Polymer 53 (2012) 5209–5231

Contents lists available at SciVerse ScienceDirect

Polymer

journal homepage: www.elsevier.com/locate/polymer

Feature article

Stimuli-responsive microgels for the loading and release of functional compounds: Fundamental concepts and applications

Daniel Klinger, Katharina Landfester*

Max Planck Institute for Polymer Research, Ackermannweg 10, 55128 Mainz, Germany

ARTICLE INFO

Article history:
Received 6 July 2012
Received in revised form
17 August 2012
Accepted 21 August 2012
Available online 1 September 2012

Keywords:
Stimuli-responsive microgels
Polymeric nanoparticles
Polymeric networks
Crosslinking
Drug delivery

ABSTRACT

Stimuli-responsive microgels represent a highly interesting and unique class of materials since they exhibit exceptional properties which stem from the particular combination of their colloidal nature with their internal network structure. While this fascinating characteristic feature has been exploited in various different research fields and applications, the essential commonality for the successful development of all those diverse materials is a precise design of the respective microgels to adjust their functionality to a specific application. Regarding the delivery of functional compounds in particular, one of the main tasks is to combine an efficient loading process with a well defined release profile. A basic requirement to achieve this goal is a profound understanding of the underlying concepts of these material's features and the impact of these basic models on the design and preparation of such highly functional materials exhibiting tailor-made properties. Therefore, in this review we present some of the important fundamental examinations on the influence of (tunable) network characteristics on loading and release profiles, basic synthetic concepts to realize these concepts and highlight several examples of different approaches to stimuli-responsive microgels for loading and release applications. By this, we wish to give the reader a broad overview of the design criteria and practical methodologies to control the functionality of microgels in order to encourage further development of highly interesting concepts and materials in this area of materials science.

1. Introduction

Life itself is certainly the most sophisticated example for the utilization of selectively tailored polymers, polymer assemblies and interfaces to provide a specific chemical function and structure. Regarding natural biopolymers such as proteins, carbohydrates, and nucleic acids, the distinct position and nature of functional groups therein not only is the driving force to a particular three-dimensional structure and the resultant function of these materials but also determines their response to external stimuli. Since these inherent features are the basis to construct and keep the complicated cell machinery running, understanding the fundamental concept means learning a lesson from the most influential expert in property control by molecular design: nature [1,2].

Even though there is still a lot to learn and discover, early insights gained in this field already enabled the transfer of several concepts of biology to the world of synthetic polymer chemistry [1]. Concerning stimuli-sensitive macromolecular materials, the gathered knowledge on the response mechanisms of biopolymers opened up new perspectives to mimic this behavior in synthetic systems. Moreover, it enabled the extension of this approach to the development of novel functional polymers exhibiting specific property changes triggered by signals non-existent in biologically-relevant areas [1,2]. While these stimuli can generally be realized either as changes in the materials environment (variations in pH, temperature, or the presence/absence of chemical and biological compounds) or the application of an external field (light, electrical- or magnetic fields), the underlying similarities of all these materials are the triggered conformational and chemical changes of the respective polymeric system. The resulting fact that small signals are able to induce a comparably huge response apparent at the macroscopic level renders these materials a fascinating research area.

Since the discovery of microgels as an intriguing class of such polymeric nanoscale materials, particularly the incorporation of stimuli-responsive properties into gel nanoparticles has gained increasing attention. In particular, regarding their utilization in loading and release applications, microgels, in comparison to other polymeric structures, exhibit exceptional properties. These stem from the unique combination of their colloidal nature (e.g. colloidal stability, high surface area, facile synthesis and control over particle

* Corresponding author.
E-mail address: landfester@mpip-mainz.mpg.de (K. Landfester).

0032-3861
http://dx.doi.org/10.1016/j.polymer.2012.08.053

size) with the inherent features of macroscopic hydrogels, i.e. their internal network structure (e.g. structural integrity in combination with fluid-like transport characteristics). Since the latter is characterized by such parameters as mesh size, polymer volume fraction, or the interaction with embedded functional compounds, the ability to control these factors by the application of external triggers represents the underlying concept to stimuli-responsive microgels.

Here, we want to highlight some of the important fundamental examinations on the influence of (tunable) network characteristics on loading and release profiles as well as basic synthetic approaches to realize these concepts. These *general* considerations (obtained from fundamental research in this field) have shown that a specific stimuli-sensitive profile is the result of highly cooperative interactions and can be realized by precisely regulating not only the type but also the localization of a vast number of functional groups in a polymeric system. Since microgels for a *specific* loading and release application call for such a well defined stimuli-responsive behavior precisely adjusted to the respective requirements and settings, it can be stated that the key point to the successful development of such materials is to understand the importance of transferring fundamental insights in structure- and composition-property relationships to the design of specific synthetic pathways.

On the basis of these considerations, it becomes obvious that the development of novel and highly sophisticated artificial materials in this area is a result of a combination of specific foundations, concepts, and tools from diverse disciplines. In particular, the synergy between organic synthesis, polymer chemistry, colloidal science, highly accurate physical characterization methods as well as not only inorganic chemistry and nanotechnology but also the biomedical field, led to a multitude of stimuli-responsive microgels for a broad variety of applications such as catalysis, optics, sensors, and drug delivery [3–6].

Therefore, the aim of this review is to give the reader (especially from research fields different than the classic colloidal science) a broad overview of the design criteria and practical methodologies to control the functionality of microgels in order to encourage the further development of highly interesting concepts and materials in this area of materials science.

2. Polymeric gels: from macro to micro

Gelation of polymers was first investigated in 1931 by Carothers who polymerized multifunctional monomers by polycondensation. He suggested that this phenomenon is a result of linking polymer molecules into a three-dimensional network of infinitely large size [7]. Since the polymer network consists of inter- and intramolecularly connected polymer chains, the entire gel network can indeed be considered as one macroscopic molecule whose size is theoretically only limited by the dimensions of the containing vessel [8].

Starting from these early investigations, the development of new polymerization techniques and the understanding and resulting utilization of specific molecular interactions dramatically expanded the field of polymeric gels over the years. Hence, three-dimensional networks are no longer only formed by polycondensation reactions in the presence of multifunctional branching units, but nowadays one can choose from a vast variety of different methods and concepts for the preparation of polymeric gels. These, in general, consist of (organic) polymer components which are crosslinked by either covalent or physical connecting points. Moreover, an additional prerequisite for gels is the ability of such networks to swell upon absorbing specific (solvent) molecules, thereby distinguishing them from other crosslinked polymeric materials such as resins, elastomers or thermoset polymers.

Depending on the absorbed media, gels can be further classified into lyogels and aerogels. While the latter represent lightweight highly porous materials, the unique combination of the structural integrity of a solid with the ability to store fluids and the mobility of functional groups in the swollen networks renders polymeric lyogels a unique class of materials. The resulting softness, elasticity and the fluid-like transport characteristics for molecules smaller than the gel pores give rise to a broad variety of different applications. Probably the most simple and widespread examples for hydrogels include their utilization as super-absorbers and contact lenses [9]. Furthermore, the combination of more complex and highly functionalized polymeric architectures with diverse crosslinking methods has been realized by a tremendous variety of different approaches, resulting in a large number of sophisticated materials with specific functionalities tailored for particular applications. Nowadays, polymeric gels are applied in the fields of e.g. self-healing materials [10], drug delivery [11,12], chemical separation [13], sensors [14], shape-memory materials [15,16] and (self-driven) actuators [17,18].

The spatial stability of gels in the presence of a variety of solvents is based on their crosslinked network structure. Linkages can be introduced by several processing routes resulting in various materials with specific properties determined by the crosslinking density, the polymer type, and the chemical nature of the crosslinks. A detailed overview over a multitude of crosslinking methods can be found in the review of Hennink and van Nostrum [19].

A widely investigated approach to chemically crosslinked gels is the radical polymerization of low molecular weight monovinylic monomers in the presence of multivinylic crosslinking agents. In the case of water swellable materials, Wichterle and Lim were the first to describe the polymerization of 2-hydroxyethyl methacrylate (HEMA) in the presence of ethylene glycol dimethacrylate as crosslinker. Detailed investigations on the reaction conditions yielded such a hydrogel as the first material for soft contact lenses [20]. From here on, the additional introduction of functional groups resulted in stimuli-responsive gels as a new class of materials. A different method to obtain covalently crosslinked networks is based on the linkage of polymeric precursors. Here, the radical polymerization of polymers derivatized with polymerizable groups is a well examined concept. As an example, the formation of hydrogels from polymerizable dextrans was pioneered by Edman et al. by reacting dextran with glycidyl acrylate and polymerizing a solution of the resulting compound [21]. In the group of Hennink, this approach was further developed and the investigated hydrogels from (meth)acrylated dextrans served as enzymatically degradable gels for enzyme immobilization [22]. This concept of crosslinking polymeric precursors via the reaction of pendant reactive groups has recently been transferred to the rising field of "click" chemistry by preparing reactive, multifunctional polymer gel films through thermal crosslinking of orthogonal click groups [23]. Moreover, the crosslinking of polymer precursors can also be achieved by the utilization of low molecular weight crosslinking agents which react with functional groups on the polymeric backbones [24]. Examples include the crosslinking of synthetic polymers such as polyvinylalcohol [25] as well as biopolymers such as gelatin with glutaraldehyde [26].

Physically crosslinked polymeric gels represent another type of gels where the network formation is achieved by non-covalent attractive interactions between the individual polymer chains. In contrast to chemically crosslinked gels, the use of often toxic crosslinking agents is avoided, thus rendering them interesting materials for biological applications. Various interaction mechanisms can be used to create crosslinking points. A very prominent example for physically crosslinked gels is the utilization of hydrophobic interactions or partial crystallization of respective domains in amphiphilic block and graft copolymers. Adapting this concept from the classical ABS rubber has led to an interesting class of materials. As an example,

Kim et al. described the utilization of "Biomer" as a segmented polymer consisting of a polyurethane and a polyether. Aggregation of the hard hydrophobic polyurethane segments served not only as crosslinks but also enabled the loading of a hydrophobic drug into the overall hydrophilic gel [27]. A similar approach was described by Bezemer and coworkers who used multiblock copolymers of poly(ethylene glycol) (PEG) and poly(butylene terephthalate) (PBT) to form hydrogels with incorporated lysozyme as hydrophilic model protein [28]. Another concept to physical crosslinks is the utilization of ionic interactions between charged polymers and their respective counterions. As an interesting example, the crosslinking of chitosan as cationic biopolymer with glycerol-phosphate disodium salt was found to depend on the temperature. Liquid mixtures containing biological materials below room temperature were injected subcutaneously in rats and gel formation occurred *in situ* [29]. Additional approaches to physically crosslinked gels include, among others, the pH-dependent crosslinking by hydrogen bonds in mixtures of poly((meth)acrylic acid) and PEG [30] or the crosslinking by antigen–antibody interactions [31].

Comparing physical and chemical approaches, it can be stated that chemically crosslinked networks are normally characterized by an increased stability compared to the physically crosslinked gels. This feature is based on the covalent linkage of the polymer chains which ensures a good structural integrity. Nevertheless, crosslinking polymerizations also bear some drawbacks influencing the properties of the final material. Among other parameters, the structure of the crosslinking agent as well as the difference in hydrophilicity of monomer and crosslinker can play a crucial role regarding the crosslinking efficiency and homogeneity [32].

Besides the field of macroscopic gels which represent insoluble polymeric materials, it was in 1935 when Staudinger was the first who found a crosslinking polymerization of a dilute solution of divinylbenzene to result in a soluble polymer of low viscosity [33]. The conclusion that *this polymer is a product consisting of strongly branched, 3-dimensional molecules* was the groundbreaking perception which led to the development of colloidal macromolecules of globular shape as a new class of materials [31,33,34]. These inter- and intramolecularly crosslinked macromolecules dispersed in either normal or colloidal solutions are termed microgels, (hydro)gel nanoparticles or nanogels. It is noteworthy that no clear correlation between the actual size of the gel particles and the nomenclature has been established and the terms microgels and nanogels are mostly used interchangeable in the literature. Due to the lack of proper characterization techniques, microgels were playing a negligible role in science and technology of polymers until the beginning of the 1970s. Since then, the progress in chemical design and understanding of the physico-chemical properties of microgels increased steadily and significantly, as can be seen from the rising number of publications [34].

3. Synthesis of microgels

As in the case of macroscopic polymeric gels, similar crosslinking methods can be applied for the formation of microgels. Preparation methods can be generally categorized in two main concepts: (a) the formation of microgels in homogeneous phase and (b) the formation of microgels in heterophase.

3.1. Microgel preparation in homogeneous phase

Among others, this concept can be realized by two main approaches. Based on the first investigations made by Staudinger, the free radical crosslinking copolymerization of mono- and bisunsaturated monomers in dilute solutions results in the formation of inter- and intramolecularly crosslinked microgels. In this solution polymerization the internal structure of the prepared microgels crucially depends on the ratio of the amount of the inert good solvent to the monomers [34]. Hence, a higher dilution increases the probability of intramolecular crosslinks. Even though the resulting internal structure of microgels prepared by this method is not well defined, investigations conducted on these systems represent an important step to get insight into the mechanism of gel formation in radical crosslinking polymerizations [8,35–37].

An alternative approach to microgels includes coacervation and desolvation techniques which are both based on the phase separation of readily formed polymers during the preparation step, thus forming nanoparticles which are subsequently crosslinked. This method has been widely investigated for the formation of microgels from biopolymers such as e.g. (modified) gelatin or chitosan which contain a large number of functional groups available for crosslinking. Hence, the preparation of pH-sensitive chitosan nanoparticles by complex coacervation can be achieved e.g. by physical crosslinking due to electrostatic interactions of the cationic polymer with either anionic poly(ethylene imine) [38] or tripolyphosphate [39]. Gelatin nanoparticles were successfully prepared by a two-step desolvation route including the chemical crosslinking with glutaraldehyde [40]. Nevertheless, since in these synthetic procedures crosslinking occurs after the nanoparticles formation, the resulting microgels are only crosslinked at the surface due to a hindered diffusion of the crosslinking agent into particle interior [41].

3.2. Microgel preparation in heterogeneous phase

Another class of synthetic routes to microgels is the heterophase copolymerization of monomers with crosslinking agents in aqueous solution. Here, three main concepts can be distinguished: (a) precipitation polymerization, (b) polymerization and (physical or chemical) crosslinking of preformed polymers in inverse mini- and (c) microemulsions.

3.2.1. Microgels by dispersion/precipitation polymerization

Dispersion and precipitation polymerizations in general, start with the initiation of polymerization in a homogenous solution of monomers and crosslinkers [42–44]. While the polymerization proceeds, the monomer and the formed oligomers are soluble until the growing chains reach a critical length and phase-separate from the continuous medium by enthalpic precipitation, thus forming particle nuclei. In the case of crosslinkers present in the mixture, entropic precipitation occurs favorably and crosslinking prevents the polymer and solvent from freely mixing even in good solvents for the polymer [45]. Here, the boundary to the previously described radical crosslinking polymerization in solution is blurred [34]. Nevertheless, in both cases, the resulting nuclei aggregate into larger particles that continue to grow by capturing other particles, newly formed polymer chains or by absorption and polymerization of monomer. In this context, dispersion polymerizations are characterized by the addition of a stabilizer to control the size and to narrow the distribution of the particles [46]. The described mechanism is schematically illustrated in Fig. 1.

The method of precipitation polymerization represents a widely investigated and elegant approach for the preparation of water swellable thermo-sensitive microgels. Especially *N*-isopropylacrylamide (NIPAAm) [47] or *N*-vinylcaprolactam (VCL) [48] are the most applied monomers [49]. In these cases, the enthalpic precipitation of growing oligoradicals occurs due to the unfavorable polymer–solvent interaction at high reaction temperatures. Since the monomers are completely water soluble but the resulting PNIPAAm and PVCL polymers exhibit a lower critical solution temperature (LCST) above which they become insoluble in water, initiation of an aqueous monomer/crosslinker solution results in

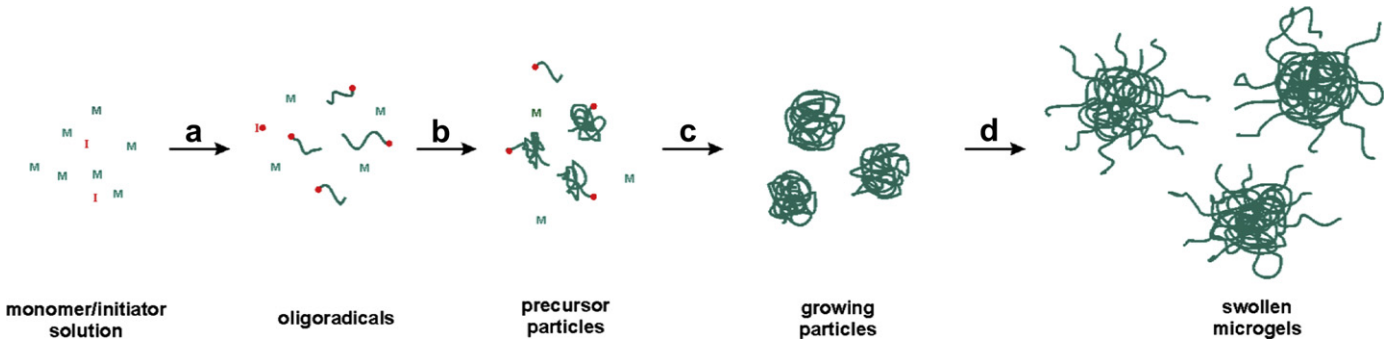

Fig. 1. Microgel formation by precipitation polymerization: (a) initiation and chain growth, (b) precipitation and nucleation, (c) particle growth and (d) transfer to good solvent or decrease of temperature below the volume phase transition temperature.

the formation of precursor particles consisting of collapsed polymer chains if the reaction temperature is above the LCST. These nuclei further react to the microgels in analogy to the mechanism described above. Stabilization of the collapsed microgels at elevated temperatures can be achieved by e.g. the utilization of potassium peroxodisulfate (KPS) as initiator. The electrostatic stabilization is based on sulfate groups from initiator fragments which are incorporated into polymer chains during the nucleation and growth process [3]. Different ways of stabilization, which in addition offer the possibility of particles size control, are the utilization of either ionic [50] or steric [51] stabilizers or ionic comonomers [52,53] analogous to surfactant-free emulsion polymerizations. After complete polymerization, the microgels dispersion is cooled down to room temperature resulting in a swelling of the networks ($T <$ LCST) and a steric stabilization of the microgels by dangling chains of the outer swollen particle layer. It is obvious that the use of crosslinking agents is a necessary requirement in order to prevent microgels from dissolution at low temperatures [54]. A tremendous variety of thermo-sensitive microgels has been prepared by this method demonstrating the high impact of this preparation route. Moreover, modifications of the synthetic protocol enabled the formation of more complex microgels structures. As an example, temperature- and pH-sensitive microgels were obtained by copolymerization of NIPAAm with e.g. acrylic acid [55], vinyl acetic acid [56,57], allyl acetic acid [58] or aminoethyl methacrylate hydrochloride [59]. In addition, precipitation polymerization of functional monomers in the presence of preformed seed particles was demonstrated to yield such complex structures as e.g. (multi-responsive) [60] core/shell microgels [3,61] or hollow hydrogel spheres [62].

Even though this preparation method is one of the most widely investigated routes to hydrogel nanoparticles, several limitations have to be taken into account. On one hand, the incorporation of comonomers can only be achieved to a certain extent depending on the hydrophilicity of the respective compound. Hence, for the synthesis of thermo-sensitive hydrogel nanoparticles, the additional introduction of strongly hydrophilic comonomers such as e.g. (meth)acrylic acid is limited as a successful precipitation during the chain growth crucially depends on the overall hydrophobicity of the resulting copolymer [3]. Furthermore, since batch copolymerization of monomers of different reactivity and hydrophilicity was shown to result in core/shell morphologies [48,63–65], the preparation of copolymer microgels exhibiting a homogenous distribution of all functionalities is hindered.

3.2.2. Microgel synthesis in dispersed droplets

As described in the previous section, precipitation polymerization for the formation of microgels bears some serious drawbacks. In order to evade these limitations, microgels synthesis in droplets dispersed in a continuous phase represents a well examined alternative. Here, the restriction of the network-forming reaction to the droplets renders the latter as “nanoreactors”, thus in principle enabling to exploit most of the crosslinking (physically and chemically) methods described for macroscopic gels for the preparation of microgels.

3.2.3. Microgel synthesis in microemulsions

Microemulsions in general can be prepared as direct oil-in-water (O/W) or inverse water-in-oil (W/O) emulsions. For the formation of microgels, especially the W/O emulsion methods are widely investigated to yield water swellable hydrogel nanoparticles [66,67]. In this approach, the dispersed phase contains the respective compounds for the network formation such as e.g. polymerizable monomer or crosslinkable prepolymers dissolved mostly in water as solvent. The microemulsion is then formed by adding this solution to a continuous organic medium containing large amounts of an oil-soluble surfactant. By stirring this mixture, the thermodynamically stable microemulsion is formed. Microgel formation can then be achieved by e.g. free radical polymerization of mono- and divinylic monomers in the droplets upon intiation from either the droplets interior or the continuous phase [68,69]. This preparation method allows the preparation of microgels with a high content of ionic groups [70].

3.2.4. Microgel synthesis in miniemulsions

In general, miniemulsions are kinetically stable emulsions, thereby distinguishing them from microemulsions [71]. The advantage is the considerably less amount of surfactant needed for successful droplet stabilization as well as the versatility of this approach with respect to the utilization of different monomers [72], the incorporation of functional compounds [73–76] and the precise adjustment of the droplets and particles size [77]. Miniemulsification is generally achieved by applying high shear forces to a pre-emulsion of droplets in a continuous phase. During this procedure, a fission and fusion process of broadly distributed (macro)droplets leads to uniform well defined nanopdroplets in the size range between 50 and 500 nm [71,77,78]. While the presence of either a sterically or electrostatically stabilizing surfactant prevents these droplets from coalescence, the kinetic stabilization is accomplished by the suppression of Ostwald ripening by the addition of a costabilizer to the dispersed phase [79,80]. The negligible solubility of this compound in the continuous phase creates an osmotic pressure in the droplets, thus counteracting the Laplace pressure. As a result the net diffusion between the droplets is inhibited and therefore, stable droplets of the same composition as the dispersed phase prior to emulsification are obtained and can be classified as “nanoreactors” [72]. As a result, the composition of the polymeric particles after polymerization resembles the composition of the monomer phase, thus enabling the equal

distribution of all different functionalities in each particle. Similar to microemulsions, also miniemulsions can either be realized as direct (O/W) or inverse (W/O) systems. While the first case is a well established approach to solid polymeric latexes by free radical polymerization of hydrophobic monomers [71,78], the inverse method gives rise to the formation of hydrogel nanoparticles by diverse synthetic pathways [41].

One approach is the free radical copolymerization of hydrophilic monomers with crosslinking agents in dispersed droplets of either aqueous solutions of these compounds or their mixture without additional solvent. This pathway results in hydrogel nanoparticles after transferring the polymerized latexes to water as continuous phase. Examples include the formation of polyacrylamide (PAAm) [81,82] and PHEMA [82,83] based microgels. Moreover, since the only main requirement for copolymerization of different monomers is their immiscibility with the continuous phase, this approach is highly tolerant to a broad variety of monomers and can be used to prepare e.g. highly charged microgels [84]. Fig. 2 illustrates the described synthetic pathway schematically.

An alternative to the crosslinking copolymerization of hydrophilic monomers is the gel formation upon crosslinking of preformed polymers in inverse miniemulsion. This concept has been realized by a sophisticated approach which is based on the ultrasonication of a mixture of two inverse miniemulsions A and B containing a solution of a preformed polymer and a solution of the crosslinking agent, respectively. Upon the appliance of high shear forces a fission and fusion process between the individual droplets of both systems results in the mixing of the components which induces the crosslinking reaction. This method was successfully applied to the formation of well defined gelatin microgels covalently crosslinked by glutaraldehyde [78,85]. In addition, more complex gel structures can be obtained as well. As an example the formation of crosslinked starch capsules was achieved by the interfacial crosslinking polycondensation in inverse miniemulsion. Here, the dispersed droplets consisted of an aqueous solution of starch which was covalently crosslinked only at the droplets surface by the addition of 2,4-toluenediisocycanate (TDI) to the continuous phase [86].

3.2.5. Microgel synthesis in other compartments

Based on the concept of closed nanoreactors, different supplementary approaches are described in the literature. A highly interesting method was described by Kazakov et al. [87]. By using liposomes as compartments for the photo initiated polymerization of monomers, hydrogel nanoparticles were obtained after solubilization of the lipid double layer. Despite that, the formation method of microgels using micro-fluidics gives rise to well defined spherical particles in the size range of several micrometers [88].

4. Stimuli-responsive microgels: features and applications

Stimuli-responsive or environmentally-sensitive micro- or nanogels are intra-molecularly crosslinked polymeric nanoparticles where a specific molecular design of the polymeric network structure allows the incorporation of stimuli-sensitive properties into the gel. In this context, a broad variety of triggers finds application. These can be classified into two main categories: (a) physical stimuli such as e.g. changes in temperature, the appliance of light, electric or magnetic fields and (b) chemical stimuli including changes in pH, ionic strength or the presence of chemical or biological compounds. Since the latter are often characteristic features of a specific location, microgels changing their properties upon their (re)location in such an environment are often called “smart” materials since they respond to an inherent feature of the location rather than to an externally applied trigger.

The broad selection of triggers and response mechanisms of stimuli-responsive microgels has led to a tremendous variety of applications including, among others, sensors [89], optics [90] and colloidal crystals [91,92]. In the biomedical field these materials are of high interest for e.g. microgel films as cell culture substrates [93] and loading and release applications [94].

4.1. Microgels for loading and release applications

In the field of biomedical applications, the controlled delivery of pharmaceutically active substances holds promise to be a key concept for future treatment of diseases. Here, hydrogel nanoparticles represent an outstanding approach, since they allow the incorporation of water-soluble drugs, including proteins and nucleic acids, in the gel [95]. The utilization of biocompatible and non-antiogenic materials enhances the protection of the payload from hostile enzymes until the delivery to targeted tissues [96]. Due to their chemically crosslinked structure and hydrophilic chains on the

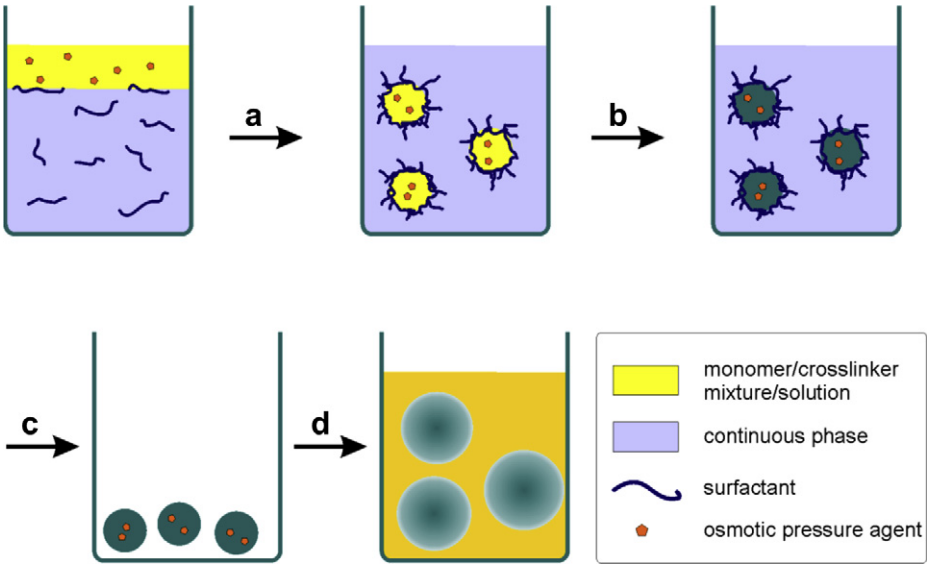

Fig. 2. Schematic representation of microgel preparation by radical crosslinking polymerization in (inverse) miniemulsion: (a) emulsification and homogenization, (b) polymerization, (c) removal of excess surfactant by washing/dialysis and subsequent freeze-drying and (d) redispersion of microgels in a good solvent for the network-forming polymer by swelling.

surface (preferably non-charged polymers such as e.g. PEG or poly(*N*-vinylpyrrolidone), microgels are characterized by a higher stability for prolonged circulation in the blood stream [97]. This unique feature is often referred to as a "stealth" effect, since opsonisation by macrophages and non-specific binding to cells is dramatically decreased [98]. Moreover, due to their soft architecture, microgels are able to flatten themselves onto vascular surfaces, thus simultaneously anchoring in multiple points. Thereby, their retention in the targeted disease site is enhanced [96]. Additional advantages include the ease of preparation, high stability and the good dispersibility in water [99]. In the special case of stimuli-responsive microgels, the drug loading and release profile can be modulated by external stimuli, thus greatly improving the loading efficiency and enhancing the bioavailability to reduce side effects [3].

Especially the ability to control the loading and release of functional compounds into and from microgels renders stimuli-responsive microgels highly interesting materials not only for the application in biomedical fields but also e.g. in the area of triggered catalysis. Here, the embedding of catalysts in a particulate stimuli-responsive (hydro)gel network allows one to trigger the activity of the catalytically active compound. This can either be achieved by a triggered release of the catalyst from the microgels upon swelling or degradation of the network, or by modulating its accessibility to specific reagents. In the latter case the catalyst remains in the network and the diffusion of substrates to and from the catalyst is triggered e.g. by the swelling of the surrounding gel. Examples realizing these described concepts are based on immobilized enzymes [100,101] or catalytically active metal nanoparticles embedded in microgels [101–103].

4.1.1. Network characteristics

Polymeric (micro)gels are networks that absorb large quantities of solvent while remaining insoluble due to the crosslinking of the individual polymer chains. As a result of the crosslinking, properties of individual polymers become visible on a macroscopic scale, thus rendering polymeric gels a unique class of materials. A key feature to the determination of the nanoscopic structure of gel networks is the understanding of the solvent-sorption capabilities since the latter is crucially dependent on the molecular interactions between the network-forming polymer and the solvent as well as e.g. the crosslinking density. Investigations on and mathematical descriptions of the parameters defining the structure of swollen networks were widely studied for the case of macroscopic hydrogels [9,32,104]. Even though the developed models can mostly be transferred to their microgels analogs, specific deviations may result from inherent features of these nanoscale materials such as e.g. their high surface area.

In general, for the description of the nanostructure of polymeric gel networks, three parameters are crucial: (1) the polymer volume fraction in the swollen state: $\nu_{2,s}$, (2) the number average molecular weight between crosslinks: $\overline{M_c}$. d (3) the network mesh size: ξ [9].

For loading and release applications of non-porous (micro)gels, the network mesh size is the most important parameter determining the mobility of embedded functional substances and their rates of diffusion within a swollen (hydro)gel matrix. Especially the comparison of the mesh size of the network relative to the hydrodynamic diameter of the compound to be delivered is of high interest since theoretically, the diffusion of the latter is hindered when mesh sizes approach the size of the payload as shown in Fig. 3 [105]. This state can e.g. be beneficial for an efficient loading of functional compounds upon entrapment in the network.

The most important factors that influence the mesh size of a gel are the degree of crosslinking and the interaction of the network-forming polymer with the solvent. Since in stimuli-responsive (micro)gels these properties can be influenced by external triggers (degradation of crosslinks, change in physico-chemical parameters of the polymers), a determination of the mesh size before and after the appliance of the respective stimulus enables to predict the loading and release profile of a specific compound. Therefore it is generally of high importance to be able to derive the mesh size from measurable macroscopic features of the respective (micro)gels. The mesh size (ξ) can be described as follows [106]:

$$\xi = \nu_{2,s}^{-1/3}\left(\overline{r_0^2}\right)^{1/2} = Q^{1/3}\left(\overline{r_0^2}\right)^{1/2} \quad (1)$$

Here, $\nu_{2,s}$ is the polymer volume fraction in the respective swollen state and describes the amount of solvent imbedded in the network. It can either be described as the ratio of the polymer volume (V_p) to the swollen gel volume (V_g) which is the reciprocal of the volumetric swelling ratio (Q). Moreover, Q can be related to the densities of solvent (ρ_1) and polymer (ρ_2) and the mass swollen ratio (Q_m) [104]:

$$\nu_{2,s} = \frac{V_p}{V_g} = Q^{-1} = \frac{1/\rho_2}{Q_m/\rho_1 + 1/\rho_2} \quad (2)$$

In the case of microgels, Q can be calculated from the hydrodynamic particle diameters in the swollen and non-swollen state. As these can be determined by e.g. dynamic light scattering (DLS) measurements under different conditions (solvents, temperatures, etc.) or combinations of DLS and electron microscopic investigations (scanning electron microscopy (SEM), transmission electron microscopy (TEM)), this parameter is easily accessible. In the case of macroscopic gels, the swelling ratio can be determined by gravimetrical analysis as the mass swollen ratio.

The next factor to be determined in order to calculate the gel mesh size based on Equation (1) is the root-mean-squared end-to-end distance of network chains between two adjacent crosslinks in

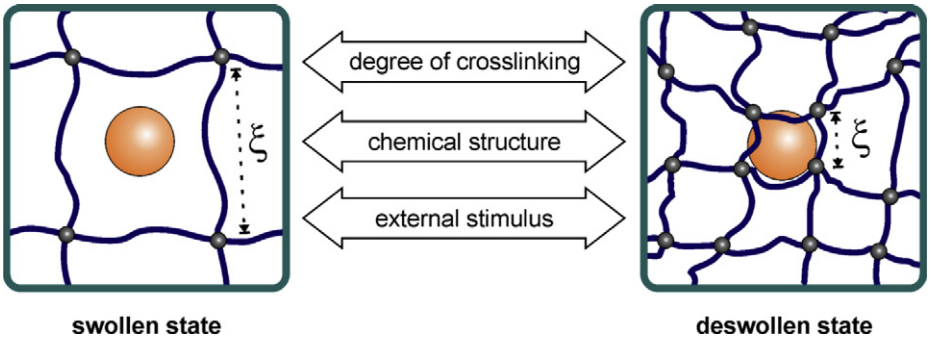

Fig. 3. Schematic representation of mesh sizes in swollen and deswollen gel networks. (Reproduced from [105], with permission from Elsevier.

the unperturbed state. As described by Canal and Peppas, it can be calculated as follows [106]:

$$\left(\overline{r_0^2}\right)^{1/2} = l(C_n N)^{1/2} = l\left(C_n \frac{2\overline{M_c}}{\overline{M_r}}\right)^{1/2} \quad (3)$$

C_n is the Flory characteristic ratio, l is the bond length along the polymer backbone, N is the number of bonds between two adjacent crosslinks, M_r is the molecular weight of one repeating unit (monomer) of the respective network-forming polymer and M_c is the average molecular weight between two adjacent crosslinks. While for a specific network-forming polymer l, C_n and M_r can be obtained from the literature, M_c is the last key parameter to be determined in order to calculate the mesh size accordingly to Equation (1). In general, this factor can be obtained from the Flory-Rehner equation [35]:

$$\frac{1}{\overline{M_c}} = \frac{2}{\overline{M_n}} - \frac{\left(\frac{\overline{\nu}}{V_1}\right)\left[\ln(1-\nu_{2,s}) + \nu_{2,s} + \chi_{12}\nu_{2,s}^2\right]}{\nu_{2,s}^{1/2} - \frac{\nu_{2,s}}{2}} \quad (4)$$

Here, M_n is the number average molecular weight and is the specific volume of the polymer prior to crosslinking; χ_{12} the solvent-polymer interaction parameter. Since this equation was originally derived for neutral divinyl crosslinked networks for which the molecular weight M_n of the respective polymer is known, more complex derivations of this equation have been developed by Peppas et al. in order to expand the theory to ionic gels or gels crosslinked during polymerization [9]. As a useful approximation, it has been shown by Mason et al. [107] that in the case of highly swollen ($Q > 10$) neutral polymeric gels, M_c can be derived directly from the swelling ratio as:

$$Q = \left[\frac{\overline{\nu}\left(\frac{1}{2} - 2\chi_{12}\right)\overline{M_c}}{V_1}\right]^{3/5} = \beta\left(\overline{M_c}\right)^{3/5} \quad (5)$$

By combining Equation (5) or (4) with (3) and subsequently combining (3) and (1), a simple model is available to determine the mesh sizes of polymeric gels from facile equilibrium swelling experiments. Data obtained from these calculations not only allow one to predict and tailor the loading and release profiles of (micro) gels, but also enable to give information about physical properties of the network such as e.g. mechanical strength and degradability [106–108].

4.2. Release from microgels

In general, the high solvent sorption capabilities of microgels induce completely different release mechanisms compared to non-swollen matrices. The release from (micro)gels is governed by passive diffusion of the payload through the (hydro)gel network and – depending on the rate limiting step of the controlled release – three main categories of release mechanisms can be distinguished:

1) Diffusion-controlled
2) Swelling-controlled
3) Chemically-controlled

Since an efficient delivery of (pharmaceutically) active compounds from microgels crucially depends on the particular release mechanism, it is of high interest to be able to predict the respective time-dependent release profile. Several simple and sophisticated models have been developed to achieve this goal and are based on the understanding of the underlying mechanism and the identification of the key parameters which govern the release. In the following sections, these considerations are described for the above mentioned different release mechanisms.

4.2.1. Diffusion-controlled release systems

In this case, two main concepts can be distinguished depending on the internal structure of the gel network. Considering porous gels, the pore sizes of the gels are typically much larger than the hydrodynamic diameters of the embedded compounds. As a result, the diffusion coefficient of the specific substance is rather governed by the porosity and tortuosity of the gel than the internal network structure, thus resulting in limited control over the release [109]. In contrast, in homogeneous networks or porous gels with pores sizes comparable to the dimensions of the payload, the polymeric chains in the crosslinked network provide a steric hindrance to the compound to be delivered and therefore influence its diffusion coefficient [109,110]. Here, the mean free diffusion path length of the embedded compound is decreased as the average free volume per molecule available to the compound is decreased. As a result, the diffusion coefficient is decreased compared to the solute state [108]. This general dependency of the diffusivity on fundamental network characteristics, such as mesh sizes and solvent content, is the underlying concept of several theoretical models which have been developed for predicting diffusion coefficients of active compounds in gels. In general, the relationship between diffusivity in gels and in solution can be described as follows [104]:

$$\frac{D_g}{D_0} = f(r_s, \nu_{2,s}, \xi) \quad (6)$$

In this equation, D_g and D_0 represent the diffusion coefficients of the respective substance in the gel network and in solution while r_s is the hydrodynamic radius of the substance to be delivered, $\nu_{2,s}$ is the polymer volume fraction and ξ is the mesh size of the network. While several theories have been developed to describe this relationship, a prominent example for the description of the correlation between diffusivity and network structure is based on a free-volume approach and was developed by Lustig and Peppas as follows [111]:

$$\frac{D_g}{D_0} = \left(1 - \frac{r_s}{\xi}\right)\exp\left(-Y\left(\frac{\nu_{2,s}}{1-\nu_{2,s}}\right)\right) \quad (7)$$

Here, Y is the ratio of the critical volume which is required for a translational movement of the embedded compound and the average free volume per molecule of solvent [104].

It becomes obvious that this approach takes into account the assumed crucial influences of the gel mesh sizes and the polymer volume fraction (the degree of solvent sorption) on the diffusivity. Regarding now typical mesh sizes of biomedical hydrogels, values between 5 and 100 nm are reported [97]. Since these size scales are considerably larger than hydrodynamic diameters of most low molecular weight compounds (e.g. drugs), their diffusion is not significantly retarded. Therefore, a controlled delivery by a simple diffusion controlled pathway is not applicable. In contrast, this mechanism finds application for the delivery of (biologically active) macromolecules such as e.g. peptides, proteins, or oligonucleotides since their release can be sustained due to their larger hydrodynamic diameters. Here, it is of special interest to design the internal network structure appropriately to achieve a desired rate of macromolecular diffusion [95]. As a result, a kinetically controlled release system can be obtained [112,113].

Based on these considerations, the utilization of stimuli-responsive (micro)gels represents a highly advantageous

approach to controlled delivery applications since it allows for the adjustment of network properties such as mesh size and polymer volume fraction upon the appliance of an external trigger. According to Equation (7), the diffusion coefficient can thus be triggered externally, thereby significantly enhancing loading efficiencies and control of the release. As an example, a functional macromolecular compound can be embedded in a gel network exhibiting initial mesh sizes smaller than the hydrodynamic diameter of the compound to be delivered, thus ensuring efficient loading by hindered diffusion. By utilization of a stimuli-responsive network-forming polymer, the polymer volume fraction ($\nu_{2,s}$) as well as the mesh size (ξ) can be controlled by external triggers. If the change results in an increased mesh size and/or a decreased polymer volume fraction, the diffusion coefficient of the active substance in the gel (D_g) is increased according to Equation (7). This response is widely realized by changes in the physico-chemical parameters of the network-forming polymer which induce an increase in swelling of the network.

Obviously, the mesh size as an important factor governing the diffusion coefficient of the embedded compound is known to crucially depend on the crosslinking density (see Equations (1) and (3)) as well. Hence, by the utilization of cleavable crosslinking points in a network, the mesh size and thereby the diffusivity can be triggered by degradation of crosslinking points. This particular example represents the border to chemically-controlled delivery systems which will be discussed in a following section.

As a last point, it has to be mentioned that diffusion controlled release is, in a lot of cases, much more complex as described in these simplified models. Here, especially interactions between the embedded compound and the polymer network (electrostatic, hydrophobic–hydrophobic, etc.) can cause severe deviations from the predicted release profiles. In order to take these phenomena into account, several theoretical models based on empirically determined diffusion coefficients have been developed to predict the release profile [104].

4.2.2. Swelling-controlled release systems

The mechanism of swelling-controlled release systems is based on the swelling of the gel as the rate limiting step for the release, meaning that the diffusion of the embedded compound is faster than the swelling of the gel matrix. Originally, this concept was widely investigated for macroscopic hydrogels which undergo a swelling-driven phase transition from a glassy/dry state to a rubbery state [114,115]. The embedded compounds are immobilized in the initial non-swollen network whereas their rapid diffusion is enabled in the swollen gel [104].

Transferring this concept to the nanoscale can be achieved by the utilization of stimuli-responsive microgels. Investigated approaches include the embedding of small-molecule compounds into collapsed gels and their swelling-induced release upon the appliance of an external trigger [4,116]. A detailed description of different stimuli-responsive microgels is discussed in a following section.

However, in these cases a necessary requirement to distinguish the resulting release mechanism from the chemically-controlled alternative is the fast response to the respective trigger. Only if the change in the chemical nature/physico-chemical properties of the network-forming polymer is faster than the resulting swelling, the release mechanism is still governed by the time-scale of gel swelling and is defined as swelling-controlled.

4.2.3. Chemically-controlled release systems

In chemically-controlled release systems, the release of a functional compound from the gel network is determined by chemical reactions in the matrix. The time-scale of the respective reaction has to be considerably slower than the diffusion of the compound from the gel and therefore represents the overall rate limiting parameter [104]. Several approaches to chemically controlled delivery systems can be distinguished by the type of chemical reaction inducing the release. Among others, the cleavage of network-forming polymer chains resulting in surface-erosion or bulk-degradation of the (micro)gel and the cleavage of pendant chains between the polymeric network and the compound to be delivered are the most common ones. These mechanisms can be further categorized into (a) purely kinetically-controlled release systems and (b) reaction-diffusion-controlled systems. While in the first case, the (polymeric) bond degradation is the rate limiting step and the diffusion term is comparably negligible, in the second case, polymer/crosslinker degradation and diffusion terms have to be taken into account in order to be able to predict the release profile.

One example for purely kinetically-controlled systems is the pendant chain approach which is based on the covalent linkage of active compounds to the gel network by cleavable linkers. Especially in the field of drug delivery, these prodrugs or polymer-drug conjugates are widely investigated to enhance the therapeutic efficacy of drugs [117] The realization of this approach is mostly achieved by the utilization of either hydrolytically [118,119] or enzymatically [120] degradable linkers where the release rate is determined by the degradation rate of the respective linker (e.g. simple first-order kinetic relationships for hydrolytic degradation) [104]. If diffusion of the liberated compound is then comparably fast, these systems are purely kinetically-controlled.

Another example of kinetically-controlled release systems is the release of a compound as a result of the surface erosion of a polymeric matrix. Here, the rate of transport of the eroding reagent into the polymer is much slower than the rate of bond hydrolysis. This can be found in the case of hydrophobic (biodegradable) polymeric networks or enzymatically degradable hydrogels. In the latter case the transport rate of the enzyme through the hydrogel layer is significantly retarded in comparison to the enzymatic bond cleavage [121].

In contrast to the kinetically-controlled release systems, reaction-diffusion-controlled systems are characterized by the influence of multiple parameters on the release profile. The theoretical considerations described above mainly consider only one mechanism to dominate the release. For many cases these simplified models either purely based on diffusion, swelling or degradation mechanisms provide a good correlation between experimental data and the predicted release profiles. In reality however, different mechanisms can occur simultaneously. As an example where the deviations from these simplified models become obvious, bulk degrading release systems have to be mentioned [104]. Here, the drug release is governed by both network/crosslink degradation and molecule diffusion, thus defining them as reaction-diffusion-controlled systems. Bulk degradation can either be achieved by the cleavage of the backbones of the network-forming polymers or the crosslinking points. In the latter case, the cleavage of crosslinking points correlates with a decreasing crosslinking density and an increasing mesh size. Therefore, the diffusivity of an embedded compound is no longer constant but increases with propagating crosslinker degradation (i.e. time-dependent mesh size). As a result the respective diffusivity correlation as described in Equation (7) can be simplified during the initial stages of degradation to [107]:

$$1 - \frac{D_g}{D_0} = \frac{r_s}{\xi} \sim e^{-7/5 j k'_E t} \tag{8}$$

Here, jk'_E is the pseudo-first-order reaction constant for the cleavage of a degradable crosslinking bond. It can be concluded that, upon degradation, the diffusion coefficient D_g increases and that the diffusivity depends on the bond cleavage kinetics [122].

4.3. Loading of microgels

In general, the incorporation of active compounds into (micro) gel matrices can be realized by either one of the two following concepts:

1) post-formation loading
2) *in-situ* loading

In the first case, functional compounds are embedded in preformed (micro)gel networks. For that, the respective materials are mostly soaked in a concentrated solution of the respective substance and loading is achieved by absorption. Regarding the second approach, the embedding of substances to be released is achieved during the network formation [104].

As described in the previous section, the release from microgels is mainly governed by the diffusion of the embedded compound from the network. Several release mechanisms, based on controlling the diffusivity, have been examined. In order to ensure an efficient loading of microgels, an underlying requirement is the prevention of diffusion (i.e. leakage) until the targeted site or time point for release is reached. In dependency on the desired release mechanism, this can be achieved by several different strategies [95,123].

4.3.1. Loading pathways for diffusion-controlled release systems

If the diffusivity of embedded compounds is only controlled by adjusting the network parameters to fixed values and thus decreasing the diffusion coefficient of the substance to be released in the gel, a solely kinetically-controlled release mechanism is realized. An efficient loading is hereby dependent on the level of retardation of diffusivity, meaning that only for very slow release kinetics a prevention of leakage can be realized. As a result the diffusion into the gel is very slow as well. Therefore, an *in situ* loading pathway is the concept of choice here [124]. In contrast, the loading efficiency can be increased by exploiting hydrophobic interactions between drug and network or – dramatically more – by the utilization of stimuli-responsive materials. Especially the use of polyelectrolytes as network-forming polymers gives rise to an enhanced loading efficiency by the exploitation of electrostatic interactions between the functional compound and the gel network [125–127]. In addition, Hoare and Pelton recently demonstrated the importance of hydrophobic partitioning in regulating drug–microgel interactions [125]. A release can be achieved by triggering these interactions by e.g. additional ions competing with the compound to be delivered thus weakening their attraction to the network [128]. Loading can generally either be achieved *in situ* or in a *post-formation* step by adjusting the ionic strength and pH of the dispersion accordingly [129]. If more complex binding ligands are used to ensure attachment of a compound to the network, models for release description have to take both compound–polymer interaction and diffusion into account. Another highly prominent example for a loading concept based on preventing diffusion of compounds by dynamically changing network properties is the entrapment of functional macromolecules such as proteins [130,131] or oligonucleotides [132]. In this case, the mesh size of a (collapsed) network is smaller than the hydrodynamic diameter of the compound to be delivered, thereby hindering its diffusion. While theoretically this could be achieved by an *in situ* loading approach, the integrity of biomacromolecules during the gel preparation can be damaged under the used reaction conditions. To overcome these limitations, a widely investigated approach is a *post-formation* method whereby the stimuli-sensitive properties of the gel allow the entrapment of a compound by a triggered contraction of the network (i.e. a reduction of mesh sizes) [131,132]. Using either the same reverse stimulus or an orthogonal second one, the substance can be released by increasing the mesh sizes as a result of (micro) gel swelling [133].

4.3.2. Loading pathways for swelling-controlled release systems

An efficient loading of functional compounds is here realized by their embedding in non-swollen glassy, dry or collapsed gel networks, thus hindering an unwanted diffusion. This can be achieved either *in situ* by the formation of microgels dispersed in a non-solvent for the polymer chains or by the *post-formation* approach which is based on a (stimulus-) induced network collapse or drying of the loaded (micro)gels [134]. While the respective release mechanism is different from diffusion controlled systems, loading can obviously be achieved in a similar way.

4.3.3. Loading pathways for chemically-controlled release systems

Depending on the respective release mechanism, this concept can be realized by different pathways. In the case of bulk-degrading release systems, the entrapment of functional compounds is mainly realized either by (radical) crosslinking copolymerization of monomers and crosslinking agents or covalent or physical crosslinking of preformed polymers in the presence of the substance to be released [135–137]. An efficient loading is ensured by a hindered diffusion due to mesh sizes in the size range of the hydrodynamic diameter of the active molecules. For pendant chain degrading systems, a functional compound is attached to the polymeric network by a degradable linker therefore preventing leakage. In the case of macroscopic hydrogels, it was demonstrated that this can either be realized by the functionalization of the compound with a reactive unit linked to the active molecule via a cleavable linker and the *in situ* co-reaction with the network-forming building blocks and crosslinking agents, or by the *post-formation* attachment of the substance to be delivered to the preformed gel network [138]. The transfer of these methods to the nanoscale by using microgels is not yet fully exploited and represents a highly promising perspective for future research.

4.3.4. Different approaches to stimuli-responsive microgels

As mentioned before, stimuli-responsive microgels are a unique class of materials since they combine specific interesting features: (a) The characteristics of gel networks such as e.g. the structural integrity and a high solvent content in their swollen state gives rise to loading and release applications of functional compounds. (b) Transferring these inherent gel features to the nanometer scale bears the advantages of a high surface area and a high diffusibility and mobility of the gel nanoparticles, thus allowing their facile distribution in specific environments. (c) In the case of stimuli-responsive microgels, a big advantage compared to their macroscopic analogs is their comparably much faster response to a stimulus [139]. Early investigations on the swelling kinetics of spherical macroscopic polyacrylamide gels by Tanaka and Fillmore revealed that the speed of swelling is inversely proportional to the square of the gel radius [140]. In other words, the relaxation time of the volume change of a gel is proportional to the square of its radius [141].

The response mechanism of these nanomaterials crucially depends on two important features. First, the overall sensitivity of microgels is defined by the specific type of stimulus to which these materials respond. This is governed by the particular nature of functional groups responsible for the sensitivity of the gel. Second, the location of these moieties in the network structure as well as their specific triggered response mechanism determines the overall stimuli-dependent behavior of the microgels. In this chapter, different approaches to stimuli-responsive microgels are

introduced and their potential for loading and release applications is reviewed. Fig. 4 schematically depicts possible mechanisms for changes of stimuli-responsive microgels characteristics. The respective response of microgels to a specific external trigger is often characterized by a change in the physico-chemical properties of the polymeric network, thus resulting in a controlled swelling and deswelling of the particles. This effect is generally accompanied by changes of numerous important features of the microgels such as, e.g., the solvent content, the mesh size, the refractive index, and the interior network permeability (see Fig. 4a). Even though this (reversible) volume phase transition is often described as the defining characteristic for stimuli-responsive microgels in the literature, several additional response mechanisms of microgels to an external trigger can be found. Alternatively, the utilization of cleavable crosslinking points in a gel represents a method for triggering the complete (reversible) decomposition of the network architecture by using external stimuli (Fig. 4b). Moreover, the introduction of stimuli-sensitive groups onto microgels surfaces can be used to trigger the interaction either between the gel nanoparticles and external compounds or among each other (Fig. 4c). As a last example, the utilization of linkers - degradable upon the appliance of an external trigger - between functional compounds and the microgel network is worth mentioning (Fig. 4d).

Since a discussion of all stimuli-responsive mechanisms would extend the scope of these remarks, the following section deals with the description of stimuli-responsive microgels for loading and release applications by either exploiting triggered changes in the physico-chemical parameters of the network-forming polymer or labile crosslinking points.

4.3.5. *Stimuli-responsive microgels based on triggered changes in the physico-chemical parameters of the network-forming polymer*

The fundamental concept of stimuli-responsive microgels based on triggered changes of the physico-chemical parameters of their network-forming polymers is a (reversible) volume phase transition of the gel nanoparticles as response to an external trigger. This behavior is induced by a changed solvation of the polymer chains as a result of reversibly triggered variations in the interplay between solvent–solvent, solvent-polymer and polymer–polymer interactions.

4.3.6. *Temperature-sensitive microgels*

Probably the most prominent approach to thermo-sensitive microgels is based on the utilization of poly(*N*-isopropylacrylamide) (PNIPAAm) as network-forming polymer [3]. This polymer exhibits a lower critical solution temperature (LCST) in water. The underlying mechanism is an endothermic, entropically driven phase transition from a random coil to a collapsed globule at temperatures above ~32 °C [142]. Two main effects influence this behavior: on one hand, the hydrogen bonding between amide groups and water molecules and on the other hand, the entropically driven polymer–polymer interactions due to the hydrophobic isopropyl groups. At low temperatures the polymer–water interactions are favored, thus resulting in a random-coil configuration of the polymer. If now the temperature is increased, the hydrophobic polymer–polymer interactions become more dominant, water is expulsed due to the weakened hydrogen bonds and the polymer chain reorganizes to a collapsed globule [143]. In the case of microgels formed from crosslinked PNIPAAm polymers, the inherent thermo-sensitive characteristic of the network-forming polymer is transferred to the entire network. Here, at temperatures below the LCST of the polymer, the gel nanoparticles are swollen and shrink upon increasing the temperature. This phase transition occurs at the so called volume phase transition temperature (VPTT) which is close to the LCST of the PNIPAAm chains [144]. Nevertheless, compared to free PNIPAAm chains, the phase transition of microgels consisting of the same crosslinked polymer is not as sharp and shifted to higher temperatures. This deviation, more exactly, the width of the volume phase transition was shown to depend on the crosslinker content [145]. While the question of the exact origin of this phenomenon is still under discussion, Wu et al. proposed that this phenomenon is a result from the heterogeneous crosslinking apparent in the network. Since in the case of PNIPAAm microgels longer polymer segments between two crosslinking points collapse at lower temperatures as shorter ones, the observed broad volume phase transition can be considered as a superposition of all phase transition temperatures of the different polymer segments [3,146].

PNIPAAm based microgels are mostly prepared by precipitation polymerization exploiting the LCST of the polymer for nanoparticles formation. Since the first synthesis of such structures reported by Pelton and Chibante in 1986 [147], the well defined response of these materials to temperature as external trigger has led to a broad variety of sophisticated applications over the years. In this context, the VPTT of PNIPAAm close to the physiological temperature renders these systems highly interesting for biomedical fields such as e.g. drug delivery. However, even though the VPTT of PNIPAAm microgels around 33 °C is already slightly higher than the LCST of the free polymer, the value is still lower than the body temperature. Therefore, various attempts have been performed to increase the VPTT by the incorporation of either anionic [53] or cationic [148] hydrophilic comonomers. Although these attempts were successful to increase the VPTT of the resulting microgels, the volume transition was broadened as well. This effect can be influenced by the exploitation of different crosslinking

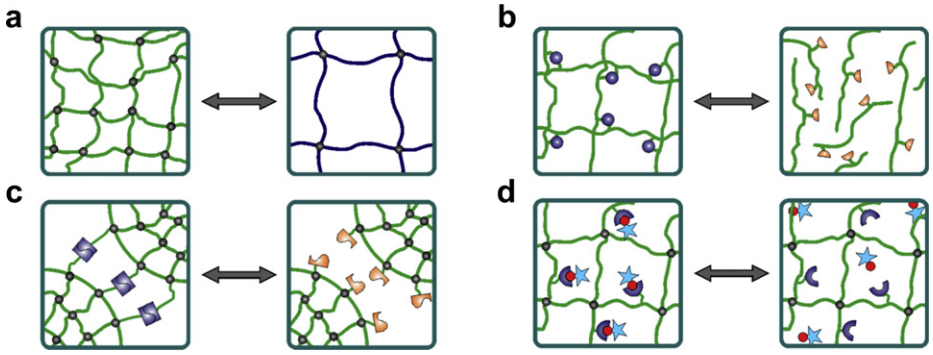

Fig. 4. Schematic representation of different mechanisms of stimuli-responsive microgels: (a) gel swelling/deswelling due to stimuli-induced changes in physico-chemical parameters of the network-forming polymer; (b) gel dissolution/formation upon triggered degradation/formation of crosslinking points; (c) externally triggered degradation/formation of intramicrogel aggregates and (d) triggered attachment/release of functional compounds onto/from the interior microgels network.

techniques such as the self-crosslinking of PNIPAAm [149] or the utilization of inorganic clay as crosslinker [150] but results in microgels of a reduced VPTT again. It can be seen that the specific adjustment of the VPTT and the transition broadness to values suitable for applications in biomedical fields is rather complex and still an important research area.

In the context of temperature-controlled drug delivery systems based on PNIPAAm microgels, several loading and release concepts have been investigated. In general, it can be distinguished between release mechanisms based on a gel collapse upon increases in temperature and mechanisms based on gel swelling upon lowering the temperature.

As examples for the release of entrapped drugs in response to increases in temperature, either passively exploiting the higher temperature of some pathological tissues or cells [151] or actively triggering the release by hyperthermia [152] are two concepts worth mentioning. Here, the release is based on a squeeze-out mechanism: as the microgels collapse due to an increased temperature, water is expelled from the network and the drug is released. This concept of drug release upon microgel deswelling was described by Nolan et al. for the delivery of insulin from PNIPAAm microgels [153]. The same volume transition of PNIPAAm microgels can be used for cancer therapy by exploiting the inherent volume change of the microgels even without the need for additionally incorporated pharmaceutically active compounds. As recently described by Lyon and coworkers, the aggregation of collapsed folate functionalized PNIPAAm microgels in the cytosol at elevated temperatures resulted in temperature-dependent cytotoxicity [154].

In contrast to the previously mentioned squeeze-out mechanism induced by heating, the release of entrapped molecules upon cooling-induced particle swelling is based on an increased diffusivity of the embedded compounds in a gel network of increased mesh sizes. A sophisticated approach described by Nayak et al. is based on thermo-sensitive hollow PNIPAAm microspheres prepared by the removal of a sacrificial core from core/shell microgels [62]. In a similar template-assisted synthetic pathway, Gao et al. prepared thermo-sensitive PNIPAAm nanocapsules loaded with FITC as model protein. While the fluorescent macromolecule was efficiently entrapped in the cavity at temperatures above the LCST of the shell-forming polymer, decreasing the temperature enabled its release [155]. Exploitation of the externally triggered temperature-dependent swelling of microgels for drug delivery applications was recently described by Park et al. using a brief cold-shock treatment to induce the swelling of crosslinked Pluronic (PEO-*b*-PPO-*b*-PEO) based microgels [156].

Although lowering the local temperature is an interesting novel concept to temperature-triggered cancer therapy based on thermo-sensitive materials, the utilization of elevated temperatures as stimulus is comparably more facile to realize by established methods such as e.g. hyperthermia. As mentioned above, the utilization of the "squeeze out" mechanism of negatively temperature-sensitive microgels based on polymers exhibiting an LCST (e.g. PNIPAAm, PVCL [157], etc.) is a widely investigated approach to realize this concept but bears some serious drawbacks. Here, the formation of a skin layer on the deswelling microgels can significantly hinder the desired drug release upon heating [96], thereby restricting this concept to low molecular weight drugs (biomacromolecules such as e.g. proteins are entrapped in the network which can be used as a loading pathway). As a consequence, the utilization of materials exhibiting a positive volume phase transition – i.e. an increase in swelling upon increasing temperature – is assumed to dramatically enhance the efficiency of thermo-sensitive microgels for drug delivery applications.

Polymeric materials fulfilling this criterion are polymers exhibiting an upper critical solution temperature (UCST). A widely investigated example is based on copolymers of acrylamide and acrylic acid. In these polymers, the volume change is driven by hydrogen bonds between the macromolecules. At temperatures below the UCST the polymer–polymer interaction is favored, thus resulting in a collapsed coil structure of the macromolecule. Upon increasing the temperature, these interactions are weakened due to the breakage of hydrogen bonds and the polymer–solvent interaction becomes dominant. As a result, the polymer exhibits a random-coil morphology [158,159]. While the preparation of macroscopic hydrogels from these materials was achieved by hydrophobic association crosslinking by Yang et al. [160], the transfer of this concept to the nanoscale was recently demonstrated by Echeverria et al. by radical crosslinking copolymerization of acrylamide with acrylic acid in the presence of *N,N′*-methylenebis(acrylamide) in inverse emulsion [161]. Microgels prepared by this synthetic pathway represent an interesting alternative to the widely examined systems exhibiting a negative volume phase transition.

Moreover, thermo-sensitive microgels containing functional compounds not only find application in release applications. The temperature-dependent volume phase transition and the corresponding change in mesh sizes of the gel network can also be used to trigger the accessibility of embedded active compounds to substances in the microgel environment. As example, Park et al. described the embedding of β-galactosidase in PNIPAAm-*co*-PAAm microgels and demonstrated the possibility to trigger the enzymatic hydrolysis of *o*-nitrophenol-*p*-D-galactopyranoside (ONPG) by changing the temperature in either a batch mode or a packed bed reactor [100]. In addition, Ballauff and coworkers investigated the immobilization of catalytically active metal nanoparticles in PS/PNIPAAm core/shell nanoparticles and demonstrated the temperature triggered catalytic activity [102].

4.3.7. Microgels sensitive to pH and ionic strength

Microgels prepared by crosslinking of weak polyelectrolytes exhibit a pH-dependent volume phase transition. Depending on the composition of the polyelectrolyte, it can be distinguished between acid containing cationic, base containing anionic or acid and base containing amphoteric microgels. The underlying mechanism of the swelling/deswelling of these materials as response to changes of the pH of the surrounding medium is the protonation/deprotonation of the weakly acidic or basic groups along the chain of the network-forming (co)polymers. The key parameter determining the swelling behavior of pH-responsive microgels is the respective critical pH value (pH_c) at which the phase transition occurs. Although this parameter is governed by the chemical nature of the polyelectrolyte, the respective pH value is defined as the point where the degree of ionization of the network-forming polymer changes. As a result of either an increased or decreased osmotic pressure within the microgels, swelling or deswelling occurs. Even though the pH_c is correlated to the respective pK_a or pK_b values of the acidic or basic groups on the polymer backbone, it has been demonstrated that the apparent pH_c value of microgels can deviate from the values of their low molecular weight analogs [96]. Especially the introduction of more hydrophobic (alkyl) moieties to the polyelectrolyte backbone can shift the pH_c values [162].

4.3.8. Anionic microgels

In the case of anionic microgels composed of weak acidic polymers such as e.g. poly(meth)acrylic acid, the gel network is collapsed at pH values below the pK_a of the polyelectrolyte due to the absence of charges and the resulting comparably hydrophobic character of the network. Increasing the pH above the pK_a of the polymer, the acidic groups are deprotonated, thus increasing the hydrophilicity of the polymer. Moreover, due to the presence of

generated anionic groups in the network, their electrostatic repulsion and the generated osmotic pressure, the swelling is significantly enhanced. In general, the swelling profile of anionic microgels depends on various parameters of the network-forming polymer such as the amount of acidic groups, their respective pK_a values and the crosslinking density.

The influence of the pK_a value of different acidic moieties attached to a polymeric backbone in the microgel network on the swelling profile was examined by Needham and coworkers [163]. It was shown that in microgels containing a fixed amount of methacrylic acid groups together with similar amounts of different acidic moieties, the pH range of the swelling response shifted by an amount proportional to the solution pK_a's of the different functional groups.

In order to examine the influence of the crosslinking density, detailed investigations on the pH-dependent swelling behavior of poly(methacrylic acid-*co*-acrylic acid) microgels have been performed by Eichenbaum et al. [130]. Microgels were prepared by crosslinking precipitation copolymerization of methacrylic acid and 4-nitrophenyl acrylate in the presence of various amounts of MBA as crosslinking agent. The subsequent hydrolysis of the nitrophenyl groups represents a sophisticated synthetic pathway to highly charged microgels which is challenging by precipitation polymerization of the respective acid monomers. It was demonstrated that the microgels exhibit a volume phase transition from the deswollen to the swollen state upon increasing the pH above pH 5.3. Furthermore, it was observed that the maximum degree of swelling (Q_{max}) at pH values $>$ 5.3 decreased linearly with an increasing degree of crosslinking (i.e. the feed ration of MBA). Fig. 5 shows the respective plots adapted from the publication [130].

Another highly important factor to be considered in the field of microgels based on polyelectrolytes is the dependency of the swelling profile on the ionic strength of the surrounding medium. Here, the addition of NaCl to highly swollen microgels at pH values above the pHc was found to result in a decrease of the swelling ratio. This effect can be explained by a shielded electrostatic repulsion of the anionic groups due to the presence of the positively charged sodium counterions. The respective plot derived from the publication is shown in Fig. 6 [130].

Since the observed swelling profiles in dependency on the crosslinking density (see Fig. 5) were found to be in good agreement with a model derived from the Flory-Huggins thermodynamic theory for the swelling of ionic networks [130], these findings represent an important step to be able to optimize microgels for their specific utilization in loading and release applications.

Loading of anionic microgels can e.g. be achieved via a post-formation pathway by exploiting the electrostatic interactions between anionic groups in the swollen gel network and positively charged macromolecules [131] or low molecular weight drugs [129]. An important factor to be considered is the careful adjustment of the pH of the microgel dispersion to guarantee an efficient loading. In this context, three parameters defining the optimum pH value for loading have to be taken into account: (1) the microgels should be in their swollen state to ensure the diffusion of the compound into the network, (2) the acidic groups of the network should be deprotonated to enable the loading due to electrostatic interactions with the functional compound, and (3) for the same reason, the functional compound should exhibit a positive net charge. While the loading of functional macromolecules such as e.g. proteins into the microgels is further determined by permeability of the gel network and respective size exclusion experiments can even be used to estimate the mesh size of the network [130], the loading of small molecules is dependent on their partition coefficients and resulting binding affinities, molecular packing and the condensation of the network upon loading [130].

Ionic microgels loaded with functional compounds can be used for drug delivery applications by responding to pH changes at the targeted delivery site. Here, it is exploited that the extracellular pH of tumor tissues is more acidic than the pH of the surrounding healthy tissues [164]. Moreover, the pH of intracellular lysosomes or endosomes is shifted to more acidic values than the cytosolic pH [165]. Based on these considerations, Das et al. demonstrated the utilization of PNIPAAm-*co*-PAA microgels for the delivery of doxorubicin [128]. The microgels surface was functionalized with transferrin to enable an enhanced uptake in cancer cells. The drug was released in the slightly acidic cytosol of HeLa cells due to the protonation of the carboxylic acid groups and the corresponding deswelling of the microgels and the weakened electrostatic interaction between drug and polymer network.

In contrast to this release mechanism upon lowering the pH, a pH-induced swelling of particles can also be used to enable drug diffusion out of the network. As example, poly(methacrylic acid-*co*-ethylacrylate) microgels were described by Tan et al. for the swelling induced release of incorporated procaine hydrochloride [166].

4.3.9. Cationic microgels

Regarding cationic microgels based on polymers containing weak basic groups such as e.g. amino moieties, the critical parameter determining their swelling behavior is the pK_b value of the respective basic groups. These cationic microgels exhibit a pH-

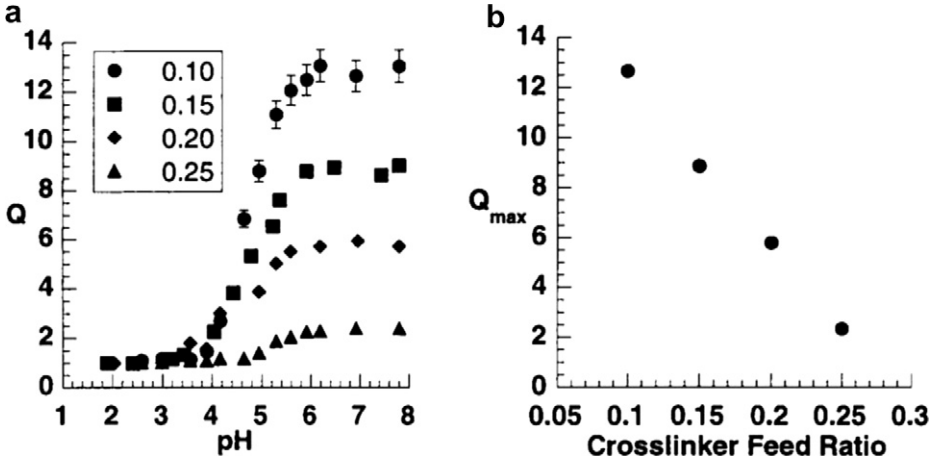

Fig. 5. (a) Plot of the equilibrium swelling ratio (Q) for the different cross-link density microgels versus the pH of the external solution. (b) Plot of the maximum swelling (Q_{max}) for the different cross-link density microgels versus the feed ratio for the pHs greater than 5.3. (Reproduced from [130], with permission from American Chemical Society.)

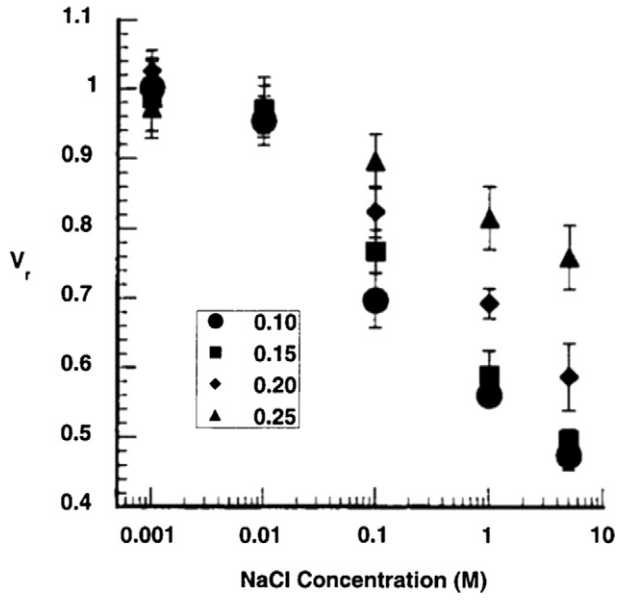

Fig. 6. Plot of V_r for the different cross-link density microgels versus NaCl concentration in the high pH regime (pH > 5.3). (Reproduced from [130], with permission from American Chemical Society.)

dependent volume phase transition orthogonal to the anionic analogs. While at pH values above the pK_b value the network is collapsed due to the absence of charges, decreasing the pH below the pK_b of the incorporated basic groups induces the protonation of the latter and the positively charged groups cause the swelling of the network.

Microgels exhibiting an increase in their degree of swelling as response to a decrease of the pH represent interesting candidates for drug delivery applications based on a "smart" response to such inherent features as the acidic environment of tumor tissues and intracellular compartments. The respective release mechanism is based on enhanced drug diffusion from the network as a result of increased mesh sizes. Hence, these systems are an orthogonal approach to the previously mentioned squeeze out mechanism of anionic microgels.

The positive charges responsible for microgel swelling can be introduced to the network by various different approaches and by the utilization of several functional groups. In the field of drug and gene delivery, these cationic groups bear several advantages. On one hand, the cellular uptake of cationic compounds by adsorptive endocytosis is assumed to be enhanced by their electrostatic interaction with the negatively charged cell membrane [167]. On the other hand, cationic polyelectrolytes represent a well examined system for the complexation of DNA and oligonucleotides. In this context, poly(ethylene imine) (PEI) represents the "golden standard" for polyelectrolyte gene delivery systems [168].

Cationic microgels based on PEI as pH-sensitive polyelectrolyte were prepared by crosslinking of bis-activated poly(ethylene oxide) (PEO) (both ends activated with 1,1′-carbonyldiimidazol) with PEI in an emulsification-evaporation method [169]. The cationic PEO-*cl*-PEI microgels exhibited a pH-dependent swelling profile and were used for the loading and release of either anionic amphiphilic molecules or oligonucleotides.

Another approach for the loading and release of oligonucleotides was recently described by Deka et al. [132]. Radical crosslinking copolymerization of various compositions of 2-vinylpyridine (VP) and divinylbenzene (DVB) in an emulsion polymerization yielded cationic microgels in the size range of 90–220 nm. The microgels exhibited a sharp increase in diameter upon decreasing the pH of the surrounding medium below pH 4.3. The correlating increase in network permeability was used for the simultaneous post-formation loading of the microgels with iron oxide nanoparticles and oligonucleotides. An efficient loading was hereby achieved by the entrapment of the comparably large compounds by increasing the pH. The simultaneous release was demonstrated to occur as a response to a decrease in pH.

Despite their potential for the loading and release of functional compounds of large hydrodynamic diameters, cationic microgels have also been described for the delivery of small molecule drugs. Zhang et al. reported on the synthesis of cationic microgels based on chitosan ionically crosslinked by sodium tripolyphosphate [170]. The gel nanoparticles were conjugated with apo-transferrin, and loaded with methotrexate disodium as cytotoxic drug for cancer treatment. Loading was achieved by electrostatic interactions between the negatively charged drug and the positively charged polymer network. The loaded microgels were found to enter HeLa cells via receptor mediated endocytosis and release the drug as a response to the intracellular low-pH environment by swelling, thus killing the immortalized cancer cells.

4.3.10. Light-sensitive microgels

As described in the previous sections, a (reversible) volume phase transition in hydrogel nanoparticles as a result of a triggered change in the physico-chemical parameters of the network-forming polymer is often induced by the alteration of the hydrophilicity of polymer bound functional groups. In the case of light as external stimulus, several chromophores are known to change their polarity upon irradiation, thus being potential candidates for such approaches. Here, especially light-triggered isomerization reactions are worth mentioning. As an example, azobenzenes undergo a *trans-cis* isomerization upon the irradiation with UV light whereby the *cis* state of the molecule is characterized by an enhanced dipole moment resulting in an increased hydrophilicity. Since this reaction is reversible either via temperature or visible light induced relaxation, attaching these chromophores to a polymeric backbone represents an interesting approach for the light-triggered alteration of the overall hydrophilicity of the respective polymer. This concept was successfully applied for the formation of light-sensitive hydrogels changing their degree of swelling upon irradiation with UV light. These were synthesized by the crosslinking copolymerization of acrylamide with *trans*-4-methacroyloxyazobenzene in the presence of MBA as crosslinking agent [171]. Moreover, double stimuli-responsive materials exploit the influence of the light-triggered isomerization of azobenzenes on the response range of the respective orthogonal trigger. Examples include the irradiation-induced shift of the LCST of copolymers from NIPAAm and an azobenzene group containing monomer [172] as well as the shrinking of initially swollen p(acrylic acid-*co*-acrylamido azobenzene) hydrogels (at pH values above the pK_a of the AA) by the *cis-trans* isomerization of the azobenzene moieties [173]. Even though these examples represent promising approaches to light-triggered materials (based on direct changes of the physico-chemical parameters of the polymer), their utilization in the nanoscale is up to now mainly applied to light-sensitive block copolymer micelles [174] or polymerosomes [175,176].

Nevertheless, light-sensitive microgels can be realized by an indirect approach based on hybrid materials. These systems are composed of a photo-sensitive moiety embedded in a temperature-sensitive polymeric network. The underlying concept is the photothermal effect, meaning that upon irradiation at the resonance wavelength of the respective light-responsive compound, the light energy is translated to heat by non-irradiative relaxation. As a result to the locally increased temperature of the surrounding thermo-sensitive network, the latter exhibits a volume phase

transition. Although this concept can be achieved by the utilization of a broad variety of photo-sensitive compounds and temperature-sensitive polymers, the embedding of dyes [177,178] or metal nanoparticles (NP) [179] into PNIPAAm gels are the most widely examined approaches.

Especially the incorporation of Au or Ag nanoparticles or −rods into thermo-sensitive microgels is a highly interesting concept and the preparation of such materials can be achieved by different pathways including the *in situ* reduction or precipitation of NP in the network [180], the network formation around NP seeds by precipitation polymerization [181], the adsorbance of NPs onto microgels surfaces [89] or their absorbance into the network [182]. Despite their different origin, the final materials exhibit the similar light-induced volume phase transition of the network. Fig. 7 depicts the irradiation-induced variation of the relative volume of hybrid Au nanorods containing p(NIPAAm-*co*-AA) microgels in comparison to pure polymeric microgels as described by the group of Kumacheva [183].

Regarding the potential of these materials for biomedical applications it is highly beneficial that the absorption spectra of nobel metal NPs can be tuned over a wide spectral range to absorb light in the NIR window (650–900 nm) while exhibiting a large optical cross-section and no self-quenching effects [184]. The inherent advantage of this particular resonance wavelength is its minimal absorbance by skin and tissue, therefore enabling a penetration depth of several hundreds of micrometers up to centimeters [175]. Another big advantage of thermo-sensitive hybrid microgels in the field of cancer treatment is the combination of their inherent ability to influence cell viability due to the photo-induced hyperthermia [185] with the potential of the externally triggered release of specific chemotherapeutics [186], thereby dramatically enhancing the therapeutic efficacy [96].

Multi-responsive microgels, complex stimuli-responsive microgel architectures and stimulus-induced transformation of hydrophobic polymeric nanoparticles to microgels.

Multi-responsive microgels in general are materials responding to more than one external trigger. The previously described Au@PNIPAAm hybrid microgels are an example for a special class of these materials, since the response to one trigger A (light) results in the creation of a second stimulus B (heat) which finally induces a response of the polymeric material. Therefore, the pure microgels are only sensitive to one trigger (B, heat). In contrast, actually double-stimuli responsive materials can be divided into gels responding orthogonally to either one of the single stimuli (type "A or B") and in networks which exhibit a response only if all stimuli are applied simultaneously (type "A and B") [96]. Since the latter are characterized by an enhanced selectivity of their response to the respective specific combination of triggers, the type "A or B" microgels are potential materials for new loading and release techniques based on the subsequent appliance of different stimuli. For the preparation of such compounds it has to be taken into account that the co-existence of various stimuli-responsive components within one microgel can result in an interference of the respective sensitivities. This effect can be desired and was already described earlier in the context of the influence of pH-sensitive groups on the VPTT of thermo-sensitive microgels [56]. However, a differentiation between the individual responses to different stimuli can be achieved by the spatial separation of the specific functional groups responsible for the respective sensitivities. A sophisticated approach to realize this concept is the preparation of core/shell microgels consisting of different stimuli-responsive network-forming polymers in the core and the shell. Jones and Lyon described the preparation of PNIPAAm (core)/PNIPAAm-*co*-PAA (shell) microgels and demonstrated the appearance of a temperature-dependent multistep volume phase transition for high pH values where the PAA component is highly charged [60]. In a similar approach, core/shell microgels consisting of two different thermo-sensitive polymers were prepared by Berndt and Richtering [187]. Since the core was formed from PNIPAAm and the shell from poly(*N*-isopropylmethacrylamid) (PNIPMAAm) exhibiting a comparably higher LCST of 45 °C, the temperature-dependent swelling profile revealed two phase transitions corresponding to the two LCSTs of the respective polymers. These materials are potential systems for the independent release of different compounds from separated compartments within one carrier.

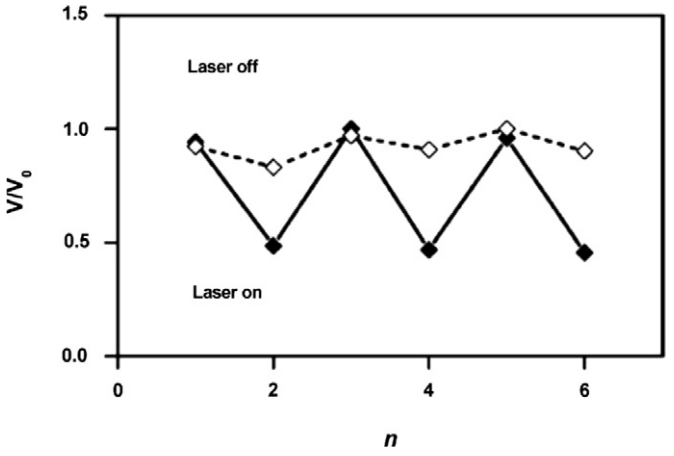

Fig. 7. Variation in volume of pure (◇) and hybrid (◆) p(NIPAAm-*co*-AA) microgels plotted as a function of the number of *laser on* and *laser off* events *n*. Both microgel systems were irradiated at $\lambda = 810$ nm. (Reproduced from [183], with permission from American Chemical Society.)

As mentioned above, multi-responsive microgels of complex architectures give rise to new loading and release mechanisms. An interesting example in this research area was described by Needham et al. [188]. They prepared pH-sensitive PMAA microgels and exploited the anionic character of the gel for the loading of positively charged doxorubicin upon electrostatic interactions with the network. The drug was efficiently entrapped in the carrier system by lowering the pH and by subsequently coating the collapsed microgels with a lipid double layer. The latter served as a diffusion barrier for the loaded drug and prevented the core/shell particles from swelling upon immersing these in aqueous medium of a pH > pK_a of PMAA. A successful release was achieved by selectively disrupting the shell upon the appliance of short electric pulses as second trigger orthogonal to the pH-sensitivity used for loading.

If it comes to release applications of microgels in the biomedical field, most investigated systems are limited to the incorporation of hydrophilic active compounds into hydrophilic networks. In order to extend this concept to the delivery of hydrophobic compounds, hydrophobic nanoparticles consisting of poly(lactic-glycolic acid) represent an approach based on the biodegradability of the polymer backbone. Nevertheless, these materials cannot be assigned to the field of microgels. An alternative mechanism for the delivery of hydrophobic compounds from hydrophilic microgels was recently demonstrated by Griset et al. [116]. The described concept is based on the generation of swollen hydrogel nanoparticles from hydrophobic polymer latexes as a result of a stimulus-induced transition of the hydrophilicity of the network-forming polymer from hydrophobic to hydrophilic. To this extend, crosslinked particles consisting of a hydrophobic polymer containing acetal protected diol groups in the backbone were prepared by inverse miniemulsion polymerization. As a response to a pH change to slightly acidic conditions (pH 5), these nanoparticles expanded several hundred-fold in volume due to the acid catalyzed deprotection of the diol groups, thus generating the swollen hydrogel. Fig. 8 depicts

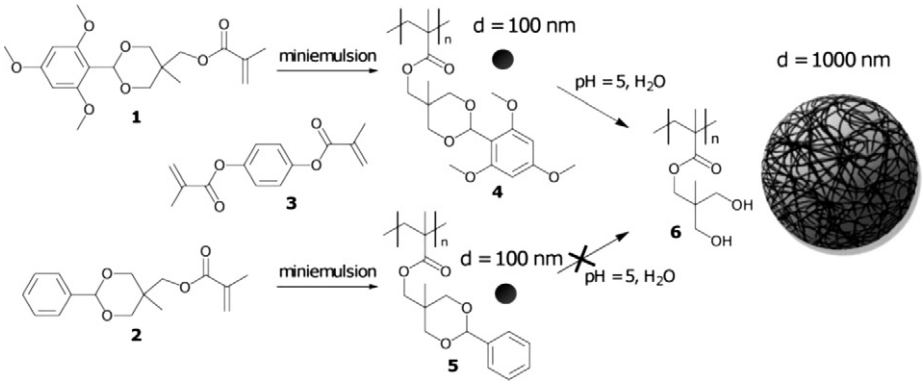

Fig. 8. Synthesis of nanoparticles with differing pH responsiveness obtained from crosslinking polymerization of either 1 or 2 in the presence of 3. The protecting group of nanoparticle 4 but not 5 is cleaved at a pH of ~5. This transformation reveals the hydrophilic hydroxyl groups and formation of nanoparticle 6 with resulting expansion of the hydrogel nanoparticle in water. (Reproduced from [116], with permission from American Chemical Society.)

the underlying mechanism. If loaded with paclitaxel as poorly water soluble anti cancer drug, this drug delivery system was shown to prevent establishment of lung cancer *in vivo*.

Based on a similar concept, Klinger et al. recently demonstrated the facile preparation of hydrophobic nanoparticles consisting of a photo-resist polymer by free radical polymerization of a photo-labile *o*-nitrobenzyl ester of methacrylic acid in miniemulsion [189]. These latexes were designed to be degradable upon a light-induced change of the hydrophobicity of the respective material. De-protection of the methacrylic acid groups on the polymeric backbone was easily achieved by the photolytic cleavage of the *o*-nitrobenzyl esters. As a result, conversion of the initial hydrophobic polymer into hydrophilic PMAA induced *in situ* particle dissolution in water which was shown to be applicable for the light-induced release of embedded nile red as a hydrophobic model compound and fluorescent probe. Fig. 9 schematically depicts the described concept.

In comparison to the potential release of hydrophilic compounds from photo-sensitive hydrogel nanoparticles the liberation of hydrophobic substances in aqueous medium dramatically extents the field of potential applications for light-responsive carriers. Moreover, the confinement of a photo-resist material to nanoscale structures is assumed to give rise to potential new ways of surface patterning by e.g. colloidal lithography.

4.3.11. Stimuli-responsive microgels based on cleavable crosslinking points

The fundamental concept of stimuli-responsive microgels based on either complete or partial cleavage of crosslinking points is the resulting increase in swelling or total dissolution of the microgels. As a result of the decreasing crosslinking density during this process, the mesh sizes in the network are increased, thus giving rise to enhanced diffusivity of embedded compounds which can be used for release applications. Several different triggers have been examined to induce the crosslinker's degradation and will briefly be discussed in this section.

4.3.12. Microgels containing hydrolytically cleavable crosslinkers

A widely investigated concept to degradable microgels is based on the utilization of crosslinking agents containing hydrolytically cleavable groups [135]. In this area, a facile approach is based on ester moieties, since their hydrolysis can be triggered by several changes of the chemical environment such as the presence of hydroxide ions, protons or enzymes. While this inherent feature has been exploited for the preparation of biodegradable nanoparticles from e.g. poly(lactic-*co*-glycolic acid) [190], Kim and Graham successfully transferred this concept to hydrogel nanoparticles. The microgels prepared from crosslinked poly(-caprolactone diol) were shown to be degradable upon incubation in acidic, alkaline or enzymatic solution of esterase at physiological temperature [191]. Even though these materials represent interesting candidates for release applications, their degradation is rather unspecific with respect to the types of external triggers and, as a result, the degradation rate is comparably low. However, for many applications a fast and precise response to a specific stimulus is desired in order to increase the respective efficacy. Thus, increasing the hydrolytic lability of crosslinking points represents an active research area and can be achieved by the precise adjustment of the molecular structure of crosslinkers (i.e. the incorporation of highly sensitive labile groups). Jhaveri and Carter exploited the well known acid-sensitivity of tertiary esters and prepared degradable PMMA microgels with 2,5-dimethyl-2,5-hexanediol dimethacrylate (DHDMA) as crosslinking agent [192]. These polymeric microgels were found to be degradable upon the addition of *p*-toluenesulfonic acid even in the absence of water,

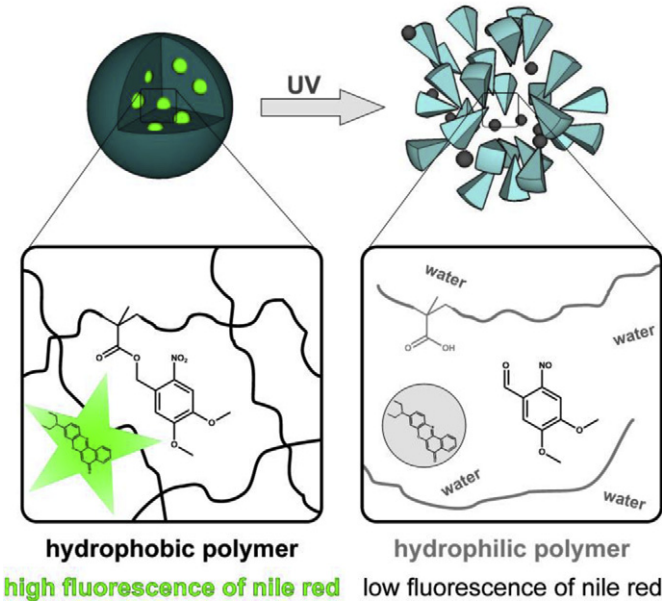

Fig. 9. Schematic illustration of the concept of light degradable polymeric photo-resist nanoparticles. Overlapping of schematic polymer chains in the initial particles refers to entanglement not to crosslinking. (Reproduced from [189], with permission from Wiley.)

thereby representing an example for the specific degradability in dependency on one particular trigger (i.e. the presence of protons).

Transferring this conception to drug delivery applications, it is of special interest to design carrier systems responding to an inherent feature of the site targeted for the release. As mentioned before, this can be achieved by taking advantage of the slightly acidic pH values in cancer tissues and intracellular compartments. In comparison to tertiary esters, requiring elevated temperatures and a highly acidic pH for their fast degradation, acetals as protection groups are well known to be hydrolyzable under mild conditions, thus rendering them beneficial for their utilization as labile moieties in biomedical applications. Fréchet and coworkers prepared acid-degradable polyacrylamide microgels containing hydrolyzable acetal moieties in the crosslinkers for the encapsulation and acid-triggered release of proteins [136]. Fig. 10 depicts the followed concept.

Similar acid-degradable microgels containing ovalbumin as an example of protein-based vaccines prepared in the same group were demonstrated to release their payload upon degradation under mildly acidic conditions in the phagosomes of antigen-presenting cells. The release of the protein was shown to activate ovalbumin-specific cytotoxic T lymphocytes *in vitro* [193]. By increasing the hydrophilicity of the labile crosslinker structure the loading efficiency and the resulting antigen presentation levels could be dramatically enhanced. Moreover, preliminary *in vivo* experiments proved enhanced survival rates for tumor-challenged mice [194]. In addition, the versatility towards the encapsulation of different biologically active macromolecules such as e.g. plasmid DNA [195] clearly demonstrates the great potential of these delivery systems. Especially the divinyl functionalization of the used crosslinkers enables their utilization in radical copolymerizations with a broad variety of monomers thus giving rise to the facile preparation of a multitude of acid-degradable polymeric microgels [196]. The concept of acid-labile crosslinkers can even be extended to more complex structures such as e.g. hollow microgel capsules prepared from polyvinylamine (PVAm) with ketal moieties containing crosslinks described by Shi et al. [197].

In addition to pH changes throughout the body, another biological relevant trigger is the presence of certain reducing agents in specific compartments. As an example, the accumulation of glutathione in the cytosol is worth mentioning [198]. Therefore, delivery vehicles based on functional groups cleavable in the presence of high reducing agents concentrations are particularly attractive for intracellular delivery [199]. In this context, the utilization of crosslinking molecules containing disulfide bonds hydrolyzable in the presence of reducing agents have gained increasing interest for the preparation of degradable microgels [200]. Bromberg et al. prepared microgels based on poly(acrylic acid) covalently bonded to Pluronic (PEO-*b*-PPO-*b*-PEO) polyether copolymers [201]. Crosslinking of these microgels was achieved by permanent and stable ethylene-glycol-dimethacrylate (EGDMA) groups together with degradable *N,N′*-bis(acryloyl)cystamine. Degradation of the disulfide linkers upon the incubation with tris(2-carboxyethyl-phosphine) (TCEP) was found to result in an increased degree of swelling due to a decreased crosslinking density of the network. An additional advantage of disulfide based crosslinkers is their reversible formation/degradation, which can be used not only for the release of functional compounds upon microgel degradation [202] but also for their entrapment based loading upon crosslinker formation. Regarding the latter, Ryu et al. recently described the formation of self-crosslinked polymer nanogels by chemically induced crosslinking of self assembled copolymers containing oligo(ethylene glycol) (OEG) and pyridyldisulfide (PDS) units [203]. Cleavage of a certain amount of PDS groups by dithiothreitol to the corresponding thiol functionalities and their subsequent reaction with uncleaved PDS groups leads to disulfide-crosslinks. Prepared microgels containing doxorubicin were found to release the drug *in vitro*, thus achieving cytotoxicity upon degradation of crosslinking points.

4.3.13. Microgels containing enzymatically cleavable crosslinkers

In the field of release applications, it is generally of interest to design carrier systems exhibiting a highly specific response to one particular stimulus. In this context, the utilization of enzymatically degradable microgels represents an attractive approach to ensure hydrolytic stability of the network – and thereby the prevention of leakage or degradation of the embedded functional compound – until the targeted site or time point is reached. Here, especially the localization of certain enzymes in distinct compartments of the body enables the design of materials with site specific stimulus-responsiveness. Based on these considerations, hydrogels containing enzymatically cleavable crosslinking points are investigated examples taking advantage of the presence of e.g. azoreductase in the colon [204]. While labile azobenzene moieties for covalent crosslinking are well examined for site specific drug delivery via macroscopic gels [205], the transfer of this concept to the micro-/nanometer scale is assumed to enhance the therapeutic efficacy due to a higher surface area of the carrier systems and a corresponding faster response to the trigger. Hatton and coworkers described the exploitation of 4,4′-di(methacryloylamino)azobenzene as crosslinker for the preparation of enzymatically degradable microgels consisting of a PAA network covalently bound to Pluronic polyether copolymers [201].

Another highly interesting approach to degradable (micro-)gels is based on the utilization of dextrans as naturally occurring polysaccharides that can be cleaved upon incubation with dextranase [206]. Covalent functionalization of dextran chains with either polymerizable vinyl groups [207] or thermal initiators [208] enables the formation of hydrogels by free radical (co)polymerization in aqueous media. The resulting networks can then be degraded by the addition of dextranase, inducing the release of

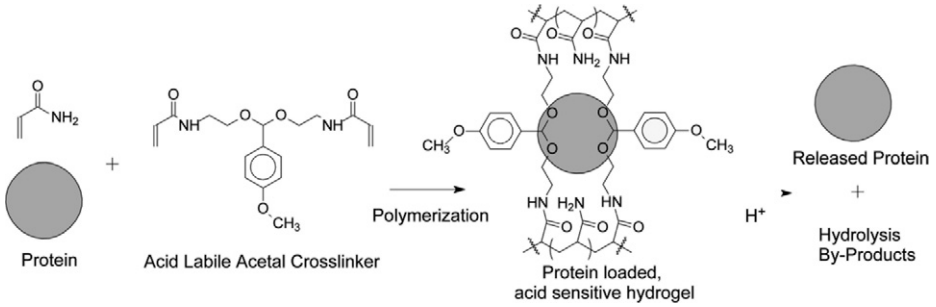

Fig. 10. Schematic representation of the concept of microgels containing acid-degradable crosslinkers. Loading of proteins *in situ* during microgel formation and their release upon acid-catalyzed hydrolysis of crosslinking points. (Reproduced from [136], with permission from American Chemical Society).

embedded active compounds [22]. Here, the dextran polymer can be considered as a macromolecular crosslinker. Microgels based on dextran methacrylates have been investigated by Hennink and coworkers for the release of immunoglobulin G as a model protein [209]. In this case the co-entrapment of dextranase into the network renders the bulk-degradation of these gels rather an inherent feature than the response to an external trigger. As shown in Fig. 11, it was observed that the rate of degradation and thereby the release of IgG is dependent on the degree of substitution (*DS*) (Fig. 11a) of the dextran chains with methacrylate groups as well as on the amount of embedded enzyme (Fig. 11b). Thus, a high loading efficiency as characterized by the reduction of the initial burst release to about 10% was achieved by a high *DS*.

De Geest et al. further extend this concept and demonstrated that coating of these self-degrading microgels with a semi-permeable shell yields self-exploding capsules as promising materials for pulsatile release applications [210]. The degradation of the microgel core leads to an increase in the swelling pressure until the latter exceeds the tensile strength of the surrounding membrane, thus causing shell rupture and release of the payload.

Even though these materials bear a high potential for delivery applications, the used preparation method of precipitation polymerization bears such drawbacks as resulting large particle diameters of 4–10 μm [135,209] and hindered incorporation of strongly hydrophilic comonomers [3]. Based on these considerations, Klinger et al. recently demonstrated the formation of enzymatically degradable nanogels (decreased in size to around 150 nm) consisting of highly hydrophilic polyacrylamide crosslinked with dextran methacrylates by an inverse miniemulsion approach. The crosslinking efficiency as well as the rate of enzymatic degradation was shown to be adjustable by the amount and degree of substitution of the functionalized polysaccharide crosslinker [211].

Another example for more complex microgel structures based on degradable dextrans is the combination of the enzyme-sensitive polysaccharide with thermo-responsive network-forming polymers. Kumashiro et al. demonstrated that the degradability of microgels containing dextrans covalently attached to PNIPAAm and poly(*N,N*′-dimethylacrylamide) (PDMAAm) is adjustable by temperature [212]. Since the network is swollen only at temperatures between the different LCSTs of PNIPAAm and PDMAAm, the enzymatic accessibility of the dextran chains (and thereby the overall degradability) is only given in this temperature range.

4.3.14. Microgels containing photo-degradable crosslinkers

Response mechanisms of microgels containing light-cleavable crosslinking points can generally be divided into two categories: (1) irradiation-induced cleavage of physical crosslinks and (2) photo-degradation of covalent crosslinking points.

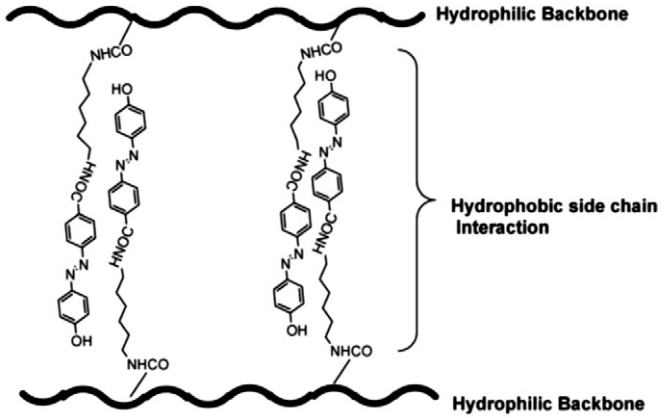

Fig. 12. Schematic representation of non-covalently crosslinked azo-dextran microgels. (Reproduced from [213], with permission from Elsevier.)

Materials of the first category are typically crosslinked by physical aggregates of hydrophobic chromophores attached to a hydrophilic polymer backbone. Utilization of photo-reactive moieties able to change their polarity upon irradiation-induced isomerization renders these hydrophobic interactions sensitive to light as external trigger. Moreover, if the isomerization process is reversible, the crosslinking density can be reversibly adjusted as well. In general, several chromophores exhibiting the described features are reported in the literature. An example for the light-triggered changes in the physical crosslinking density of hydrophilic microgels was reported by Patnaik et al. by the utilization of azobenzenes as photo-reactive chromophores [213]. As shown in Fig. 12, microgel networks were prepared by the self aggregation of pre-formed azobenzene-dextran polymers due to hydrophobic interactions of trans-azobenzene groups attached to the hydrophilic polymer backbone.

As mentioned before, these molecules are able to undergo a *trans-cis* isomerization upon the irradiation with UV light. Since this change in configuration is accompanied by a change in the dipole moment of the molecule, the *cis*-isomer is significantly more hydrophilic, thus weakening the hydrophobic interactions responsible for network-formation. The resulting increase in the degree of swelling of these microgels corresponds to increased mesh sizes. Hence, the release of embedded compounds such as rhodamine and aspirin was found to proceed faster for irradiated microgels containing azobenzene moieties in the Z-configuration.

A similar approach was described by Akiyoshi and coworkers and is based on the microgel formation upon the self-assembly of spiropyran-bearing pullulan [214]. The physical crosslinks are

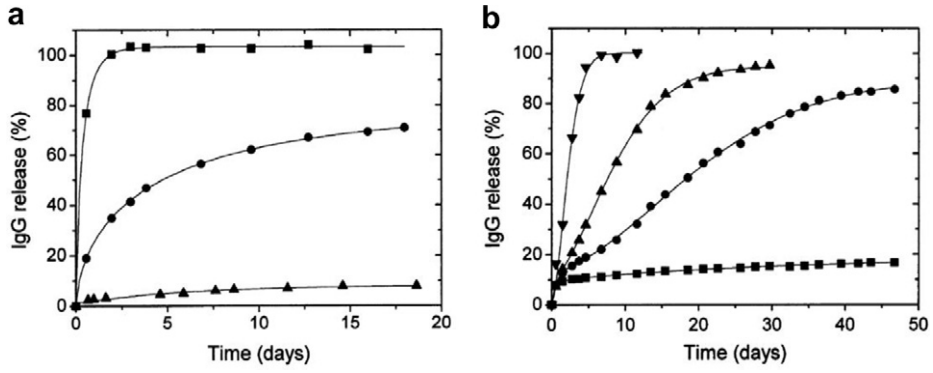

Fig. 11. Cumulative release of IgG versus time from degrading dextran microgels: (a) as a function of the DS: *DS* 4 (■), *DS* 7(●) and *DS* 13 (▲); (b) as a function of the amount of incorporated dextranse (*DS* 4): 2 U/g solid (▼), 0.7 U/g solid (▲), 0.2 U/g solid (●) and 0 U/g solid (■). (Reproduced from [209], with permission from American Chemical Society.)

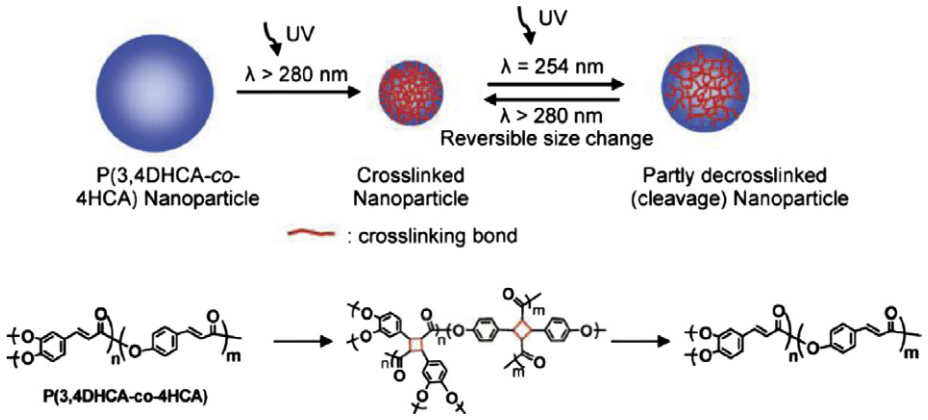

Fig. 13. Schematic representation of size change behavior of microgels with UV irradiation; chemical structure of UV-induced [2 + 2] cycloaddition formation (crosslinking) and deformation (cleavage). (Reproduced from [222], with permission from American Chemical Society.)

formed by the aggregation of hydrophobic spiropyran groups. In analogy to the example described above, these molecules are known to undergo a light-triggered change in configuration to a hydrophilic zwitter-ionic merocyanine form, thereby reducing the hydrophobic interactions, destroying the physical crosslinking points and changing the solution properties of the microgels.

Regarding covalently crosslinked microgels, photo-dimerization reactions – especially [2 + 2] cycloaddition reactions–in combination with macroscopic polymeric gels are well established for the formation of crosslinks by the application of UV light [215,216]. Transferring this concept to the nanoscale gives rise to a broad variety of interesting materials such as thermo- and pH-sensitive microgels [217] prepared by e.g. photo-crosslinking of poly(*N,N'*-dimethylaminoethyl methacrylate-co-dimethylmaleimidoethyl methacrylate) [218]. Extending this concept by the exploitation of reversible photo-dimerization reactions, of e.g. cinnamoyl, anthracene and coumarin derivatives, is an attractive approach to both the light-triggered formation and cleavage of covalent crosslinks. The forward dimerization reaction of such chromophores is widely investigated for the preparation of (stimuli-responsive) [219] microgels by photo-crosslinking of e.g. cinnamoyl side groups in micellar aggregates of (amphiphilic) block copolymers [220] or photo-crosslinking of graft-copolymers containing the photo-reactive moieties in the polymer backbone [221].

Nevertheless, only little attention has been paid to the utilization of the photo-induced backwards reaction as light-cleavable

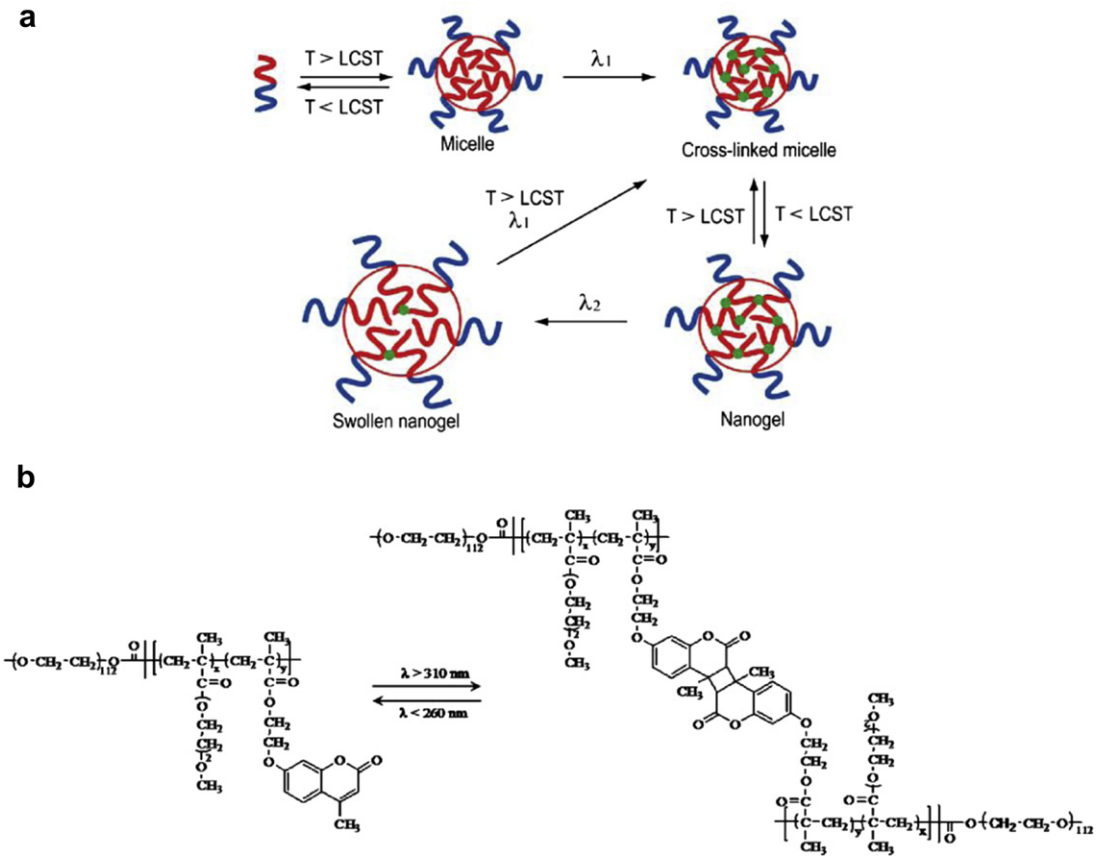

Fig. 14. (a) Schematic representation of the preparation and photo-controlled volume change of thermo- and light-sensitive microgels. (b) Designed diblock copolymer bearing coumarin side groups for the reversible photo-crosslinking reaction. (Reproduced from [223] with permission from American Chemical Society.)

crosslinks in microgels. Akashi et al. demonstrated the light-induced crosslinking and de-crosslinking of microgels prepared from poly(3,4-dihydroxycinnamaic acid-*co*-4-hydroxycinnamaic acid) [P(3,4DHCA-*co*-4-HCA)] copolymers containing cinnamates in the polymeric backbone [222]. As shown in Fig. 13, the reversibility of the photo-reaction was found to enable a controlled swelling/deswelling of the gel networks upon irradiation-induced adjustment of the crosslinking density.

In contrast to the non-degradable microgels prepared by photo-crosslinking of micellar (block) copolymer aggregates, the utilization of reversible photo-dimerization reactions in a similar approach allows to control the crosslinking density by light-induced formation/cleavage/re-formation of crosslinking points. As an example, thermo- and light-responsive nanogels were prepared by photo-crosslinking the core of hydrophilic block copolymer micelles containing a polymer displaying an LCST and bearing coumarin moieties [223]. After micelles were formed by heating a polymer solution above the LCST of the core forming block, crosslinking was achieved by the photo-dimerization of the chromophore upon illumination at λ_1. Upon cooling below the LCST crosslinked nanogels were obtained. It was demonstrated that the degree of crosslinking can be reduced by irradiating a dispersion thereof at λ_2 leading to swelling of the gel network. Furthermore, the initial crosslinking density can be restored by photo-induced re-crosslinking at $T >$ LCST. Fig. 14 depicts the described concept.

Recently, this concept has been extended to pH- and light-responsive microgels following a similar approach which is based on exchanging the thermo-sensitive moieties in a comparable crosslinkable block with pH-sensitive 2-(diethylamino)ethyl groups [224]. In addition, the utilization of a water soluble block copolymer containing pH-sensitive carboxylic acid moieties in one block and thermo-sensitive groups along with coumarin moieties in a second block led to multi-responsive microgels [225]. In dependency on the preparation route, either shell-crosslinked or core-crosslinked gel structures were obtained.

Even though these examples represent sophisticated approaches to controllable nanocarriers, cleavage of crosslinks obtained from [2 + 2] cycloadditions requires harsh irradiation conditions such as UV-C light ($\lambda = 200-280$ nm), thus limiting their application to fields where photo-degradability of functional compounds is not an issue [96].

In order to overcome these limitations, photo-sensitive microgels based on light-cleavable crosslinkers containing *o*-nitrobenzyl derivatives as the photo-reactive chromophores have recently been reported by our group to be degradable under mild irradiation conditions. Here, the light-sensitivity of photo-degradable PMMA microgels (prepared by free radical copolymerization of monomer and crosslinker in direct miniemulsion) was shown to be tunable by adjusting the molecular structure of the crosslinking molecules respectively. The latter were designed to exhibit significant

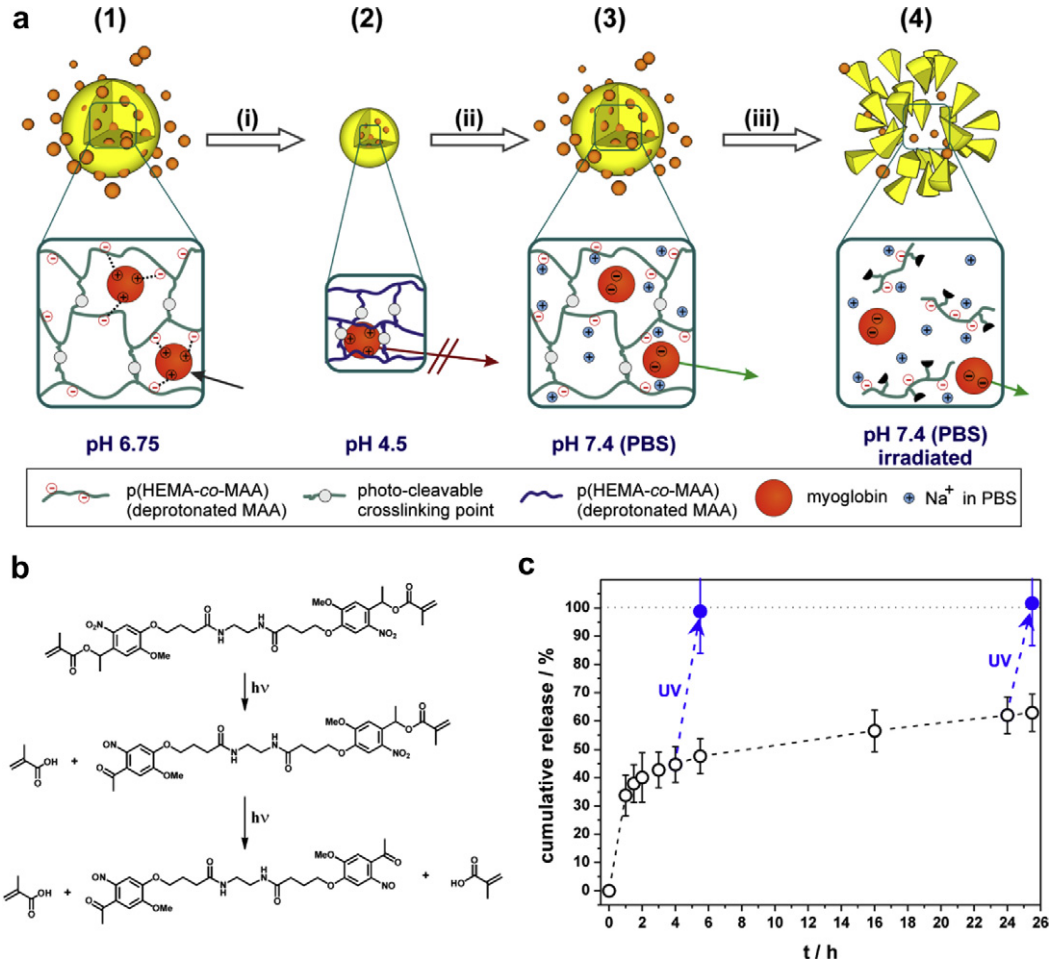

Fig. 15. a) Schematic illustration of the loading and release strategy for p(HEMA-*co*-MAA) microgels. (1)–(2) Loading of large cationic functional compounds into the anionic gel: (i) entrapment by pH-induced deswelling. (3) Diffusion controlled release: (ii) reswelling in PBS. (4) Degradation controlled release: (iii) irradiation in PBS. b) Photoreaction of the used photo-clevable crosslinker. c) Investigations on the loading of myoglobin and its subsequent release from light-sensitive microgels: cumulative release dependency on the incubation and irradiation time. The dotted line is a guide to the eyes. (Reproduced from [133] with permission from American Chemical Society.)

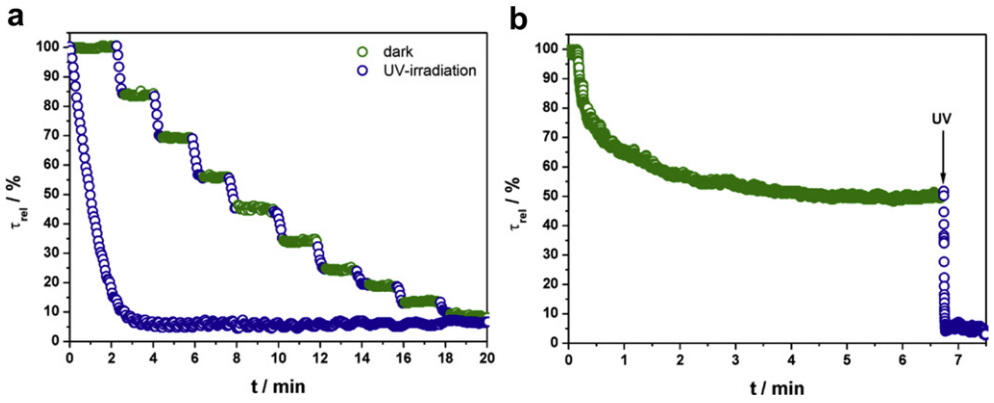

Fig. 16. Investigations on the swelling/degradation of p(AAm-*co*-Dex-PL-A) microgels by time-dependent turbidity measurements: a) photolytic particle degradation and b) swelling induced by incubation with dextranase and subsequent complete degradation upon irradiation. (Reproduced from [227] with permission from Wiley.)

differences in their photolysis rates depending on the irradiation conditions, therefore enabling the independent and successive degradation of the resulting microgels following either a wavelength-controlled or an irradiation-time controlled approach [226]. This concept of light-degradable microgels was successfully transferred to water swellable gel nanoparticles by copolymerizing the photo-cleavable crosslinkers with HEMA and MAA in an inverse miniemulsion which resulted in double stimuli-responsive p(HEMA-*co*-MAA) microgels exhibiting a pH-dependent swelling and light-induced degradation behavior [133]. This unique swelling/degradation profile was successfully used for the loading of the hydrogel nanoparticles with myoglobin as a model protein and its subsequent release. It was found that a *post-formation* loading method by electrostatic interactions between protein and gel network in combination with a pH-induced entrapment was very efficient. The observed diffusion controlled release profile upon swelling the particles under physiological conditions followed an initial burst release in combination with a slow release over a prolonged period of time. In addition, it was demonstrated that a subsequent fast and quantitative on-demand release could be realized by the application of UV light, thereby representing a novel two-step release profile. Fig. 15 schematically depicts the described loading and release mechanism, the crosslinker structure and its photolytic cleavage reaction, as well as the cumulative release of the protein upon particle swelling/degradation.

In a different approach, the concept of photo-sensitive nanoscale hydrogel networks was further extended to double stimuli-responsive enzymatically- and light-degradable microgels. Here, acrylate functionalized dextrans containing a photo-labile linker between the polymerizable vinyl group and the polysaccharide backbone (Dex-PL-A) were used as macromolecular crosslinkers for the preparation of p(AAm-*co*-Dex-PL-A) microgels [227]. It was shown that irradiation with UV light enabled either complete particle degradation or the adjustment of a desired specific degree of swelling by tuning the irradiation time accordingly. In addition, a two-step degradation profile based on the subsequent appliance of the two orthogonal stimuli was realized. Fig. 16 depicts the time-dependent turbidity curves used to monitor the particle degradation either induced by irradiation or by incubation with dextranase and subsequent irradiation.

This behavior renders these materials promising candidates for either triggered release or accessibility applications in aqueous media. Especially, the water solubility of the light-cleavable crosslinkers is assumed to give rise to a potential *in situ* embedding of functional water soluble compounds already during microgel formation by free radical (co)polymerization in the aqueous droplets.

5. Conclusions and outlook

As shown above, stimuli-responsive microgels represent a highly interesting and versatile class of artificial polymeric materials for loading and release applications in the nanometer range. Even though the common underlying concept of these approaches is to control the diffusion of embedded functional compounds in the respective networks by the application of an external stimulus, the number of different approaches to realize this goal is enormous. Here, a profound knowledge of the underlying network characteristics enables to design a well defined response mechanism – determined by the type and location of stimuli-sensitive moieties in the gel network – to perfectly suit a specific application. It becomes obvious that the development of such highly sophisticated materials can only be achieved by the synergy of different research areas and thus combining foundations, concepts and tools from a multitude of fields including organic synthesis, polymer and physical chemistry, biomedical areas and nanotechnology. Since this cooperation between disciplines is nowadays a crucial requirement for the successful pursuit of new nano- and microstructured materials in general, it is obvious that this endeavor towards new stimuli-responsive microgels for loading and release applications is highly interesting from both an academic and industrial point of view.

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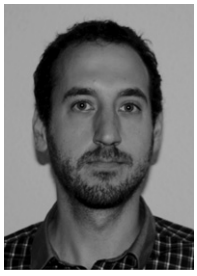

Daniel Klinger obtained his diploma degree in chemistry from the University of Mainz in 2008. He received his PhD in the end of 2011 at the Max Planck Institute for Polymer Research in Mainz in the group of Prof. Dr. Katharina Landfester, focusing on the development of photo-responsive microgels and polymeric nanoparticles for loading and light-triggered release applications. Daniel joined the group of Prof. Dr. Craig J. Hawker at the University of California Santa Barbara as a postdoc in early 2012 and his current main research interests are in the area of functional polyethers and the generation of stimuli-responsive nanotubes from self-folding hydrogel bilayers.

Katharina Landfester studied chemistry at the technical university of Darmstadt. For her diploma thesis, she was at the Ecole d'Application des Hautes Polymères in Strasbourg (Prof. M. Lambla). In 1995, she received her doctoral degree in physical chemistry from the Johannes Gutenberg University of Mainz after working with Prof. H.W. Spiess at the Max Planck Institute for Polymer Research on the synthesis and characterization of core–shell latexes by transmission electron microscopy and solid state NMR. After spending another year as a group leader at the institute, she moved for a postdoctoral stay at the Lehigh University (Prof. M. El-Aasser) where she first came in contact with the miniemulsion technique. She returned to Germany in 1998 joining the group of Prof. M. Antonietti at the Max Planck Institute of Colloids and Interfaces in Golm where she led the miniemulsion group working on new possibilities in the synthesis of complex nanoparticles. In 2002, she got her habilitation in physical chemistry at the University of Potsdam. In 2003, she accepted a chair (C4) of Macromolecular Chemistry at the University of Ulm. Since 2008, she is director at the Max Planck Institute for Polymer Research.