DOI: 10.1021/acs.jced.9b00738

Independent citations: 6

SJR indicator: Q1

IF<sub>2020</sub>: 2.694

7. Paul Rouster, Marko Pavlovic, Tianchi Cao, **Bojana Katana**, and István Szilágyi

*Stability of Titania Nanomaterials Dispersed in Aqueous Solutions of Ionic Liquids of Different Alkyl Chain Lengths*

JOURNAL OF PHYSICAL CHEMISTRY C 123 (2019) 12966–12974.

DOI: 10.1021/acs.jpcc.9b03983

Independent citations: 7

SJR indicator: D1

IF<sub>2019</sub>: 4.189

### Scientometric Data

Sum of peer reviewed publications: **11**

In relation to the theses: **4**

Cumulative impact factor: **47.013**

In relation to the theses: **11.163**

Sum of independent citations: **78**

In relation to the theses: **34**

### Oral and poster presentations related to the theses

1. **Bojana Katana**, Dóra Takács, István Szilágyi (oral presentation)

*Halloysite Nanotube Colloids – Ion Specific Effects and Enzyme Immobilization*

18th European Student Colloid Conference, June 26-30, 2022, Szeged, Hungary

2. **Bojana Katana**, Dóra Takács, István Szilágyi (oral presentation)

*Effect of inorganic salts and ionic liquid constituents on the stability of halloysite nanotubes particle dispersions*

28th EUCHEM Conference on Molten Salts and Ionic Liquids, June 5-10, 2022, Patras, Greece

3. **Bojana Katana**, Dóra Takács, István Szilágyi (oral presentation)

*Halloysite Nanotube Colloids - Role of Ion Specificity, Functionalization with Polyelectrolytes and Enzyme Immobilization*

33rd Australian Colloid and Surface Science Student Conference, 31 January-2 February 2022, Mawson Lakes, South Australia (online)

4. **Bojana Katana**, Dóra Takács, István Szilágyi (poster presentation)  
*Ion specific effects on the stability of halloysite nanotubes colloids*  
Geneva Colloids 2021, 8-9 April 2021, Geneva, Switzerland (online)
5. **Bojana Katana**, Paul Rouster, Gábor Varga, Szabolcs Muráth, Karine Glinel, Alain M. Jonas, István Szilágyi (oral presentation)  
*Self-assembly of biomacromolecules on halloysite nanotubes*  
XLII. Kémiai Előadói Napok, 28-30 October 2019, Szeged, Hungary
6. **Bojana Katana**, Paul Rouster, István Szilágyi (poster presentation)  
*Immobilization of superoxide dismutase on protamine-functionalized halloysite nanotubes*  
33rd Conference of the European Colloid and Interface Society (ECIS), 8-13 September 2019, Leuven, Belgium
7. **Bojana Katana**, Paul Rouster, István Szilágyi (poster presentation)  
*Immobilization of enzymes for development of biocatalytic systems in stable dispersions*  
56th Meeting of the Serbian Chemical Society, 7-8 June 2019, Nis, Serbia