Ergänzungen zur Dissertation

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Motivation and goals

The chemical synthesis of nanomaterials has been a powerful tool for the progress of nanoscience. Many detailed recipes have been reported for the preparation of a wide variety of nanoscale materials like iron oxide,[1–7] titanium oxide[8] and manganese oxide[9] typically in the form of colloidal dispersions, within an equally wide range of available particle sizes and shapes.

Still, even established synthetic recipes are known to fail. It can be challenging to identify the reasons, resulting in significant loss of time and resources. Impurities, variations between batches and vendors, unintended reactions of chemicals but also changes in parameters like "room" temperature can lead to undesired and unexpected results.[2,10–12] Unfortunately, problems like this are rarely discussed and published. Therefore, a detailed knowledge of the synthesis parameters is essential for making a synthesis reproducible and to allow for easy upscaling and adapting.

Although tungsten oxide-based NCs show promising properties in different fields for example (photo)catalysis[13–15] and plasmonics,[16–18] detailed analysis of their formation behavior is still scarce. Only a few publications are available that provide insight into the mechanisms of tungsten oxide NC formation.[18–20]

The scientific objective of this work was to gain a fundamental understanding of the formation of tungsten oxide-based NCs synthesized by a standard heat-up synthesis using oleic acid and oleylamine as solvents and ammonium metatungstate as precursors. The experimental variables had to be evaluated in detail to build up ideas regarding the nucleation process and the phase selectivity of the two resulting products, WO_{3-x} NRs and hexagonal ammonium tungstate bronze NCs. Based on these findings, refinement of the NCs synthesis for other systems was explored. Reduced tungsten bronze NCs could be prepared by addition of alkali metal salts and (transition) metal tungstates were obtained with divalent metal cations. The respective reaction routes had to be evaluated, based on the synthetic approach and the relevant reaction parameters. A main objective of this study was to further investigate the elementary reactions of products and possible intermediates and the effect of impurities on the outcome of reactions.

Based on the surface reactions of tungsten oxides, applications in oxidation catalysis,[21,22] to utilize their localized surface plasmon resonance (LSPR)[23,24] and in terms of antimicrobial behavior[25] have already been investigated. In this context, the catalytic activity of WO_{3-x} NRs as catalysts for the oxidation of organic sulfides was investigated first. These catalytic properties were the basis to explore the activity in oxidative halogenation reactions (haloperoxidase-like reactions) of WO_{3-x} NRs and various tungsten bronze NCs. The final goal of these studies was to show that biofilm formation of nosocomial bacteria can be suppressed by quorum quenching using those NCs. The LSPR, especially of tungsten bronze

NCs was investigated with respect to growth inhibition of bacteria using near-infrared light (NIR) by hyperthermia.

Further studies using metal tungstate NCs were carried out with the aim to investigate their behavior regarding biofilm formation of different bacterial species. Based on the knowledge that transition metals, especially iron and manganese, are nutrients for bacteria, a conceptional understanding of the effects had to be delivered.

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Summary of Chapter 2

Tungsten oxides display a variety of materials with promising properties for different applications. The main goal presented in this chapter was to establish an adaptable way for the synthesis of different reduced tungsten oxide NCs and to ensure reproducibility and comparability. The work in this chapter lays the foundation for all other synthetic methods on metal tungstate NCs (chapters 4 and 6) and tungsten bronze NCs (chapter 5) presented in this thesis.

Chapter 2 describes the synthesis of Magnéli-type WO_{3-x} nanorods and hexagonal ammonium tungsten bronze NCs. Both nanomaterials form side by side as products of a classic NC synthesis via seeded growth in oleic acid and oleylamine, but the formation of each product depends on the reaction conditions. Concentration of ammonium metatungstate precursor, heating rate, solvent ratio and stirring speed were identified as relevant parameters the affect the phase selectivity. WO_{3-x} nanorods are favorized for slow heating rates, low precursor concentrations and fast stirring speed, while the opposite conditions lead to enhanced formation of ammonium tungsten bronze NCs. Changing the oleic acid to oleylamine ratio from 1:1 results in reduced tungsten bronze formation and changes the aspect ratio of the nanorods. Further investigations revealed, that the solvents oleic acid and oleylamine undergo a condensation reaction with oleyl oleamide as reaction product. A kinetic experiment revealed that the formation of the amide was independent of the presence of tungsten oxide NCs. Additionally, the amide has a strong effect on the phase selectivity, as it suppresses the formation of tungsten bronzes. A transmission electron microscopy study revealed that the formation of WO_{3-x} nanorods and hexagonal ammonium tungsten bronzes occurs simultaneously. Evidence was found that larger nanocrystals form *via* aggregation of nanorods during the early steps of the reaction. This behavior was exploited to show that the formation of WO_{3-x} nanorods and ammonium tungsten bronzes is based on the concept of non-classical nucleation by oriented attachment of smaller primary NCs.

The binding situation of ammonium cations located inside the channels of the hexagonal tungsten bronzes was elucidated using solid state NMR spectroscopy. It was shown that annealing of the nanocrystals at elevated temperatures leads to oxidation of tungsten, which leads to a release of ammonia. At 500 °C a phase transformation to monoclinic tungsten oxide occurred that is associated a complete release of ammonia. All tungsten bronze nanocrystals show strong absorption in the NIR regime, which is associated with localized surface plasmon resonance. The maximum of the plasmon band depends on the aspect ratio of the WO_{3-x} nanorods and can be tuned synthetically.

The utilization of WO_{3-x} NRs as oxidation catalyst is presented in chapter 3.

Summary of Chapter 3

Oxidation catalysis is a promising field for the low-cost production of different chemicals. The goal of the research described here was to utilize WO_{3-x} NRs (presented in chapter 2) as catalyst for the selective oxidation of sulfides to sulfoxides without overoxidation to sulfones. The influencing parameters were analyzed and discussed in detail, to establish a well-defined, reusable and adoptable system.

Thioanisole was used as model compound and hydrogen peroxide as oxidant. The catalysis was performed in methanolic solution and investigated by *in-situ* NMR. The oxidation of thioanisole to methyl phenyl sulfoxide occurred on a timescale of one hour. Only minor further oxidation to methyl phenyl sulfone was observed. The key parameters affecting the reaction were identified. Increasing concentrations of water in the solvent methanol led to exponential quenching. This was rationalized by the similar affinity of water and hydrogen peroxide towards the WO_{3-x} surface. Higher concentrations of hydrogen peroxide did not enhance the reaction rate, but higher concentrations of the catalyst and reactants increased the turnover rate significantly. The optimum temperature for annealing WO_{3-x} NR prior to the oxidation reaction is 300 °C. This allows removal of passivating surface ligands such as oleylamine water without oxidizing reduced WO_{3-x} NRs to the pure oxide WO_3. WO_{3-x} NRs can be reused at least five times without loss of activity.

To demonstrate the variability of the catalytic response, a screening of sulfides with different chemical structure and properties was performed. Aliphatic, non-steric sulfides exhibit a significantly faster reaction than thioanisole, while the reaction of sterically hindered sulfides was more slowly. Conjugated aromatic systems decreased the reaction rate due to their electron-withdrawing effect. This effect was enhanced when an electron-withdrawing substituent was in the conjugated position of the aromatic system. Electron-donating substituents increased the reaction rate. Even for the most rapid reactions no significant overoxidation to the corresponding sulfones was observed.

The activity of various compounds such as hexagonal ammonium tungsten bronze, ceria, and titania NCs, as well as various reference materials such as commercial tungsten oxide and soluble sodium tungstate, were compared with WO_{3-x} NRs. Only sodium tungstate showed acceptable catalytic activity, but with significant oxidation to sulfones. Because of the formation of surface peroxo-complexes, WO_{3-x} NRs can form soluble tungstate frames with hydrogen peroxide, which may be responsible for the overoxidation to sulfones. A more comprehensive analysis of this phenomenon is presented in chapter 5. Ceria nanocrystals showed a pronounced selectivity in the oxidation of sulfoxides to sulfones, while the oxidation of sulfides to sulfones was slow. ζ-potential offers a plausible explanation, since negatively charged WO_{3-x}-NR surfaces have a higher affinity for sulfides than the more negatively charged sulfoxides. Ceria NCs with positive ζ-potential showed higher affinity for sulfoxides than sulfides.

The results of this chapter 3 show the catalytic activity for the WO_{3-x} NRs prepared in chapter 2 and conceptionally lay the foundation for the oxidative bromination catalysis and antimicrobial behavior of WO_{3-x} NRs tungsten bronze NCs discussed in chapter 5.

Summary of Chapter 4

The results of this chapter are directly based on the findings from the synthesis of WO_{3-x} NRs and hexagonal ammonium tungsten bronzes presented in chapter 2. It shows the necessity of detailed investigation of impurities and side-products, which can ultimately lead to new research prospects.

Oleic acid that had been stored in an approximately 2-year-old opened steel canister had a brownish appearance and showed, compared to colorless oleic acid that was stored in a glass bottle, a significant effect on the synthesis of WO_{3-x} NRs. The WO_{3-x} NRs were shorter, and no hexagonal ammonium tungsten bronze was formed at higher ammonium metatungstate precursor concentrations. Further analysis revealed that iron leached from the steel canister into the oleic acid. This effect could be reproduced purposefully by addition of iron oleate to non-contaminated oleic acid. Iron tungstate NCs with ferberite structure was formed as side product for higher concentrations of iron oleate. Pure ferberite NCs were obtained for an iron to tungsten ratio of 1:1. Size and morphology of those $FeWO_4$ NCs could easily be tuned by adjusting the ratio of the solvents oleic acid and oleylamine, as well as by the heating rate, comparable to the synthesis of WO_{3-x} NRs (chapter 2).

When iron acetylacetonate was used as iron precursor, a new iron tungstate with a crystal structure different from ferberite was identified. The PXRD profile of this iron tungstate did not match that of any known iron tungstate structure. The new iron tungstate was obtained in pure form when oleyl oleamide (as described in chapter 2) was used as solvent. Mössbauer spectroscopy revealed that ferberite and the new iron tungstate structure differ in their iron(II) environment. The crystal structure of the new iron tungstate could not be solved from powder diffraction data so far. However, the powder diffractogram of a metastable magnesium tungstate (with an unknown trigonal or tetragonal structure) resembles the diffractogram of the new iron tungstate. As the thermodynamically stable structure of $MgWO_4$ is similar to that of ferberite, a structural relationship between the metastable $MgWO_4$ and the new iron tungstate was assumed. The metastable $MgWO_4$ polymorph could be synthesized via the standard synthesis in oleic acid/oleylamine mixtures starting from magnesium acetylacetonate and ammonium metatungstate. The crystal structure of this metastable magnesium tungstate and the new iron tungstate remains unresolved in this work. In ongoing research, structural analysis with 3D electron diffraction is being pursued.

The synthesis of ferberite NCs and of new iron and magnesium tungstates shows that the synthetic approach outlined in chapter 2 is useful for the syntheses of different tungsten oxide-based nanocrystals. Several transition metal tungstate NCs based on the foundation set in this chapter show a pronounced antibacterial activity, which is introduced in chapter 6.

Summary of Chapter 5

Microbial resistances against antibiotics are a major issue in medicine. Combat strategies that do not cause new resistances are highly desired. Based on the synthesis of hexagonal ammonium tungsten bronze NCs (chapter 2) alkali metal tungsten bronzes could be prepared, as detailed in this chapter 5. Inspired by the oxidative properties of WO_{3-x} NRs (chapter 3) the haloperoxidase-like properties of WO_{3-x} NRs and tungsten bronzes were established. The antimicrobial applications based on these findings were explored in a cooperation project with the microbiology in the [redacted].

Hexagonal cesium tungsten bronze NCs were prepared in a straightforward manner with cesium carbonate in the generalized synthesis (Cs:W ratio = 0.3:1) which was described in chapter 2. WO_{3-x} NR formation is suppressed completely when cesium carbonate was used is starting compound. A slow heating rate led to the formation of rectangular and disk-like shaped hexagonal cesium tungsten bronze NCs. With increasing oleylamine to oleic acid ratio, NCs of a pyrochlore-type cubic cesium tungsten bronze NCs were formed next to the hexagonal NCs, while the NC size was reduced. For a Cs:W ratio of 0.6:1 pure cubic cesium tungsten bronzes formed, independent of the oleic acid to oleylamine ratio. In analogy to the LSPR of WO_{3-x} NRs (chapter 2) the hexagonal alkali metal tungsten bronzes showed a strong absorption or NIR regime, with an intense tailing into the visible range due to the low anisotropy of the NCs. The cubic cesium tungsten bronze showed significantly less absorption than the hexagonal counterpart.

The haloperoxidase-like properties of WO_{3-x} NRs and alkali metal tungsten bronzes were established with a phenol red assay. Likewise, soluble sodium tungstate showed a reasonable catalytic activity. As haloperoxidase activity may lead to antibacterial activity for Gram-negative bacteria based on the quorum quenching, biofilm formation of the nosocomial bacterium *Pseudomonas aeruginosa* was explored with WO_{3-x} NRs and cubic cesium tungsten bronzes as haloperoxidase mimics. Cubic cesium tungsten bronzes showed the most promising suppression of the biofilm formation, while the effect was slightly less pronounced for WO_{3-x} NRs. A positive control with sodium tungstate showed less retardation of biofilm formation. The stability of different tungsten oxide bronzes was investigated in water and hydrogen peroxide. WO_{3-x} NRs showed moderate leaching in water and high leaching in the presence of hydrogen peroxide due to surface binding of peroxo-groups and subsequent formation of water-soluble tungstate species. All other tungsten bronze NCs showed negligible leaching in water and only slight leaching in the presence of hydrogen peroxide. Therefore, tungsten leaching is certainly not the prime reason for the catalytic and antibacterial properties of the tungsten bronzes.

In addition to biofilm inhibiting properties tungsten bronzes inhibit the growth of the fungus *Fusarium graminearum* in the presence of hydrogen peroxide. The highest activity was observed for cubic cesium tungsten bronzes. WO_{3-x} NRs showed a slightly lower activity, but additionally reduced the mycelium growth.

The LSPR prevent growth of the bacteria *Pseudomonas aeruginosa*, *Phaeobacter gallaeciensis* and *Staphylococcus aureus*. Mixed hexagonal/cubic cesium tungsten bronzes showed reduction of bacterial growth under irradiation of NIR light (λ=950 nm). Growth reduction was low for WO_{3-x} NRs and hexagonal ammonium tungsten bronzes, although they show significant absorption at λ=950 nm.

Summary of Chapter 6

Colonizing bacteria can form biofilms on different surfaces. Dispersion of such biofilms is a potential strategy to remove bacteria from such surfaces. Chapter 6 deals with the synthesis of various (transition) metal tungstate NCs. The results are partly based on the findings in chapter 4 for ferberite-type iron tungstate NCs. Their properties regarding biofilm formation were investigated in collaboration with the microbiology in the [redacted].

Starting point of the investigations was the study of the biofilm formation of *Phaeobacter gallaeciensis* on various surfaces, especially on structural steel. It was shown that biofilm formation on steel surfaces is significantly more pronounced than on simple laboratory glass. With polymers as reference materials, it was shown that less polar polymers significantly suppressed biofilm formation compared to more polar polymer surfaces and glass.

The concept of biocorrosion can explain the formation of bacterial films on steel surfaces. Salts of various biologically relevant metals that are typical constituents of structural steel (e.g. iron, manganese, zinc, cobalt and magnesium) were investigated for their influence on biofilm formation. Iron and manganese promote biofilm formation by up to 25 %. The effect is probably due to the essential properties of these metals for the metabolism of bacteria. The effect was concentration-independent for iron, while for manganese a decrease in biofilm increase was observed with increasing concentration. Magnesium showed a similar effect to manganese, but less pronounced. The other metals resulted in a decrease in biofilm formation, especially at higher concentrations.

Ferberite-type iron tungstate NCs and hübnerite-type manganese tungstate NCs synthesized based on the results in chapter 4 were investigated for their impact on biofilm formation. Surprisingly, a significant reduction of biofilm in both cases was observed, with the manganese tungstate NCs showing a stronger influence. On the other hand, the number of planktonic cells in the supernatant was significantly increased compared to the reference without NCs. Iron tungstate NCs with different morphologies were synthesized and tested. They showed no significant difference in their effect on biofilm formation. Moreover, different precursors were used for the synthesis of magnesium tungstate NCs to test for dependence. All NCs studied showed similar behavior. The effect of all NCs on the biofilm formation of *Pseudomonas aeruginosa* was investigated and the effect was similar to that for *Phaeobacter gallaeciensis*. Other metal tungstate NCs based on cobalt, magnesium, strontium, and barium were synthesized but had no effect on biofilm formation. Zinc tungstate NCs showed evidence of antimicrobial behavior, corresponding to the antibacterial properties of zinc salts.

The impact of magnesium and iron tungstate NCs leads to the assumption that these NCs cause a dispersion of the biofilm. Since the matrix and dispersion of the biofilm is a rather complex issue, only several plausible reasons are discussed, e.g. binding of relevant siderophores to NC surfaces, which would prevent the bacteria from extracting biorelevant cations.

The results of this chapter show a non-toxic way to reduce biofilm formation and thus reduce the risk of increasing bacterial resistance.