DOCTORAL THESIS

Magnetic Nanomaterials Synthesis and Functionalization for Biomedical Applications

Maria Volokhova

TALLINNA TEHNIKAÜLIKOOL
TALLINN UNIVERSITY OF TECHNOLOGY
TALLINN 2022

TALLINN UNIVERSITY OF TECHNOLOGY
DOCTORAL THESIS
39/2022

Magnetic Nanomaterials Synthesis and Functionalization for Biomedical Applications

MARIA VOLOKHOVA

TALTECH PRESS

TALLINN UNIVERSITY OF TECHNOLOGY
School of Science
Chemistry and Biotechnology

The dissertation was accepted for the defence of the degree of Doctor of Philosophy on 6 June 2022

Supervisor: Dr. Liis Seinberg
Laboratory of Chemical Physics
National Institute of Chemical Physics and Biophysics
Tallinn, Estonia

Opponents: Associate Professor Yoji Kobayashi
King Abdullah University of Science and Technology
Thuwal, Saudi Arabia

Associate Professor Janno Torop
University of Tartu
Tartu, Estonia

Defence of the thesis: 4 July 2022, Tallinn

Declaration:
Hereby I declare that this doctoral thesis, my original investigation and achievement, submitted for the doctoral degree at Tallinn University of Technology, has not been submitted for any academic degree elsewhere.

Maria Volokhova ______________________
signature

ISSN 2585-6898 (publication)
ISBN 978-9949-83-864-6 (publication)
ISSN 2585-6901 (PDF)
Printed by Koopia Niini & Rauam

TALLINNA TEHNIKAÜLIKOOL
DOKTORITÖÖ
39/2022

Magnetiliste nanomaterjalide süntees ja funktsionaliseerimine biomeditsiiniliste rakenduste jaoks

MARIA VOLOKHOVA

TALTECH KIRJASTUS

List of Publications

The present Ph.D. thesis is based on the following publications that are referred to in the text by Roman numbers.

I **Volokhova, M.**; Boldin, A.; Link, J.; Tsujimoto, M.; Stern, R. and Seinberg, L.; Synthesis of Ni@SiO_2 and Co@SiO_2 Nanomagnets After Formation of NiO and Co_3O_4 Nanoparticles at Low Temperatures Using CaH_2. *Journal of Materials Research and Technology* **2022**, *16*, 988-992.

II Xie, R.; **Volokhova, M.**; Boldin, A.; Seinberg, L.; Tsujimoto, M.; Yang, M.; Rasche, B.; Compton, R.; (2020).Electro-catalytic oxidation of hydroxide ion by Co_3O_4 and Co_3O_4@SiO_2 nanoparticles both at particle ensembles and single particle level. ChemElectroChem, 7 (5), 1261-1276.

III **Volokhova, M.**; Shugai, A.; Tsujimoto, M.; Kubo, A.-L.; Telliskivi, S.; Nigul, M.; Uudekull, P.; Vija, H.; Bondarenko, O.M.; Adamson, J.; Kahru, A.; Stern, R.; Seinberg, L. Cubic Iron Core–Shell Nanoparticles Functionalized to Obtain High-Performance MRI Contrast Agents. *Materials* **2022**, *15*, 2228.

IV **Volokhova, M.**; Boldin, A.; Link, J.; Shugai, A.; Pehk, T.; Stern, R.; Seinberg, L. Synthesis of zwitterionic dopamine sulfonate coated cubic $\alpha - Fe@SiO_2$ nanoparticles and their magnetic properties analysis [Appearing].

Author's Contributions to the Publications

I The author was carrying out and planning the experiments, conducted, interpreted, and presented the powder x-ray diffraction measurements. The author wrote the manuscript, with contributions from the co-authors, and compiled the supporting information.

II The author was carrying out nanoparticles synthesis. Conducted, interpreted, and presented powder x-ray diffraction measurements. The author wrote nanoparticles synthesis and x-ray diffraction results in the manuscript and supporting information.

III The author was carrying out and planning the experiments, conducted, interpreted, and presented the powder x-ray diffraction measurements. The author wrote the manuscript, with contributions from the co-authors, and compiled the supporting information.

IV The author was carrying out and planning the experiments, conducted, interpreted, and presented the powder x-ray diffraction measurements. The author wrote the manuscript, with contributions from the co-authors, and compiled the supporting information.

Other publications by the author (Not discussed in this thesis)

1. **Industrial property:** Invention: METAL-BASED CORE NANOPARTICLES, SYNTHESIS AND USE; Owners: LSMedical ; Authors: Liis Seinberg, **Maria Volokhova**; Patent application nr WO2021/144006 A1 (2021)

2. Kiolein, L; Kaare, K; **Volokhova, M**; Tsujimoto, M; Palgrave, R; Seinberg, L; Kruusenberg, I (2022). Silica Encapsulated Cobalt and Nitrogen Co-doped Carbon Nanotubes as a Catalyst for Oxygen Reduction Reaction. ACS Applied Nano Materials [Appearing].

3. Barghi, B; Jürisoo, M ; **Volokhova, M**; Seinberg, L; Reile, I; Mikli, V; Niidu, A (2022). Process Optimization for Catalytic Oxidation of Dibenzothiophene over UiO-66-NH_2 by Using Response Surface Methodology. ACS Omega (2022).

Introduction

Magnetic Resonance Imaging (MRI) has developed into a very effective technique for imaging live specimens noninvasively. The contrast agents (CA) that contain paramagnetic ion gadolinium (III) are currently used to improve visibility of tissues and cells that are magnetically similar but histologically distinct. To maximize the efficiency of CA, it is necessary to design agents that are sensitive to biological features and directed to particular locations.

Preparing nanoparticles with homogeneous size and shape, especially for large-scale production remains challenging. Superparamagnetic NPs with size less than 50 nm are found to be promising materials for biomedical application as a MRI T_2 CA. Usage of metal hydride reduction reaction (at the temperatures <200°C) offers to synthesize new metal superparamagnetic NPs. This could be a significant leap forward in the MRI field.

Superparamagnetic metal magnetic NPs with size 50 nm synthesis and application as MRI CA continues to suffer from several challenges, such as long-term magnetic stability, aggregation, coagulation and lack of crystallinity in the materials. This work is focused on the synthesis and functionalisation of NPs with stable superparamagnetic properties in water solution and with functionalised nontoxic and biocompatible organic shells. Such NPs overcome aggregation and coagulation and would be applicable as improved MRI CA. NPs improved magnetic properties in the field of MRI CA and early disease diagnostics. Therefore, there is still a strong need for improved CA that are more specific, give higher signal and are less retained. It is important to synthesise NPs that can serve not only as transverse relaxation T_2, but could also influence longitudinal relaxation T_1 CA.

This thesis contains a modified synthesis method of NPs, their design as well as their characteristics. The experimental part depicts a variety of instruments, as well as characterisation and analytical procedures, reagents, and their solutions. Iron, cobalt and nickel oxide NPs were synthesized through a modified solvothermal synthesis process. Different single source precursors were used to regulate the composition. Several reaction stoichiometric ratios were observed, as well as reaction temperatures. Secondary chalcogen source were all examined under various conditions. As a result, Co_3O_4, NiO (**Publ. I and Publ. II**) and Fe_2O_3 NPs were obtained (**Publ. III and IV**). Those NPs were coated with SiO_2, and later reduced with CaH_2. Metal cores of Co and Ni NPs with SiO_2 shell were formed.

In addition Co_3O_4 and Co_3O_4@SiO_2 NPs were synthesised and studied separately (**Publ. II**). This part of research was done with help from Prof. Compton group from Oxford University. In this collaborative work we wanted to study nanoparticle-mediated electrochemical oxidation. Particles with core-shell structure were synthesised and their electrochemical behaviour was studied and compared with oxide nanoparticles.

NPs crystal structure and morphology were analyzed using powder X-ray diffraction and transmission electron microscopy. Vibrating-sample magnetometry was used to evaluate the mass magnetization and coercivity at room temperature, which is superior in comparison to oxide cores. After studying reaction properties, Fe NPs were synthesised (less than 500°C) for the future biomedical application. α-Fe@SiO_2 NPs surface was chemically modified with NH_2 - silane (**Publ. III**) and zwitterionic dopamine sulfonate (**Publ. IV**). This was done to improve their water solubility for biomedical application, such as MRI contrast agent.

Abbreviations

NPs	Nanoparticles
MRI	Magnetic Resonance Imaging
PXRD	Powder X-Ray Diffraction
TEM	Transmission Electron Microscopy
VSM	Vibrating Sample Magnetometry
PPMS	Physical Properties Measurement System
NMR	Nuclear Magnetic Resonance
DLS	Dynamic Light Scattering
ZDS	Zwitterionic Dopamine Sulfonate
CA	Contrast Agents
CSF	Cerebrospinal fluid
SC fat	Subcutaneous fat
Ms	Saturation Magnetization
PVP	Polyvinylpyrrolidone
DMF	N,N-Dimethylformamide
EtOH	Ethanol
MeOH	Methanol

Abstract

Magnetic nanomaterials synthesis and functionalization for biomedical applications

Nanomaterials can be prepared via several different synthesis methods. To obtain stable material it is crucial to optimize the synthesis conditions. In nanotechnology, most common way to synthesise NPs is by using high temperature reactions. In contrast, using the synthesis that is carried out at low temperatures(<500°C), chance is higher to obtain nanomaterials with desired properties and functionality. This thesis is divided into four chapters.

The first study describes the synthesis of Ni and Co based NPs, that are widely used because of their magnetic and electric properties. They can be used as efficient electrocatalysts for oxygen reduction reactions to replace fuel cell cathode materials. In this work NiO and Co_3O_4 NPs were synthesized using one-pot pyrolysis. Oxide NPs were covered with SiO_2 and reduced at a low temperature using CaH_2 in a vacuum-sealed tube, and as a result core-shell structures with metal Ni and Co cores and SiO_2 shell were obtained. Both Ni and Co metal oxides and their metal NPs crystal structure and morphology were analyzed using powder X-ray diffraction (PXRD) and transmission electron microscopy (TEM). Furthermore, Ni@SiO_2 and Co@SiO_2 NPs magnetic behaviour was measured.

The second study was based on Co_3O_4 and Co_3O_4@SiO_2 NPs that were synthesised and studied separately with the help of Prof. Compton's group from the University of Oxford. Particles with core-shell structure were synthesised and their electrochemical behaviour was studied and compared with oxide NPs. Co_3O_4@SiO_2 NPs showed similar electrochemistry results as a Co_3O_4 NPs. NPs had electro-catalytic activity towards OH^- oxidation, as well as activity towards the chemical decomposition of H_2O_2, which gave us an opportunity to use Co_3O_4 cored NPs as a bifunctional electrochemical and chemical catalysts.

The third study presents cubic iron core NPs with SiO_2 coating (α-Fe@SiO_2). These were found to have saturation magnetization very close to the highest possible value of any iron containing NPs and the bulk iron saturation magnetization. Further studies showed that they possess better MRI contrast agent potential than iron oxide NP-s on a clinical 3T MRI. (r_2 - 55 $s^{-1}mM^{-1}$ spherical γ-Fe_2O_3@SiO_2, 109 $s^{-1}mM^{-1}$ cubic core α-Fe@SiO_2).

The forth study focuses on synthesising NPs coated with a layer of chemically bound organic ligands. α-Fe core NPs were successfully synthesised with a SiO_2 shell. Their crystal structure and morphology were analysed using PXRD and TEM. Zwitterionic dopamine sulfonate (ZDS) was synthesised and used as a coating material for the cubic core α-Fe@SiO_2NP to improve their water solubility. Polydispersity index (PDI) was measured for Fe@SiO_2@ZDS NPs and was found to be 0.342, which is suitable for biomedical application.

Kokkuvõte

Magnetiliste nanomaterjalide süntees ja funktsionaliseerimine biomeditsiiniliste rakenduste jaoks

Nanomaterjale saab valmistada erinevate sünteesimeetodiga. Stabiilse materjali sünteesiks on oluline sünteesitingimusi optimeerida. Nanomaterjalide sünteesimisel on levinumad kõrge temperatuuriga reaktsioonid, kuid sünteesides madalatel temperatuuridel, on võimalus kontrollida materjali omadusi ja nende funktsionaalsust suurem. Selle doktoritöö tulemused on jagatud nelja uuringuga.

Esimeses uuringus kirjeldatakse Ni- ja Co-põhisid nanomaterjali, mida kasutatakse laialdaselt nende sootsatemagnetiliste ja elektriliste omaduste tõttu. Näiteks saab neid kasutada elektrokatalüsaatoritena hapniku redutseerimise reaktsioonis, et asendada kütuseelemendi katoodmaterjale. Selles töös sünteesiti NiO ja Co_3O_4 NP ühe-poti pürolüüsi abil. Oksiid-nanoosakesed kaeti SiO_2-ga ja redutseeriti madalal temperatuuril (<500°C) CaH_2 abil vaakumkinnitusega torus, mille tulemuseks saavutati Ni- ja Co-metalse tuuma ja SiO_2-katetega tuum-kest struktuurid. Nii Ni- kui ka Co-metallioksiide ja nende metallide nanoosakeste kristallstruktuuri ja morfoloogiat analüüsiti pulberröntgendifraktsiooni (PXRD) ja transmissioonelektronmikroskoobi (TEM) abil. Lisaks mõõdeti Ni@SiO_2 ja Co@SiO_2 nanoosakeste magnetkäitumist.

Teine uuring põhines Co_3O_4 ja Co_3O_4@SiO_2 NP-del, mis sünteesiti ja uuriti eraldi Oxfordi ülikooli prof Comptoni rühma abiga. Sünteesiti tuum-kest struktuuriga osakesed ning uuriti nende elektrokeemilisi käitumisi ja võrreldi nende oksiidiga. Co_3O_4@SiO_2 NP-d näitasid sarnaseid elektrokeemilisi tulemusi kui Co_3O_4 NP-d. NP-del oli elektrokatalüütiline aktiivsus OH^- oksüdatsiooni suhtes, samuti aktiivsus H_2O_2 keemilise lagunemise suhtes, mis annab võimaluse kasutada Co_3O_4 tuumaga NP-sid bifunktsionaalse ainena, ehk elektrokeemilise ja keemilise katalüsaatorina.

Kolmas uuringus esitletakse SiO_2 kattega kuubikujulisi metalse raua tuumaga nanoosakesi. Leiti, et nende küllastusmagnetiseerumine on väga lähedane kõigi Fe sisaldavate nanoosakeste kõrgeimale võimalikule väärtusele ja metalse raua küllastusmagnetiseerituse-le. Edasised uuringud näitavad, et neil on parem MRI kontrastaine potentsiaal kui raudoksiidi nanoosakestel. (r_2 - 55 $s^{-1}mM^{-1}$ sfääriliste γ-Fe_2O_3@SiO_2, 109 $s^{-1}mM^{-1}$ kuubiliste α-Fe@SiO_2 puhul).

Neljas uuring keskendub keemiliselt seotud orgaaniliste ligandite kihiga kaetud NP-de sünteesimisele. α-Fe tuuma nanoosakesed sünteesiti edukalt SiO_2 kestaga. Nende kristallstruktuuri ja morfoloogiat analüüsiti PXRD ja TEM abil. Zwitter dopamiin sulfonaati (ZDS) sünteesiti ja seda kasutati kuubilise tuuma α-Fe@SiO_2 nanoosakeste kattematerjalina, parandades nende vees lahustuvust. Polüdisperssuse indeks (PDI) mõõdeti Fe@SiO_2@ZDS NP jaoks ja leiti, et see on 0.342. See näitab, et NP-d on bioloogiliselt ühilduvad.

Curriculum Vitae

Personal data

Name	Maria Volokhova
Date of birth	05.08.1994
Nationality	Russian

Contact information

E-mail	Maria.Volokhova@kbfi.ee

Education

2018–2022	Tallinn University of Technology, Applied Chemistry and Biotechnology, PhD studies
2016–2018	Tallinn University of Technology, Applied Chemistry and Biotechnology, MSc
2013–2016	Tallinn University of Technology, Applied Chemistry and Biotechnology, BSc
2007–2013	Tallinn Tynismae Science School
2001–2007	Multidisciplinary Lyceum №4, Russia, Pskov

Language competence

Estonian	fluent
English	fluent
Russian	native

Professional employment

2018– …	National Institute of Chemical Physics and Biophysics, Early-stage researcher (PhD student) (1,00)
2017–2018	National Institute of Chemical Physics and Biophysics, Engineer (0,50)

Honours and awards

- 2020, Estonian National Culture Foundation, Tamkivi Real Sciences Scholarship
- 2019, Maria Volokhova, Dora Pluss (student mobility)
- 2018, Maria Volokhova, Kristjan Jaak Scholarship for Short Study Visit

Scientific work

AVS Conference 2018 - Pacific Rim Symposium on Surfaces, Coatings and Interfaces (PacSurf 2018 USA)

2018 - Workshop “From Nanotechnology to Nanomedicine” (Estonia, Tallinn, Nordic Hotel Forum)

France, Workshop - NMR Relaxometry for Interdisciplinary and Industrial Applications. (COST 2019)

JSPS-ETAg Joint Seminar (2019, Centennial Hotel Tallinn, Estonia)

3rd Workshop of the “EURELAX” COST Action (CA15209). (Co-Organizer, COST Tallinn 2020)

Elulookirjeldus

Isikuandmed

Nimi	Maria Volokhova
Sünniaeg	05.08.1994
Kodakondsus	Vene

Kontaktandmed

E-mail	Maria.Volokhova@kbfi.ee

Haridus

2018–2022	Tallinna Tehnikaülikool, Rakenduskeemia ja biotehnoloogia, PhD
2016–2018	Tallinna Tehnikaülikool, Rakenduskeemia ja biotehnoloogia, MSc
2013–2016	Tallinna Tehnikaülikool, Rakenduskeemia ja biotehnoloogia, BSc
2007–2013	Tallinna Tõnismäe Reaalkool
2001–2007	Multidistsiplinaarne Lütseumi №4, Venemaa, Pihkva

Keelteoskus

eesti keel	kõrgtase
inglise keel	kõrgtase
vene keel	emakeel

Teenistuskäik

2018– ...	Keemilise ja Bioloogilise Füüsika Instituut, nooremteadur (PhD) (1,00)
2017–2018	Keemilise ja Bioloogilise Füüsika Instituut, Insener (0,50)

Autasud

- 2020, Maria Volokhova, Eesti Rahvuskultuuri Fond, Tamkivi Reaalteaduste stipendium
- 2019, Maria Volokhova, Dora Pluss (lühiajaline õpiränne)
- 2018, Maria Volokhova, Kristjan Jaagu välislähetuste stipendium

Teadustegevus

AVS konverents 2018 – Pacific Rim Symposium on Surfaces, Coatings and Interfaces (PacSurf 2018 USA)

2018 - konverents “Nanotehnoloogiast nanomeditsiinini” (Eesti, Tallinn, Nordic Hotel Forum, Tallinna Tehnikaülikool, Keemilise Füüsika ja Biofüüsika Instituut)

Prantsusmaa, konverents – NMR-relaksomeetria interdistsiplinaarseteks ja tööstuslikeks rakendusteks. (COST 2019)

JSPS-ETAg ühisseminar (2019, Centennial Hotel Tallinn, Eesti)

EURELAXi COST Aktsiooni konverents (CA15209). “NMR ja MRI relaksomeetria keemias ja biomeditsiinis” (Kaaskorraldaja, COST Tallinn 2020)

ISSN 2585-6901 (PDF)