Zinc Oxide Based Polymer Hybrid Materials

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vorgelegt von

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Publikationen in Fachzeitschriften


Beiträge zu Konferenzen


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<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>AMPA</td>
<td>2,2’-Azobis-(2-methylpropion-amidin) Dihydrochloride</td>
</tr>
<tr>
<td>BIS</td>
<td>Methylene-bis-acrylamide</td>
</tr>
<tr>
<td>°C</td>
<td>Degree Celsius</td>
</tr>
<tr>
<td>CDCl₃</td>
<td>Deuterized Chloroform</td>
</tr>
<tr>
<td>CdS</td>
<td>Cadmium Sulfide</td>
</tr>
<tr>
<td>CdSe</td>
<td>Cadmium Selenide</td>
</tr>
<tr>
<td>cm</td>
<td>Centimeter</td>
</tr>
<tr>
<td>Dₜ</td>
<td>Hydrodynamic Diameter</td>
</tr>
<tr>
<td>DEG</td>
<td>Diethylene Glycol</td>
</tr>
<tr>
<td>Diglyme</td>
<td>Diglycole Dimethyl Ether</td>
</tr>
<tr>
<td>DLS</td>
<td>Dynamic Light-Scattering</td>
</tr>
<tr>
<td>DMF</td>
<td>Dimethyl Formamide</td>
</tr>
<tr>
<td>EEGE</td>
<td>Ethoxyethyl Glycidyle Ether</td>
</tr>
<tr>
<td>EtOH</td>
<td>Ethanol</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier-Transform-Infrared Spectroscopy</td>
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<tr>
<td>GPC</td>
<td>Gel Permeation Chromatography (Size Exclusion Chromatography)</td>
</tr>
<tr>
<td>h</td>
<td>Hour</td>
</tr>
<tr>
<td>hd-FB</td>
<td>Human Dermal Fibroblasts</td>
</tr>
<tr>
<td>HR-TEM</td>
<td>High Resolution Transmission Electron Microscopy</td>
</tr>
<tr>
<td>IA</td>
<td>Itaconic Acid</td>
</tr>
<tr>
<td>IADME</td>
<td>Dimethyl Itaconate</td>
</tr>
<tr>
<td>ICP-MS</td>
<td>Inductive Coupled Plasma – Mass Spectrometry</td>
</tr>
<tr>
<td>kV</td>
<td>Kilovolt</td>
</tr>
<tr>
<td>L</td>
<td>Litres</td>
</tr>
<tr>
<td>L₄</td>
<td>Laureth-4-Carboxylic Acid</td>
</tr>
<tr>
<td>L₄-Na</td>
<td>Sodium Salt of Laureth-4-Carboxylic Acid</td>
</tr>
<tr>
<td>LCST</td>
<td>Lower Critical Solution Temperature</td>
</tr>
<tr>
<td>LDH</td>
<td>Lactate Dehydrogenase</td>
</tr>
<tr>
<td>MACl</td>
<td>Methacryloyl Chloride</td>
</tr>
<tr>
<td>Mal</td>
<td>Maltose</td>
</tr>
<tr>
<td>MeOH</td>
<td>Methanol</td>
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## List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>MFRT</td>
<td>Microfluidics Reaction Technology</td>
</tr>
<tr>
<td>mL</td>
<td>Millilitres</td>
</tr>
<tr>
<td>nm</td>
<td>Nanometer</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear Magnetic Resonance</td>
</tr>
<tr>
<td>NIPAAm</td>
<td>N-Isopropylic Acrylamide</td>
</tr>
<tr>
<td>PCL</td>
<td>Poly(ε-caprolactone)</td>
</tr>
<tr>
<td>PEG</td>
<td>Poly(ethylene glycole)</td>
</tr>
<tr>
<td>PEG-MA</td>
<td>Poly(ethylene glycol) Methacrylate</td>
</tr>
<tr>
<td>PEI</td>
<td>Poly(ethylene imine)</td>
</tr>
<tr>
<td>PG</td>
<td>Poly(glycidole)</td>
</tr>
<tr>
<td>PDI</td>
<td>Polydispersity Index</td>
</tr>
<tr>
<td>PIPES</td>
<td>Piperazine-N,N’-bis(2-ethanesulfonic acid)</td>
</tr>
<tr>
<td>$P_n$</td>
<td>Degree of Polymerization</td>
</tr>
<tr>
<td>PVCL</td>
<td>Poly(N-Vinylcaprolactam)</td>
</tr>
<tr>
<td>$R_g$</td>
<td>Radius of Gyration</td>
</tr>
<tr>
<td>$R_h$</td>
<td>Hydrodynamic Radius</td>
</tr>
<tr>
<td>Rh-B</td>
<td>Rhodamine-B</td>
</tr>
<tr>
<td>RT</td>
<td>Room Temperature</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>T</td>
<td>Temperature</td>
</tr>
<tr>
<td>TGA</td>
<td>Thermogravimetric Analysis</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TEM</td>
<td>Transmission Electron Microscopy</td>
</tr>
<tr>
<td>(FE)SEM</td>
<td>(Field Emitter) Scanning Electron Microscopy</td>
</tr>
<tr>
<td>UV-Vis</td>
<td>Ultraviolet – Visible Light</td>
</tr>
<tr>
<td>VCL</td>
<td>N-Vinylcaprolactam</td>
</tr>
<tr>
<td>VPTT</td>
<td>Volume Phase Transition Temperature</td>
</tr>
<tr>
<td>wt.-%</td>
<td>Weight-Percentage</td>
</tr>
<tr>
<td>ZnO</td>
<td>Zinc Oxide</td>
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<tr>
<td>ZnS</td>
<td>Zinc Sulfide</td>
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</table>
Aim of the Work

The scope of this work is the preparation of wound dressings for improved wound healing. Hence, the thesis presented here describes the bottom-up approach to generate functional ZnO-polymer hybrid materials and their deposition on electrospun fibers (Scheme 1).

The first chapter focuses on the preparation and characterization of functional zinc oxide (ZnO) nanoparticles, by precipitation in organic solution as well as by use of microfluidic technology, with controlled size and morphology. Furthermore, the surface-modification with two different polymer-systems for colloidal stability improvement of nanoparticles in water is discussed, leading also to a desired additional functionality of the particles.

The next chapter comprises the synthesis of functional microgels and the integration of ZnO into them in order to prepare nanocomposites, which were characterized in aqueous dispersion.

The work described in the last chapter details the preparation procedure for coating microfibers with microgel/ZnO nanocomposites, the investigation of zinc ion release from the fibers and the evaluation of the fiber-biocompatibility.

The work leading to the experimental results presented in this thesis was funded by the DFG project (SPP 1327), primarily concerned with the preparation of a wound-dressing for improved healing of burn wounds. The novel idea was to use a physical, laser-based method in order to prepare ZnO-microgel nanocomposites by combining laser-ablation of nanoparticles from metal-foils in aqueous medium and in-situ polymerization of microgels. The laser-ablation process was accomplished by our partners at the university of Essen-Duisburg. The project also involved the collaboration with the Medical School of Hanover, responsible for biocompatibility tests for the composite microgel-based fibers (see Scheme 1).
Scheme 1: The structure of the present thesis. The thesis consists of three parts, building up on each other. Starting with ZnO nanoparticle synthesis, composites are prepared by the incorporation of nanoparticles into the microgels. These nanocomposites are further processed in order to obtain microfibers, forming non-wovens on the macrometer-scale, acting as wound-dressings with zinc-releasing properties.
Abstract

ZnO nanoparticles were successfully synthesized by a one-pot precipitation method and their surface modified by use of a fatty acid. It was shown that by switching the base amount between two different concentrations, two morphologies can be obtained, namely nanospheres and nanorods. ZnO nanorods showed improved colloidal stability in water and higher antibacterial activity compared to nanospheres. Further characterization revealed a double layer assembly of the surfactant-molecules on the nanoparticle’s surfaces. Moreover, the surfaces of the nanorods were successfully modified after reacting with a novel PEI-maltose compound. The synthesis of ZnO nanospheres was successfully transferred to the inside of a PVCL-based microgel system, i.e. the precipitation of ZnO occurred in the microgel network, as demonstrated by TEM, DLS and zetapotential measurements. Different PVCL-based microgels were tested concerning their use as ZnO hosts, including a microgel that was prepared using a novel macro monomer consisting of iso-Eugenol and poly(glycidole). The PVCL-itaconic acid and PVCL-iso-Eugenol systems show the most promising results within the framework of this thesis. Further on, novel PVCL-PEG microgels were synthesized under various conditions. DLS, zetapotential and calorimetric measurements revealed nanosized hydrogels; no turbidity was observed during the wet-chemical synthesis. These nanogels were successfully used for the in-situ conjugation of laser-generated nanoparticles, in cooperation with Essen University, by reproducing the synthesis during laser-ablation. The PVCL-itaconic acid and –PEG based microgels loaded with ZnO nanoparticles were further processed to obtain PCL microfibers by means of electrospinning. FESEM and TEM characterization proved the successful deposition of microgel-ZnO composites onto the PCL microfibers. Further studies showed that these novel three-component systems can release zinc ions and are non-toxic for human dermal fibroblasts.
1. Synthesis of ZnO Nanoparticles with Variable Morphologies and Reactive Surface

1.1. Introduction

1.1.1. General Properties of ZnO

Zinc oxide (ZnO) is a semi-conducting material with a direct band-gap of 3.4 eV. ZnO has been widely investigated and proved to be a useful material for many industrial applications. The most important application of ZnO (bulk) worldwide is its use in the rubber industry where it is applied as vulcanization additive aiming improved thermal resistivity and mechanical properties of rubber products\textsuperscript{[1–3]}. ZnO is a basic oxide with amphoteric properties in aqueous environment and shows very low solubility in neutral pH-aqueous solutions, whereas it degrades easily at acidic and basic pH. Due to the low water solubility of ZnO in combination with the low toxicity of zinc ions, applications in cosmetics industry have reached a high level of interest among scientists in order to investigate the capabilities of this material regarding, for example, ointments and tapes. Besides its application as a white-pigment ZnO also acts as an anti-septic agent due to the toxicity of zinc against microbes. Furthermore, studies were done in order to investigate the wound healing properties of ZnO-based topical wound therapy leading to increased wound-closure. The reason for the latter application is due to the physiological importance of zinc as trace metal in human organisms where it is utilized by many enzymes\textsuperscript{[4]}. Moreover, zinc offers possibilities for applications in burn injury therapy due to its antioxidant properties (for more information concerning biological properties, see chapter 3).
1. Synthesis of ZnO Nanoparticles with Variable Morphologies and Reactive Surface

Figure 1: The three different crystalline structures of ZnO. Thermodynamically stable at standard conditions is the wurtzite Structure (c). Reprinted with permission from Reference [5]. Copyright (2014) AIP Publishing LLC.

ZnO crystallizes under standard conditions in a wurtzite type configuration that is thermodynamically the most stable structure. The elementary cell includes one oxygen atom tetrahedrally surrounded by four zinc atoms and vice versa, leading to a zinc to oxygen ratio of one to one. Furthermore and under special conditions, ZnO can crystallize to a zinc blende configuration - a cubic body centered type - and to a rocksalt configuration (see Figure 1).
1. Synthesis of ZnO Nanoparticles with Variable Morphologies and Reactive Surface

1.1.2. Properties of Nanocrystalline ZnO

Decrease of size in case of an inorganic metal oxide below a certain dimension leads to a dramatic change of physical properties, more specifically, properties related to electron energy changes between the Highest Occupied Molecular Orbital (HOMO) and the Lowest Unoccupied Molecular Orbital (LUMO). The reason why these effects are specific for metal oxides is due to their semi-conducting nature and the fact that they own a fully occupied valence-band and an empty conduction band separated by a band gap.

Different works of Weller, Spanhel and co-workers extensively investigated the physical and chemical properties of II-VI semiconducting quantum dots\(^6\textendash\textsuperscript{10}\). They demonstrated the formation of ZnO nanoparticles in alcoholic solution by use of zinc acetate, and tested their optical and catalytic properties in size ranges of 1-10 nm. One of the main features of ultrasmall semi-conductors are their luminescent and photocatalytic properties\(^9,11\).

When ZnO nanoparticles are excited by light radiation with wavelengths in the UV spectral range, two emission signals will usually be observed: the exciton signal which corresponds to the band gap energy and the fluorescence signal arising in the visible spectral region. Koch et al. observed that the exciton signal of ZnO shifts depending on the size of the particles, i.e. the band gap differs with particle sizes\(^6\). Thereupon, Bahnemann et al.\(^12\) investigated this behavior (see Figure 2) more detailed. Several years ago, the behavior of nanosized materials had already been investigated and it was postulated that reducing crystal size of ZnO below the “q-size” level leads to dramatic physical changes due to band gap diminishment. “Q” stands for “Quantum” and q-size defines a size dependent state of a crystalline material that exists in a “transition range between molecular and bulk properties (in case of ZnO: 1-10 nm)”\(^12\textendash\textsuperscript{15}\).
1. Synthesis of ZnO Nanoparticles with Variable Morphologies and Reactive Surface

Figure 2: UV-Vis and Fluorescence spectra of aqueous ZnO colloids. Reprinted with permission from Reference [12]. Copyright (2014) American Chemical Society.

The nature of these quantification effects due to size, so-called “quantum-dot” – nanoparticles showing the q-size effects -, are nicely explained by Weller: It is like a “particle-in-a-box” where charge carriers “feel the walls”, in contrast to a macrocrystalline material where the same charge carriers are “free to move”[8]. There have been many works that mathematically describe the quantum mechanic particle-in-a-box model mainly based on CdS as model compound[7,13,14,16]. The method introduced by Nosaka for example offers a general method of calculating excited states of ultrasmall semiconductors based on the particle-in-the-box approach[16]. In contrast, there are other works following different approaches, e.g. the tight-binding approach[17],[18].

One of the main characteristic of ZnO nanoparticles is the existence of fluorescence in the visible range under irradiation with UV light at an excitation wavelength of ca. 360 nm. The reason for the fluorescence of ZnO nanoparticles has been widely and controversially discussed. The largely recognized explanation is that oxygen vacancies act as electron traps from which emission at visible wavelengths occurs, with relatively long lifetimes[19]. Works of van Dijken et al. showed in which way the mechanism of electron trapping by oxygen vacancies act[20–22]. In essence, during irradiation of a colloidal suspension electrons will be excited and transit into the conducting band while at the same time an electron hole remains in the valence band. The theory assumes the existence of anion (oxygen) vacancies deep within the bulk volume (\(V_0^-/V_0^{++}\)). Fluorescence occurs after electron holes are getting trapped
1. Synthesis of ZnO Nanoparticles with Variable Morphologies and Reactive Surface

within surface related vacancies subsequently followed by falling into a trap located deep in the bulk volume of the nanocrystall. After recombination with the excited electron light emission in the visible domain occurs\(^{[21]}\).

Figure 3: Transitions of an electron via excitation with UV light irradiation of the conduction band. The corresponding electron hole (+) remains in the HOMO of the valence band. The energy-band schemes additionally show the energy distribution of \(\text{O}^/-\text{O}^{2-}\) at the surface and deep trap levels \((\nu_0^-/\nu_0^{\ldots})\). Reprinted with permission from Reference [21]. Copyright (2014) American Chemical Society.

The hypothesis supports several conclusions drawn from afore done observations, like for example the fact that reductive atmosphere and so oxygen vacancies lead to
increased fluorescence intensity, as well as that intensity is dependent on the surface\[^{21}\]. The paths of possible recombinations of electrons and electron holes are shown in Figure 3. Reference [21] gives following explanation for this phenomenon: There are three possible competitive relaxation processes (A-C). The transitions are marked by the T-superscripts below each scheme. The first relaxation process shows radiative recombination of electrons and holes under emission of the exciton energy without any trapping (A). Secondly, an electron hole is trapped into a surface anion vacancy (B) followed by subsequent transition into a deep trap hole (D) where recombination with the excited electron lead to photons emission (G). Alternatively, the surface trapped hole recombines with the electron non-radiatively at the surface (E-H). The third possibility describes trapping of an electron in a surface vacancy (C) followed by non-radiative recombination with the corresponding hole in the surface (F-H)\[^{21}\].

ZnO is a semiconductor with a direct bandgap. The direct bandgap allows electron transitions from the valence to the conduction band and vice versa. This is why only for direct energy gap semiconductors, e.g. CdS and ZnO, excitons can be observed while semiconductors owning an indirect bandgap, e.g. silicon, do not show exciton emission in luminescence spectra (cf. Figure 4)\[^{23}\]. Direct bandgap and a large exciton energy of ca. 60 meV make ZnO a promising material for light emitting diodes, as comprehensively reviewed by Özgür et al.\[^{5}\] and Lee\[^{24}\].

Photocatalytic properties of nanocrystalline semiconductors have been investigated for ZnO as well as other materials, mainly TiO\(_2\). Both semiconductors show promising catalytic efficiency under UV light irradiation when suspended in liquid media like water and alcohols. As shown above, under irradiation of ZnO nanoparticles with UV light, electrons will be excited leaving holes in the valence band. During this process and in presence of hydroxy group containing compounds, excited electrons and corresponding holes can react with the compounds, leading to the formation of hydroxy-radicals. The oxygen radicals are commonly summarized under the term: “reactive oxygen species” (ROS).
1. Synthesis of ZnO Nanoparticles with Variable Morphologies and Reactive Surface

Figure 4: Absorption (left) and fluorescence (right) spectra of CdS (a) - shown for two different CdS samples – and silicon (b). CdS is a direct semiconductor, silicon is an indirect semiconductor. Reprinted with permission from Reference [23]. Copyright (2014) American Chemical Society.

The catalytic properties are attributed to the ROS which subsequently react with other chemical compounds and offer even other applications due to their reactivity, e.g. antibacterial properties (see below). The photocatalytic properties are usually measured by dye degradation that occurs when surface adsorbed dye molecules react with ROS\textsuperscript{[25–29]}. Other applications concentrate on the electrons transfer via dyes into the electron bands of the nanocrystalline semiconductors. Such hybrid materials are thought to produce electric energy and might find their use in a special kind of photocells, so-called dye-sensitized photocells (DSSC)\textsuperscript{[30–36]}. 
1. Synthesis of ZnO Nanoparticles with Variable Morphologies and Reactive Surface

1.1.3. ZnO Nanoparticles in Solution

Besides the effect on physical properties, another important change occurs when the size-dimensions are reduced dramatically, namely the behavior in liquid media like alcohols or water. While bulk metal oxides usually sedimentate in solvents, the nanocrystals may form a relatively stable colloidal dispersion due to surface charges. The colloidal stability allows the homogenous distribution of nanoparticles in the solvent and gives them the ability to diffuse through the medium. Both properties will counteract the aggregation tendency of nanocrystals – due to their high specific surface. If stabilization is not sufficient agglomeration and sedimentation will occur. Nevertheless, the colloidal stability of nanoparticles without any additional surface modification has led to discussions which concern the toxicity of these materials. On the one hand, the ability of diffusion within a nano-colloidal dispersion could be neutralized by agglomeration and formation of microscale clusters\cite{37}. On the other hand, non-agglomerated colloids might harm biological environments including human body after diffusing into these systems\cite{38}.

Hence, bringing nano-colloidal dispersions and biological systems together might lead to uncontrolled effects and undesired consequences, when nanoparticles are allowed to diffuse into the biological system. Therefore, it is of great importance to prohibit the diffusion of nanoparticles. One possibility to avoid uncontrolled diffusion is integrating nanoparticles into a polymeric matrix where additional functional groups might increase the anchoring by interactions with the polymer chains.

There are a number of interesting applications if nanoparticles form well-stabilized colloidal dispersions. Applications can directly result from using the nanoparticle-dispersion or indirectly by applying the dispersion to prepare a composite in which the particles are well-distributed, e.g. antibacterial coatings or UV-light absorbing films. Among these applications there are many which focus on the special properties offered by reduction of the semiconducting materials size to nanometer scale, e.g. zinc ion release\cite{39} or photocatalytic properties\cite{40–42}. Amongst related applications, antibacterial properties are often implied. The antibacterial properties of ZnO nanoparticles are discussed in the literature and the proposed mechanism considers ROS and hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) that are formed on the particles surface due to the photocatalytic properties\cite{40–42}. This mechanism correlates with the special
1. Synthesis of ZnO Nanoparticles with Variable Morphologies and Reactive Surface

properties of the nano-size material mentioned above and it has already been shown that ZnO nanoparticles have higher antibacterial efficiency with decrease in size\cite{43,44}. Brayner et al. were able to demonstrate that small ZnO nanoparticles are capable to enter cells of E. Coli. in Diethylene glycol (DEG), acting as effective antibactericidal agents\cite{45}.

Antibacterial mechanism of metal oxide nanoparticles leading to cell death is not explained by one general theory. However, many different theories are discussed. Whether there are ROS harming the cell by changed redox potentials within the mitochondria, metal ions simply absorbed leading to apoptosia or cells internalizing whole quantum dots, it is not fully understood how cell death might occur. Usually, in case of small nanocrystals, two basic mechanisms are discussed: the photocatalytic mechanism and the metal release mechanism. In addition, Brayner et al showed that cell-internalization also occurs in case of well dispersed quantum dots and in DEG\cite{45}.

While antibacterial properties are usually favorable, they are accompanied by cytotoxic properties. Both properties are linked to analogues mechanism reasons, especially metal ion release and photocatalytic properties. Metal oxide nanoparticles applied in cosmetics are usually in a state of agglomerated microstructures which are known for being non-harming to human organism (when applied topically, e.g. by ointments or sun creams)\cite{37}.

Nevertheless, cytotoxicity is the most problematic aspect that is discussed when contact between cells of non-bacterial or -microbial origin and ZnO nanoparticles is considered. It has been shown that human and algae cells can be harmed by ZnO nanoparticles\cite{38,46}. Furthermore, ZnO nanoparticles can release toxic zinc ion concentrations\cite{47}, depending on the solvation conditions. Even if the surfactants would be removed in time, e.g. by hydrolysis, electrostatic stabilization - in the way as explained by DLVO theory – could still be sufficient enough to permit particles diffusion without complete sedimentation through an aqueous medium\cite{48}. Thus, all these aspects must be considered when nanocolloidal material is supposed to be applied in biological systems. However, the antibacterial properties along with relative low toxicity of ZnO are worth the effort. Biofilm formation on surfaces is an important aspect within the framework of antibacterial applications, since bacteria and more general microbes show increased resistance against antimicrobial
1. Synthesis of ZnO Nanoparticles with Variable Morphologies and Reactive Surface

Due to the problem of high aggregation tendency of nanoparticles in water\textsuperscript{[51]}, a lot of research has been done in the fields of stabilization of ZnO nanoparticles and their antibacterial properties in aqueous suspensions. Since agglomeration leads to decreased specific surface by the formation of micrometer-scale particle aggregates, it remains a challenge to utilize ZnO nanoparticles as well-dispersed aquatic antibacterial agents.
1. Synthesis of ZnO Nanoparticles with Variable Morphologies and Reactive Surface

1.1.4. Synthesis Methods of Nanocrystalline ZnO

A vast number of synthesis approaches concerning the preparation of ZnO nanoparticles has been published. The variety of the synthesis-approaches goes along with the variety of applications that have been proposed. Many of them only focus on the preparation of nanoparticulate spheres without any stabilizing additives. Others discuss surfactants for stabilizing ZnO nanoparticles, mainly in organic solutions, while only a few numbers of works treat the colloidal stability of ZnO nanocrystals in water. During the early 1980s working groups were already able to achieve the formation of quantum dots, spherical nanoparticles with diameters of ca. 1-10 nm.

One of the most important wet-chemical synthesis approaches is the sol-gel preparation method that involves reaction of zinc acetate dissolved in alcohol, e.g. ethanol or propanol. The name: “Sol-Gel” describes the mechanistic background of this approach. After dissolving a precursors or colloids in a certain solvent a “Sol” is formed. Reaction occurs after heating or adding a catalyst at room temperature, e.g. sodium or lithium hydroxide. First, smallest clusters are formed by aggregation and/or Ostwald rippening followed by formation of a three dimensional cluster-network and the viscosity increases. The colloidal dispersion with low viscosity (“Sol”) transforms into a viscous “Gel”\[^{9,11,52}\]. All theoretical aspects of zinc acetate reacting in alcohol has been extensively reviewed by Spanhel\[^{11}\] and Niederberger\[^{53}\] and shall not be explained here in detail. Pacholski et al reported that annealing of ZnO colloidal dispersion in alcohol for several hours will lead to nanorods formation through alignment of particles along the c-axis\[^{10}\].

Moreover, the preparation of ZnO nanoparticles by use of THF as solvent has also been investigated by Chaudret and co-workers\[^{54–56}\]. Different morphologies of ZnO nanoparticles were synthesized and stabilized with surfactants, mainly long chained amines and carboxylic acids. The preparation method succeeds by degradation of a organometallic zinc precursor, zinc \textit{cyclo}-hexyl, by moisture or addition of water at ambient temperature. In one work, reaction led to the preparation of ZnO nanoparticles by use of Laureth carboxylic acid\[^{57}\].

In this work, the formation of water dispersible ZnO nanoparticles was achieved with a lauryl-carboxylic acid under formation of a surfactant double layer at the
1. Synthesis of ZnO Nanoparticles with Variable Morphologies and Reactive Surface

nanoparticle's surface. The synthesis procedure introduced here was adopted from a patent [58] and chosen because it showed good results in case of nanoparticles preparation and modification with a surfactant as a one-pot approach. The obtained ZnO nanoparticles are highly crystalline products with well-defined morphology and shape. The use of Laureth-4 carboxylic acid as surfactant yields stability in water [58]. The developers pronounced the good stabilizing effect for this group of surfactants [58].
1. Synthesis of ZnO Nanoparticles with Variable Morphologies and Reactive Surface

1.2. Results and Discussion

1.2.1. ZnO Nanoparticles

1.2.1.1. Wet-chemical Synthesis and Characterization

The basic principle of the here introduced synthesis-procedure was mentioned in a patent\textsuperscript{[58]}. The patent was published by a group of developers who invented different formulations in the field of cosmetics, especially ointments. Amongst others, Laureth-4 carboxylic acid (L4) was introduced and declared to be sufficient in order to afford water stability to the ZnO nanoparticles. Synthesis and stabilization by surface modification was achieved by a two-step approach. The first step was done by precipitating zinc hydroxide from a zinc chloride containing methanol solution after adding sodium hydroxide dissolved in the same solvent. Afterwards, the methanol had to be removed completely and the precipitation transported into aqueous medium where the reaction was performed at 80 °C for several hours in presence of the surfactant.

Here, the synthesis approach was chosen and optimized to achieve the same or even better quality by a one-pot method. Scheme 2 shows the synthesis-process of ZnO nanoparticles for two different morphologies depending on the amount of NaOH. Furthermore, the synthesis approach allows modification of the ZnO surface in-situ as well. Therefore and with respect to the patent mentioned above, Laureth-4 carboxylic acid (trade name) was used. Besides L4, L11 had already been investigated as sufficient surfactant in the diploma thesis of P. Nachev and was found to be less sufficient due to lower solubility of L11-modified ZnO nanoparticles in organic medium compared to L4.
1. Synthesis of ZnO Nanoparticles with Variable Morphologies and Reactive Surface

\[
\text{ZnCl}_2 + 0.55 \text{ L}_4 + 2 \text{ NaOH} \quad \xrightarrow{\text{THF/ ROH}} \quad \text{Zinc Oxide-L}_4 \text{ nanoparticles} \\
\text{ZnCl}_2 + 0.55 \text{ L}_4 + 2.7 \text{ NaOH} \quad \xrightarrow{\text{THF/ ROH}} \quad \text{Zinc Oxide-L}_4 \text{ nanorods}
\]

R: Me,

\[ \text{L}_4 (x: 2, n: 12) \]

Scheme 2: Scheme of the wet-chemical synthesis of ZnO nanoparticles and the in-situ surface modification by use of Laureth-4 carboxylic acid.

Table 1 gives an overview of all approaches regarding the synthesis of ZnO nanoparticles. Yields and organic fractions were determined by means of thermogravimetric analysis (TGA) after purifying the samples with acetone. The lines for which none such value is given, no precipitation was achieved after washing with acetone (**), or agglomeration occurred, which yielded these tests worthless. The samples were purified with acetone in order to remove surfactant that was not strongly bound to the surface and to evaluate the organic fractions. As shown in Table 1 samples were characterized by TGA (exemplarily shown for ZOSP and ZONR; marked with * in Table 1 and in Figure 5).
1. Synthesis of ZnO Nanoparticles with Variable Morphologies and Reactive Surface

![Graph showing TGA curves of ZnO nanoparticles after treatment with acetone.](image)

Figure 5: TGA curves of ZnO nanoparticles after treatment with acetone. Samples were degraded in nitrogen atmosphere at a heating rate of 5 K/min.

The experiments were done in nitrogen atmosphere where the surfactant degraded leaving a residue as shown by the Reference. The quantity of residue remaining is approximately 5 wt-% and was introduced in the calculation of the organic fraction for all samples. Determination of the organic fraction gave the inorganic amount, i.e. the amount of ZnO. Taking the ZnO amount into account, the yields related to the initial amount of zinc chloride were calculated.

Table 1: Preparation parameters of ZnO nanoparticles after treatment with acetone.

<table>
<thead>
<tr>
<th>ID</th>
<th>$c_0$(L-4) [mol L$^{-1}$]</th>
<th>$c_0$(NaOH) [mol L$^{-1}$]</th>
<th>Alcohol</th>
<th>Morphology</th>
<th>Organic fraction</th>
<th>Yields</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZnO-1</td>
<td>0.01</td>
<td>0.2</td>
<td>MeOH</td>
<td>Spheres</td>
<td>28 %</td>
<td>51 %</td>
</tr>
<tr>
<td>ZOSP</td>
<td>0.05</td>
<td>0.2</td>
<td>MeOH</td>
<td>Spheres*</td>
<td>32 %</td>
<td>52 %</td>
</tr>
<tr>
<td>ZnO-2</td>
<td>0.05</td>
<td>0.2</td>
<td>EtOH</td>
<td>Spheres</td>
<td>26 %</td>
<td>56 %</td>
</tr>
<tr>
<td>ZnO-3</td>
<td>0.25</td>
<td>0.2</td>
<td>MeOH</td>
<td>Aceton soluble**</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ZnO-4</td>
<td>0.01</td>
<td>0.27</td>
<td>MeOH</td>
<td>Agglomerates</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ZnO-5</td>
<td>0.05</td>
<td>0.27</td>
<td>MeOH</td>
<td>Rods</td>
<td>22 %</td>
<td>65 %</td>
</tr>
<tr>
<td>ZONR</td>
<td>0.05</td>
<td>0.27</td>
<td>EtOH</td>
<td>Rods*</td>
<td>16 %</td>
<td>74 %</td>
</tr>
<tr>
<td>ZnO-6</td>
<td>0.25</td>
<td>0.27</td>
<td>MeOH</td>
<td>Rods</td>
<td>34 %</td>
<td>64 %</td>
</tr>
</tbody>
</table>
1. Synthesis of ZnO Nanoparticles with Variable Morphologies and Reactive Surface

The yields in case of the spherical ZnO nanoparticles are slightly higher than 50 wt-% and do not show any dependence on the investigated parameters. In comparison, the yields of the nanorods are improved, which might be caused by the additional amount of sodium hydroxide. When ethanol is used as dispersant for NaOH, the yield increases even more. As will be shown below, TEM measurements show that the alcohol does have an influence on the shape of the rods and so on the surface-area.

First, measurements were done in order to investigate the influence of the solvents on the crystallization process. If the synthesis is done in water only, large hexagonal crystals will be observed (Figure 6a). The crystals shown in the TEM image are belonging to a small fraction while most of the product agglomerated and was not suitable for TEM measurements. So, the shown image shall give us an idea about how the synthesis in water leads to highly increased crystall growth compared to organic medium. If the reaction is performed in THF and the base is added in aqueous solution, the crystal-dimensions lie in between the preparation method with organic solvents only (shown below) and water only (Figure 6b).

![Figure 6: ZnO microparticles after preparation in water (a) and ZnO nano/microparticles after preparation in a THF-water mixture (b).](image)

Both reactions including the use of water reveal the moderating properties of both organic solvents, i.e. THF and alcohol. If THF is exchanged by dichloromethane, – in which zinc chloride is soluble at the same concentration – small ZnO nanoparticles
1. Synthesis of ZnO Nanoparticles with Variable Morphologies and Reactive Surface

show a broader size dispersity compared to the THF based synthesis of the same particles (see Figure 7).

Figure 7: ZnO nanoparticles prepared in chloroform-methanol.

After proving the importance of the alcohol/THF solvent-mixture, this system was taken into focus. The variation of surfactant concentration by factors of 5 and 0.2 revealed an optimum at 0.05 mol/L with respect to the molar mass given by the distributor (Sigma Aldrich, Mn ~360 g/mol). On the one hand, low amount of surfactant leads to decreased dispersibility or even agglomeration, while high amount of surfactant can prevent particle formation. The latter is supposed to be caused due to high amount of organic acid in the system. ZnO is well known to degrade in acidic medium consequenting in zinc salt formation.
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Figure 8: TEM images of ZnO nanorods prepared using methanol.

In contrast Figure 8 shows ZnO nanorods prepared under analogous conditions as ZONR except when using methanol instead of ethanol (see Table 1, ZnO-5). Comparison of methanol and ethanol (ZONR, Figure 9b) based nanorod preparation indicates a more controlled reaction mechanism in case of ethanol due to the regular shape and monodispersity of the rods. The reason can only be found in the moderating role of the alcohol that somehow must be involved in the crystallization process.

Figure 9 shows TEM (a and b) and HR-TEM (c and d) images of ZOSP and ZONR as marked in Table 1. Regular TEM images show monodisperse spherical ZnO nanoparticles (ZOSP) having a mean diameter of 4-5 nm and nanorods (ZONR) of polydisperse length and cross-sections of 5 to 10 nm or higher. HR-TEM measurements verify the existence of nanocrystals which show a preferred growth direction for nanorods along one plane, which takes place along the [001] direction. This direction corresponds to the c-axis in hexagonal wurtzite type structures as proofed by XRD and related to the typical wurtzite structure of ZnO.
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Figure 9: TEM images of ZOSP-A (a) and ZONR-A (b) as well as the HR-TEM\(^1\) images of ZOSP-A (c) and ZONR-A (d). XRD Patterns of ZOSP-A (e) and ZONR-A (f).

\(^1\) HR-TEM images were recorded at Gemeinschaftslabor für Elektronenmikroskopie, RWTH Aachen.
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However, all investigations so far led to two samples, one for each morphology, that show ideal properties and thus were selected for more detailed characterization. These samples are named ZOSP (nanospheres) and ZONR (nanorods). The amount of ZnO after synthesis and before treatment of acetone was investigated in case of ZONR and ZOSP via ICP. Therefore, the ZnO nanoparticless were redispersed in water and treated with semi-concentrated HNO₃. The mass amount of nanoparticles compared to the initial amount of ZnO is 18 (for ZOSP) and 15 wt-% (for ZONR). Compared to the initial amounts of the reactants the conversion of zinc chloride to ZnO is 27 (ZOSP) and 23 % (ZONR).

XRD measurements of ZnO nanoparticles (Figure 9 e, f) show reflections at angles of $2\Theta = 31.8^\circ, 34.5^\circ, 36.25^\circ, 47.6^\circ, 56.6^\circ, 62.9^\circ, 67.9^\circ$, corresponding to the reflections from 100, 002, 101, 102, 110, 103 and 112 crystalline planes. The XRD pattern confirms the existence of hexagonal wurtzite structure having space group P63mc in case of ZONR. In comparison, smaller nanospheres show broader peaks compared to the larger nanorods, as expected$^{[10,59]}$. The XRD patterns additionally show signal coming from a ~20° scattering angle, where characteristic ZnO peaks are usually not expected. Hence, further investigations were carried out with regard to the surfactant (L4) to clarify if it could be the cause of these. Since L4 is a liquid at room temperature, the sodium salt of L4 was prepared according to the synthesis method of ZONR but in absence of zinc chloride. The resulting product, a brown and waxy like material, was characterized by $^1$H-NMR and FTIR spectroscopies (Figure 10).
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Figure 10: $^1$H-NMR spectra of L4 (a), and L4-Na (b), as well as the FTIR spectra for L4 (c) and L4 (d).

The results of both spectroscopic methods give evidence that formation of the waxy-like product having the characteristic brown colour derives only from the sodium salt formation. We couldn’t find a reasonable explanation for the strong colour of the sodium salt. However, all spectra proof that under the circumstances of ZONR synthesis, L4 suffers no chemical transformation except the mentioned salt formation as seen by FTIR spectroscopy and outlined in Scheme 3.

Scheme 3: L4 (left) reacting to the corresponding sodium salt (right).

L4-sodium was characterized by XRD. The result is compared with the XRD patterns of ZOSP-A and ZONR-A in Figure 11. L4-sodium shows a broad signal appearing at 20°. The broadening is due to the amorphous character, as one would expect for a waxy-like fatty acid salt. Interestingly, the XRD signals are much more sharper and defined in case of the remaining surfactant attached to the nanorod’s surface. This observation could be explained by a dense assembly of surfactant molecules on the
1. Synthesis of ZnO Nanoparticles with Variable Morphologies and Reactive Surface

The question on why the surfactant shows such behavior when attached on nanorods but not on nanospheres naturally arises. One explanation might be the geometrical properties of the surface. Where the surface is expected to show a plane like geometry in case of a cylindrical rod, one could imagine a bended surface in case of a sphere. The former geometry might possibly be favored by L4 in order to form a dense brush like assembly of surfactant.

DLS measurements of ZOSP revealed that ZnO nanoparticles are not well dispersed in water due to their aggregation behavior. The measurements show that the particles tend to form aggregates which exist in dispersion. It was therefore necessary to treat the samples in an ultrasonic bath for 3 minutes and to filter them with syringe-filters to enable the characterization of the smallest clusters.

The measurements were performed after dispersing nanoparticles in neutral water
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and adding 0.01 M NaOH, immediately following the ultrasonic treatment of the samples. The DLS results for ZOSP show that the minimum hydrodynamic radius ($R_h$) is 44 nm at pH 7. The $R_h$, as measured in solution by DLS, compared to the result of the TEM analysis clearly shows a ten-fold to twenty-fold larger size. This can only be explained by aggregation of nanoparticles. Due to the anisotropic morphology, the ZONR sample was not characterized via DLS.

Figure 12: TEM images of ZOSP after coating a carbon/formvar grid from THF. The images indicate the presence of free L4 which did not sufficiently attach to the ZnO surface, forming aggregates.

TEM of ZOSP (Figure 12) before treatment with acetone shows the presence of excess surfactant which remains free in the waxy-like product. The non-attached molecules form aggregates which possibly remain in aqueous dispersion leading to the high hydrodynamic radii. At the same time, well dispersed nanospheres surround aggregates after coating a TEM-grid by use of THF-dispersed samples.

To analyze the colloidal stability, both samples were resuspended in water and centrifuged at 6000 rpm for 10 min after which they were analyzed by TEM. In case of ZOSP precipitation occurs almost instantly and TEM measurement shows the formation of aggregates, indicating that the surfactant is insufficient to stabilize the spherical particles in water. Figure 13 shows TEM images of the post-water treated ZnO samples including the non-water-stable fraction of ZONR (Figure c, d). All TEM
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measurements were recorded after resuspending the samples in THF and good coverage of the carbon/formvar grid was achieved via drop-cast coating the grid. ZOSP (Figure a, b) shows that the presence of water leads to the formation of small aggregates, compared to the sample before water addition (see Figure 9 a). This indicates that the surfactant is insufficient to hinder the small and highly active particles from aggregation in water. In comparison, the nanorods are mostly stabilized (Figure 13 e, f) while a smaller fraction of rods undergoes an alteration of their surface conditions as seen by TEM (Figure 13 c, d). However, these tests clearly reveal the improved water stability of ZONR compared to ZOSP.

![TEM images](image13.png)

Figure 13: TEM images of ZOSP after dispersion and centrifugation out of aqueous medium (a, b) and ZONR (e, f), as well as the water stable nanorods in transparent dispersion (c, d). All samples were dried and coated on carbon/formvar grids by drop-casting from a THF dispersion in order to achieve good distribution of nanoparticles on the grid.

To get a better understanding in which way the surfactant assembles on the ZnO
nanoparticles surface, zeta-potentials were measured at basic pH values for both samples and additionally for two different NaCl concentrations. The samples were equally treated as mentioned in the case of DLS measurements (see above). The results are listed in Table 2 and indicate a different behavior of ZOSP and ZONR. After adding HCl in low concentrations the samples showed quenched fluorescence signal at pH 4.5 (see below) which is caused by complete degradation of the nanoparticles in acidic medium.

All samples exhibited only negative surface charge. ZOSP show an increase of the negative surface charge at higher pH values as well as after increasing the NaCl concentration. Compared to the typical zeta-potential values reported for ZnO nanoparticles\(^{[60]}\), there is no isoelectric point at \(\sim \)pH 9.4. These results can be explained by the formation of a double layer of surfactant molecules on the ZnO nanoparticles surface. Similar results had been reported in the literature\(^{[57]}\).

Table 2: Zeta-potential measurements at different pH values.

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH 7</th>
<th>pH 10.2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>c(NaCl) 0 M</td>
<td>c(NaCl) 0.01 M</td>
</tr>
<tr>
<td>ZOSP</td>
<td>-18.4 mV</td>
<td>-27 mV</td>
</tr>
<tr>
<td>ZONR</td>
<td>-47.3 mV</td>
<td>-43.1 mV</td>
</tr>
</tbody>
</table>

Figure 14 shows schematically the assembly of the surfactant molecules on the ZnO nanoparticles surface for both samples. It is assumed that the second layer of surfactant molecules is bearing carboxylate groups as a consequence of the synthesis conditions, including the increased amount of NaOH, which also explains the increased water stability of ZONR compared to ZOSP. In case of ZOSP a similar assembly of the surfactant is assumed, with higher amount of acid groups, as seen in the FTIR spectrum (Figure 16 a).
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![Diagram](image)

Figure 14: Hypothetical double layer assembled from surfactant molecules on ZnO nanoparticles surface, forming a first layer of organic molecules attached on to the surface by carboxylic functions. In case of ZOSP a double layer of carboxylic acids is assumed (a). Meanwhile, a layer of carboxylates is likely to form the double layer structure in case of ZONR (b).

The low colloidal stability of ZOSP could be explained by high surface activity of the spherical particles due to its high specific surface for equal masses compared to ZONR. Possibly, the double layer is just not sufficient to prevent the nanoparticles against aggregation. According to investigations of Bian et al who showed that ZnO nanoparticles stabilized electrostatically tend to lose colloidal stability with increasing ionic strength and the decrease of zeta-potential from higher positive values to low positive values. The result was explained by increased compression of the electronic double layer (EDL) at increased ionic strength\(^{[48]}\). In our system we observed that the negative charge increases in the case of ZOSP which are comparable in size and morphology to those investigated by Bian et al. The comparison emphasizes that the EDL forming at the particles surface does not play a major role in case of surface charge. Further characterization of ZnO nanoparticles was performed by using solid state \(^{13}\text{C}\)-NMR and FTIR spectroscopy. Figure 15 shows the results of solid state \(^{13}\text{C}\)-DPMAS NMR (high power decoupling) performed before and after purification of the samples. The ZOSP and ZONR spectra show a peak at \(~170\ \text{ppm}\) which is undoubtedly related to the carboxylic function. The signal disappears after treating the samples with acetone, while the backbone signals,
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according to the multiplet of singlets between 14 to 40 ppm and the singlet at 70 ppm, remain, emphasizing that the surfactant remains at close distances to the ZnO nanoparticles surface, which might lead to a different magnetic susceptibility of the carboxylic groups and consequently lead to a shift out of the measurable range.

Figure 15: Solid state $^{13}$C-NMR of ZOSP (a), ZONR (b), ZOSP-A (c) and ZONR-A (d).

To support this assumption, FTIR measurements were performed. The FTIR spectra show modes appearing at 1630 cm$^{-1}$ and 1615 cm$^{-1}$ before and after treating with acetone. Investigations in case of glycin$^{[61]}$ and pectin$^{[62]}$ carboxylate species identified the same wavenumbers of ionized carbonyl groups. An additional peak at ca. 1759 cm$^{-1}$ in the ZOSP spectrum (Figure 16 a) corresponds to carboxylic acid groups$^{[63]}$ and does not appear in the ZONR spectrum, indicating an assembly as figured above (Figure 15). The reason for the small amount of acid found might be the lower amount of NaOH used during the synthesis of ZOSP.

After partially removing the surfactant with acetone the signal at 1759 cm$^{-1}$ disappears (see Figure 16 c) while a new signal appears at 1537 cm$^{-1}$ in the ZONR spectrum (see Figure 16 d). This peak has already been reported as being related to
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zinc ion-carboxylates interactions\(^{[63]}\). However, it is difficult to judge if the peak at 1537 cm\(^{-1}\) is due to a zinc-acid salt of free zinc ions remaining from the synthesis or if it is caused by a different kind of carboxylate-ZnO interactions which become better detectable after acetone treatment.

![Diagram of carboxylate interactions](image)

Figure 16: FTIR spectra of ZOSP (a), ZONR (b), ZOSP-A (c) and ZONR-A (d). Scheme on top of the spectra explains the shift of the carboxylate groups of L4 due to surface attachment on ZnO.

Solid state \(^{13}\)C-NMR performed after acetone treatment clearly showed that the surfactant-backbone remains after removing excess surfactant and therefore must be attached to the surface, while the carboxylic peak completely disappears. In comparison with FTIR spectroscopy, the peaks at 1615 cm\(^{-1}\) and 1630 cm\(^{-1}\) can be observed, proving the existence of carboxylate groups. This observation strongly supports the assumption that backbone and particle surface are linked via these ionic
1. Synthesis of ZnO Nanoparticles with Variable Morphologies and Reactive Surface Carboxylate Groups

Besides the attachment of ZnO and surfactant, further carboxylate functions might exist due to the basic conditions of the synthesis. In order to obtain more information about the nanoparticle’s behavior in aqueous medium, UV-vis spectroscopy was performed at various times during the synthesis. ZnO is known to show absorption at ~355 nm. Both samples were buffered with PIPES at pH 7.5 to avoid any effects correlating to the pH variation occurring due to zinc ion release. Figure 7 clearly shows that nanorods remain stable for at least 24 hours. Meanwhile, the nanospheres show the characteristic onset in aqueous medium right after dispersing and shortly after the signal starts to collapse, vanishing completely after ~5 hours. All nanosphere measurements were performed after shaking the cuvette in order to avoid sedimentation affecting the results. The nanorods in contrast were characterized without shaking before each measurement.

![UV-Vis spectra of nanospheres and nanorods](image)

Figure 17: UV-Vis spectra of nanospheres (a) and nanorods (b) recorded in buffered medium (pH 7.5) at 37 °C for different times of the synthesis. In case of nanospheres, samples were shaken before each measurement.

1.2.1.2. Optical and Photocatalytic Properties

Since some of the most discussed effects related to the antibacterial properties are optical properties, fluorescence spectroscopy measurements were performed for ZOSP and ZONR in water at concentration of 0.1 gL⁻¹ and different pH values (Figure 18). The fluorescence spectra were recorded at an excitation wavelength of 355° nm, as reported by Singla et al. This time, treatment with ultrasonic waves and filtering...
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were not done, in order to investigate the behavior of the samples as prepared for antibacterial tests.

The results show the characteristic emission peaks at ca. 560° nm. In both samples decreased fluorescence intensity in basic medium and a complete quenching in acidic medium was observed, indicating hydrolysis of the surfactant and disintegration of the nanoparticles. The relatively low fluorescence emission intensity of ZOSP can be explained by their low stability and so their aggregation in water, which decreases the specific surface.

The results show similar behavior and optimal efficiency at pH 7. The reason for this phenomenon seems not to have been clarified yet, but it is assumed that due to the amphoteric character of ZnO, the surface of nanoparticles might become passivated by reactions with acid or base. This explanation assumes that the nanoparticles surface has not completely been coated by the surfactant.

Figure 18: Fluorescence spectra of (a) ZOSP and (b) ZONR.

Further tests were performed in order to investigate whether the photo-catalytic activity is dependent on the shape and surface area of ZnO nanoparticles. As model system the photo-degradation of Rhodamine-B (RhB) was used.
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Scheme 4: Degradation reactions of dyes due to the catalytic formation of reactive oxygen species at metal oxide surfaces (e.g. TiO$_2$, ZnO). Reprinted with permission from Reference [29]. Copyright (2014) American Chemical Society.

Scheme 4 depicts a map which is useful for explaining the way in which the here applied model system works. Metal-oxides, especially titanium dioxide and ZnO, are well known for their photocatalytic properties. These properties are caused by the semiconducting nature of the material as explained above. Due to the high specific surface of this material, the band-gap of the nanocrystals is relatively large and can only be vanquished by exciting electrons with UV radiation. The excited electrons will afterwards be able to cause fluorescence (as shown in Figure 18) or to react as shown in Scheme 4. Here, the excited electrons will react with oxygen in water. The excited oxygen will then keep on reacting until hydrogen peroxide will be formed, which can be considered to be a reactive oxygen specie (ROS). ROS are well known in medical topics where they are considered to be toxic. The toxicity of ROS is the reason why nanocrystalline semiconductors are, amongst others, described as toxic for biological enviroments, even for humans, e.g. in case of cosmetical applications. This is why the evaluation of such properties is very important. The model system introduced here determines the kinetic properties of the degradation of a dye caused by ROS. Basically, a Langmuir-type equation is used to calculate the efficiency of different systems, making it possible to compare them with each other. By applying
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the Langmuir type expression, the system becomes dependend on the surface of the catalysts and one may imagine the ZnO nanoparticles as heterogeneous catalysts where free surface sites will be at first occupied by adsorbing dye molecules, followed by their degradation due to harmful reactions with ROS which will directly be formed on the surface.

For the photocatalytic tests\(^2\), ZOSP-A and ZONR-A were suspended in water and stirred during the reaction with RhB added to an overall concentration of \(2 \times 10^{-5}\) M. The degradation was investigated under UV-light irradiation at wavelength ranging between 280-360 and 460-510 nm without monochromatic filter, followed by UV-VIS spectroscopy for different concentrations.

\[\text{Figure 19: Degradation of Rhodamine-B and exemplarily the UV-VIS spectra of the degradation in presence of ZnO and under UV irradiation. Chemical structure of Rhodamine-B (a). Time dependent decrease of the Rh-B absorption peak intensity due to degradation (ZONR-A; c = 0.25 g/L) (b).} \]

\[\text{Figure 19 shows the degradation of the dye visualized by the decrease of the absorption’s peak intensity. Exemplarily, the signal evolution of the UV-Vis spectrum}\]

\(^2\) The photocatalytic tests and the relating result as shown below were performed and evaluated by Dr. Yan Lu, Helmholtz Institut Berlin.
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is shown in case of ZONR-A. The concentration of the dye decreases as seen by it's characteristic absorption in the visible region. After 2.5 hours the signal collapses as the concentration moves towards zero. From plots like this the apparent rate constant was determined assuming first order kinetics. Since the average surface of nanorods is important for the coming discussion of the results, the surface was calculated by evaluating the lengths and cross-sections shown by TEM (Figure 20).

Figure 20: TEM micrograph that was used to determine the length to cross-section ratio of the nanorods.

In order to determine the adsorption rate constant ($k_0$) and the equilibration constant ($K$) the apparent rate constants were plotted against the surface balanced concentration of the catalyst. The plot and the corresponding equation are shown in Figure 21. The equation is derived from the Langmuir adsorption theory and rearranged to its reciprocal form which offers the advantage of determining $k_0$ and $K$. The determination of both values was done after calculating the surface-weighted concentration ($S$) from the concentrations of ZnO and with known density (5.606 g cm$^{-3}$) and surface area.
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\[
\frac{1}{k_{app}} = \frac{1}{k_0} \frac{1}{[ZnO]} + \frac{1}{k_0}
\]

Figure 21: Plots of apparent rate constants against specific surface. The resulting lines follow the reciprocal form of the Langmuir expression.

In case of ZOSP spherical morphology and a diameter of 4 nm was assumed, while for ZONR the ratio of length-to-cross-section was determined by evaluation of the TEM image shown in Figure 20, giving a mean length of 69 nm and a mean cross-section of 10 nm. The area was calculated assuming the rods are cylindrically shaped. The resulting values of \(k_0\) and \(K\) are listed in Table 3.

<table>
<thead>
<tr>
<th>Sample</th>
<th>(k_0) [min(^{-1})]</th>
<th>(K) [L m(^{-2})]</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZOSP</td>
<td>0.041</td>
<td>0.04</td>
</tr>
<tr>
<td>ZONR</td>
<td>0.037</td>
<td>0.16</td>
</tr>
</tbody>
</table>

The results are expected and clarify in which way the efficiency of ZONR increases compared to ZOSP. While the rate constants remain equal for the same material, the equilibrium constant increases in case of the nanorods leading to the assumption of an increased adsorption of dye molecule on the catalyst surface.
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![Figure 22: Surface dependent photocatalytic efficiency of ZOSP and ZONR.](image)

The results are highlighted in Figure 22 where all apparent rate constants are plotted against S. The plot clearly shows that ZONR-A has a higher photocatalytic activity compared to ZOSP-A and this effect is related to the difference in the surface area, due to an increased efficiency of the adsorption of RhB occurring at the nanorod surface. This observation is in good agreement with the zeta-potential values discussed above. Since Rhodamine-B bears a positively charged nitrogen atom (see Figure 19), one would expect enhanced adsorption on the ZONR surface that exhibits more negative surface potential compared to ZOSP. Furthermore, one must consider the low water stability of ZOSP which will lead to decline in specific surface during the measurement in aqueous suspension. Nevertheless, ZONR has improved catalytic activity due to its characteristic surface properties related to its likewise improved water stability.

1.2.1.3. Antibacterial Properties

To prove if the antibacterial properties are influenced by the photocatalytic activity, dead stop tests under radiation from a polychromatic lamp as well as in darkness were performed (Figure 23). We decided to determine the photocatalytic influence of the antibacterial activity by use of a polychromatic source to proof the effect of ROS production over a long time period (20 h). Since UV light offers antibacterial
1. Synthesis of ZnO Nanoparticles with Variable Morphologies and Reactive Surface

properties we used the daylight tests as a non-harming model system to determine photocatalytic effects. Besides, a similar system was used in the studies of Jones et al. Instead of a polychromatic lamp, they observed increased antibacterial activity of ZnO against E.coli under “visible light prevalent in the laboratory”[44].

![Figure 23: Dead-stop method to evaluate antibacterial properties of ZnO nanoparticles in darkness as well as under daylight irradiation at concentrations of 0.1 g/L. Images a and b show the well-plates after incubating with ZOSP (a: darkness; b: daylight). Images d and e show the results for ZONR (d: darkness; e: daylight). Image c shows positive control and image f negative controls for both samples.]

The tests clearly show that there is no significant difference of antibacterial efficiency in case of ZOSP and ZONR depending on light irradiation and there is no evidence that ROS are parent for the antibacterial properties. In comparison, Talebian et al. accomplished similar investigations after accomplishing similar tests with different measurements.

3 Measurements were performed by Nina Keusgen, DWI at RWTH Aachen.
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ZnO morphologies with respect to their antibacterial properties\(^{[65]}\). In this work, the antibacterial efficiency of rod-like, spherical and flower-like nanoparticles were tested against \(E.\) coli and \(S.\) aureus. All tests were done under UV-light (365 nm) irradiation for short-time period up to 80 minutes. In this work, spherical nanoparticles showed a higher activity than rod-like nanoparticles under irradiation of UV-light\(^{[65]}\). However, photocatalytic activity of ZnO nanoparticles cannot be responsible for the antibacterial behavior in our model system. The antibacterial properties of both ZnO nanoparticles samples were tested with respect to the minimum inhibiting concentrations (MIC) of nanoparticles by growth inhibiting tests after adding an aqueous dispersion of ZnO nanoparticles to a strain containing culture medium. The MICs were measured by optical density and so the test-system had to be treated in darkness. The experimental results are listed in Table 2. The MIC was calculated based on the total mass of the composites.

The antibacterial properties of L4-Na were also tested to check if the treatment with additional amount of sodium hydroxide leads to different antibacterial behavior of the surfactant itself. The result of Table 4 show that treatment with sodium hydroxide even seems to slightly lower the antibacterial efficiency in case of L4-Na.

The suspensions were filtered by use of a centrifugation tube in which a membrane (MCWO 10000) is incorporated. After centrifugation, two times at 8000 g for about 10 min, the filtrates were tested in the \(E.\) coli system only where no activity was observed, clarifying that besides of the surfactants there was no other antibacterial active substance in the \(E.\) coli test-system, e.g. inorganic impurities. In case of the \(S.\) aureus system the test was omitted since we did not see any evidence to test it due to the high activity of the surfactants against \(S.\) aureus.

According to the experimental data summarized in Table 4 ZONR show fivefold lower MIC than ZOSP. Since ZONR shows increased water stability compared to ZOSP, we assume that the ZnO nanorods exhibit increased antibacterial properties due to the increased amount of water stable nanoparticles of ZnO compared to ZOSP. Since the photocatalytic properties can be excluded as to be reasonable for the antibacterial properties, we assume zinc ion release to be responsible for the antibacterial behavior in case of \(E.\) coli. Otherwise, the remaining antibacterial activity of ZOSP – where aggregation must have occurred during the test – cannot be
explained in a different way as by zinc ion release only. The size of the nanoparticles plays a major role related to the efficiency as has been investigated elsewhere\cite{43,44,65} and will be usually associated with photocatalytic effects or interaction with cell membrane\cite{66}. Besides, size is also known to be responsible for zinc ion release\cite{39}. However, in our work the efficiency of antibacterial properties was observed to be in opposition of what would be expected due to the increased water-stability of the ZnO nanorods.

Table 4: MIC against S. aureus and E. coli\textsuperscript{4}.  

<table>
<thead>
<tr>
<th>Sample</th>
<th>MIC against S. aureus [mg/mL]; $6 \times 10^5$ [cfu/mL]</th>
<th>MIC against E. coli [mg/mL]; $2 \times 10^5$ [cfu/mL]</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZONR</td>
<td>0.03</td>
<td>0.05</td>
</tr>
<tr>
<td>ZOSP</td>
<td>0.14</td>
<td>0.20</td>
</tr>
<tr>
<td>L4</td>
<td>0.02</td>
<td>0.50</td>
</tr>
<tr>
<td>L4-Na</td>
<td>0.05</td>
<td>&gt; 0.50</td>
</tr>
</tbody>
</table>

In case of the S.aureus system – where ZnO has already been proven to show antibacterial properties – the surfactant starts to play a major role related to the antibacterial activity of the composites. Nevertheless, it can be seen that the use of ZOSP compared to ZONR and L4 leads to a decrease of activity, while we do not observe a difference between ZONR and L4. This behavior might be caused by the uneffective ZnO amount in the ZOSP system.

\textsuperscript{4} Measurements were performed by Nina Keusgen, DWI at RWTH Aachen.
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1.2.2. Synthesis of ZnO Nanoparticles in Aqueous System by Use of MFRT

1.2.2.1. Precipitation Route

Synthesis of ZnO nanoparticles in aqueous medium has been done mainly aiming on non-dispersible nano- and microparticles. Still, no method worth mentioning has been established that facilitates the preparation of monodisperse, colloidally stable ZnO nanoparticles. In this chapter, synthesis of ZnO nanoparticles in water is described by use of an innovative new device already proven to be efficient in biological and emulsion based chemistry, namely Microfluidic Reaction Technology (MFRT). It is a high force emulsifier working at pressures up to 3000 bar, pushing the solvent streams through capillars with diameters of minimum 70 µm. A special flow battery forces the stream to collide with inner walls, giving rise to extremely high shear forces.

The aim is to prepare waterborne monodisperse ZnO nanoparticles precipitating in a solvent stream within the microfluidic reactor, stabilized by additional electrosteric modifiers that are shown below. The first approach of the MFRT mediated synthesis was reproducing the organic synthesis introduced above. The amount of all compounds was held constant compared to the standard reaction. The reaction pressure was set to 3000 bar and the canule through which the reaction medium is sent was effectively cooled by an ice-bath.

<table>
<thead>
<tr>
<th>Nanospheres</th>
<th>Nanorods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction Time [min]</td>
<td>c₀(L4) [mol/L]</td>
</tr>
<tr>
<td>1.5</td>
<td>0.05</td>
</tr>
<tr>
<td>2.5</td>
<td>0.05</td>
</tr>
<tr>
<td>5.0</td>
<td>0.05</td>
</tr>
</tbody>
</table>

The reaction was performed for nanospheres as well as for nanorods. Since the MFRT is an open system all nanospheres were synthesized by use of ethanol to
1. Synthesis of ZnO Nanoparticles with Variable Morphologies and Reactive Surface

avoid methanol evaporation. The synthesis of ZnO nanoparticles was easily and rapidly achieved. In case of nanospheres the reaction time does not show an observable effect concerning shape, size and dispersity of the nanospheres. In contrast, the nanorod sample giving the most homogeneous cylindrical shape was achieved after 5 minutes. Hence, the reaction after 5 minutes was repeated for two additional amounts of L4 surfactant relative to the standard amount shown above. Results after 5 minutes and L4 amount of 0.05 mol/L are demonstrated by TEM images shown in Figure 24.

Figure 24: Nanospheres (a) and nanorods (b) prepared in THF/ethanol mixtures by use of MFRT and after 5 minutes reaction time.

The nanospheres having a diameter of ca. 6-7 nm are slightly larger, compared to the spheres prepared under normal conditions. The nanorods show a comparable aspect ratio and dispersity when prepared by MFRT. UV-Vis, luminescence and FTIR spectroscopies showed that both type of particles are analogues with respect to the surfactant to oxide properties compared to the samples prepared in standard reaction vessels and under reflux for three hours.

After reproducing equivalent particles under comparable conditions except for the MFRT, the synthesis was redesigned in order to prepare particles in water. Since anisotropic nanoparticles have already been prepared especially by hydrothermal methods, the experiments were aiming on well dispersed nanospheres or quantum
1. Synthesis of ZnO Nanoparticles with Variable Morphologies and Reactive Surface

dots, respectively. Two modifications brought to the preparation method so far were done for certain reasons: First, zinc chloride was exchanged by zinc acetate because acetate is known for its stabilizing properties which were assumed to moderate the nanosphere synthesis in water. Secondly, the surfactant was exchanged by o-PEA for further two reasons. On the one hand, L4 is a fatty acid lowering the pH value in aqueous medium and is only weakly soluble at the same time. On the other hand, Pich et al. had already proven o-PEA as sufficient stabilizer for quantum dots prepared in water\textsuperscript{[67]}.  

![Chemical structure of o-PEA](image)

Figure 25: Chemical structure of o-PEA

The chemical structure of o-PEA shows a phosphoric acid function perfectly suited for attachment on metal oxide surfaces and an amine end-group causing electrostatic surface charge in water due to protonation. All experiments are listed in Table 6.

<table>
<thead>
<tr>
<th>Sample</th>
<th>c (ZnAc) [g/L]</th>
<th>n (o-PEA) [mmol]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17.5</td>
<td>1.6</td>
</tr>
<tr>
<td>2</td>
<td>17.5</td>
<td>4.5</td>
</tr>
<tr>
<td>3</td>
<td>3.5</td>
<td>4.5</td>
</tr>
<tr>
<td>4</td>
<td>8.8</td>
<td>4.5</td>
</tr>
<tr>
<td>5</td>
<td>17.5</td>
<td>16</td>
</tr>
</tbody>
</table>

Table 6: Parameters of the precipitation based synthesis of ZnO nanoparticles by use of MFRT in water.

All approaches led to the formation of a colorless precipitation that shows no stability in water after a reaction time of five minutes. A TEM image of dried samples at 100 °C under vacuum is exemplarily shown (Figure 26). The image indicates the
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formation of nanospheres somehow embedded in a film. Meanwhile, a XRD pattern (not shown) clearly proves the non-existence of nanoparticulate zinc oxide. Both results give evidence that the synthesis is not sufficient to produce ZnO nanoparticles. However, up to this point it has not been possible to prepare nanocrystalline material and thus another approach had to be conceived.

![Image of TEM image of sample 1 recorded after drying for 48 hours at 100 °C under vacuum.](image)

Figure 26: TEM image of sample 1 recorded after drying for 48 hours at 100 °C under vacuum.

1.2.2.2. Peroxide Route

The new approach was derived from already established preparation methods that emanated from a peroxide based mechanism. A typical approach starts from zinc acetate and hydrogen peroxide reacting in aqueous medium leading to zinc peroxide precursors. The challenge of controlling the formation of small crystals is similarly large compared to the precipitation method, since zinc peroxide will be formed immediately after mixing zinc salt and hydrogen peroxide forming a water insoluble solid. A Reference synthesis of zinc acetate and hydrogen peroxide in presence of o-PEA following the attempt of sample 1 in Table 7 was done in standard reaction vessels. The result showed that it would not be efficient in order to prepare nanoparticles of monodisperse size distribution in presence of the stabilizer.
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Table 7: Synthesis parameters of ZnO nanoparticles by use of MFRT in water.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Zn(ac)$_2$:o-PEA [mol:mol]</th>
<th>Diameters [nm]</th>
<th>Yields ZnO$_2$ [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1/1</td>
<td>8.1 ± 2.3</td>
<td>44.0</td>
</tr>
<tr>
<td>2</td>
<td>1.7/1</td>
<td>2.9 ± 0.9</td>
<td>22.6</td>
</tr>
<tr>
<td>3</td>
<td>5/1</td>
<td>1.8 ± 0.4</td>
<td>17.6</td>
</tr>
<tr>
<td>4</td>
<td>10/1</td>
<td>6.0 ± 1.2</td>
<td>38.3</td>
</tr>
</tbody>
</table>

When Microfluidic Reaction Technology is used as reaction system, the formation of small nanoparticles and quantum dots respectively is achieved easily and after short reaction times, comparable to those of the precipitation route in organic medium, i.e. five minutes. The results for two samples are given in Figure 27. The TEM images show well dispersed nanoparticles of small size (cf. Table 7) and excellent monodispersity.

Figure 27: TEM Image of zinc peroxide nanoparticles prepared by the MFRT technique in water.

Further investigation by RAMAN spectroscopy revealed in which way the surfactant attaches on the particles surface while XRD unexpectedly proves the existence of zinc peroxide nanoparticles (see Figure 28). Thus, it was found that the use of MFRT
1. Synthesis of ZnO Nanoparticles with Variable Morphologies and Reactive Surface

led to the formation of colloidally stable ZnO₂ quantum dots in water. α-PEA attaches on the peroxide surface as expected for any other oxide surface: the phosphoric acid links up while the amine group remains idle.

Figure 28: RAMAN spectra (a) and XRD patterns (b) proof the formation of zinc peroxide nanoparticles stabilized by α-PEA.

Zetapotential measurements of zinc peroxide reveal an isoelectric point at pH 8. At pH values lower than 8 the surface charges become positive. The behavior of zinc peroxide is similar to ZnO having its isoelectric point at pH 9.5. Interestingly, zetapotential measurements done exemplarily for one sample at various pH values did not lead to positive potentials for a pH lower than 8. Beside the absence of the typical zinc peroxide behavior, one would expect the amine groups forming protonated end groups carrying a positive charge. Instead, Figure 29 shows a zero charge for pH values of 8 or lower.

This is obviously an unexpected result and might possibly be caused by the formation of protonated groups where counter ions, e.g. sodium, are locally linked to the NH₄-groups somehow masking the positive charge. This is just speculation and surely more work has to be done here to understand the exact mechanism.
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![Diagram of zeta potential vs pH](image)

Figure 29: Zetapotentials of zinc peroxide (red) and sample 2 from Table 8 (structure sketched at top).

At last, the organic amount after reaction and purification of the o-PEA modified zinc peroxide nanoparticles was characterized by use of thermogravimetric techniques. The results are listed in Table 8 and the corresponding thermogrammes are shown in Figure 30. The measurements were done in nitrogen atmosphere so that only degradation occurred. Hence, the organic amount can be calculated by subtracting the mass loss of ZnO₂ (11.2 wt-%) from each sample assuming the remaining mass loss is caused by the surfactant.

Table 8: Thermogravimetric measurements.

<table>
<thead>
<tr>
<th>Sample</th>
<th>T₁ [°C]</th>
<th>T₂ [°C]</th>
<th>Mass Loss [wt-%]</th>
<th>ZnO₂:o-PEA [m/m]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>227.8</td>
<td>468.4</td>
<td>27.5</td>
<td>3/1</td>
</tr>
<tr>
<td>2</td>
<td>210.7</td>
<td>-</td>
<td>25.3</td>
<td>3.5/1</td>
</tr>
<tr>
<td>3</td>
<td>194.9</td>
<td>-</td>
<td>22.6</td>
<td>4.4/1</td>
</tr>
<tr>
<td>4</td>
<td>191.4</td>
<td>-</td>
<td>22.0</td>
<td>4.6/1</td>
</tr>
<tr>
<td>ZnO₂</td>
<td>193.6</td>
<td>-</td>
<td>11.2</td>
<td>-</td>
</tr>
<tr>
<td>o-PEA</td>
<td>263.9</td>
<td>527.3</td>
<td>52.8</td>
<td>-</td>
</tr>
</tbody>
</table>
1. Synthesis of ZnO Nanoparticles with Variable Morphologies and Reactive Surface

Since o-PEA does not fully degrade, only 52.8 wt-% of the remaining mass loss of each sample reflects the half of the surfactant amount, so the remaining mass losses have to be doubled. Afterwards, the organic amount is expressed as the ZnO₂:o-PEA ratio.

The resulting inorganic to organic ratios show only low variation compared to starting ratios indicating that the attachment of surfactant is affected by particle size. Another interesting observation is that with increasing surfactant amount the onset of the peroxide degradation temperature shifts towards higher values (Figure 30a). Temperature shift and surfactant amount is in linear correlation as shown by Figure 30b.

From this point, nanoparticles synthesis by processing inorganic salts with hydrogen peroxide has not been investigated in more detail within the framework of this thesis but is in progress by works of C. Bergs under the supervision of Prof. A. Pich.

Figure 30: TGA curves of four different zinc peroxide samples modified with o-PEA. Curves show the characteristic ZnO₂ degradation at ca. 200 °C for all samples at a ZnO₂:o-PEA-ratio of 1:1 (a). Temperature shift and surfactant amount is in linear correlation (b).
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1.2.3. PEI-Based ZnO Nanocomposites

ZnO nanoparticles are known for their antibacterial activity. First tests showed that the antibacterial activity (see above) is tremendously influenced by the behavior of the particles in water and their specific surface. Small nanospheres show less activity compared to larger nanorods though their specific surface is higher and so a higher surface related activity, i.e. zinc release or photocatalytic properties, is expected.

Scheme 5: Chemical structures of PEI-Mal (a), implied hyperbranched PEI (b) and implied Maltose (c). Self-Assembly of positively charged PEI-Maltose (PEI-Mal) units on Nanorods surfaces which bear negative charges. The scheme shows two possible scenarios: formation of single core-shell particles and formation of aggregates (d).

However, larger nanorods showed a fourfold higher activity due to their increased water stability caused by a higher surface potential. The electrostatic stabilization was caused by a double layer assembly of fatty acid (L4) which shows potential as
1. Synthesis of ZnO Nanoparticles with Variable Morphologies and Reactive Surface

modifier but without any other specific properties. The approach shown in this chapter goes one step further. The aim is to introduce an additional surfactant assembling on the double layer surface. The compound that was selected is a PEI polymer that is covalently grafted with Maltose forming a core-shell morphology (PEI-Mal). At this juncture, the shell exists as open shell.

![Figure 31: Comparison between nanorods and nanospheres after reaction with PEI-Mal and purification.](image)

The PEI-Mal compounds have been synthesized and characterized by D. Appelhans and co-workers\(^\text{[68–70]}\). Since the core consists of PEI, the amine groups lead to positive charge within the frame of the PEI network. Scheme 5 holds the proposed mechanism for the self-assembly strategy. First, nanorods and nanospheres were tested to set a proof of principle in order to find out whether both samples are operational compounds in water. The synthesis was done by dispersing the nanoparticles and dissolving a given amount of PEI-Mal in water in different containers. Then, the nanoparticles dispersions were dropwise added into the PEI-Mal solutions under vigorous stirring at room temperature. After one hour the water was removed by freeze drying. The samples were then purified by washing them 3 times with acetone in order to remove free L4. After drying, the samples were washed with water and isolated by ultracentrifugation at 14000 rpm. The products were analyzed by TEM (Figure 31). Only in case of the
1. Synthesis of ZnO Nanoparticles with Variable Morphologies and Reactive Surface

nanorods a polymer shell is visible. It was expected that the rods would act more efficiently for two reasons. First of all, the rods are simply better dispersible. Secondly, the nanorods show a higher negative surface potential. FTIR spectroscopy confirmed the existence of PEI-Mal after purification with water in case of the nanorods, while in case of the nanospheres no PEI-Mal specific signal was observed (Figure 32). The spectra focus on wavenumbers between 1000 and 1200 cm\(^{-1}\). The nanoparticles show a signal caused by the PEO function of L4, the PEI-Mal composite shows the characteristic Maltose related signal. Only in case of nanorods both signals are occurring after reaction and purification.

Figure 32: FTIR spectra of nanorods (a) and nanospheres (b) focusing on 1000-1100 cm\(^{-1}\). Spectra show PEG-signal belonging to L4 attached on ZONR-A and ZOSP-A (1), Maltose groups bonded to PEI-Mal (2) and the product (NR/PEI-Mal and SP/PEI-Mal) after purification in the center. Comparison of each reaction product proves addition of PEI-Mal on the nanorod surface but not on the nanosphere surfaces.

Since only the nanorods showed sufficient reactivity concerning the self-assembly approach with PEI-Mal, the following studies were performed by reacting nanorods together with PEI-Mal. Table 9 lists, from a large number of experiments the relevant
1. Synthesis of ZnO Nanoparticles with Variable Morphologies and Reactive Surface

ones, including water purification. The products were prepared and purified as explained above. Afterwards, the particles were characterized by TEM, DLS and zetapotential measurements. Figure 33 shows TEM images of NRPM-4 before and after purifying it with water and ultracentrifugation. The images show in which way the rough treatment affects the composite. The nanorods show homogeneous distribution on the TEM-grid after their application from water. In contrast, all L4-only modified samples always had to be applied from organic medium, e.g. THF or toluene, in order to achieve good distribution on the grid. After reaction with PEI-Mal water became a sufficient solvent for such procedures.

Figure 33: TEM images of NRPM-4 before (a) and after (b, c) treatment with water and centrifugation. Due to the purification procedure, products lose their smooth plane-like shape.
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However, the PEI-Mal assembly on the nanorod/L4 surface is not able to resist the high force of ultracentrifugation. Instead, the composites show an irregular topography due to twisting (Figure 33 b) or formation of aggregates (Figure 33 c). The hydrodynamic radii shown in Table 9 proof what is assumed and schematically shown in Scheme 5: with increased PEI-Mal amount the formation of non-aggregated, single core-shell nanorod/PEI-Mal conjugates increases giving a lower mean radius. All The zetapotentials show a positive charge value but with unsystematic appearance.

Table 9: Synthesis parameters.

<table>
<thead>
<tr>
<th>Sample</th>
<th>ZnO:PEI-Mal [mg:mg]</th>
<th>Rₚ [nm]</th>
<th>ZP [mV]</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZONR</td>
<td>-</td>
<td>-</td>
<td>- 40</td>
</tr>
<tr>
<td>NRPM-1-aq</td>
<td>70/7</td>
<td>220</td>
<td>+ 11</td>
</tr>
<tr>
<td>NRPM-2-aq</td>
<td>50/10</td>
<td>168</td>
<td>+ 10</td>
</tr>
<tr>
<td>NRPM-3-aq</td>
<td>30/10</td>
<td>139</td>
<td>+ 21</td>
</tr>
<tr>
<td>NRPM-4-aq</td>
<td>30/30</td>
<td>150</td>
<td>+ 3</td>
</tr>
<tr>
<td>NRPM-5-aq</td>
<td>90/30</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Sample NRPM-5-aq was unable to be redispersed in water after purification. Furthermore, the sample showed a different behavior during the preparation of one hour since precipitation occurred. That’s why the experiment was repeated, the precipitate removed by smooth centrifugation at 2000 rpm and the supernatant stored at -20 °C.

It was found that after purification of nanorod-PEI-Mal composites by centrifugation from water at high velocity, the composites exhibit changes in shape and properties. One great disadvantage after centrifugation was the lack of water solubility. Thus, more work was done to avoid centrifuging the composites from water and therefore were only washed with acetone in order to remove excess L4-surfactant. To guarantee the absence of eventual excess PEI-Mal, we performed ITC titration at the highest ZONR:PEI-Mal ratio investigated here.
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The result is shown in Figure 34 and proves that after complete addition of PEI-Mal containing solution to a ZONR dispersion, the exothermic reaction will not be finished at all. In order to assure that no side reaction occurred during that experiment, the dispersion gained from the ITC experiment was checked by TEM proofing the composite formation visually (see Figure S1). Thus, we assume that all of the here discussed composites do not contain any free PEI-Mal and so do not have to be purified by washing with water.

Figure 34: ITC measurement of NRPM-4. After complete titration of PEI-Mal containing solution to a ZONR containing reservoir, exothermic peaks keep exiting. The result proves that self-assembly of positively charged PEI-Mal on negatively charged ZONR surfaces in water was not completed. TEM images proof the existence of composite within the sample after performing ITC measurement.

Hydrodynamic diameters and zetapotentials of ZONR modified with PEI-Mal and washed with acetone were recorded and are listed in Table 9. As expected, diameters decrease with increasing PEI-Mal amount. Meanwhile, surface potentials show increased shifts to positive region with increased PEI-Mal amount. For the
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highest ZnO:PEI-Mal ratio of 1:3 the zetapotentiel shows positive values as proposed.

Table 10: Synthesis parameters and colloidal data after purification with acetone.

<table>
<thead>
<tr>
<th>Sample</th>
<th>ZnO/PEI-Mal [mg:mg]</th>
<th>Dₜ [nm]</th>
<th>ZP [mV]</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZONR</td>
<td>-</td>
<td>-</td>
<td>-40</td>
</tr>
<tr>
<td>NRPM-1</td>
<td>10/1</td>
<td>173</td>
<td>-26</td>
</tr>
<tr>
<td>NRPM-2</td>
<td>3/1</td>
<td>118</td>
<td>-10</td>
</tr>
<tr>
<td>NRPM-3</td>
<td>1/1</td>
<td>101</td>
<td>-4</td>
</tr>
<tr>
<td>NRPM-4</td>
<td>1/3</td>
<td>81</td>
<td>+6</td>
</tr>
</tbody>
</table>

Hydrodynamic diameters and zetapotentials of ZONR modified with PEI-Mal and washed with acetone were recorded and are listed in Table 9. As expected, diameters decrease with increasing PEI-Mal amount. Meanwhile, surface potentials show increased shifts to positive potentials with increased PEI-Mal amount. TEM images of NRPM-1, -2, -3 and -4 show decreased aggregation behavior with increasing PEI-Mal amount (see Figure 35).

So far, successful self-assembly driven by electrostatic attraction between nanorods and PEI-Mal was proven by DLS, Zetapotentiel and TEM. Comparable results have been proposed by Klementieva et al. who demonstrated formation of aggregates consisting of amyloids and similar PEI-based dendrimers. The morphology of aggregates was controlled by dendrimer concentration\(^{[70]}\).
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Figure 35: TEM images of NRPM-1 (a), NRPM-2 (b), NRPM-3 (c), NRPM-4 (d). Progression of decreasing aggregation behavior from images a to d was achieved by increased PEI-Mal modification of the rods.
1. Synthesis of ZnO Nanoparticles with Variable Morphologies and Reactive Surface

1.3. Conclusion

A protocol for the synthesis of ZnO nanoparticles in THF and alcohol was successfully established, following a one-step approach. The method is capable of preparing nanospheres as well as nanorods, depending on the amount of NaOH used for the precipitation of nanoparticles. Nanoparticles were in-situ surface-modified by adding a fatty acid (L4) to the reaction. TEM, HR-TEM and XRD measurements proved that indeed nanocrystalline structured particles were prepared. FTIR, $^{13}$C-NMR spectroscopy and zetapotential measurements revealed that surface modification leads to formation of a double layer. The double layer remains stable in water only in the case of nanorods, while nanospheres do not show sufficient water-stability. After treatment with acetone, nanorods also lost their water solubility. Spectroscopy revealed the absence of second layer after acetone-washing that was responsible for this behavior. Nanospheres and nanorods were tested regarding their photocatalytic properties in water and in the presence of rhodamine-B as a model compound. Results showed that nanorods are more efficient than nanospheres. Taking Langmuir’s expression into account, the reason is due to increased negative surface potential of nanorods compared to nanospheres. Moreover, antibacterial properties were analyzed. Here, nanorods showed four-fold higher activity in case of E. coli and S. aureus. Tests indicated the reason for the increased efficiency is due to increased water-stability of nanorods and thus increased membrane-particle interaction with bacteria. Besides preparation in organic medium, attempts were done in order to achieve ZnO nanoparticle synthesis in aqueous medium. We found that monodisperse nanoparticle formation was able to be accomplished when zinc acetate and hydrogen peroxide were introduced. However, after characterizing the product by XRD it was found that zinc peroxide nanoparticles were formed during synthesis. At last, nanorods after organic synthesis were additionally modified with a novel polymer. This polymer consists of PEI – forming a hyperbranched core – and maltose which is covalently grafted, forming an open-shell system. Nanorods were found to be successfully modified by electrostatic attraction to the positively PEI-Mal compound. The improved efficiency of nanorods can be traced back to their suitable water-stability and particularly to their high negative surface charges.
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So far, the ZnO nanorods preparation method has been established, producing anisotropic nanocolloids suitable for further reactions in aqueous medium. Meanwhile, the synthesis approach established in case of nanospheres serves as principle for the following studies focusing on ZnO nanoparticle precipitation within porous host systems, namely microgels.
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1.4. Experimental Part

Synthesis of ZnO Nanoparticles

In a typical reaction 1 g (7.3 mmol) of zinc chloride and 1.5 g (4.2 mmol) of L4 were dissolved in 75 mL THF. For the preparation of ZOSP, 0.6 g (2.1 eq) NaOH was suspended in 7.5 mL methanol at room temperature and for the preparation of ZONR 0.8 g (2.7 eq) NaOH was suspended in 10 mL ethanol by stirring at 60 °C. The basic suspensions were added to the THF solution under stirring and then refluxed for 3 hours. After reaction the solvents mixture was evaporated and the residue re-suspended in ca. 80 mL toluene. The suspension was treated by centrifugation at 5000 rpm for 5-10 min and the liquid phase isolated. After evaporating the solvent, a waxy colorless (ZOSP) or brownish (ZONR) precipitant remained, which was dried in a vacuum oven at 100 °C for 48 hours.

For further purification the waxy product was washed with acetone for at least three times. The colorless precipitant was isolated by decantation.

Characterization of ZnO nanoparticles by $^{13}$C-solid state NMR

**ZOSP:** $\delta$(ppm) = 14.6 -32.7 (Backbone carbons, 12-C), 70.1 (PEO-intermediate, 4-C, methylene bridge between PEO and backbone, 1-C, $\alpha$-methylene, 1-C), 170.5 (carbonylic carbon, 1-C).

**ZOSP-A:** $\delta$ (ppm) = 14.7 -32.7 (Backbone carbons, 12-C), 41.9 (unknown, 0.6-C), 71.3 (fragment of PEO-intermediate signal, 3.4-C).

**ZONR:** $\delta$ (ppm) = 14.6 -32.7 (Backbone carbons, 12-C), 59.0-70.9 (PEO-intermediate, 4-C, methylene bridge between PEO and backbone, 1-C, $\alpha$-methylene, 1-C), 177.1 (carbonyl carbon, 1-C).

**ZONR-A:** $\delta$ (ppm) = 14.7 -32.7 (Backbone carbons, 12-C), 41.9 (unknown, 1.2-C), 71.3 (fragment of PEO-intermediate signal, 3.3-C).

Characterization of ZnO nanoparticles by FTIR-Spectroscopy

**ZOSP:** $\nu$ (cm$^{-1}$) = 480 (Zn-O), 1119 (-C-O-C-), 1635 (-C(=O)-O), 1759 (-C(=O)-OH).
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ZOSP-A: \( \nu \text{ (cm}^{-1}\) = 476 (Zn-O), 1115 (-C-O-C-), 1620 (-C(=O)-O').

ZONR: \( \nu \text{ (cm}^{-1}\) = 512 (Zn-O), 1120 (-C-O-C-), 1620 (-C(=O)-O).

ZONR-A: \( \nu \text{ (cm}^{-1}\) = 490 (Zn-O), 1117 (-C-O-C-), 1531 (-C(=O)-O' +Na), 1614 (-C(=O)-O').

Investigation of Photocalatytic Activity

The photocatalytic activity of ZnO nanoparticles was evaluated by the degradation of Rhodamine-B under UV-radiation. The UV source was a 150 W Hg lamp (44 mm long) with higher radiation intensity level in wavelength ranges 280 - 360 and 460 - 510 nm, which was surrounded by a circulating water jacket (Heraeus) to cool the lamp. The distance between the Hg lamp and the reactor was 10 cm for each experiment. For a typical run, ZnO particles were dispersed in 20 ml of an aqueous solution of RhB (2×10^-5 M) in a quartz glass reactor with stirring. This dispersion was stored in the dark for ca. 30 min prior to irradiation to establish the adsorption/desorption equilibrium of the dye on the catalyst surface. After a given irradiation time, UV-Vis spectra were taken from the sample in the wavelength range of 400–650 nm. The rate constant of the reaction was determined by measuring the change in the intensity of the peak at 554 nm with time.

Bacterial Culture

The bacteria strains employed in this work were the gram-negative and the gram-positive *Escherichia coli* (ATCC 23716) bacteria and the *Staphylococcus aureus* (ATCC 6538) bacterium. The nutrient solution at pH 7 contained 5 g peptone, 3 g meat extract per L bidistilled water. All solutions were autoclaved for 15 min at 120 °C prior to use.

Antibacterial Assessments of ZnO Solutions

A suspension of strains with known amount of colony forming units (CFU) was incubated at 37 °C in nutrient solution to which the respective ZnO was added in
1. Synthesis of ZnO Nanoparticles with Variable Morphologies and Reactive Surface

different concentrations. The growth of the bacteria was followed over 20 h by measuring every 30 min the optical density at 612 nm using a microplate reader/incubator. From these data, a minimal inhibitory concentration (MIC) was ascertained, equal to the concentration of the test substance at which a log 4 reduction of the growth of the inoculated bacteria relative to a control sample was observed. This test does not discriminate whether the substance is bactericidal or bacteriostatic. Experiments were triplicated.

A thermal shaker (Heidolph), a Microplate Incubator/Reader, an Infinite M200 Pro (Tecan), a Photometer Cary 100 (Varian), a drying oven, and a clean bench (Kendro) were used for the antibacterial assay.

For the daylight experiments a suspension of strains with known CFU was incubated in a thermal shaker at 37 °C in nutrient broth including the respective amount of ZnO. The 96 well plates were incubated under a daylight lamp. As a control a second plate was incubated in the same shaker in darkness and a third plate was incubated as described above. After 20h of incubation the solution was inoculated to nutrient agar plates and the amount of CFU/mL was determined after incubation over night at 37 °C.

Thermal shaker (Heidolph), Microplate Incubator/Reader, Infinite M200 Pro (Tecan), Photometer Cary 100 (Varian), drying oven, and clean bench (Kendro) were used for the antibacterial assay.

Analytical Methods

Transmission electron microscopy (TEM) measurements were performed with a Zeiss LIBRA 120 microscope. To prepare the specimen, the samples were dispersed in toluene or THF. Subsequently, one drop of the dispersion was added on a Formvar/carbon grid which was placed before on a filter paper. An organic solvent was used in order to optimize the coating on the carbon grids by drop casting. The samples were left at room temperature for a few minutes until they were completely dried and then were measured with an acceleration voltage of 80 kV in high vacuum (10^-6-10^-7 mbar).

High-resolution transmission electron microscopy (HRTEM) images were obtained by
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using a FEI Tecnai F20 electron microscope operated at 200 kV. Improvement of image contrast of HRTEM images for extremely small nanoparticles was obtained by applying an *a posteriori* Fourier lattice filtering using the implemented routines in the DigitalMicrograph package (Gatan, Inc.).

X-Ray Diffractometry (XRD) measurements were performed by using a Siemens D5000HR diffractometer with copper Kα radiation (λ=0.15406 nm). Trace element analysis was performed by use of inductively coupled plasma (ICP) accomplished with the Emissions Plasma Spectrometer 400 from Perkin Elmer.

Solid state nuclear magnetic resonance (NMR) measurements were performed by use of a AV700 Bruker NMR spectrometer operating at 1H frequency of 700.234 MHz and 13C frequency of 176.079 MHz. The number of scans was 2048 at a dwell time of 4 microseconds and a recycle delay of d1 = 10 seconds. The 13C rf pulse duration was 4 microseconds with a power of 87.2 W. Proton high-power decoupling was performed with a spinal 64 pulse sequence. The method used to measure 13C high-resolution spectra was based on direct polarization (DP) with proton high-power decoupling. The sample was rotating at 5 kHz under magic angle spinning (MAS). Chemical shift calibration was made using adamantane, a plastic solid. Finally, the calibration is on 13C resonance of TMS.

Fourier Transformation Infrared (FTIR) Spectroscopy measurements were recorded with a Thermo Nicolet Nexus 470 instrument. Low amounts of the dried samples were mixed with KBr to form a pellet which acts as specimen.

The zetapotential of ZnO nanoparticles were measured with Zetasizer NanoZS (Malvern) after dispersing solid samples in HPLC-grade water.

Fluorescence spectra were recorded with a Perking Elmer LS-50 photoluminescence spectrometer.
2. Synthesis of Microgel-ZnO Nanocomposites

2.1. Introduction

2.1.1. Microgels

Microgels have become a group of macromolecular compounds of high interest due to their very unique properties combining hydrogel-properties together with their ability to form stable colloidal dispersions in several solvents like water and alcohols. Basically, a microgel is an intramolecularly cross-linked macromolecule with diameters up to ca. 100 µm. Chemically, microgels are designed like any macro-gel, namely by crosslinking of polymer-chains. The difference is the colloidal nature of the microgel synthesized by precipitation polymerization in which all compounds are first dissolved and precipitate shortly after initiating the chain propagation. Methodologically, precipitation polymerization of microgels belongs to the heterophase polymerization.

The reaction scheme shown in Figure 36a exemplarily shows the NIPAM polymerization initiated by use of potassium peroxo-disulfate performed by Pelton and Chibante for the first time\cite{71}. The proposed mechanism involves the formation of microgels as stable colloids, comparable to micelles, but intramolecularly cross-linked. Stability is assured by surface charges which are usually induced by single charged polymer chains which act as stabilizer in the early state of colloid formation\cite{71,72}. Later, Saunders and Vincent proposed a general concept consisting of a core-shell structure based on the work of Wu and Pelton\cite{73,74}.

Wu and Pelton investigated the kinetics of NIPAM polymerization in presence of methylenebisacrylamide (BIS), acting as crosslinker. They found that BIS conversion occurs at a faster rate compared to NIPAM conversion. Additionally, Wu and Pelton found out by comparison of DLS and CPS data that larger particles consist less solvent (acetonitril) compared to smaller and hence larger particles consist of higher BIS amount compared to smaller particles. Moreover, the larger particles were formed during the early stage of reaction due to high BIS consumption while smaller particles bearing less BIS were formed at a later stage. Thus, Saunders and Vincent proposed the general structure shown in Figure 36b, taking into account that particle formation comes along with particles aggregation. The consequence is a
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heterogeneous microgel shape with core and shell segmentation showing higher density in the core region compared to the shell region. Moreover, this concept explains the swelling properties controlled by the shell while light scatter behavior and turbidity are controlled by core observed for NIPAM-styrene microgels\textsuperscript{75} in case of NIPAM-BIS microgels.

Figure 36: (a) Microgel formation by the “precipitation polymerization” mechanism. The Initiator (K$_2$S$_2$O$_8$) will degrade thermally and form an anionic species (steps a-c). After initiation, the first polymer-chains will bear a radical group as well as an anionic group. The charged polymers act as surfactants during synthesis, moderating the colloidal growth (steps d-g). Reprinted with permission from Reference [71], page 7. Copyright (2014) Elsevier. (b) Spatial relations between core and shell areas within a microgel. Reprinted with permission from Reference [73], page 254. Copyright (2014) Elsevier.

NIPAM is the most versatile compound investigated by many different groups concerning microgels and their properties. Besides NIPAM homopolymer-microgels,
there have been many works reporting NIPAM based copolymers crosslinked and forming microgels\textsuperscript{[76–78]}. NIPAM is so popular in this scientific field due to its thermosensitive properties providing microgels with the ability to react to temperature changes.

There have been many reviews enumerating many different synthesis approaches like for example inverse-emulsion polymerization, anionic polymerization, synthesis by radiation methods, mini-emulsions, addition of functional oligomers and polymers, controlled radical polymerization and microfluidic technology\textsuperscript{[71,79–84]}. The physical properties of microgels are based upon the various monomers used for polymerization, their polymer-like behavior as well as their colloidal properties. As explained above, colloidal gels are assembled heterogeneously depending on the polymerization rate of the monomers (including crosslinker) applied. Hence, microgel suspensions consist of polymers which are intramolecularly crosslinked, forming stable colloids. These colloids have unique properties regarding the interaction with solvent molecules and due to their softness (see below). The interaction with solvents has been mentioned before and states the fact that polymer to solvent interaction dramatically changes by varying temperature around a critical value. This temperature is known as Volume Phase Transition Temperature (VPTT) and associates to the Lower Critical Solution Temperature (LCST) of the corresponding linear homopolymer\textsuperscript{[85]}. Below this temperature, polymer to solvent interaction dominates leading to swollen microgel colloids which consist mainly, ca. 80-90%, of water. Above the VPTT microgel colloids deswell due to polymer-polymer interactions which become favorable at higher temperature\textsuperscript{[75]}. The loss of water usually leads to an increase of density within the microgel particles. This increase of density results in a dramatic change of the colloidal stability because in the swollen state Van-der-Waals attractions are negligible due to the high amount of solvent molecules inside the network\textsuperscript{[71]}. During deswelling, Van-der-Waals interaction becomes more important. Additionally, a repulsive force is built-up in the swollen state caused by higher amount of expanded hydrophilic-chains providing steric repulsion\textsuperscript{[73]}. Swelling-/de-swelling-processes can be followed by differing turbidity that is scaled by the network density\textsuperscript{[86]}. 

2. Synthesis of Microgel-ZnO Nanocomposites
2. Synthesis of Microgel-ZnO Nanocomposites

In contrast to hard spheres, compressibility also plays an important role. “In case of hard-spheres, an increase in viscosity is observed as the concentration of particles approaches close to that for random close packing of spheres. For soft particles, this limit no longer applies”[85]. Works of Senff and Richtering investigated the behavior of PNIPAM suspensions regarding their rheological properties. For dilute solutions, the relative viscosity ($\mu_{rel}$) is expressed by the following equation[87]:

$$\mu_{rel} = 1 + 2.5\phi_{eff} + 5.9\phi_{eff}^2$$

The effective volume ($\phi_{eff}$) shown in the equation above is the theoretical packing density of hard spheres in suspension and shows a theoretical maximum at ca. 0.5. $\phi_{eff}$ can be substituted with $kc$ where $c$ is the mass concentration and $k$ the shift factor. By plotting the relative viscosity against $c$, $\phi_{eff}$ can be determined[87].

![Figure 37: Plots show relative viscosity of PNIPAM suspensions at different temperatures and concentrations (a), as well as shear rates (b). Reprinted with permission from Reference [87]. Copyright (2014) AIP Publishing LLC.](image)

When the concentration of PNIPAM suspension is varied at low values and for different temperatures the gradient decreases with increasing temperature and so do the shift factor $k$ and $\phi_{eff}$, respectively (cf. Figure 37 a) [87]. Hence, by decreasing the size of the microgel-particles with increasing temperature, the packing-density for a specific concentration decreases.
The shear viscosity was determined and plotted against the shear rate using the function given by Cross\cite{87,88}:

$$\frac{\mu - \mu_\infty}{\mu_0 - \mu_\infty} = \frac{1}{1 + (\kappa \gamma)^m}$$

Figure 37 b shows that microgels tend to behave more like suspensions containing hard spheres when temperature increases up to 30 °C, that is very close to the VPPT of PNIPAM\cite{87,88}.

When the relation of ($\mu_0$) versus ($\phi_{\text{eff}}$) of microgels suspensions are compared with that for hard spheres (Figure 38), two important statements have to be made depending on the critical $\phi_{\text{eff}}$ of 0.5\cite{87,88}:

For $\phi_{\text{eff}} < 0.5$ microgels and hard spheres show analogous behavior, indicating that solvent molecules within the microgel networks are immobilized and thus resulting in hard-sphere-like behavior. For $\phi_{\text{eff}} > 0.5$ microgel suspensions show an ongoing increase of viscosity. Hence, this behavior was shown to be caused by the elasticity of the microgel-spheres which is tunable by cross-linking densities\cite{87,89}.

Works of Cloitre et al. additionally investigated the behavior of polyelectrolytes based on acrylates at very high volume fractions\cite{90-92}. So far, most of the properties related to thermosensitive microgels have been investigated for PNIPAM as model system. With respect to this work, another thermosensitive polymer is of main interest, namely poly(N-vinylcaprolactam) (PVCL). PVCL shows similar VPTT compared to PNIPAM but is favored for applications focusing on biomedical application due to its relative low-toxicity\cite{93}. 

2. Synthesis of Microgel-ZnO Nanocomposites
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Figure 39: Plot shows relative shear viscosity against effective volume of microgels suspension for different temperatures. The marked area shows behavior of microgels for $\phi_{\text{eff}} > 0.5$. Compared to hard spheres, this behavior is subjected to microgels only. Reprinted with permission from Reference [87]. Copyright (2014) AIP Publishing LLC.
2. Synthesis of Microgel-ZnO Nanocomposites

2.1.2. Microgels as Hosts for Metal Nanoparticles

As has already been described in chapter 1, the sufficient modification of nanoparticle surfaces is mandatory for application in aqueous environment. One idea was to modify ZnO nanoparticles directly on the particles surface by use of an organic acid in organic suspension. Here, another facile approach is the encapsulation of nanoparticles in porous networks. Microgels in this regard promise several features becoming of interest for several applications. The most important feature is their colloidal stability in several solvents, e.g. alcohols and water, their high specific surface which is interesting for drug-release applications and their responsiveness to external stimuli. Microgels show sensitivity to external stimuli like temperature, pH and salt concentrations. These properties come into account when the nanoparticle stabilization is supposed to be linked with drug release as it is in case of this work.

However, with respect to nanoparticle-microgel composite formation, several attempts have already been done in this field. ZnO was prepared in presence of polystyrene latex particles by use of zinc acetate. Zinc acetate is used as a common zinc salt known for its good capability to react to ZnO nanoparticles in alcoholic solutions. By pre-reacting zinc acetate and latex particles followed by basic treatment, two different composites can be prepared simply. The outcome of the reaction is controlled by base concentration and leads to raspberry-like morphology or core-shell composites. Moreover, additional inorganic material can be incorporated\[^{94,95}\].

In other approaches, ZnO and zinc sulfide nanoparticles were precipitated in presence of PVCL microgels copolymerized with AAEM. AAEM consists of a 1,3-diketonic group, known for well-chelating properties of metal-ions, acting as fixative of ZnO and ZnS nanoparticles. In both attempts, microgels were immersed in zinc acetate containing iso-propanol solutions. By ultrasonic treatment, zinc salt was incorporated. After treatment with sodium hydroxide the corresponding nanoparticles were successfully prepared\[^{96,97}\].
Further investigation revealed in which way zinc sulfide nanoparticles interact with their host network. A positive charge of the PVCL-AAEM network was presumed and then demonstrated by zetapotential measurements. Hence, at low pH – 2 to 6 – positively charged zinc sulfide particles interact with slightly positively charged microgel networks leading to the increase of the radius of gyration ($R_g$). Between pH 6 and 7 hydrophobic interactions between nanoparticles and polymer-chains were found. At this point, no changing of $R_g$ was detected. Further increase of pH (>7) led to collapse of hybrid particles due to attractive interactions between negatively charged zinc sulfide nanoparticles and positively charged polymer-chains (see Figure 40). In comparison, PVCL-AAEM microgels usually do not show an influence of diameter for different pH values$^{[96]}$. Comparable measurements were not performed in case of ZnO incorporated microgels, possibly due to the lability of this material against bases and acids.

Concerning the possible application of in-situ prepared and fixed metal nanoparticles within microgel networks and the water stability of the hybrid colloids, many applications are found in biological fields of interest. Lanthanide based nanoparticles were incorporated in PVCL-AAEM microgels as cell-tags for biodiagnostics$^{[67]}$. Moreover, TiO$_2$, silver and gold nanoparticles were incorporated due to their catalytic and antimicrobial properties$^{[95,99–101]}$. Looking beyond the applications, it was also shown in which way the combination of specific properties can influence physical properties of a colloidal system. Karg et al. demonstrated how thermosensitive PNIPAM based microgels can tune the plasmon resonance of gold nanorods. The

Figure 40: Behavior of hybrid microgels-zinc sulfide colloids with increasing pH values. Reprinted with permission from Reference [96]. Copyright (2014) American Chemical Society.
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rods were linked to the surface of the microgel spheres and then simply manipulated by capitalizing the diameter change of the thermosensitive colloids by changing the temperature (see Figure 41)\textsuperscript{[102]}.  

Figure 41: Absorbance of Au-nanorods (fixed on a PNIPAM-co-allylacetic acid microgel) increases and shifts towards increased wavelengths by increasing the temperature (15 °C, 42 °C, 60 °C); shown for two different samples. Dashed lines show Au-nanorods in absence of microgel. Reprinted with permission from Reference [102]. Copyright (2014) American Chemical Society.

Softness, in comparison to hard-spheres, is mandatory for such kind of applications. Without compressibility in combination with thermosensitive properties, the behavior investigated by Karg et al. would not have been possible, e.g. if gold nanorods had been fixed on hard-spheres. Besides applications focusing on metal nanoparticles, another way of utilizing soft microgel is the application as drug-delivery and drug-release systems.
2. Synthesis of Microgel-ZnO Nanocomposites

2.1.3. Biomedical Applications

The most interesting biomedical application of hydrogels, in general, can directly be linked to their unique properties corresponding to their softness and sensitivity to external stimuli, i.e. their ability to act as a drug releasing agent. Drug-release is a term widely used in literature, describing the controlled uptake and release of a chemical compound, “drug”, from a host-system. It is prerequisite that the host-system must be able to fix a certain compound under controlled conditions as well as to release it under preferred conditions, e.g. by changing the physical properties of the environment. With respect to this fact, it becomes clear why hydrogels are favored in this context.

On the one hand, their porous structure, achieved by chemical crosslinking, offers space for drug incorporation. Moreover, the network guarantees a non-linear response of external stimuli for the whole gel-system. At last, during polymerization of the hydrogel, a variety of other polymers can be linked to the backbone by copolymerization. Alternatively, additional polymer chains can be linked by surface-grafting methods after preparing the hydrogel-foundation. Additional polymers extend the hydrogel-properties physically and chemically in order to extend their activity as host-system in a way as prerequisited for interacting with different compounds (see Figure 42)\cite{103}.

Figure 42: Schematic representation of the mesh size of the gel ($\zeta$) and its behavior in swollen (left side) and deswollen state (right side) Reprinted from Reference [103]. Copyright (2014), with permission from Elsevier.

By diffusing into a host system, drugs will diffuse into the swollen hydrogel offering a volume fraction of $[\text{Volume of polymer}] / [\text{Volume of swollen gel}] = \left( \frac{V_p}{V_q} \right)$. The volume
2. Synthesis of Microgel-ZnO Nanocomposites

depends on the pore size that is represented by the correlation length between two adjacent crosslinks, given by the mesh size ($\zeta$). The mesh size correlates to the molecular weight between crosslinks ($\bar{M}_c$) as described by the following equation. Furthermore, the molecular weight of the polymer repeating unit ($\bar{M}_r$), the Flory characteristic ratio ($C_N$) and the length of the bond along the backbone (l) are of importance:\textsuperscript{[104–106]}

$$\zeta = \left( \frac{V_p}{V_q} \right)^{-1/3} \left( \frac{2C_N \bar{M}_c}{\bar{M}_r} \right)^{1/2} l$$

The equation shown here simply combines the pore size of a hydrogel, consisting of crosslinked polymer of a certain molecular weight, and the swelling behavior\textsuperscript{[105]} as demonstrated by others\textsuperscript{[107,108]}.

In contrast to hydrogel films, nano- and microparticles offer an additional advantage. Colloidally dispersed hydrogels are able to act as systems for drug-release due to their gel-like nature combined with the ability to remain colloidally stable in appropriate solvents. Diffusing into a biological environment in order to deliver drugs to certain location inaccessible for any film is a great tool for biomedical applications. The correlating field of science is called “nanomedicine” and discusses mainly the permeability and retention of nanoparticles. Nanoparticles can internalize cells by five different ways. Citing Petros and DeSimone, the five different ways contain:

“Internalization of large particles is facilitated by phagocytosis. Nonspecific internalization of smaller particles (>1 μm) can occur through macropinocytosis. Smaller nanoparticles can be internalized through several pathways, including caveolar-mediated endocytosis, clathrin-mediated endocytosis and clathrin-independent and caveolin-independent endocytosis, with each being subject to slightly different size constraints”\textsuperscript{[109]}.

In case of microgels, one would expect internalization by macropinocytosis or in case of small microgels, clathrin-mediated endocytosis. However, regarding the softness and compressibility of microgels, they might show additional features. This idea has been under investigation by Nguyen et al. who proofed that uptake of nano- and microgels via phagocytosis was amongst others dependent on the shear stress modulus of the medium\textsuperscript{[110]}.
2. Synthesis of Microgel-ZnO Nanocomposites

2.2. Results and Discussion

2.2.1. Synthesis of Microgel Systems

Different monomers were examined aiming the preparation of viable comonomers for the stabilization of ZnO nanoparticles by their microgel-incorporation. Six different microgels were investigated focusing on their ability to host ZnO by in-situ precipitation. The preparation method of nanoparticles is the same as that introduced in chapter 1, except for omitting L4.

Table 11: Investigated PVCL-based microgels.

<table>
<thead>
<tr>
<th>Microgel Type</th>
<th>Successful</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-Vinylcaprolactam, IA - via dimethyl itaconate route</td>
<td>Yes</td>
</tr>
<tr>
<td>N-Vinylcaprolactam, AAEM</td>
<td>Yes</td>
</tr>
<tr>
<td>N-Vinylcaprolactam, iso-Eugenol-PG</td>
<td>Yes</td>
</tr>
<tr>
<td>N-Vinylcaprolactam, PEG</td>
<td>Yes</td>
</tr>
<tr>
<td>N-Vinylcaprolactam, AAEM, IA</td>
<td>No</td>
</tr>
<tr>
<td>N-Vinylcaprolactam, AAEM, PAHM</td>
<td>No</td>
</tr>
<tr>
<td>N-Vinylcaprolactam, AAEM, VPA</td>
<td>No</td>
</tr>
</tbody>
</table>

Table 11 contains a list of all microgels which are based on VCL. Co-polymerization occurred by adding the comonomers and performing free radical polymerization. Referring to Table 11, four microgel-systems were successfully loaded, namely those containing itaconic acid (IA), AAEM, PEG and an iso-Eugenol end-capped linear poly(glycidole)-methacrylate polymer. The latter was synthesized in our laboratory as part of the thesis-related efforts and is not commercially available. In case of itaconic acid containing microgels, the first and successful system was prepared following the method developed by S. Schachschal[111]. Polymerization occurs by a two-step
2. Synthesis of Microgel-ZnO Nanocomposites

approach starting with the addition of dimethyl itaconate, followed by subsequent hydrolysis. As explained in details below, this route assures core-shell morphology with itaconic acid groups primary located within the core-area. The other attempts were unsuccessful regarding the formation of stable hydrogel-colloids at the micrometer scale and so the ZnO inclusion could not be attempted. These microgels will not be discussed further. All chemical structures are shown in Figure 43.

![Chemical structures of monomers and macromonomers used as comonomers for the microgel synthesis.](image)

Figure 43: Chemical structures of monomers and macromonomers used as comonomers for the microgel synthesis. The scheme shows acetoacetoxyethyl-methacrylate (AAEM), itaconic acid (IA), vinyl phosphoric acid (VPA), phosphoric acid 2-hydroxyethyl methacrylate ester (PAHM) and poly(glycidole)-methacrylate-iso-Eugenol (PGM-iso-E).
2. Synthesis of Microgel-ZnO Nanocomposites

2.2.2. PVCL-PAAEM Microgels

The polymerization of PVCL based microgels including co-polymers has been intensively investigated by A. Pich\textsuperscript{[96,111,112]}. PVCL based microgels have also been intensively investigated regarding the nanoparticle inclusion and their applications, including ZnO nanoparticles (see above). Hence, in this work PVCL-AAEM microgels were chosen as model-system in order to evaluate optimal parameters for ZnO nanoparticle incorporation by the method established in chapter 1.

Scheme 6: Reaction equation of the microgel synthesis by co-polymerization of VCL and AAEM.

The microgel synthesis follows the standard procedure as published by Pich and others. The approach is very simple: after mixing all monomers and the cross-linker (BIS) in aqueous solution, the polymerization will be started by the addition of an initiator (AMPA) and annealing up to 70 °C. Due to different hydrophobicity of all reactants, the free-radical polymerization approach occurs well controlled, leading to monodispersive core-shell colloids bearing PAAEM in the core and PVCL in the shell. In order to determine the possible influences of the AAEM core and additional interactions between the ZnO surface and AAEM functions on the ZnO nanoparticles capping within the dense gel, three different amounts of AAEM were adjusted. The three amounts of AAEM used were 1.25 mol-%, 5 mol-% and 10 mol-%.
2. Synthesis of Microgel-ZnO Nanocomposites

After dialyzing and freeze-drying, the microgels were loaded with ZnO nanoparticles by redispersing a given amount of gel in THF. Subsequently, the microgels were loaded with zinc chloride followed by precipitation of ZnO through the addition of sodium hydroxide which was beforehand suspended in ethanol (see Scheme 7). Dispersion of the microgels in THF occurs after their treatment in THF for 30 to 60 seconds in an ultrasonic bath and by stirring with a magnetic stirrer for several hours at room temperature.

The dispersion in THF is not comparable in its stability than that in an aqueous one but, however, sufficient for the upload procedure which starts by first adding zinc chloride and then ongoing stirring at 50 °C or room temperature for 3, 8 or 18 hours (overnight). The addition of the zinc salt does not negatively inflict the stability of the dispersion at any time. The ZnO nanoparticle formation will be started after adding sodium hydroxide and annealing up to 90 °C oil bath temperature, following the analogues procedure described for L4 stabilized ZnO nanoparticles (see above).

Scheme 7: Deposition of ZnO into microgels by controlled growth of ZnO nanoparticles in an organic medium.

During the formation of ZnO nanoparticles which lasts about 3 hours, increased precipitation of the microgels in THF/alcohol can be observed in some cases. After formation of ZnO nanoparticles the microgels composites were characterized without any purification. TEM images are shown in Figure 44, below, after preparation under different conditions. Images a, b and c depict composites with different PAAEM amounts, namely 1.25 mol-%, 5 mol-% and 10 mol-%, all with a constant starting mass of zinc chloride which was 4 wt%. Image d shows exemplarily the increased nanoparticles amount when the starting mass of zinc chloride is increased up to 8 wt%, as expected. The ZnO nanoparticles distribute in all four cases in the core...
and in the shell – which appears as a grey carpet – regions of the microgel. To verify if the ZnO nanoparticles assemble as seen when capped within the microgel network, a Reference test was done in order to visualize what happens when ZnO nanoparticles do not form inside the microgel colloids.

Shortly after the ZnO precipitation reaction was started, PVCL-PAAEM was added and the final product micrographed by TEM. The resulting image is shown in Figure 44e. obviously, the ZnO nanoparticles and the microgel particles start to aggregate after drop casting on a TEM grid or maybe even during the reaction. The nanoparticles assemble mainly at the periphery of the microgels and not homogenously in the whole matrix.

The following section describes the evaluation of optimum co-monomer amount. Further investigations of the composites were done by fluorescence spectroscopy. The spectra were recorded at excitation wavelength of 325 nm, giving similar results as the prior tests done in case of L4 modified ZnO nanoparticles. Here, the main point of interest lies in the relative intensity change. All samples were measured from the reaction medium, i.e. at equal microgel-concentrations. Fluorescence spectroscopy is an adequate technique to reveal the ZnO nanoparticle’s dispersibility within the microgel spheres. Polymer-capped ZnO nanoparticles and non-capped ZnO nanoparticles show different behavior, as shown by their fluorescence emission spectra as presented in the work of Jetson et al.\textsuperscript{[113]}. The authors chose MEH-PPV since the quenching of the luminescence and the surface properties of zinc oxide are related. The oxygen deficiencies (see chapter I) produce excited electrons under UV light exposure, which, rather than relaxing to their ground state and emitting green light observed as fluorescence, will preferentially interact with the polymer that comprise the capping material. Due to the tremendous decrease of the green light emission, the related peak at 550 nm quenches in the zinc oxide-capped spectrum while the characteristic exciton peak at 380 nm becomes visible. The MEH-PPV compound has to be seen as model system which achieves best results and so was chosen by the authors for their investigations.
2. Synthesis of Microgel-ZnO Nanocomposites

Figure 44: PVCL-PAAEM microgels loaded with ZnO nanoparticles. (a) AAEM content is 1.25 mol-%; ZnO amount is 4 wt%. (b) AAEM content is 5 mol-%; ZnO amount is 4 wt%. (c) AAEM content is 10 mol-%; ZnO amount is 4 wt%; (d) AAEM content is 5 mol-%; ZnO amount is 8 wt%. Reference shows microgels added to a solution in which ZnO synthesis had already been started before (e).

Figure 45 shows the fluorescence spectra of microgels with different PAAEM content (1.25 mol-%, 5 mol-% and 10 mol-%), focusing on the ZnO nanoparticles typical emission signals arising at ca. 500 nm after exciting at 325 nm. Comparison of PAAEM content shows no significant difference, while the peak evolution with increasing starting amounts of zinc chloride shows that, after reaction with NaOH, ZnO is formed in all cases. Further comparison shows that when doubling the zinc chloride amount from 2 wt% to 4 wt%, the emission intensity increases only slightly, as compared to its doubling when going from 4 wt% to 8 wt%. This tremendous jump of intensity can be explained by the increase of ZnO surface which remains unaffected by the molecules from the matrix. Thus, the increasing lack of interaction
2. Synthesis of Microgel-ZnO Nanocomposites

between ZnO nanoparticles and AAEM groups could explain the dramatic increase of intensity when introducing 8 wt-% zinc chloride.

Figure 45: Fluorescence spectra of PVCL-AAEM-ZnO nanoparticles composites bearing 1.25 mol-% PAAEM (a), 5 mol-% PAAEM (b) and 10 mol-% PAAEM (c).

It is well known that if ZnO nanoparticles are capped by polymer matrices, the emission signal will be quenched due to interactions with the nanoparticles surface that is responsible for the emission properties (see above). When increasing the ZnO amount by increasing the starting amount of zinc chloride from 4 wt% to 8 wt% leads to a dramatic increase of the emission intensity due to formation of particles that are insufficiently capped by the microgel-matrix, probably due to over-saturation of ZnO nanoparticles in relation to the microgel-hosts.

To evaluate the influence of the AAEM content on fluorescence emission of ZnO nanoparticles, the emission spectra were rearranged regarding the emission peak at 500 nm for different AAEM content, giving two spectra series for 4 wt% and 8 wt%.
2. Synthesis of Microgel-ZnO Nanocomposites

ZnCl$_2$ concentrations (Figure 46 a and b). Both plots show spectra that were normalized with respect to the exciton peak intensity at ~355 nm. In case of the ZnCl$_2$ 4 wt% composites (Figure 46 a) the highest peak is gained for the 5 mol-% AAEM composite, followed by the 1.25 mol-% composite and the 10 mol-% composite. The intensity decline is not directly proportional to the amount of AAEM, as it is in case of the ZnCl$_2$ 8 wt% composites (Figure 46 b). This behavior is unexpected and might be explained by non-homogenous distribution of ZnO nanoparticles inside of the 5 mol-% composite.

Figure 46: Fluorescence spectra of ZnO nanoparticles incorporated in PVCL-PAAEM microgels with different AAEM content. The starting concentration of ZnCl$_2$ is 4 wt% (a) and 8 wt% (b). The shoulder arising at the marked position (*) is caused by AAEM.

So far, microgels were investigated right after synthesis. To purify the composites, dialysis was performed resulting in an almost complete loss of ZnO nanoparticles. It was impossible to detect ZnO nanoparticles after dialysis for ca. 4 days by TEM (image not shown). This speaks for a high dissolution rate of ZnO nanoparticles. Since purification by dialysis leads to “empty” microgel due to ZnO degradation, the composites were purified by washing and centrifugation with water. So far, the use of PVCL-AAEM microgels as hosts for ZnO nanoparticles revealed an optimum amount of co-monomer of 1.25 mol-% or 5 mol-%. For further experiments, co-monomer amount of 5 mol-% was chosen. After loading PVCL-AAEM microgels containing 5 mol-% AAEM with a very high amount of zinc chloride, 33 wt-%, purification as
explained above was attempted. The results show that PVCL-AAEM microgels are limited with regard to the amount of ZnO which they can take up. The amount was evaluated by ICP-MS and showed a ZnO concentration of 26.4 wt-%. This value is much too high compared to the theoretical ZnO amount of 19.7 wt-% for the hypothetical case of 100 % zinc chloride conversion. The experiment was repeated a second time, revealing a comparable ZnO amount. The only possible explanation for this observation is that during purification, ZnO nanoparticles will be accumulated by centrifugation, leaving “empty” microgels in the supernatant.

Figure 47: TEM images of PVCL-AAEM after purification. An overview shows the presence of loaded (red) and non-loaded microgels together with ZnO aggregates (blue) (a). Close-up reveals deformation occurred regarding the microgel’s spherical shape (b).

TEM measurements of PVCL-AAEM microgels with high ZnO content were recorded after purification. Figure 47a pictures a larger region with loaded and non-loaded microgels, while Figure 47b reveals that microgels somehow were affected showing changed shape by TEM. So far, it remains unclear why the PVCL-PAAEM microgel shows this behavior after the synthesis-process. However, this microgel is valuable as model-system for the now to follow host-systems, regarding the co-monomer amount of 5 mol-%.
2. Synthesis of Microgel-ZnO Nanocomposites

2.2.3. PVCL-PIA Microgels

After the AAEM modified microgels, other microgels were investigated to achieve ZnO encapsulation. The method of synthesis is based on the preparation protocol established by Schachschal et al.\[^{[111]}\]. She developed a PVCL-based microgel including itaconic acids assembled in the core. Though itaconic acid is hydrophilic, the controlled incorporation within the core was achieved by using a precursor, dimethyl itaconate. The methyl-ester of itaconic acid is significantly more hydrophobic leading to similar core-shell formation compared to PVCL-AAEM. Afterwards, the ester will be hydrolyzed, forming the corresponding acid.

![Scheme 8: Reaction equation of PVCL-PIA microgels by a two-step approach including the formation of PVCL-PIADME precursors which react to the aimed product by hydrolysis in basic medium.](image)

First tests with itaconic acid were done and characterized regarding the retaining of ZnO nanoparticles after dialysis. The starting concentrations of zinc chloride were chosen to be analogues compared those used for the AAEM microgels. Qualitative characterizations of the products with fluorescence and DLS revealed that in case of itaconic acid, ZnO nanoparticles are retained within the microgels. Fluorescence spectra measured in water showed characteristic exciton signals of ZnO nanoparticles after dialysis (Figure 48). DLS measurements showed a decrease of diameters with increasing upload of particles (Figure 49).
2. Synthesis of Microgel-ZnO Nanocomposites

![Fluorescence spectra](image)

Figure 48: Fluorescence spectra of product with 8 wt-% zinc chloride. Spectra shown for unpurified product, measured in THF (a), dialyzed product measured in water (b) and water-only Reference (c).

In contrast to the AAEM microgels, qualitative investigations clearly showed an improvement concerning ZnO encapsulation when AAEM co-monomer was exchanged by itaconic acid. The following chapter treats more detailed and quantitative studies focusing on itaconic modified PVCL microgels.

![Hydrodynamic radii and PDIs](image)

Figure 49: Hydrodynamic radii (a) and PDIs (b) of PVCL-PIA (5 mol-%) microgels loaded with different amounts of zinc chloride. All data were collected after dialysis.

Itaconic acid is a bicarbonic organic acid that is produced by fermentation of molasses on industrial scale. It was established as a microgel aqueous stabilizer due to its negative charge leading to increased electrostatic repulsion between the
colloidal particles. Here, itaconic acid was chosen as potential candidate for the stabilization of ZnO nanoparticles within the microgel network. Since L4 has already proven its stabilizing properties, carbonic acids were favored. Besides, phosphonic acid derivatives were investigated as another class of potential co-monomers but were not found to be suitable due to low dispersibility of the microgels in water. The synthesis of PVCL-co-poly(itaconic acid) (PIA) microgels was first demonstrated, as mentioned, by S. Schachschal. She developed a synthesis approach starting with the dimethyl ester of the itaconic acid, dimethyl itaconate (IADME). The reason herefor is the decreased hydrophobicity of the ester compared to the acid which makes it favorable for the formation of core-shell microgel colloids, containing the ester in the core while VCL remains mainly in the shell region. After microgel formation, the acid will be added after hydrolysis in basic medium. The microgels will be purified by dialysis, freeze-dried and applied for the ZnO nanoparticles loading procedure, as the PVCL-PAAEM microgels were applied before (see above).

Itaconic acid bears two carbonic acid functions leading to a chelating ligand which is known to complex cations or interact with metal oxide surfaces efficiently\cite{114–116}. After the first loading procedure with zinc chloride, sodium hydroxide, which is previously dissolved in ethanol at low concentration, is added to the mixture. After reaction, further purification combined with a transport of the dispersion into aqueous solution will be done by washing with water and isolation of the product by centrifugation. Dialysis was not attempted since the first results show a dramatic loss of inorganic material during dialysis. Even though the itaconic acid-modified microgels showed improved properties regarding ZnO encapsulation, the loss of mass was not acceptable. Moreover, as shown in chapter 3, ZnO nanoparticles will completely degrade forming zinc ions as seen during cumulative release studies. In comparison of cumulative release studies – where the medium will be completely exchanged against fresh medium – dialysis follows a comparable mechanism. Due to these reasons and the fact that mass amounts cannot be reproduced, dialysis has not been performed again.
2. Synthesis of Microgel-ZnO Nanocomposites

Figure 50: TEM images of PVCL-PIA microgels (IA content is 5 mol-%) with ZnO contents of 0.6 wt-% (a), 1.4 wt-% (b), 6.3 wt-% (c) and 15.9 wt-% (d).

Figure 50 shows TEM images of the product after ZnO synthesis in THF and purification by washing with water for three times. Microgels show the characteristic core-shell morphology: dark drawn core and grey shaded shell. Within the microgels, nanoparticles are capped after ZnO precipitates in the organic solution. The ZnO synthesis was accomplished for four different starting concentrations of zinc chloride. To evaluate the ZnO amount after preparation, photospectrometric measurements were performed following the method introduced by Säbel et al\[117\]. The results are
2. Synthesis of Microgel-ZnO Nanocomposites

listed in table 1. The transformation of zinc chloride to ZnO is characterized by a conversion of approximately 50 % for all samples.

Table 12: ZnO contents of PVCL-PIA microgels.

<table>
<thead>
<tr>
<th>Sample</th>
<th>ZnCl₂-amount [wt%]</th>
<th>ZnO-content [wt%] Photospectrometry</th>
<th>ZnO-content [wt%] ICP-MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>MG-0.5</td>
<td>1.0</td>
<td>0.6</td>
<td>0.5</td>
</tr>
<tr>
<td>MG-1.4</td>
<td>3.3</td>
<td>1.4</td>
<td>1.4</td>
</tr>
<tr>
<td>MG-5.5</td>
<td>10.0</td>
<td>6.3</td>
<td>5.5</td>
</tr>
<tr>
<td>MG-16.9</td>
<td>33.0</td>
<td>15.9</td>
<td>16.9</td>
</tr>
</tbody>
</table>

The photospectrometric measurements were done after treating the microgel dispersion with half concentrated nitric acid, followed by ultracentrifugation and dilution of the zinc containing filtrate by adding borate/urea buffer at a pH of 9.0 to the solution. The zinc ion concentration was evaluated after addition of zinc-complexing ligand, namely Zincol[117]. After Zincol addition, UV VIS spectra were recorded giving characteristic signals bearing a peak at 628 nm (see Reference [117] for detailed information). Furthermore, the ZnO amounts in the microgel samples were investigated by ICP-MS\textsuperscript{5}. Both methods were used for assure the accurate and quantitative determination of the ZnO amount in the microgels.

FTIR spectra show an increase of a characteristic signal at ca. 480 cm\textsuperscript{-1}, caused by zinc-oxygen vibration, with increasing ZnO amount (Figure 51). FTIR spectroscopy qualitatively proves the increasing amount of zinc within the microgels by the increased absorbance at the wavenumber characteristic for the zinc-oxygen bonding so demonstrating the formation of crystalline ZnO (see chapter 1).

\textsuperscript{5} All ICP-MS measurements were performed by Milen Nachev at Aquatic Ecology and Centre for Water and Environmental Research (ZWU), University of Duisburg-Essen.
2. Synthesis of Microgel-ZnO Nanocomposites

Figure 51: FTIR spectra of microgel/ZnO composites focusing on the absorption band at 500 cm\(^{-1}\), associated with the Zn-O group vibration bonding at 500 cm\(^{-1}\). The signal increases with increasing ZnO amount.

The reason why itaconic acid was chosen as comonomer is the increase of negative surface charge of the microgel and the known ability to bind heavy metal cations\(^{[116]}\). The negative charge of the microgel is supposed to support the precipitation of ZnO nanoparticles via electrostatic attraction of positively charged zinc(II)-cations, dissolved in THF, into the porous microgels where the highest content of itaconic acid is located in the core. Electrophoretic mobility measurements were done to show the influence of ZnO on the negative charge (Figure 52).

Figure 52: Electrophoretic mobility recorded at 25 °C for several microgel/ZnO composites and different ZnO contents (25 °C, pH 5.8).
2. Synthesis of Microgel-ZnO Nanocomposites

As expected, after measuring the composites in HPLC-grade water at 25 °C, the negative charge of the microgels decreases with increasing ZnO amount, since ZnO does have a positive surface charge at pH < 7. DLS measurements of microgel-ZnO composites reveal the decrease of the hydrodynamic diameter and the VPTT of the microgels with increased ZnO nanoparticles amount. The size quenching can be explained by the electrostatic attraction between positively charged ZnO nanoparticles and negative acid groups. Likewise, the hydrophobic nature of ZnO might lead to an increased water exclusion from the microgels\cite{118-120}. This is typical for hydrogels, e.g. the size mainly being dependent on the water content of the gels. In case of PNIPAM-microgels in the swollen state, it was found that the gels bear up to 90 wt-% water within their network, depending on temperature\cite{121}.

Figure 53: Hydrodynamic radii at different temperatures (a) and for different ZnO amounts at 25 °C (b).

The decreasing hydrodynamic radii might be explained by increasing electrostatic interaction between positively charged ZnO surfaces and negatively charged itaconic acid groups. Consequently, this kind of interaction would reduce the free volume within the porous structure due to polymer chain-ZnO attraction (see Scheme 9).
2. Synthesis of Microgel-ZnO Nanocomposites

Scheme 9: Influence of ZnO incorporation on the zetapotential and the hydrodynamic radii. After Incorporation, ZnO nanoparticles introduce positive surface-charges and further reduce free volume within pores due to increased electrostatic interaction.

The measurements were done in HPLC-grade water at the pH-level existent in the water as received. To get a deeper understanding of the swelling properties of the composites at a pH level found in biological environments, measurements were repeated at pH 7.5. Therefore, the pH of the HPLC-grade water was adjusted by the addition of an aqueous solution of NaOH. The results confirm that at 37 °C and pH 7.5 all nanocomposites are not fully collapsed. This behavior is desired, since microgels are supposed to swell in bodily fluids and facilitate ion exchange with wound tissue.

Figure 54: Hydrodynamic radii at different temperatures in HPLC-grade water with the pH value adjusted to 7.5.
2. Synthesis of Microgel-ZnO Nanocomposites

So far, after showing that PVCL-AAEM microgels are feasible as model systems for basic investigations, a PVCL-PIA microgel was constructed for hosting ZnO nanoparticles. It was shown that ZnO nanoparticles will remain within the microgel spheres after purification by their threefold washing with water. This purification approach sufficiently substituted the standard dialysis method. All microgels showed increasing surface potential and decreasing hydrodynamic radii due to incorporation of ZnO\textsuperscript{[97]}, which is known to have positive electrostatic potential for pH $< 9.4$\textsuperscript{[122]}. In addition, DLS measurements revealed decreasing hydrodynamic radii of the composites compared to empty microgels, also proving incorporation of nanoparticles. The influence of ZnO nanoparticles on both zetapotential and decreasing radii are sketched in Scheme 9.
2. Synthesis of Microgel-ZnO Nanocomposites

2.2.4. PVCL-iso-Eugenol Microgels

Further improvement of ZnO encapsulation in combination with additional antibacterial applicability was attempted by preparation of iso-Eugenol modified PVCL-microgels. The aim is to attach Eugenol to the microgels, which has been shown to form antibacterial polymer-films\textsuperscript{[123,124]}. It is of great importance to include spacers in order to enable the iso-Eugenol groups to interact with bacterial cells through the fringe-like microgel-edge. Only a hydrophilic spacer is supposed to assemble at the outer rim of the microgel-colloids during the precipitation polymerization. Thus, poly(glycidole) was chosen as spacer-group, since controlled spacer lengths can be adjusted simply by anionic polymerization. In addition, Eugenol is well-known for its zinc complexing properties and thus interesting for systems which are designed to cap ZnO\textsuperscript{[125]}.

2.2.4.1. Synthesis of iso-Eugenol Based Microgels

The synthesis of iso-Eugenol containing macromonomers with controlled spacer length was accomplished by a three step approach. First, Eugenol acts as initiator of the controlled anionic polymerization of glycidole. The synthesis method is a standard method of glycidol polymerization and follows the ring opening approach by nucleophilic addition of the initiator at the epoxy group. Therefore, Eugenol is activated by using potassium tert-butoxide, and the polymerization started after addition of specific amounts of glycidol. The anionic approach offers a great advantage regarding the control of spacer length.
2. Synthesis of Microgel-ZnO Nanocomposites

Scheme 10: Route of iso-Eugenol consisting macromonomers with adjustable length for the preparation of Eugenol containing PVCL-microgels.

Since the iso-Eugenol groups are assumed to be assembled at the outer rim of the microgel colloids, two features offered by this approach are important: the hydrophilic nature of poly(glycidol) as well as the chain length of linear poly(glycidol). The synthesis was performed for three different degrees of polymerization ($P_n$). It is important to start the glycidole polymerization after protecting the alcohol function with vinyl ether. The protected glycidol is called ethoxy-ethyl glycidyl ether (EEGE) and well known for linear glycidol polymerization. If glycidol is used instead of EEGE, hyperbranched poly(glycidol) is formed.

After EEGE polymerization of the desired chain length, methacryloyl chloride is added which stops the anionic polymerization on the one hand and introduces a new chemical function on the other hand, namely a methacrylic group. At last, the EEGE protection group will be easily removed by acidic hydrolyzation.
Table 13: Synthesis parameters of iso-Eugenol macromomers.

<table>
<thead>
<tr>
<th>ID, Name</th>
<th>P_n</th>
<th>E/Z-Ratio</th>
<th>Yield/%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoeug-PEEGE₄</td>
<td>4.09</td>
<td>82/18</td>
<td>92</td>
</tr>
<tr>
<td>Isoeug-PEEGE₆</td>
<td>6.15</td>
<td>84/16</td>
<td>91</td>
</tr>
<tr>
<td>Isoeug-PEEGE₁₂</td>
<td>12.06</td>
<td>92/8</td>
<td>92</td>
</tr>
<tr>
<td>Isoeug-PEEGE₄-MAE</td>
<td>4.09</td>
<td>81/19</td>
<td>70</td>
</tr>
<tr>
<td>Isoeug-PEEGE₆-MAE</td>
<td>6.15</td>
<td>83/17</td>
<td>70</td>
</tr>
<tr>
<td>Isoeug-PEEGE₁₂-MAE</td>
<td>12.06</td>
<td>63/37</td>
<td>50</td>
</tr>
<tr>
<td>Isoeug-PG₄-MAE</td>
<td>4.09</td>
<td>66/34</td>
<td>92</td>
</tr>
<tr>
<td>Isoeug-PG₆-MAE</td>
<td>6.15</td>
<td>85/15</td>
<td>99</td>
</tr>
<tr>
<td>Isoeug-PG₁₂-MAE</td>
<td>12.06</td>
<td>63/37</td>
<td>88</td>
</tr>
</tbody>
</table>

Table 13 contains all important results of the iso-Eugenol macromonomer synthesis. In each case, the yield is related to the pre-step. Table 13 shows that addition of methacryloyl chloride leads to decreased yield, depending on the degree of polymerization. The higher P_n, the lower the yields.

Figure 55: 1H-NMR spectrum of Isoeug-PEEGE₆ (a), the scheme of E-/Z-isomerization (b) and the 1H-NMR spectrum focusing on isomer-signals (c).

Polymerization of EEGE initiated by Eugenol was followed by ¹H-NMR spectroscopy. The spectrum of Isoeug-PEEGE₆ is shown exemplarily in Figure 55. The figure shows the complete spectrum including signals of Eugenol (blue color) and polymer (red color). The spectrum holds several important informations. First, the peaks between
2. Synthesis of Microgel-ZnO Nanocomposites

2.4-2.6 ppm belong to the epoxy group of the glycidol monomer and have shifted to a multiplet at around 3 ppm. This proves successful ring opening and polymerization. The degree of polymerization can be computed by calculating the area under peak 14 of the polymer spectrum (red). At last, the formation of signals at 5.6-5.7 ppm and 6.0-6.1 ppm are related to the isomerization of Eugenol, forming iso-Eugenol (Figure 55 b). The isomerization splits the iso-Eugenol group into E- and Z-isomers. Z-isomer is seen by signals 5.6-5.7 ppm, while E-isomer is seen at chemical shifts of 6.0-6.1 ppm (Figure 55 c). From the ratio of their integrals, the E/Z isomer ratio was computed and listed in Table 13. In addition, $^{13}$C-NMR and FTIR spectroscopy confirmed successful polymerization as well as E/Z isomerization for all three Pn (spectra are not shown). The isomerization of Eugenol is usually caused under basic conditions and could not be avoided. After successful addition of Methacrylolyl chloride, the spacer groups were left unprotected under acidic conditions. Decrease of protection was done in THF at room temperature and stirring for 3 hours after adding HCl (37 wt-%). The vanished signals of purified product Isoeug-PG$_6$-MAE 8 and 9 at ~1.2 ppm confirm that the spacer groups were left unprotected (Figure 56).

![Figure 56: $^1$H-NMR spectrum of Isoeug-PEEGE$_6$-MAE and Isoeug-PG$_6$-MAE. Vanishing peaks at ca. 1.2 ppm proof successful removal of protection groups.](image_url)
2. Synthesis of Microgel-ZnO Nanocomposites

Analogues results were achieved for Isoeug-PG₄-MAE and Isoeug-PG₁₂-MAE. Unfortunately, in all three cases, the products were not completely relieved of impurities. Even after intensive attempts by several steps of precipitating the products from diethyl ether, the impurities remained. However, the macromonomers introduced so far were subsequently used for microgel synthesis in combination with VCL and BIS.

Table 14: Preparation parameters of the isoeug-PGₙ-MAE microgels⁶.

<table>
<thead>
<tr>
<th>n</th>
<th>x</th>
<th>R_H at 20°C [nm]</th>
<th>PDI at 20°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>142</td>
<td>0.094</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>367</td>
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<td>4</td>
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<td>6</td>
<td>0.1</td>
<td>58</td>
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</tr>
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<td>6</td>
<td>1</td>
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</tr>
<tr>
<td>6</td>
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<td>69</td>
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<td>10</td>
<td>66</td>
<td>0.522</td>
</tr>
<tr>
<td>6</td>
<td>20</td>
<td>6</td>
<td>0.247</td>
</tr>
<tr>
<td>12</td>
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</tr>
<tr>
<td>12</td>
<td>10</td>
<td>71</td>
<td>0.420</td>
</tr>
</tbody>
</table>

The following studies concerning microgel synthesis and characterization were done by Claudia Dähling under the supervision of Michael Kather. They prepared and

⁶ Microgel-synthesis reactions were performed and characterized by C. Dähling under the supervision of M. Kather.
2. Synthesis of Microgel-ZnO Nanocomposites

categorized several microgels using macromonomers with different $P_n$ (4, 6, 12) and moreover, varied the mol-amount of the macromonomers. The overview is important for discussing the reason why isoeug-PG$_6$-MAE was chosen for ZnO encapsulation and in which way it behaves in water.

Table 14 shows all relevant results of C. Dähling. The table contains the hydrodynamic radii and PDI at 20 °C for all microgels. The table reveals highest monodispersity for isoeug-PG$_6$-MAE microgels with 0.1 mol-% co-monomer, followed by 1 and 5 mol-% of the same system. The isoeug-PG$_6$-MAE with 0.1 mol-% co-monomer was of no interest due to the extremely low iso-Eugenol content. Comparison of surface potentials in case of isoeug-PG$_6$-MAE for different mol-amounts reveals the highest negative charges in neutral medium for 5 and 20 mol-% (see Figure 57). As shown in the chapter before, high negative zetapotential supports the ZnO precipitation within the microgel spheres. Interestingly, the 10 mol-% microgel shows a dramatic shift of its iso-electric point that cannot be explained so far. Nevertheless, all obtained results led to the conclusion that isoeug-PG$_6$-MAE with 5 mol-% is a promising microgel for ZnO encapsulation.

Figure 57: Zetapotentials for different pH and mol-amounts of co-monomer$^7$.

$^7$ Microgel-synthesis reactions were performed and characterized by C. Dähling under the supervision of M. Kather.
2. Synthesis of Microgel-ZnO Nanocomposites

2.2.4.2. ZnO Encapsulation

PVCL-iso-Eugenol consisting of spacers with 6 units length was chosen for subsequent investigations. The ZnO upload procedure was the same as for the PVCL-PIA nanocomposites. After preparation in organic medium, starting with the addition of zinc chloride and sodium hydroxide, purification with water was done. TEM images were recorded for the PVCL-iso-Eugenol nanocomposite with highest amount of zinc chloride used here (33 wt-%).

Figure 58: PVCL-Eugenol-6 microgel containing 10.6 wt-% ZnO nanoparticles. Images show distorted microgels and/or microgel-aggregates engulfing ZnO nanoparticles. The microgels have a more irregular shape compared to PVCL-PAAEM and PVCL-PIA, since no core-building co-monomer like AAEM or IADME was used here.

The isoeug-PG₆-MAE PVCL-based microgel system was loaded with three different amounts of zinc chloride and characterized regarding the amount of ZnO after purification by use of ICP-MS (analogues to PVCL-PIA systems). Table 15 shows all results, including the results of the PVCL-PIA systems for comparison, since the motivation of using Eugenol-based co-monomers was to insert a ZnO retaining group similar to bicarbonate acids. Thus, the comparison is mandatory in order to draw conclusion regarding which system is favorable.
2. Synthesis of Microgel-ZnO Nanocomposites

Table 15: ZnO amounts of the Eugenol-PVCL systems as well as the PVCL-PIA systems for comparison.

<table>
<thead>
<tr>
<th>ID</th>
<th>ZnCl$_2$-amount [wt%]</th>
<th>PVCL-Itaconic acid [wt%]</th>
<th>PVCL-iso-Eugenol [wt%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>A</td>
<td>3.3</td>
<td>1.4</td>
<td>0.5</td>
</tr>
<tr>
<td>B</td>
<td>10.0</td>
<td>5.5</td>
<td>5.3</td>
</tr>
<tr>
<td>C</td>
<td>33.0</td>
<td>16.9</td>
<td>10.6</td>
</tr>
</tbody>
</table>

The amounts of ZnO showed significant differences. The use of a zinc chloride starting concentration of 33 wt-% especially showed lower conversion when iso-Eugenol co-monomer is used, in contrast to itaconic acid. However, the successful incorporation of ZnO nanoparticles was investigated by use of DLS and zetapotential measurements (see Table 16). Both microgels show zetapotentials shifting with increasing ZnO amount to positive values, due to the positive surface potential of ZnO under these conditions (25 °C, HPLC-grade water without treatment and pH of 5.8). In comparison to itaconic acid containing microgels, no systematic change of hydrodynamic diameters can be ascertained here.

Table 16: Comparison of zetapotentials, hydrodynamic radii and PDIs between PVCL-itaconic acid and PVCL-iso-Eugenol.

<table>
<thead>
<tr>
<th>ID</th>
<th>PVCL-Itaconic acid</th>
<th>PVCL-iso-Eugenol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zetapotential [mV]</td>
<td>$R_h$ [nm]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>- 28.1</td>
<td>299</td>
</tr>
<tr>
<td>A</td>
<td>- 18.5</td>
<td>271</td>
</tr>
<tr>
<td>B</td>
<td>- 15.8</td>
<td>256</td>
</tr>
<tr>
<td>C</td>
<td>- 12.9</td>
<td>252</td>
</tr>
</tbody>
</table>
2. Synthesis of Microgel-ZnO Nanocomposites

At last, the properties of the microgel-colloids in water were tested by DLS (see Figure 59). Interestingly, with increasing ZnO amount the swelling/de-swelling ability becomes more explicit. The reason for this finding is cannot be explained by detail here. Possibly, an increased amount of ZnO deactivates the hydrophilic components (as discussed before) of the microgel network, thus having weaker hydrophilic properties. Hydrophilic properties, vice versa, might be the reason for the linear plots for decreased ZnO contents and have been discussed for PVCL-PAAEM-PEG microgels elsewhere[128].

![Figure 59: DLS temperature trends for PVCL-iso-Eugenol microgels. ZnO amounts of 0.5 and 5.5 wt-% show no variation with varied temperature. In contrast, ZnO amount of 10.6 wt-% shows a characteristic S-curve.](image)

In summary, both PVCL-based microgels (IA and iso-Eugenol) show great potentials as host systems for ZnO nanoparticles due to good encapsulation properties, primarily caused by high negative zetapotentials supporting the zinc(II) cation inclusion and the ZnO retaining properties. The advantages of PVCL-Eugenol-ZnO composites result from the combination of bioactive ZnO nanoparticles and the antimicrobial properties of Eugenol. This novel material might become useful as improved drug-release system of surface-coatings for materials focusing on antimicrobial applications, e.g. wound therapy or antifouling-coatings.
2. Synthesis of Microgel-ZnO Nanocomposites

2.2.5. PVCL-PEG Microgels for the Laser-Ablation Process

As an alternative approach, in collaboration with the university of Essen-Duisburg and the working group of Prof. Barcikwoski, the polymerization of microgels was performed during the laser ablation process (see Scheme 11). The reaction chamber is circularly arranged and made from steel. Within the chamber, there is a channel in which a solvent-stream flows circularly. At one end, the channel passes an area where a Quartz window is enclosed with the outer rim of the chamber. In opposite direction, the target is mounted on the inner metal-part. Light can pass through the quartz window, penetrate the stream and hit the target, in this setup. The metal targets are chosen with regard to the application demands so, concerning this work, zinc was the appropriate choice.

Scheme 11: Schematic setup of how in-situ conjugation of laser-generated ZnO nanoparticles occurs, followed by their application by means of electrospinning and release.
2. Synthesis of Microgel-ZnO Nanocomposites

The laser ablation process is accomplished by focusing light emitted by a laser on the target. The laser hits the target, which induces the formation of a plasma bubble – a so-called cavitation-bubble. The bubble collapses in the solvent stream, which steadily surrounds the target, releasing nanoparticles into the solvent stream\textsuperscript{126,127}. The particles will be present as aggregates and capped by in-situ polymerization of certain monomers. Here, the scope was the polymerization of VCL, comonomers and a crosslinker (BIS) only. The aim is to cap the nanoparticles by enclosing them into microgel colloids.

The chamber will be heated externally by a heating stage and a stirrer driven by an electric engine enables the diffusion of all reactants. The advantage of this setup is the direct production of nanoparticles in aqueous medium and the diversity of the produced materials. Besides zinc, metals like titanium, iron, magnesium, copper, gold and silver can be ablated and won as nanocrystalline aggregates. The following discussion treats the synthesis and characterization of PVCL-PEG microgels by means of wet-chemical methods. Subsequently, the synthesis approach developed is supposed to be reproduced during the laser-ablation process and within the laser-chamber. The aim is to accomplish in-situ encapsulation of the laser-generated nanoparticles by the microgels formed at the same time.

Scheme 12 shows the performed reaction. VCL reacts together with a PEG-methyl methacrylate macromonomer in the presence of BIS (the crosslinker) and initiated by AMPA. The desired morphology is spherical, as shown on the right side of the scheme. In addition, to avoid turbidity as far as possible, the concentration of VCL was reduced by a factor of 0.25, leading to a 0.025 mol/L VCL starting concentration in the reaction medium – in contrast to 0.1 mol/L, amount used as standard\textsuperscript{67,111,112}. The reason for the decreased concentration as well as the decreased turbidity is
found in the laser-ablation process. As explained above, the PVCL-PEG microgel is supposed to be reproduced in aqueous medium surrounding the metal target for the ablation process. Ablation occurs by transmitting a laser beam through the medium in which the microgels are formed simultaneously. If the turbidity of the emerging microgel dispersion is too high, the laser-beam is scattered, leading to lower ablation-efficiency. Hence, low turbidity is crucial regarding the use of this microgel. The chosen PEG amounts were 1.25 mol-%, 2.5 mol-%, 5 mol-% and 10 mol-% with respect to the VCL concentration. All products were purified by dialyzing with cellulose membranes having a MWCO of 3500 Da. In order to proof that PEG is present in the probe, the PVCL-PEG (5 mol-%) microgel was characterized. Figure 60 shows the comparison of FTIR signals and proves the existence of PEG after polymerization and dialysis. Hence, it is assumed that in case of all microgels PEG must have been successfully incorporated. This has already been shown by Pich et al.\textsuperscript{[128]} in case of PVCL-AAEM-PEG microgels.

![FTIR spectra of PVCL-PEG (5 mol-%) and PVCL. First spectrum shows characteristic PEG signals after synthesis and dialysis of PVCL-PEG (5 mol-%).](image)

Further investigations were done focusing on the colloidal properties of the microgels. The systems were characterized by DLS regarding their thermo-sensitive properties. Thus, hydrodynamic radii and PDI for different temperatures were measured in HPLC-grade water after filtering with a 1.2 µm syringe filter. Figure 61 shows the diameters on the left side. In case of the PVCL-PEG particles containing
2. Synthesis of Microgel-ZnO Nanocomposites

1.25 mol-% and 2.5 mol-%, the spectra show typical microgel-behavior. The curves show the characteristic S-shape, caused by collapsing colloids around VPTT (see above). In case of PVCL-PEG microgels containing 5 and 10 mol-% the trends proceed vice versa in contrast to the first two trends. For more details, the PDIs are shown in Figure 61, too. Here, all four trends are proceeding analogously, showing decreasing slopes.

With regards to typical microgel behavior, one would expect PDIs decreasing during collapsing since collapsed microgels are less diffuse and more hard-sphere like. This expected and intuitive picture is in contradiction with the fact that PVCL-PEG (5 and 10 mol-%) microgels are swelling at higher temperatures. Such a behavior would be expected for polymers having an UCST instead of LCST. Neither PVCL nor PEG does possess an UCST and thus swell at higher temperatures will not be discussed here. The only possible explanation remaining is aggregation.
Figure 62: Zetapotentials (a) and hydrodynamic diameters (b) of PVCL-PEG microgels versus pH.

Further studies were done to determine surface potentials and size-dependence on pH. Therefore, microgels were diluted in HPLC-grade water and characterized regarding zetapotential and hydrodynamic diameter for different pH values. Titration was done under controlled conditions by use of an autotitration system. Figure 62 gives the results, revealing only slight changes of surface potential and thus of the diameters. The zetapotential measurements show slight differences between surface potentials, with an iso-electric point at 6.2. The surface related properties of VCL and PEG groups are expectable, as they do not interact with protons or hydroxyl ions. As a result of low influence on surface potential, different proton concentrations do not influence the hydrodynamic diameters, yielding constant diameters in the whole pH range (pH 3 to pH 10).

Investigations regarding DLS at varied temperatures revealed the mentioned behavior to be reversible. In contrast, TEM measurements, so far, were not able to image microgel colloids and their behavior during size increase. What the images show are films and aggregates in case of PVCL-PEG 5 mol-% (Figure 63 a). Hence, nanogels were additionally characterized by AFM, showing single nanocolloids with heights up to 10 nm (Figure 63 b).

When PVCL-PEG nanogels are prepared in-situ during laser-ablation of ZnO nanoparticle, successful encapsulation occurs (Figure 63 c, d). However, DLS clearly shows hydrodynamic diameters that indicate the existence of colloidal particles. In
another attempt, the same sample was prepared during laser ablation. In this case, microgel colloids were observed assembling on the ZnO nanoparticle surfaces.

Figure 63: TEM images of PVCL-PEG 5 mol-%. Images show film-like hydrogels (a) while AFM proves the existence of single colloidal nanogels (b). Inset of image b exemplarily gives heights of three particles. When PVCL-PEG is added during laser ablation, encapsulation of laser-generated nanoparticles occurs (c, d).
2. Synthesis of Microgel-ZnO Nanocomposites

So, possibly when active surfaces are absent, microgels don’t aggregate and thus assemble in domains as usual. If single microgels do not form domains and show a smooth and soft consistency – due to the lack of co-monomers forming a core – flat and shade-like films during TEM measurements might be a result of coating and drying in high vacuum, typical for electron microscopy.
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2.3. Conclusion

PVCL-PAAEM microgels were first used in order to act as carrier system for ZnO nanoparticles. PVCL-PAAEM microgels have already been shown to work well as hosts for ZnO nanoparticles, as well as other metals. However, the main effort of this chapter was to find and characterize more suitable microgel than PVCL-PAAEM. Hence, after establishing PVCL-PAAEM-ZnO composites as model-system, PVCL-itaconic acid and PVCL-iso-Eugenol were found to be more suitable. After quantitative evaluation of the remaining ZnO content in the composites, PVCL-PIA and PVCL-iso-Eugenol showed optimum results concerning ZnO incorporation. In comparison with PVCL-PAAEM, the ZnO mass was not accumulated during purification by centrifugation. This fact speaks for nanoparticles being mainly precipitated within the microgel porous networks and their successful retention. Zetapotential measurements of both host systems proved this fact by showing positively increasing potentials with increased ZnO content. Moreover, the temperature trends of the microgels were affected by the incorporated nanoparticles. In case of PVCL-PIA, diameters were found to decrease with increased ZnO amount. This behavior correlates with the interactions between carboxylic functions and ZnO surface. Here, spectroscopic methods were insufficient to observe this interaction. Thus, this behavior is assumed, in contrast with the results from chapter 1, where interaction between ZnO and L4 was clearly shown. However, the behavior of ZnO and PVCL-iso-Eugenol was found to be different. Here, diameters of host systems remained unaffected.

The use of PVCL-iso-Eugenol presumed the preparation of a iso-Eugenol-poly(glycidole) macromonomer. Synthesis and characterization of the macromonomers were accomplished for different spacer (poly glycidole) lengths. Investigations of the corresponding PVCL-iso-Eugenol microgels in terms of antibacterial properties are in progress. Here, the ZnO-containing nanocomposites might be useful due to the antibacterial properties of ZnO as shown in chapter 1.

At last, microgels were utilized to encapsulate laser-generated ZnO nanoparticles. Laser ablation offers advantageous working conditions, like clean and fast preparation. In contrast, this method is only suitable of preparing nanoparticles of high polydispersity and large aggregate formation. After first attempts to utilize PVCL-
AAEM as a host system, a new microgel was taken into focus. This microgel was found to meet two important properties: on the one hand PVCL-PEG microgels of low diameters and second, low opacity, both advantageous for in-situ usage. Here, laser-scattering has to be avoided. Moreover, we found great encapsulation properties of the nanogels by their effective attachment on the large nanoparticle-aggregates. Colloidal tests of the PVCL-PEG microgels revealed typical behavior in water-based medium for low PEG amounts. In case of increased PEG amounts, nanogels were found to aggregate reversibly and for temperatures above VPTT. Surface potentials showed neutrality for all PEG amounts between pH 10-3.

In summary, this chapter has introduced two approaches suitable for the nanocomposite synthesis of ZnO nanoparticles within microgels. Scheme 13 shows that ZnO precipitation within the microgels was successfully achieved, as well as the encapsulation of ZnO nanoparticles by in-situ polymerization.

Scheme 13: Two principles of nanocomposite synthesis of microgels and ZnO nanoparticles. First route emanates from microgels in which ZnO nanoparticles are formed by means of precipitation after addition of NaOH. Second route implies the in-situ polymerization of microgels encapsulating the already existing nanoparticles.
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2.4. Experimental Part

Synthesis of PVCL-PAAEM microgels

VCL (2 g, 1 eq), AAEM (0.0125 eq, 0.05 eq, 0.1 eq) and the cross-linker, BIS (0.06 g), were added into a reactor and dissolved in 150 mL H$_2$O. The solution was annealed to a temperature of 70 °C and flushed with nitrogen for 1 h, followed by addition of 5 mL of an aqueous solution (5 g L$^{-1}$) of the Initiator, AMPA. The reaction was left stirring at 250 rpm and 70 °C for 18 h. Shortly after the initiator was added, the solution became turbid without any color change. After the reaction was finished, the products were dialyzed in regenerated cellulose in aqueous media for 4 days.

Synthesis of PVCL-PIA Microgel

VCL (2 g, 1 eq), IADME (0.0125 eq or 0.05 eq) and the cross-linker, BIS (0.06 g) were added into a reactor and dissolved in 150 mL H$_2$O. The solution was heated up to 80 °C and flushed with gaseous nitrogen for 1 h followed by the addition of 5 mL of an aqueous solution (5 g L$^{-1}$) of the Initiator, AMPA. The reaction was left stirring at 250 rpm and 80 °C for 18 h. Shortly after the initiator was added the solution became turbid without any color change occurring. For hydrolysis of IADME, the turbid dispersion was diluted with an additional amount of 150 mL H$_2$O. Subsequently, 0.1 M NaOH (10 mL or 40 mL) was added dropwise at ambient temperature and the reaction was left stirring for 18 h. The dispersion was subsequently concentrated by freeze-drying and then purified by dialysis in regenerated cellulose in water for 4 days. After dialysis, the dispersions were free-dried and stored as a slightly yellow powder.

Synthesis of iso-Eugenol-PEEGE

In a Schlenk-flask bearing stirrer, septum and nitrogen atmosphere Eugenol (1 eq) were dissolved in diglyme. Subsequently, tert-potassium butoxide (0.2 eq dissolved in THF, with a 1 M concentration) is added dropwise under stirring. The solution is stirred at room temperature for 30 minutes. Tert-butanol that formed during the
2. Synthesis of Microgel-ZnO Nanocomposites

reaction is removed at 30 °C under high vacuum. EEGE (4 eq, 6 eq, 12 eq) is added,
followed by the annealing of the mixture up to 80 °C, temperature at which it will be
stirred for 18 hours. After removing the solvent at 40 °C under high vacuum, products
are obtained as high viscous liquids. The exact amounts used for the reactions
described here are listed in Table 17.

Table 17: Synthesis parameters for the preparation of iso-Eugenol-PEEGE.

<table>
<thead>
<tr>
<th>ID</th>
<th>$P_n$</th>
<th>Eugenol [mL] (mmol)</th>
<th>KOTBu [mL] (mmol)</th>
<th>EEGE [mL] (mmol)</th>
<th>Diglyme [mL]</th>
</tr>
</thead>
<tbody>
<tr>
<td>iso-Eugenol-PEEGE$_4$</td>
<td>4</td>
<td>0,28 (1,83)</td>
<td>0,37 (0,37)</td>
<td>1,16 (7,31)</td>
<td>2</td>
</tr>
<tr>
<td>iso-Eugenol-PEEGE$_6$</td>
<td>6</td>
<td>2,81 (18,27)</td>
<td>3,65 (3,65)</td>
<td>17,42 (109,62)</td>
<td>30</td>
</tr>
<tr>
<td>iso-Eugenol-PEEGE$_{12}$</td>
<td>12</td>
<td>0,94 (6,09)</td>
<td>1,22 (1,22)</td>
<td>11,61 (73,08)</td>
<td>20</td>
</tr>
</tbody>
</table>

$^1$H-NMR: (400 MHz, CDCl$_3$)

$^1$H-NMR: (400 MHz, CDCl$_3$)

δ = 6,81-6,77 (m, 3H, CH (7,6)), 6,28 (t, 1H, CH (1')), 6,04 (dq, 0,82H, CH (5'-E)),
5,65 (dq, 0,2H, CH (5'-Z)), 4,67-4,63 (m, n-1H, CH (14)), 4,06-3,40 (m, 4+n·7H, CH$_3$
(2), CH$_2$ (10,12,13), CH (11), OH (15)), 1,82 (dd, 3H, CH$_3$ (3')), 1,26-1,23 (m, n-3H,
CH$_3$ (9)), 1,15-1,10 (m, n-3H, CH$_3$ (8)) ppm.

Here, n has the meaning of polymerization degree.

$^{13}$C-NMR: (100 MHz, CDCl$_3$)

$^{13}$C-NMR: (100 MHz, CDCl$_3$)
2. Synthesis of Microgel-ZnO Nanocomposites

\[ \delta = 149.65 \text{ (C (10))}, 147.52 \text{ (C (9))}, 131.73 \text{ (CH (1'))}, 130.60 \text{ (C (7))}, 123.89 \text{ (CH (8'))}, 118.65 \text{ (CH (6))}, 113.91 \text{ (CH (5))}, 109.23 \text{ (CH (4))}, 99.72 \text{ (CH (17))}, 79.37-77.36 \text{ (CH (14'))}, 70.67-69.17 \text{ (CH (13'))}, 65.97-64.22 \text{ (CH (16))}, 61.04-60.82 \text{ (CH (15))}, 55.81 \text{ (CH (3))}, 19.27 \text{ (CH (12))}, 18.36 \text{ (CH (3'))}, 15.27 \text{ (CH (11))} \text{ ppm.} \]

FTIR:
\[ \tilde{\nu} = 1205-979 \text{ (C-O-Ether), } 1511 \text{ (C=C-Arom.), } 1607 \text{ (C=C-Arom.), } 1639 \text{ (C=C-Arom.), } 2973-2896 \text{ (C-H-Alkan) cm}^{-1}. \]

Synthesis of iso-Eugenol-PEEGE-MAE

Iso-Eugenol-PEEGE (1 eq) is introduced into a flask which also include a stirrer and septum. The compound is dissolved in diethyl ether and flushed with nitrogen for 5 minutes. TEA (1 eq) is added and the solution stirred for another 5 minutes. Then, MACl (1.25 eq) dissolved in diethyl ether will be added dropwise over a period of ca. 30 minutes under vigorous stirring. The solution is stirred for 48 hours at room temperature. After reaction, all precipitates will be removed by filtration. Further purification will be done via extraction from a 5-% NaOH solution twice, followed by two extractions from distilled water. The aqueous phases will be removed with diethyl ether. All organic phases will be collected and dried with Na\(_2\)SO\(_4\). Then, the solvent is removed by distillation, followed by drying under high vacuum at 30 °C. The products are obtained as high viscous liquids. The exact amounts used for the reactions here are listed in Table 18.
2. Synthesis of Microgel-ZnO Nanocomposites

Table 18: Synthesis parameters for the iso-Eugenol-PEEGE-MEA preparation.

<table>
<thead>
<tr>
<th>ID</th>
<th>$P_n$</th>
<th>Isoeug-PEEGE [g] (mmol)</th>
<th>TEA [mL] (mmol)</th>
<th>MACl [mL] (mmol)</th>
<th>Et$_2$O [mL]</th>
</tr>
</thead>
<tbody>
<tr>
<td>iso-Eugenol-PEEGE$_4$-MAE</td>
<td>4</td>
<td>1,376 (1,81)</td>
<td>0,25 (1,81)</td>
<td>0,22 (2,26)</td>
<td>1,81</td>
</tr>
<tr>
<td>iso-Eugenol-PEEGE$_6$-MAE</td>
<td>6</td>
<td>5,698 (6,13)</td>
<td>0,85 (6,13)</td>
<td>0,75 (7,66)</td>
<td>6,13</td>
</tr>
<tr>
<td>iso-Eugenol-PEEGE$_{12}$-MAE</td>
<td>12</td>
<td>5,129 (2,66)</td>
<td>0,37 (2,66)</td>
<td>0,33 (3,32)</td>
<td>2,66</td>
</tr>
</tbody>
</table>

$^1$H-NMR: (400 MHz, CDCl$_3$)

\[
\delta = 6.85-6.76 \text{ (m, 3H, CH (7,6))}, 6.29 \text{ (t, 1H, CH (1'))}, 6.07 \text{ (m, 1,86H, CH (5'-E, 18))}, 5.66 \text{ (dq, 0,16H, CH (5'-Z))}, 5.51 \text{ (s, 1H, CH (17))}, 4.70-4.64 \text{ (m, n·1H, CH (14))}, 4.06-3.33 \text{ (m, 3+n·7H, CH$_3$ (2), CH$_2$ (10,12,13), CH (11))}, 1.89-1.80 \text{ (s + dd, 6H, CH$_3$ (3', 16))}, 1.26-1.22 \text{ (m, n·3H, CH$_3$ (9))}, 1.13-1.11 \text{ (m, n·3H, CH$_3$ (8)) ppm.}
\]

$^{13}$C-NMR: (100 MHz, CDCl$_3$)

\[
\delta = 166.69 \text{ (C=O (21))}, 149.66 \text{ (C (10))}, 147.52 \text{ (C (9))}, 136.34 \text{ (C (20))}, 131.72 \text{ (CH (1'))}, 131.60 \text{ (C (7))}, 125.60 \text{ (CH$_2$ (19))}, 123.88 \text{ (CH (8'))}, 118.65 \text{ (CH (6))}, 113.92 \text{ (CH (5))}, 109.22 \text{ (CH (4))}, 99.76 \text{ (CH (17))}, 78.86-78.07 \text{ (CH (14))}, 72.57-69.00 \text{ (CH$_2$ (13))}, 65.09-63.19 \text{ (CH$_2$ (16))}, 60.99-60.81 \text{ (CH$_2$ (15))}, 55.81 \text{ (CH$_3$ (2))}, 19.71 \text{ (CH$_3$ (12, 18))}, 18.34 \text{ (CH$_3$ (3'))}, 15.02 \text{ (CH$_3$ (11)) ppm.}
\]
2. Synthesis of Microgel-ZnO Nanocomposites

FTIR:
\[ \tilde{\nu} = 1205-979 \text{ (C-O-Ether), } 1511 \text{ (C=C-Arom.), } 1607 \text{ (C=C-Arom.), } 1639 \text{ (C=C-Arom.), } 1718 \text{ (C-O-Ester), } 2973-2896 \text{ (C-H-Alkan) cm}^{-1}. \]

Synthesis of iso-Eugenol-PG-MAE

In a flask including a stirrer, iso-Eugenol-PEEGE_{12}-MAE was introduced and dissolved in THF. To the solution, a 37-% HCl solution was slowly added and the solution stirred for 3 hours at room temperature. Precipitation occurred, which was isolated from the solvent by decantation and its subsequent washing with THF for at least three times. The products were dried at 30 °C in vacuum. In the cases of iso-Eugenol-PEEGE_{4}-MAE and iso-Eugenol-PEEGE_{6}-MAE, the products did not precipitate under acidic conditions and thus the solutions were neutralized by adding Na\textsubscript{2}CO\textsubscript{3}. After isolation by filtration, the solution was concentrated by rotation distillation. Afterwards, the products were washed with n-pentane and diethyl ether for several times. After drying at 30 °C in vacuum, all products were acquired as high viscous liquids. The exact amounts used for the reactions here are listed in Table 19.

Table 19: Synthesis parameters for the iso-Eugenol-PG-MEA preparation.

<table>
<thead>
<tr>
<th>Bezeichnung</th>
<th>( P_n )</th>
<th>Isoeug-PEEGE-MAE [g] (mmol)</th>
<th>HCl (37 %) [mL] (mmol)</th>
<th>THF [mL]</th>
</tr>
</thead>
<tbody>
<tr>
<td>iso-Eugenol -PG\textsubscript{4}-MAE</td>
<td>4</td>
<td>0,787 (0,91)</td>
<td>0,93 (11,24)</td>
<td>24</td>
</tr>
<tr>
<td>iso-Eugenol -PG\textsubscript{6}-MAE</td>
<td>6</td>
<td>3,581 (3,03)</td>
<td>3,10 (37,45)</td>
<td>107</td>
</tr>
<tr>
<td>iso-Eugenol -PG\textsubscript{12}-MAE</td>
<td>12</td>
<td>1,365 (0,67)</td>
<td>0,69 (8,31)</td>
<td>41</td>
</tr>
</tbody>
</table>
2. Synthesis of Microgel-ZnO Nanocomposites

\(^1\)H-NMR: (400 MHz, DMSO-d6)

\[ \delta = 6.98-6.81 \text{ (m, 3H, CH (7,6))}, 6.33 \text{ (t, 1H, CH (1'))}, 6.19-6.06 \text{ (m, 1,86H, CH (5'-E, 18))}, 5.66 \text{ (m, 1,16H, CH (5'-Z, 17))}, 4.05-3.42 \text{ (m, 3+n·5H, CH}_2(2), \text{CH}_2(10,12), \text{CH}_2(11)), 1.89-1.73 \text{ (m, 6H, CH}_3(3',16)) \text{ ppm}. \]

\(^{13}\)C-NMR: (100 MHz, DMSO-d6)

\[ \delta = 166.58 \text{ (C=O (21))}, 149.09 \text{ (C (10))}, 147.27 \text{ (C (9))}, 136.16 \text{ (C (20))}, 130.97 \text{ (CH (1'))}, 130.85 \text{ (C (7))}, 125.79 \text{ (CH}_2(19)), 124.92 \text{ (CH (8'))}, 118.69 \text{ (CH (6))}, 113.82 \text{ (CH (5))}, 109.44 \text{ (CH (4))}, 80.18-79.39 \text{ (CH (14))}, 74.42-66.12 \text{ (CH}_2(13)), 61.18-60.66 \text{ (CH}_2(16)), 55.69 \text{ (CH}_3(2)), 18.26 \text{ (CH}_3(18)), 18.12 \text{ (CH}_3(3')) \text{ ppm}. \]

Loading procedure with ZnO nanoparticles

Dry microgels (50 mg) and a certain amount of zinc chloride are added into a glass vessel and suspended in THF (25 mL). The mixture was treated with ultrasonic for a few minutes and stirred at room temperature for 1 hour. Meanwhile, 2.2 equivalents of NaOH with respect to the ZnCl\(_2\) mass are dissolved in 2.5 mL MeOH and added into the reaction mixture. Subsequently, the mixture will be refluxed for 3 hours. Afterwards, the solvent will be removed by evaporation and the product washed by suspending it in water and ultra-centrifugation at 12000 rpm. Altogether, washing and centrifugation will be done for three times and the obtained probes freeze-dried after.
2. Synthesis of Microgel-ZnO Nanocomposites

Synthesis of PVCL-PEG microgels

VCL (0.5 g, 1 eq), PEG-MA (Mn 526 g/mol, 0.0125 eq, 0.025 eq, 0.05 eq and 0.1 eq) and cross-linker, BIS (0.015 g) were dissolved in 150 mL water within a double-walled glass reactor. The solution was annealed to a temperature of 80 °C and flushed with nitrogen for 1 h. Afterwards, the initiator, AMPA (0.0125 g), previously dissolved in water, was added. The reaction was left stirring at 300 rpm and 80 °C for 8 hours. The turbidity slightly increases during annealing at 80 °C but disappears at room temperature leaving a colorless and transparent solution after reaction and cooling. The product was dialyzed for 4 days using regenerated cellulose membranes with MWCO of 3500 g/mol.

Analytical Methods

Transmission electron microscopy (TEM) measurements were performed with a Zeiss LIBRA 120. To prepare the specimen, the samples were dispersed in toluene or THF. Subsequently, one drop of the dispersion was added on a Formvar/carbon grid which was placed before on a filter paper. An organic solvent was used in order to optimize the coating on the carbon grids by drop casting. The samples were left at room temperature for few minutes until they were completely dried and measured with an acceleration voltage of 80 kV in high vacuum (10^{-6} to 10^{-7} mbar).

Solid state nuclear magnetic resonance (NMR) measurements were performed with a AV700 Bruker NMR spectrometer operating at \(^1\)H frequency of 700.234 MHz and \(^{13}\)C frequency of 176.079 MHz. The number of scans was 2048 at a dwell time of 4 microseconds and a recycle delay of d1 = 10 seconds. The \(^{13}\)C rf pulse duration was 4 microseconds with a power of 87.2 W. Proton high-power decoupling was performed with a spinal 64 pulse sequence. The method used to measure \(^{13}\)C high-resolution spectra was based on direct polarization (DP) with proton high-power decoupling. The sample was rotating at 5 kHz under magic angle spinning (MAS). Chemical shift calibration was made using adamantane a plastic solid. Finally, the calibration is on \(^{13}\)C resonance of TMS.

Fourier Transformation Infrared (FTIR) Spectroscopy measurements were recorded with a Thermo Nicolet Nexus 470 instrument. Low amounts of the dried samples
were mixed with KBr to form a pellet which acts as specimen.
The zetapotential of ZnO nanoparticles were measured with Zetasizer NanoZS (Malvern) after dispersing the solid samples in HPLC-grade water. Fluorescence spectra were recorded with a Perking Elmer LS-50 photoluminescence spectrometer.

The concentrations of zinc (Zn) in the sample solution were analyzed using inductively coupled plasma mass spectrometry (ICP-MS). The analyses were carried out with a quadrupole ICP-MS system (Perkin Elmer - Elan 6000) operating at 1000 W plasma power, 14 L/min plasma gas flow and 0.95 L/min nebuliser gas flow and an auto sampler system (Perkin Elmer AS-90) connected with a peristaltic pump with a sample flow of 1 ml/min. To avoid contamination and memory effects the wash time between measurements was set at 10 seconds (with 1% HNO₃, suprapure). Before analyses, the samples were diluted 1:10 using a solution of 1 % HNO₃ with a concentration of 10 ng/L of yttrium (Y) as internal standard. In order to control the accuracy and stability during measurements, a standard solution of Zn with concentration of 10 µgL⁻¹ (ICP Multielementstandard IV solution, Merck, Darmstadt, Germany) was analyzed after every 10 samples. The calibration was carried out with a series of 11 dilutions of a zinc standard solution (ICP Multielementstandard solution, Merck, Darmstadt, Germany). Element concentrations were calculated as mgL⁻¹ using corresponding regression lines (correlation factor ≥ 0.999).

Photospectrometric measurements were performed by adding 25 µL of each sample into a UV-vis cuvette. Additionally, dye-containing solution (25 µL) and buffer (950 µL) were added to the cuvette in the way, that adding the buffer as last step sufficiently homogenized the solution. The samples were kept at 25 °C for ca. 5 min and then put into the UV-vis spectrosoc to evaluate the absorbence at λ = 628 nm. Afterwards, the concentration was calculated by taking a calibration curve into account, that had been determined before.

The dye-containing solution was prepared by adding Zincon monosodium-salt (43.5 mg) to a flask, adding 1 M NaOH solution (1 mL) and filling-up by adding millipore water, giving a final volume of 58 mL. Buffer was prepared by dissolving boric acid (350 mg) and Urea (50.56 g) in water (100 mL). Subsequently, the pH was adjusted at 9 by adding a 5 M NaOH solution carefully.
3. Electrospun Fibers for Biomedical Applications

3.1. Introduction

3.1.1. Antioxidant and Wound Healing Supporting Properties of Zinc

To understand the role of zinc as wound-healing supporting agent, it is first important to understand in which way it can be utilized and how it can be useful in medical applications. Zinc is a very important trace element for the human organisms and part of about 300 enzymes\[^4\]. Its properties and influence on the human body has been investigated for many years. Zinc deficiencies may cause several diseases, e.g. diarrhea, retardation of children growth, increased risk of infections, etc. as has been reviewed by others\[^4,129–132\]. A clinical reason for malabsorption of zinc is Acrodermatitis enteropathica, which is an inborn error of the metabolic system\[^133\]. Of course, zinc is not the only trace element existentially important for our lives. There are elements like copper, iron, molybdenum, nickel and cobalt, which also play an important role of our survival\[^134\]. Zinc supplementation in the human body was found to be dependend on gender, age and condition (e.g. pregnancy) and can be influenced by several factors, e.g. iron supplementation or presence of scavengers, like phytic acid\[^132,135,136\]. Metallic trace elements are embedded in proteins, so-called metalloproteins, which coordinate and bind metals or inorganic metal clusters\[^134\]. Coordination of metalls is a very complex matter and has widely been studied\[^137–139\].

One of the main characteristics of trace elements like zinc and copper is its antioxidant property\[^140\]. After thermal injuries, the systemic inflammatory response syndrome (SIRS) and oxidative stress emerge. SIRS may lead to acute lung injury, shock, renal failure and multiple organ dysfunction syndrome (MODS)\[^141\]. If SIRS is caused by a confirmed infectious process (instead of thermal injury or other non-infectious processes), it is called “sepsis”\[^141\]. Oxidative stress describes the formation of ROS, caused by several sources that can be categorized into three parts: Endogenous sources, antioxidant deficiencies and exogenous sources (see Figure 64)\[^142\]. ROS are constantly produced, mainly in mitochondria, and are considered to influence the ageing process\[^15\]. Ku et al. investigated the ageing phenomenon in case of mammalian cells of several animals and found the maximum life span of oxygenyl radical species where different creatures produce ROS with
different rates\textsuperscript{[143]}.

Figure 64: ROS production and its main sources. ROS levels being too low or too high cause non-homeostatic conditions. Reprinted with permission from Reference [142]. Copyright (2014) by Nature.

As seen in Figure 64, antioxidant deficiencies and – if thermal injuries are considered to be part of the exogenous sources – burns will lead to increased ROS production. High level of ROS are going to cause cellular damage and in consequence cause ageing, disease and cell death. In case of plants, reactive oxygen intermediates (ROI which means radical species which are chemically the precursors of ROS, like H$_2$O$_2$) have been found to act as signalling molecules by signal transduction\textsuperscript{[144]}. Anyway, the use of antioxidant compounds is of great importance in order to avoid cell damage during post-burn periods. Thus, zinc, amongst others, has been widely investigated in this regard. Though zinc does not act as antioxidant, reacting with ROS by direct contact, its benefit lies in its protein expressing nature, inducing the
production of other substances that serve as antioxidants\textsuperscript{140}. Shenkin and Berger investigated in which way nutrition amounts in vivo are affected by thermal injuries\textsuperscript{145}. They found in case of 33-% of burn injury patients that their zinc level drops significantly and recommended a daily 40 mg zinc dosage for adults, applied intravenously. Moreover, copper and selenium concentrations are also affected\textsuperscript{145}. Under normal conditions, German society of nutrition (Deutsche Gesellschaft für Ernährung e.V., DGE) recommends a daily zinc suplementation of 7 mg for women and 10 mg for men\textsuperscript{146}. Main sources for zinc nutrition are beef, pork, poultry, eggs, milk, cheese, wholemeal products and legumes\textsuperscript{146}.

An interesting group of metalloproteins that have been discussed as possible antioxidants, is the group of methallotioneins (MT). Metallothioneins are low molecular (6000-7000 Da) metal-binding proteins, containing 60-68 amino acid residues, of which 25-30 % are cysteine\textsuperscript{140}. Cysteine bears thiol groups which are supposed to act effectively as radical scavengers. The antioxidant effectiveness in case of DNA degradation by hydroxyl radicals was shown and furthermore compared to another antioxidant, glutathione, showing much higher efficiency\textsuperscript{147}. The direct link between thermal injury and enhanced MT expression was found by Ding et al.\textsuperscript{148}. They showed that MT concentration in the liver increased after sever thermal injuries within a 48 hour post-burn period, reaching a maximum after 24 hours. In addition, they observed a decrease of serum zinc concentration accompanied by an increase of liver zinc concentration within the same period. In case of zinc, a concentration maximum in the liver was reached after 12 hours, while in Serum the minimum was found to appear after 12 hours. Though the authors mentioned effects like activated cytokines, these results demonstrate in which way severe thermal injury influences zinc body-level. The results become more evident when works of Andrews - in which how oxidative stress and zinc induction lead to increased MT gene expression was analyzed - are taken into account\textsuperscript{149,150}. Ding et al also observed an increased lipid peroxidation with the liver due to oxidative stress\textsuperscript{148}. Thus the authors speak of a “compensative mechanism of natural self-defense under the condition of stress”\textsuperscript{148}.

These findings were supported by investigations focusing on human keratinocytes, where increased MT expression under UVB irradiation and cell proliferation was investigated for varied zinc concentrations\textsuperscript{151}. Parat et al. and Richard et al. found a
maximum zinc concentration of 100 µmol/L to be vital for human keratinocytes, and 150 µmol/L in presence of human fibroblasts\textsuperscript{[151,152]}. Morellini et al. observed increased wound healing after topical application of a zinc-MT complex - including seven zinc atoms - compared to zinc sulfate only and controls\textsuperscript{[153]}. Burns commonly are induced to living organisms by exogeneous sources and through contact between heat source and the skin. After induction of thermal energy followed by SIRS and oxidative stress, lipid peroxide levels increase in several body parts, but mainly in the skin\textsuperscript{[154]}. From there, peroxides are released into the blood stream as proposed by Nishigaki et al.\textsuperscript{[154]}. Thus the highest concentration of cell damaging lipid peroxides are located in the skin during the first 24 hours post-burn period (see Figure 65)\textsuperscript{[154]}.

![Figure 65: Lipid peroxide levels in the skin, serum and several organs of rats after thermal injury. Arrow shows control. Reprinted with permission from Reference [154]. Copyright (2014) by Elsevier.](image)

Lansdown reported twice the importance of zinc-MT complexes, regarding the wound healing properties even exceeding the antioxidant properties. He mentioned the importance during epihitelization in scar tissue due to increased collagen expression,
as well as the use by topical application. Moreover, Lansdown mentioned the need of other elements, especially copper, though only low doses\textsuperscript{[155,156]}. Works of Lansdown, Agren and others had already demonstrated the use of topically applied ZnO during the re-epithelization process\textsuperscript{[157–161]}. ZnO is non-toxic and a zinc-donor, influencing epithelialization by means of zinc induction. It was found that the type of zinc source does play role with regard to its medical purposes. Agren demonstrated that ZnO shows increased properties compared to zinc sulfate when applied on porcine wounds (see Figure 66)\textsuperscript{[162]}. Deters et al. showed that zinc histidine accelerated normal human keratinocyte proliferation more efficiently than zinc sulfate\textsuperscript{[163]}. Due to the wound healing supporting properties, ZnO has already been implemented in a variety of different products (find an overview in Reference [162]).

![Figure 66: Epithelisation efficiency of ZnO and zinc sulfate in case of porcine wounds. Reprinted with permission from Reference [162]. Copyright (2014) Wiley. Authors of Reference [162] extracted data from Reference [158]. (Additional) Permission from Reference [158]. Copyright (2014) Elsevier.](image)

Additionally, the well-known and extensively investigated antibacterial properties of zinc and ZnO increase the medical scope of this material as it offers antibacterial properties, generally valuable in the field of wound toppings\textsuperscript{[44,45,164,165]}. It is of great importance to keep the zinc ion concentrations below a toxic level when release takes place into the human organisms. ZnO is non-soluble in water at neutral pH but can be easily degraded in acids and bases due to its amphoteric nature.
3. Electrospun Fibers for Biomedical Applications

Besides, ZnO nanoparticles have been found to be dispersible when their scale reaches very low diameters, ca. 4 nm\(^{[48]}\). This phenomenon is caused by electric charges located on the particle’s surface leading to electrostatic particle-particle repulsion and a relative hydrophilic character. This behavior of nanoparticles and the well-known photocatalytic properties of ZnO nanoparticles are fundamental for several works, treating the toxicity of such kind of nanomaterial\(^{[38,46]}\). Therefore, it is a challenge to hinder nanoparticles entering the human body when applied as drug or antibacterial agent.
3. Electrospun Fibers for Biomedical Applications

3.1.2. Electrospun Fibers and Their Applications

Electrospinning techniques in combination with ZnO or other metal oxides have been investigated regarding sensors application\textsuperscript{[166–169]}, photocatalytic properties\textsuperscript{[170–173]}, antibacterial properties\textsuperscript{[174–176]}, UV-protection\textsuperscript{[177]} and humidity sensing\textsuperscript{[178]}. The theoretical background and principles have been well summarized by Greiner and Wendorff\textsuperscript{[179]}. Briefly stated, electrospinning is a process aiming on extremely thin fibers. The diameters of the fibers range commonly between several nanometers up to the micrometer scale. To achieve such thin fibers, polymer solutions or melts of high viscosity are electrically charged within an electric field. After applying strong voltage at low conductions, the droplet formed at the tip of the syringe-nozzle will overcome its surface tension. This will result in a thin polymer stream ejected out of the droplet. The ejected polymer stream is collected by the counter electrode and received as a nonwoven of ultrathin fibers (see Figure 67).

![Figure 67](image)

Figure 67: Scheme representing the principal method of the electrospinning apparatus and the way in which a polymer containing solution or melt will be applied with strong electric fields. Reprinted with permission from Reference [179]. Copyright (2014) Wiley.
Some of the most important parameters of this procedure are the viscosity and flow-rate of the solution. Viscosity may tune the thickness of fibers, which is the greatest reason for performing this method. Interestingly, the outcome of varied viscosities seems to be independent on the polymer-type. Lim et al demonstrated that in case of PCL thin fibers with higher crystallinity, stiffness and strength result from dilute solutions\textsuperscript{[180]}. Similar results regarding fiber-diameter were demonstrated by Chowdhury and Stylios in case of Nylon-6\textsuperscript{[181]}. In case of polystyrene solutions, the flow-rate adjusted to control the polymer release out of the syringe was found to be the most important factor influencing the diameters of the fibers\textsuperscript{[182]}. Other important parameters in case of PCL are molecular weight, surface tension and other solution properties, conductivity, voltage, nature of collector, nozzle-to-target distance, temperature and humidity\textsuperscript{[183,184]}. 

To the widest investigated polymers concerning properties and applications within the framework of electrospinning techniques is PCL due to its biocompatibility\textsuperscript{[185,186]}. Electrospun fibers and meshes are usually intended for bioengineering and are commonly considered to get into contact with biological environment, i.e. the human organism. Two of the main fields of these applications envelop wound healing, implicating their use as wound dressing and tissue engineering. The latter describes the application of a scaffold as a three-dimensional template for cell adhesion, proliferation and formation of an extracellular matrix where PCL “is often blended together with natural products, e.g. chitosan, in order to improve its biocompatibility”\textsuperscript{[187]}.

Since PCL and PCL-blends are considered to be applied in-vivo\textsuperscript{[188–190]}, biocompatibility and furthermore biodegradability are mandatory for such kind of applications. Pitt et al. investigated the biodegradability of PCL and demonstrated that it takes ca. 100 weeks within a living organism until degradation of PCL reaches its plateau (Figure 68a). The tests were done for PCL and two PCL blends. Additionally, the excretion of the carboxylic containing degradation products were studied focusing on daily and cumulative rates (Figure 68b), nicely demonstrating how biodegradation is supposed to work\textsuperscript{[188]}. Biodegradation of PCL must not only occur by enzymatic reactions\textsuperscript{[191]}, but also by bacterial degradation\textsuperscript{[192]}.
3. Electrospun Fibers for Biomedical Applications

Figure 68: In-vivo degradation of subdermal PCL implants. Molecular weights were measured, after samples were surgically removed and analyzed by GPC (a). Daily and cumulative excretion of PCL and its metabolites as measured by radioactivity in the urine, feces, and expired water of rats (b). Reprinted with permission from Reference [188]. Copyright (2014) Wiley.
3.2. Results and Discussion

The purpose of this chapter is to describe the preparation of a novel fiber-based material modified by the nanocomposites synthesized before. The method takes advantage of the fact that microgels offer multiple properties for different purposes. As intensively discussed above, one purpose of using colloidal hydrogels is their suitability for drug-carrying and drug-releasing applications. Another reason is surface-coating or more specifically, the coating of electrospun microfibers. The general concept is given in Scheme 14. Wet-chemical synthesized nanocomposites as well as nanocomposites prepared by laser-ablation are further processed by means of electrospinning. The colloidal stability of microgels, even in organic solvents, is utilized by preparing a mixture including the nanocomposites and the fiber-polymer, PCL. By applying the dispersion into an electric field, electrospun microfibers will be formed that are surface-coated by the composites. Further investigations proved the capability of the fibers regarding zinc ion release and biocompatibility.

Scheme 14: Preparation-concept of PCL-microfibers suitable for zinc ion release by processing the nanocomposites previously synthesized by means of electrospinning.
3. Electrospun Fibers for Biomedical Applications

3.2.1. Fiber Preparation

In order to compare the improvement of the polymerization method including the in-situ capping as shown in the chapter above, Reference composites were prepared by electrospinning a mixture of PCL (Mw 45000 g/mol, Sigma Aldrich) and ZOSP. The target was prepared by fixing TEM grids on metal plates using Parafilm for fixation, so that at least one side of the grid remained uncapped by the film.

Figure 69 shows the result when a simple mixture of ZOSP and commercially available PCI will be electropsun. ZnO nanoparticles tend to form domains of non-aggregated nanoparticles. The domain formation is comparable to the one observed when ZOSP will be drop-casted on TEM grids and remains as a surface dependent property of the highly active particles. The reason why the nanoparticles did not agglomerate can be explained by the presence of the surfactant, which was not harmed during electrospinning, still protecting the nanoparticle against agglomeration.

![Image of electrospun PCL with incorporated ZnO nanospheres, modified with L4.](image)

Several attempts were done utilizing PVCL-PAAEM microgels by electrospinning from THF-DMF solutions. Compared to the standard synthesis approach of PVCL-PAAEM microgels, a six-fold higher amount of crosslinker was chosen in order to receive microgels with a morphology resembling that of hard-sphere. Acceleration
3. Electrospun Fibers for Biomedical Applications

voltage, distance and concentration were varied. All samples were characterized qualitatively by TEM in order to get information about the microgel assembly on the fiber surfaces.

Figure 70: PVCL-AAEM microgels in absence of ZnO nanoparticles. Sketch shows expected behavior of fiber grafting with increasing microgel concentration (a). TEM images qualitatively proof the expected behavior for starting microgels amounts of (b), (c), (d).

The aim was to determine in which way electrospinning parameters control the microgel assembly. We found that only the microgel to PCL ratio had an impact on the surface modification of fibers. As seen in Figure 70b, increasing the microgel amount by a factor of 10 leads to the formation of knob-like modified fibers (Figure 70c) and furthermore to core-shell modified fibers (Figure 70d).

The results so far showed that variation of different parameters did not significantly affect the microgel assembly, except for increasing microgel amount. Hence, one probe was taken for comparison with the method of D. Kehren\textsuperscript{193}. FESEM images after electrospinning were taken and put into contrast. By comparing two examples of equivalent electrospinning parameters (concentration, voltage, etc.), the attempt of D. Kehren was found to be more suitable for high amount surface modification. Thus, all attempts regarding electrospinning from THF-DMF were disregarded in favor of the approach developed by Kehren et al.
Kehren’s approach focusses on electrospinning PCL from toluene-methanol solutions. He found that best results are gained for a PCL and PVCL-AAEM mixture with a 7:3 ratio and a PCL concentration of 12 wt-%. He showed that under these conditions surface-assembly of microgels ontop of fibers is preferred. All of the following investigations were done by use of fibers prepared exactly following Kehren’s approach. Electrospinning was applied by use of the PVCL-itaconic acid microgels loaded with ZnO, as well as the PVCL-PEG microgels loaded with laser-generated nanoparticles. Figure 72 shows fibers of PCL and PVCL-PIA loaded with 5.9 wt-% ZnO, giving a resulting ZnO amount of 1.36 wt-% within the fibrous membrane. As seen by FESEM characterization, fibers show low homogeneity when microgel-ZnO composites are incorporated. Hence, images proof the successful fiber-surface modification and thus that Kehren’s approach works well. TEM images also prove the successful integration of ZnO nanoparticles into fibers. In contrast with L4-modified ZnO nanoparticles (see Figure 69), here we receive well distributed nanoparticles on the fiber due to controlled assembly of microgel.
Figure 72: FESEM (a, b) and TEM (c, d) images of PVCL-itaconic acid microgels containing ZnO nanoparticles. TEM proves the successful assembly of ZnO nanoparticles incorporated within the porous gels and fixed towards the fibers-surface.

The PVCL-PEG nanocomposites were received from EDU (Essen) without further purification. Here, we focussed on a very simple course of action. Hence, raw products were used and only the fibers were purified before release studies were done. Figure 73 shows analogues results in case of laser-generated composites.
3. Electrospun Fibers for Biomedical Applications

Figure 73: SEM after gold-sputtering (a, b) and TEM (c, d) images of PVCL-PEG microgels containing laser-generated Zn nanoparticles.

Fiber preparation was accomplished for several PVCL-PIA based nanocomposites including ZnO, listed in Table 20. PVCL-PIA incorporated composites were first characterized concerning their microgel retaining properties and the swelling behavior. All results were gained as triplicates. First investigations were attempted by adding non-purified fibers into water, assuring that fibers remained below water level. After 5-6 hours the fibers were wet-balanced, dried and dry-balanced. Comparison of weight before and after treatment with water is listed in the “Dried” column for all fibers in Table 21.
3. Electrospun Fibers for Biomedical Applications

Table 20: Electrospun fibers prepared from PCL and microgel-ZnO composites.

<table>
<thead>
<tr>
<th>ID</th>
<th>Nanocomposite Content [wt-%]</th>
<th>ZnO content in MG [wt-%]</th>
<th>ZnO content in Fiber [wt-%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fiber-1</td>
<td>30</td>
<td>1.4</td>
<td>0.11</td>
</tr>
<tr>
<td>Fiber-2</td>
<td>12.5</td>
<td>5.5</td>
<td>0.40</td>
</tr>
<tr>
<td>Fiber-3</td>
<td>25</td>
<td>5.5</td>
<td>0.90</td>
</tr>
<tr>
<td>Fiber-4</td>
<td>30</td>
<td>5.5</td>
<td>1.36</td>
</tr>
<tr>
<td>Fiber-5</td>
<td>30</td>
<td>16.9</td>
<td>3.38</td>
</tr>
</tbody>
</table>

Experiments were done at room temperature and 37 °C. The results clearly reveal that with increasing ZnO amount, microgels are retained much more efficiently. Meanwhile, microgels without ZnO incorporated, were almost completely removed since the relative microgel amount was adjusted to 30 wt-%. The tests showed that in all cases, except for 16.9 wt-% filled microgels, temperature doesn’t play a role.

Table 21: Water uptake and mass loss tests after water-contact for 6 hours.

<table>
<thead>
<tr>
<th>ZnO [wt-%]</th>
<th>RT</th>
<th>37 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wet [%]</td>
<td>Dried [%]</td>
</tr>
<tr>
<td>0</td>
<td>+179±3.03</td>
<td>-27±0.04</td>
</tr>
<tr>
<td>1.4</td>
<td>+161±1.20</td>
<td>-13±0.17</td>
</tr>
<tr>
<td>5.5</td>
<td>+212±7.83</td>
<td>-8±0.01</td>
</tr>
<tr>
<td>16.9</td>
<td>+223±3.47</td>
<td>-13±0.07</td>
</tr>
</tbody>
</table>

The swelling behavior of microgels was determined by comparing the wet samples from Table 21. “Wet” in this regards means: after water uptake for six hours and wiping the fiber-pads carefully with a filter-paper, in order to remove facile water. The results from this comparison test are shown in Figure 74. Here, no significant
influence of temperature for each sample was determined. Thus, water diffusion may not be controlled by the VPTT of the PVCL-PIA-ZnO nanocomposites.

![Swelling degrees of fibers with different ZnO contents and PCL at two different temperatures.](image)

Figure 74: Swelling degrees of fibers with different ZnO contents and PCL at two different temperatures.

All of these results maintain that microgels are suitable surfactants and hosts for ZnO nanoparticles leading to good nanoparticle-distribution on PCL microfibers. Moreover, Kehren’s approach is a great method for microgel-PCL fiber preparation. At last, swelling behavior indicates good release properties of the composites due to microgel porosity. The next step is to undertake cumulative zinc ion release tests. But before, it is necessary to assure that no microgel release occurs at the same time and for that, a simple test was executed.
3. Electrospun Fibers for Biomedical Applications

Figure 75: Microgels removed after six hours resting in water at RT (a). Fiber-1 with ZnO amount of 0.11 wt-% was tested regarding the efficiency of fiber-purification (shaking for 20 seconds) focusing on microgel removal. No further microgel-removal occurs after an additional period of six hours in water (b).

In order to evaluate the efficiency of microgel entanglement, fibers-1, -4, -5 and an additional fiber with “empty” microgels were analyzed. The fibers were put into water and left for 6 hours at 37 °C (Figure 75a). Afterwards, fibers were dried and balanced. Mass losses were found to be dependent on the ZnO amount (see Figure 75a).

In order to assure that no microgel leaching occurred during the release tests, fiber-1 with 0.11 wt-% ZnO (ZnO amount within microgels is 1.4 wt-%) was tested again - as triplicate – focusing on the washing procedure applied before each release test. This time fibers were washed and vigorously shaken for 20 seconds and subsequently dried and balanced. Subsequently, fibers were put into water, left for 6 hours, dried and balanced again. Here we found an equal mass loss after shaking for 20 second compared to Figure 75a (shown in Figure 75b). After resting in water for 6 hours, no further mass loss was observed (see Figure 75b).
Figure 76: FESEM images showing fiber-1 (table 20, 0.11 wt-% ZnO-content) after zinc ion release test. The images prove that microgels are not ejected from the fibers during the release studies.

FESEM images recorded after release tests in case of fiber-1 (see Table 20) revealed that the colloidal microgels remained on the surface (see Figure 76). Thus, we assume that all insufficiently attached microgels were removed by washing and so microgels had not been leached during the release studies.
3. Electrospun Fibers for Biomedical Applications

3.2.2. Zinc Ion Release Studies and Biocompatibility

Zinc Ion Release from PVCL-Itaconic Acid Microgels

Mandatory for any medical application in terms of ZnO is the release of zinc cations out of their hosting oxide crystal. Therefore, it is of tremendous importance to evaluate zinc ion release rates of the fibers in order to determine the applicability of such fiber-composites. As shown before, the fibers consist of PCL (as carrier material and fiber-template) PVCL-itaconic acid (as drug-hosts and surfactants) and ZnO nanoparticles (as drug (zinc) delivery systems). Moreover, it was shown that all fibrous composites are able to take up water and swell. This behavior was shown to be independent of ZnO content and temperature around the VPTT. In addition, it was shown that all zinc release from the fibers after washing with aqueous medium for 20 seconds before and release test, finds place from microgels firmly fixed on the fiber surfaces.

![Graph showing cumulative zinc ion release rates for two different temperatures (25 °C and 37 °C). All aliquots were characterized by photospectrometric methods.]

Since the release of chemical compounds by dissolving from a hosting crystal structure and diffusing through a porous membrane is supposed to be temperature dependent, first investigations attempted to reveal such a temperature dependence. The release was accomplished by leaving fiber-mats in PIPES buffered (2 mM) medium at 25 °C and 37 °C. The pH was adjusted at 7.5 and all tests were done as triplicates by shaking fibers in polystyrene vessels. At given times, the whole volume
was exchanged and the aliquots stored until further measurements were done. The first results (shown in Figure 77) were obtained by photospectrometric techniques (see more in experimental part).

The concentrations determined for each time were cummulated and plotted against time. The experiments were done for three different composites including microgels with different ZnO content. As shown in Figure 77, with decreasing temperature, zinc ion concentrations increases. This behavior becomes more significant for higher ZnO content within the microgels (also shown in Figure 77). One might expect that the microgels are responsible for this behavior, though it was shown in chapter 3.1 that swelling is independent from temperature. In comparison with literature, it becomes clear that such a behavior – seeming unexpected since one would intuitively think zinc would be faster released at higher temperature – has already been shown in case of ZnO. Reed et al investigated commercial ZnO nanoparticles regarding their solubility in different biological (aqueous) media. They found that – without determining a clear release study – after 24 hours, zinc was release at higher concentrations for lower temperatures\textsuperscript{[194]}. Since this behavior seems to be independent from the media they analyzed, they explained this unexpected behavior by thermodynamic circumstances, namely a negative dissolution enthalpy of ZnO. Since temperatures below and above 37 °C are of no usage regarding medical applications, no further work was done in this terms, rather, the release study was repeated and characterized by ICP-MS\textsuperscript{8}. The results show similar cumulative release curves and effective ZnO contents found on the fibers (see Figure 78a). The plots are compared to the relative release which shows the release rate in relation to the overall amount in percentage (see Figure 78b). The latter graph is important, to discuss the rates of zinc ion release. Interestingly, they are not equivalent but depend on the amount of ZnO applied in the media. Note that in all samples, for any of the here shown release studies, a constant fiber-to-volume ratio was kept at 2.5 g/L.

\textsuperscript{8} All ICP-MS measurements were performed by Milen Nachev at Aquatic Ecology and Centre for Water and Environmental Research (ZWU), University of Duisburg-Essen.
Figure 78: Cumulative release of Zn$^{2+}$ ions focusing on concentrations (a) and relative release rates (b) of fibers-1, -2,-3, -4 and -5 carrying 0.11, 0.40, 0.90, 1.36 and 3.38 wt-% ZnO. All aliquots were characterized by ICP-MS.

The slower release rates are interesting for long-term application of wound dressings. The longer the application period of a dressing, the longer the release period must be. The link between decreased rates and higher applicability, and increased ZnO content, offers another advantage: higher capacity. This advantage enables the use of dressing with high ZnO content incorporated within the microgels over long periods. An optimum condition would be an application time equal to the PCL degradation time (see 3.1).

The solubility of ZnO has already been investigated regarding the dissolution, depending on size and surface area. Mudunkotuwa et al found that ZnO particles having diameters of 4 nm and 7 nm show maximum levels of concentrations of 14.9 mg/L and 7.6 mg/L$^{[195]}$. All measurements were done in medium with the pH adjusted at 7.5$^{[195]}$. For deeper understanding in which way solubility plays a role for zinc ion release, the release plots in Figure 78a were re-plotted focusing on non-cumulated concentrations (see Figure 79). The size of the nanoparticles precipitated within the PVCL-PIA hosts is assumed to be analogues to the L4-modified ones, so 4-5 nm. Hence, the critical concentration determined by Mudunkotuwa et al for sizes of 7 nm is drawn in Figure 79 for comparison.
Figure 79: Non-cumulated concentrations of the cumulative release tests from Figure 78a. The critical concentration is taken from Reference [195] and was postulated for ZnO nanoparticles with diameters of 7 nm.

Figure 79 proves that each determined concentration is located below the critical concentration and thus the limit of solubility during the cumulative measurements had never been reached. Figure 79 clarifies that cumulative release was not influenced by critical concentration at any time.

**Zinc Ion Release from PVCL-PEG Microgels**

Cumulative release-tests were repeated with laser-generated PVCL-PEG nanocomposites containing ZnO. Here, fibers prepared by use of microgel-ZnO composites were used as received from our partners in Essen. The only purification step in this process was to wash the fibers for 20 seconds (as shown above). After purifying, zinc ion release studies were accomplished and the retained ZnO amounts evaluated. All results are listed in Table 22.

All samples were received after laser-ablation from EDU (Essen) without further purification of the microgels. Thus, ZnO content in microgels cannot be evaluated. The samples were processed as received and all results gained after purifying the fibers.
3. Electrospun Fibers for Biomedical Applications

Table 22: Electrospun fibers prepared from PCL and PVCL-PEG-ZnO composites.

<table>
<thead>
<tr>
<th>PEG-Amount [mol-%]</th>
<th>ZnO-content in composite [%]</th>
<th>Composite content in electrospinning mixture [wt-%]</th>
<th>ZnO content in Fiber [wt-%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 5</td>
<td>5.9</td>
<td>8</td>
<td>0.41</td>
</tr>
<tr>
<td>2 5</td>
<td>5.9</td>
<td>24</td>
<td>1.27</td>
</tr>
<tr>
<td>3 5</td>
<td>5.9</td>
<td>30</td>
<td>1.53</td>
</tr>
<tr>
<td>4 1.25</td>
<td>6.7</td>
<td>30</td>
<td>2.16</td>
</tr>
</tbody>
</table>

Figure 80a shows increasing cumulative concentrations of zinc with increasing amount of ZnO on the fiber. At the same time, Figure 80b shows a comparable behavior of release rates as observed before: the higher zinc content on the fibers, the lower the release rates.

Figure 80: Cumulative release focusing on concentrations (a) and relative release rates (b) of laser-generated PVCL-PEG nanocomposites. All aliquots were characterized by ICP-MS.
Comparison

In summary, PVCL-PIA-ZnO and PVCL-PEG-ZnO nanocomposites can both be simply mounted on microfibers by the method described by Kehren. Independent from the microgel-system and the synthesis approach (wet-chemical and in-situ polymerization), the nanocomposites are suitable for fiber surface-coating and furthermore for zinc ion release. Moreover, the zinc ion release properties are equally regulable by mounting nanocomposites with different ZnO-contents onto the fibers. Hence, only the method used to obtain nanocomposites differs, while the outcome of their application on fibers seems to be independent of their nature. Nevertheless, the laser-ablation approach is limited concerning the yield of ZnO nanoparticle mass compared to the wet-chemical approach. Thus, it has to be considered that high ZnO contents (incorporated with the microgels) cannot be achieved by laser-ablation as simple as by wet-chemical approach.

Biocompatibility

Since the fibers are supposed to be applied for biomedical applications where trace amounts of zinc are useful but higher concentrations may be harmful, the toxicity of the material was tested. Therefore, fiber-4 containing 1.36 wt-% ZnO was placed for 24 hours in a buffer solution. For this experiment we used three samples with fiber:buffer ratios 1 mg/mL, 2 mg/mL and 3 mg/mL. Subsequently, the eluates were taken and brought into contact with NIH3T3 fibroblasts for the cell viability tests and the results are presented in Figure 81. Microfibers without ZnO nanoparticles show excellent results in terms of cell viability indicating that the composite material contains no toxic impurities. As expected for the eluates from the ZnO-loaded fibers, the eluate from lowest fiber:buffer ratio (and consequently lowest released Zn\textsuperscript{2+} concentration) shows no toxicity after incubation of 24 hours. Contrary, the eluates from middle and high fiber:buffer ratio lead to cell death. From this we conclude that

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\[\text{Biocompatibility tests were performed by Vincent Coger at Department of Plastic, Hand- and Reconstructive Surgery, Hannover Medical School.}\]
the ZnO content in microfibers should be carefully adjusted to avoid negative effect on living cells. For our system this can be easily done by: a) the regulation of the ZnO nanoparticle amount in microgels or b) adjustment of the polymer:microgel ratio during electrospinning by keeping the ZnO loading in the microgels constant.

Figure 81: CellTiter-Blue activity tests (a) and Cytotox-One cytotoxicity tests (b) of human dermal fibroblasts NIH3T3.

Surprisingly, control samples where eluates from fibers containing no ZnO were exposed to cells, show an increasing activity with increasing fiber:buffer ratio (Figure 81a), observation that we cannot explain at this point. As shown in Figure 81b, the lactate dehydrogenase (LDH) release measurements for the control fibers are equal to culture growth controls, as well as to 1 mg/ml ZnO-fibers. However, eluates from the 2 and 3 mg/ml fiber:buffer ratio probes conduce to enhanced measure of LDH release (Figure 81b). The experimental data in Figure 81a and b are in good agreement with each other and indicate that composite microfibers do not reduce the cell activity when the concentration of the Zn ions is kept below the physiological toxicity values. To prove this we determined the amount of released Zn\(^{2+}\) ions in the eluates from three fiber:buffer ratios used for the cell tests. It was found that 2.5 mg/ml eluates of fiber-4 with 1.36 wt-% zinc oxide yield 275 µmol/L zinc concentrations after 24 hours (see Figure 78a). Extrapolations lead to zinc concentration of 110 µmol/L, 220 µmol/L and 330 µmol/L for eluates from samples with 1 mg/ml, 2 mg/ml and 3 mg/mL fiber:buffer ratios respectively. The toxicity limit of Zn\(^{2+}\) ions as shown in the literature\(^{196}\) in case of human dermal fibroblasts (hd-FB) is 150 µmol/L. This explains the negative effect of eluates from solutions of 2 mg/ml
and 3 mg/mL fiber:buffer ratios on the cell activity. Therefore, our experimental data are in good agreement with the literature and prove the importance of controlling Zn$^{2+}$ ion concentration to avoid reduction of the cell viability.

In addition we performed live/dead stains of cells seeded directly onto the surface of electrospun mats. The experimental data show the same ratio between green cytoplasm (living cells) and red nuclei (dead cells) for both types of fibers (Figure 82). In this experiment we adjusted the Zn$^{2+}$ concentration by selection of the fiber:medium ratio of 1 mg/mL to perform the experiment below the toxicity level. From this experiment we conclude that microfibers with (ZnO concentrations below the toxic level) and without ZnO exhibit very good compatibility with human dermal fibroblasts and can be potentially used in biomedical applications.

Figure 82: Live/Dead staining of hd-FB on control fiber without ZnO (a), fiber-4 containing 1.36 wt-% ZnO (b) (4x magnification).
3. Electrospun Fibers for Biomedical Applications

### 3.3. Conclusion

Synthesis of ZnO nanoparticles in organic solution was done and successfully transferred to microgel systems. Subsequently, composites consisting of microgel (host) and ZnO (drug) were successfully processed onto PCL microfibers by electrospinning, ending up with a three component drug-release system. Electrospinning of PVCL-PIA/ZnO together with PCL was found to lead to well distributed nanocomposites on PCL-fibers. All fibers were successfully tested regarding swelling behavior and zinc ion release properties. First tests revealed that swelling occurs independent in temperature ranges of interest, i.e. room temperature and 37 °C, and on the ZnO content.

Zinc ion release was demonstrated to be dependent on the amount of ZnO regarding the absolute zinc concentrations. As expected, the more inorganic material is transferred into the fiber-mats, the higher are the cumulative ion concentrations with time. Furthermore, by focusing on the relative release rates, they were found also to be dependent on the ZnO amount transferred onto the fibers. The higher the ZnO content, the lower the zinc ion release rate. This feature is very important when it comes to long-period application as wound dressings, where slow addition of zinc ions is assumed over a long time. The release tests were performed as cumulative tests and all fibers were characterized regarding the remaining ZnO content after each release study. Therefore, any fiber was collected after the test and treated with 1 v-% HNO₃ and ultrasound for ca. 20-30 minutes for three times. Tests by ICP-MS revealed zinc remaining within the fibers, indicating that no microgel leaching occurred during these tests. In addition, gravimetrical tests of fibers proved this assumption.

At last, biocompatibility tests of eluates after the fibers (ZnO content 1.36 wt-%) were left in aqueous medium for 24 hours and fibers themselves were done. Eluates were tested in presence of NHT3T fibroblasts, while fibers were tested in presence of human dermal fibroblasts. Both probes revealed no toxicity of the fibers as well as for the eluates, but showed dependence on the fiber-to-volume ratio. Hence, preparation of ZnO internalized microgels and their utilization within fiber-mats electrospun with PCL, was successfully completed. So far, release tests and biocompatibility tests proved the capability of these fibers to be applied as wound dressings. Further
studies have to be done in order to reveal cell-proliferating properties in vitro and if possible in vivo. Especially for burn-wound treatment, such tests are valuable, due to the well-known antioxidant properties of ZnO (see chapter 3.1.).
3. Electrospun Fibers for Biomedical Applications

3.4. Experimental Part

Electrospinning
Dried microgel-ZnO composite of a certain amount was mixed with PCL giving a PCL to composite ratio of 7:3. A toluene-methanol mixture (1:1) was added so that the PCL mass amount is 12-wt%. The mixture was treated by means of a vortex and/or ultrasonic to dissolve the polymer. Homogenization was done by use of OMNI TIP homogenizer with a stainless steel tip. After dispersing for about 1-3 minutes, the mixture was given into a syringe and fixed into a syringe pump. Electrospinning parameters used for a typical approach are: 0.3-0.5 mL/h flow rate, the target to spinneret distance was between 15-20 cm and the acceleration voltage was set between 22-26 kV. Targets were chosen by their application oriented demand. In case of ion release tests and FESEM measurements, the target was an aluminum foil fixed on a steel drum, rotating during electrospinning. In case of TEM measurements, a carbon/formvar grid was fixed on a SEM plate.

Zinc Ion Release Tests
The received fiber-mats were removed from the aluminum-foil by scratching them carefully off by use of a scalpel. It is necessary to work with extreme caution in order to avoid removing of aluminum-foil together with the fibers-mats. The fiber-mats were cut into pieces with masses of 2-2.5 mg. The fibers were put into polystyrene centrifugation tubes which were purified by HNO₃ (2 v-%) and Millipore water previously. PIPES (2 mM) buffered water adjusted at pH 7.5 was added into the tubes, giving a fiber-water concentration of 2.5 g/L. Each tube was then shaken for about 20 seconds and the whole volume was changed. Washing the fibers is mandatory since a certain amount of microgels is not linked to the fibers. Hence, all ion release studies concern PVCL-PIA/ZnO composite-fibers fixed on PCL fibers only.

The cumulative release was done by taking aliquots for given times by changing the whole volume with fresh buffered medium. All aliquots were stored at room temperature until zinc ion quantification was performed.
Transmission electron microscopy (TEM) measurements were performed with a Zeiss LIBRA 120. To prepare the specimen, the samples were directly spun onto the grids, that had been fixed on metal-targets before. The samples were left at room temperature for few minutes until they were completely dried and measured with an acceleration voltage of 80 kV in high vacuum (10^{-6} to 10^{-7} mbar).

Fibers were characterized by FESEM, using a Hitachi S-3000N instrument. The acceleration voltage was set to 2 kV.

The concentrations of zinc (Zn) in the sample solution were analyzed using inductively coupled plasma mass spectrometry (ICP-MS). The analyses were carried out with a quadrupole ICP-MS system (Perkin Elmer - Elan 6000) operating at 1000 W plasma power, 14 L/min plasma gas flow and 0.95 L/min nebuliser gas flow and an auto sampler system (Perkin Elmer AS-90) connected with a peristaltic pump with a sample flow of 1 ml/min. To avoid contamination and memory effects the wash time between measurements was set at 10 seconds (with 1% HNO₃, suprapure). Before analyses, the samples were diluted 1:10 using a solution of 1 % HNO₃ with a concentration of 10 ng/L of yttrium (Y) as internal standard. In order to control the accuracy and stability during measurements, a standard solution of Zn with concentration of 10 µgL⁻¹ (ICP Multielementstandard IV solution, Merck, Darmstadt, Germany) was analyzed after every 10 samples. The calibration was carried out with a series of 11 dilutions of a zinc standard solution (ICP Multielementstandard solution, Merck, Darmstadt, Germany). Element concentrations were calculated as mgL⁻¹ using corresponding regression lines (correlation factor ≥ 0.999).

Photospectrometric measurements were performed by adding 25 µL of each sample into a UV-vis cuvette. Additionally, dye-containing solution (25 µL) and buffer (950 µL) were added to the cuvette in the way, that adding the buffer as last step sufficiently homogenized the solution. The samples were kept at 25 °C for ca. 5 min and then put into the UV-vis spectroscop to evaluate the absorbence at λ = 628 nm. Afterwards, the concentration was calculated by taking a calibration curve into account, that had been determined before. The dye-containing solution was prepared by adding Zincon monosodium-salt (43.5
mg) to a flask, adding 1 M NaOH solution (1 mL) and filling-up by adding millipore water, giving a final volume of 58 mL. Buffer was prepared by dissolving boric acid (350 mg) and Urea (50.56 g) in water (100 mL). Subsequently, the pH was adjusted at 9 by adding a 5 M NaOH solution carefully.

Cell culture
NIH3T3 cells (murine embryonic fibroblast cell line obtained by ATCC CRL-1658™) were cultured in DMEM-High glucose (Biochrom AG; FG 4815), supplemented with 10 % Fetal Bovine Serum (FBS) (Biochrom AG; S0615), 1 % penicillin/streptomycin (Biochrom AG; A2213) and 0.5 % L-ascorbic acid-2-phosphate (Sigma; 113170-55-1), in a 37 °C incubator with 5 % CO₂ atmosphere.

CellTiter-Blue® & CytoTox-One®
PVCL-PIA microgels were eluted for 24 hours in the same medium used for NIH3T3 at 37 °C under rough agitation. Fibers were down centrifuged, and the medium was filtered with a 0.22 µm syringe-filter (TPP; 99722). Meanwhile, NIH3T3 were seeded in 96-wells plates (Nunc; 165305) at a concentration of 10⁵ cells/100µl/well and set overnight in culture conditions to allow cell-adhesion. The medium was supplemented with 100 µl of eluates of the different products, two times concentrated in medium. Three different eluate concentrations were tested (1 mg/ml, 2 mg/ml, 3 mg/ml). Cells were then kept under standard culture conditions for 24h. 100µl of medium was transferred to another 96-well plate to perform CytoTox-One® assay (Promega; G7892). The CellTiter-Blue® (Promega; G8082) and CytoTox-One assays were performed as prescribed by Promega with respective incubation time of 3h and 10min. Reads were made with a TECAN GENios with 590nm excitation filter and 560nm emission filter. All experiments were repeated at three independent times with N=6.

Live/Dead
10⁶ cells were taken in suspension and set onto flattened microgel pads in 12 wells plates. Cells remained one hour in a cell culture incubator to adhere to the pads. 2ml medium was then slowly added and the samples were incubated for 24h. Live/Dead
(life technologies; L-3224) was performed as promoted by life technologies. Fluorescence microscopy was performed with a Keyence Biozero after transferring the pads onto microscopy slides.

**Statistical analysis**
Statistics were performed in SPSS (IBM) to calculate the means and standard errors of the mean, (SEM) as to compare the significance with a one-way analysis of variance (ANOVA) with post oc.
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