L. A. Lyon and S. Nayak

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Nanotechnology

Soft Nanotechnology with Soft Nanoparticles

*Satish Nayak and L. Andrew Lyon**

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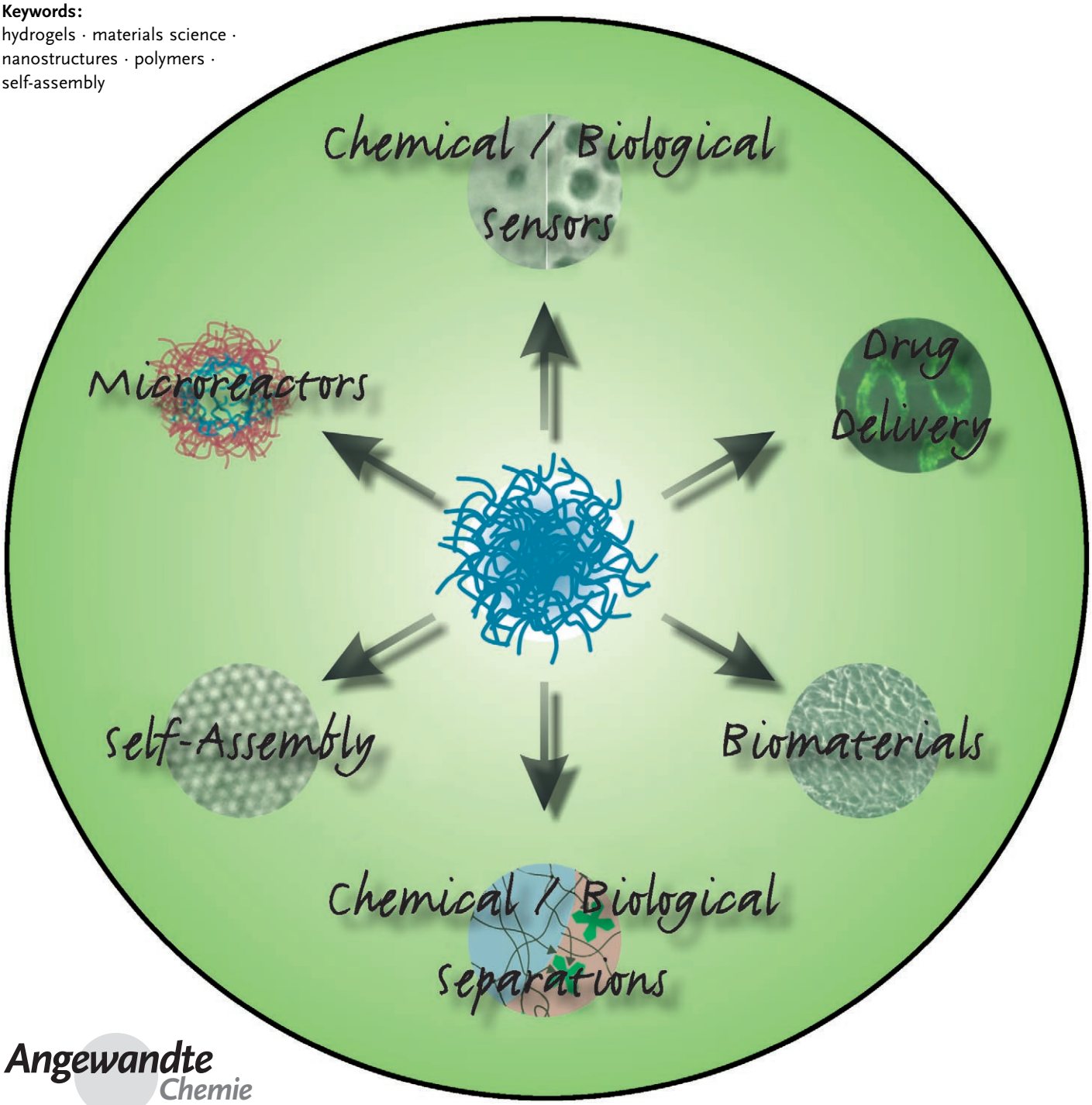


The last decade of research in the physical sciences has seen a dramatic increase in the study of nanoscale materials. Today, "nanoscience" has emerged as a multidisciplinary effort, wherein obtaining a fundamental understanding of the optical, electrical, magnetic, and mechanical properties of nanostructures promises to deliver the next generation of functional materials for a wide range of applications. While this range of efforts is extremely broad, much of the work has focused on "hard" materials, such as Buckyballs, carbon nanotubes, metals, semiconductors, and organic or inorganic dielectrics. Meanwhile, the soft materials of current interest typically include conducting or emissive polymers for "plastic electronics" applications. Despite the continued interest in these established areas of nanoscience, new classes of soft nanomaterials are being developed from more traditional polymeric constructs. Specifically, nanostructured hydrogels are emerging as a promising group of materials for multiple biotechnology applications as the need for advanced materials in the post-genomic era grows. This review will present some of the recent advances in the marriage between water-swellable networks and nanoscience.

From the Contents

1. Introduction

In a sense, the earliest investigators of synthetic and natural polymers were practicing nanoscience, that is, they were studying structures with spatial dimensions on the order of a few nanometers up to many microns. A similar statement could be made regarding the advent of research in molecular biology, as the dimensions of peptides, proteins, and oligonucleotides span the nanoscale. Thus, the nanometer length scale was as important in the early days of macromolecular science as it is today for nanoscientists. Many researchers in nanoscale phenomena rigidly define the nanoscale as that dimension where things become "different" (e.g. quantum confinement, dipolar plasmon oscillations, quantum conductance, etc.). Early polymer scientists may have also appreciated this philosophy, as they struggled to understand why certain arrangements of small molecules behaved in such a different fashion than their isolated molecular precursors. Indeed, polymers could be considered as the first demonstration of synthetic materials where the details of their length scale made them "different".

How then, in this modern age of nanoscience, do we define polymeric nanomaterials if most polymer chains are indeed nanometric? We could attempt to apply the above definition of differentness to some physical property of the polymer. That is, define, for a particular material, the point at which a decrease in macromolecular size leads to a discontinuity in some physical property. However, most scaling laws for polymer solution behavior begin to break down only when the polymer length approaches the persistence length of the chain, that is, the point where solutions begin to look "molecular" and not "macromolecular". While interesting physics arise from such situations, many of the desirable properties of polymers also disappear at this point. It seems then that this is a poor guideline for defining polymer nanoscience if functional materials are the overriding goals of our work. Given this conundrum, it may be the case that the redundant term "polymeric nanomaterials" cannot be rigorously defined. With this in mind, the goal of this Review is simply to explore current research in polymeric hydrogel particles, where the specific arrangement of the polymeric matter at the nanoscale imparts new properties that are not attainable from simple polymer solutions. The focus on polymer particles is important, as these discrete structures bridge the gap between more traditional areas of nanoscience and the world of soft matter. The focus on hydrogels arises from the growing need to understand how hydrophilic polymers can influence emerging areas of biotechnology.

1.1. Definition of Hydrogels

As a state of matter, gels are somewhat difficult to define, as they combine the properties of solids and fluids. They have structural integrity and do not flow appreciably when removed from their container. However, for molecules that are significantly smaller than the gel pore size, the transport of material through a gel is similar to mass transport in a fluid. Hydrogels are simply gels that swell strongly in aqueous media, and are typically composed of a hydrophilic organic polymer component that is cross-linked into a network by either covalent or noncovalent interactions.[1–3] It is the cross-linking that provides for dimensional stability, while the high solvent content gives rise to the fluid-like transport properties. The particular physical properties that result from this unusual state of matter make hydrogels ideal candidates for a number of applications. Perhaps the most widespread use

[*] Dr. S. Nayak, Prof. L. A. Lyon
Georgia Institute of Technology
School of Chemistry and Biochemistry
Atlanta, GA 30332-0400 (USA)
Fax: (+1) 404-894-7452
E-mail: lyon@chemistry.gatech.edu

comes in the form of simple superabsorbent materials, which act to absorb and entrap water in applications where large volumes of aqueous media must be rapidly removed from a localized source. However, to have an impact in areas such as in vivo diagnostics, drug/gene delivery, chemical separations, chemical and biological sensors, and optical materials, many groups have pushed towards the synthesis of more complex polymer architectures to obtain highly functional nanomaterials. Such materials may be designed for biocompatibility, biodegradation, encapsulation, biorecognition, environmentally switchable payload release, or directed self-assembly. A number of creative synthetic methods have thus been brought to bear on this problem. In this Review, we will focus on a wide variety of approaches towards nanostructured hydrogel materials. For example, tremendous progress has been made in the assembly of amphiphilic block copolymers into dimensionally stable micellar particles. We will also address the use of stimuli-sensitive polymers in such materials, as these polymers offer new routes to hydrogels that can sense and respond to local environmental conditions. For example, it is possible to imagine the creation of a hydrogel particle that encapsulates and protects a pharmacologically active protein, only releasing it when the particle "senses" the presence of a particular disease state.

1.2. Classification of Hydrogels

Hydrogels can be classified in many ways but in this Review we will deal mostly with the classification based on type of cross-links. Cross-links are important to maintain the network structure of the hydrogels and to prevent dissolution of the hydrophilic chains. Based on the type of cross-links there are two classes of hydrogels.[4] For more detailed information on noncrystalline polymer networks, see the excellent review by Dušek and Prins.[5]

1.2.1. Physically Cross-Linked Hydrogels

The synthetic and natural hydrogels in this class have led to the concept of reversible or degradable hydrogels that undergo a transition from the three-dimensionally stable structure to a polymer solution. Most often these hydrogels have been used to encapsulate proteins,[6] cells,[7] or drugs[8] and then release them through the dissolution of the hydrogel structure. The cross-links in this class of hydrogels arise from noncovalent attractive forces between the polymer chains (Figure 1). These forces are often hydrophobic interactions, hydrogen bonding, or ionic interactions.

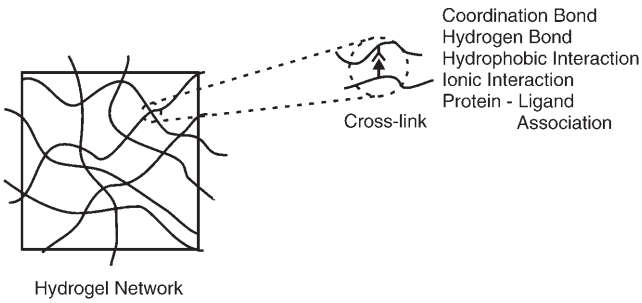
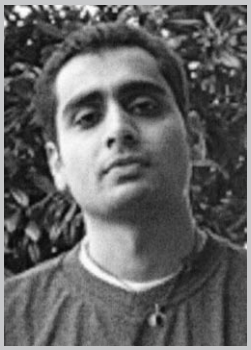


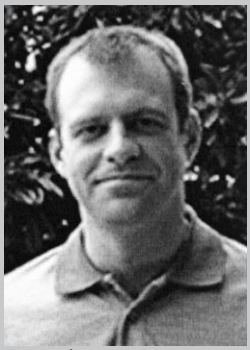
Figure 1. Physical cross-linking in hydrogels, in which the cross-links are formed by noncovalent interactions.

Alginate is a polysaccharide composed of mannuronic acid and gluconic acid, which can be cross-linked by divalent calcium ions.[4,9] The hydrogel can then be dissolved by using a chelating agent that binds to calcium ions. Alginate hydrogels have been used for encapsulating proteins and also as a matrix for the encapsulation of cells, and they often allow growth and proliferation of the cells within the encapsulating gel. Another example of cross-linking by ionic interactions is that of dextran, which lacks charged regions, but forms hydrogels in the presence of potassium ions. The reason for the cross-linking is that the ionic radius of potassium ions is such that it fits perfectly in the cage formed by six oxygen atoms of glucose units on three adjacent polymer chains.[10]

Noncovalent cross-links can also be formed in blends and interpenetrating networks of two dissimilar polymers. For example, poly(acrylic acid) and poly(methacrylic acid) form hydrogen bonds with poly(ethylene glycol), which results in formation of hydrogels. The hydrogen bond is formed between the oxygen of the poly(ethylene glycol) and carboxylic group of the poly acids. The hydrogen bonds are formed only when the acid groups are protonated, hence the hydrogel formation is pH dependent.[11,12] Oligonucleotides have also been used in the formation of hydrogels. Nagahara et al.



Satish Nayak received his BSc in chemistry from Ramnarain Ruia College, Mumbai and his MSc in analytical chemistry from the Institute of Science, Mumbai. He started his doctoral work on synthesizing and characterizing various types of hydrogel nanoparticles at Georgia Institute of Technology in 2000 under the supervision of Prof. L. Andrew Lyon. He successfully defended his thesis in 2004 and is currently working as a postdoctoral fellow at the University of Chicago.



Andrew Lyon received his PhD at Northwestern University under the direction of Professor Joseph T. Hupp (1996) and was a postdoc with Professor Michael J. Natan at Penn State (1997–1998). He began his career at Georgia Tech in 1999 and was promoted to Associate Professor in 2003. His current research involves the design of stimuli-sensitive hydrogel nanoparticles, as well as the assembly of these particles into higher order structures such as photonic crystals. His awards include the NSF CAREER Award, Beckman Young Investigator Award, Research Corporation Research Innovation Award, and the Camille Dreyfus Teacher-Scholar Award.

coupled water-soluble poly(*N*,*N*-dimethylacrylamide-*co*-*N*-acryloylsuccinimide) to a single-stranded DNA.[13] At room temperature and in presence of complementary DNA, hydrogels are formed, which dissolve at temperatures above the DNA melting point.

1.2.2. Chemically Cross-Linked Hydrogels

These types of hydrogels are usually more stable than the physically cross-linked hydrogels because the cross-links are formed by covalent bonds.[4] The hydrogels formed by such cross-links have a permanent structure unless chemical labile bonds have been intentionally added to the network.

Chemically cross-linked gels are usually formed by polymerizing monomers in the presence of cross-linking agents. Poly(2-hydroxyethyl methacrylate) is a well studied hydrogel-forming polymer. It is typically synthesized by polymerizing 2-hydroxy methacrylate ($H_2C{=}C(CH_3)COOCH_2CH_2OH$) with ethylene glycol dimethacrylate ($H_2C{=}C(CH_3)COOCH_2CH_2OCO(CH_3)C{=}CH_2$) as a cross-linker. Various physical properties, such as the swelling capacity of the hydrogels are controlled by the amount of cross-linker used.[4] Hydrogels can also be formed by cross-linking of the various functional groups present on the polymer backbone. Polymers containing hydroxy, amine, or hydrazide groups can be cross-linked by using glutaraldehyde, which forms covalent bonds with each of these functionalities.[4,14,15]

Enzymes have been used in the formation of cross-linked hydrogels. In one interesting example, Sperinde et al. employed tetrahydroxy PEG (PEG = poly(ethylene glycol)) functionalized with glutaminyl groups. This polymer forms hydrogels in the presence of the enzyme transglutaminase and poly(lysine-*co*-phenylalanine), where by the enzyme catalyses the reaction between the γ-carboxyamide group of the PEG-glutaminyl polymer and ε-amine group of the lysine to form an amide bond.[16,17]

1.2.3. Other Classifications

Hydrogels can also be classified based on their size as either macrogels or microgels. Macrogels[18–20] are bulk gels where the size can be anywhere from millimeters and larger, while microgels[21,22] are colloidally stable hydrogels, and their size can vary from tens of nanometers to micrometers. Perhaps the earliest report of microgel synthesis was by Staudinger in 1935,[23] so as with most of macromolecular science, the nanoscale has been the focus for a very long time. However, given the recent interest in such materials across multiple disciplines, as well as the continued efforts in all things "nano", microgels or hydrogel nanoparticles will be the central topic of this Review.

Hydrogels can be further classified as stimuli-responsive or non-responsive gels. Non-responsive gels, as the name suggests, are merely materials that swell upon water absorption. On the other hand stimuli-responsive gels have been called "smart" materials because they respond by a change in swelling to subtle changes in the environment.[24,25] These hydrogels can be made responsive to temperature,[19] pH value,[22,26] ionic strength,[27–29] light,[30–34] electric field,[35] and biomolecules.[36–40] The responsive behavior of the hydrogels is inherited from the type of the polymer used in making the gel and/or any post-polymerization modifications that are made.

1.3. Stimuli-Sensitive Polymers

The use of stimuli-sensitive polymers in fabricating hydrogels has led to many interesting applications; in this section we illustrate a few examples. One of the most widely studied stimuli-sensitive polymers is poly(*N*-isopropylacrylamide) (pNIPAm) formed from the monomer *N*-isopropylacrylamide ($H_2C{=}CHCONHCH(CH_3)_2$). to facilitate the understanding of some of the later parts of this Review, and as most of our group's work is based on pNIPAm, it is appropriate to provide a brief background on this polymer. For an in depth understanding see the very comprehensive review by Schild.[41]

One of the earliest studies on the solution properties of PNIPAm was carried out by Heskins et al.,[42] in which they observed that the phase transition of pNIPAm is endothermic and entropy driven. Owing to this striking thermal behavior in aqueous media, pNIPAm has been widely used to make responsive hydrogels. As with most olefin-based monomers, pNIPAm has been synthesized by a variety of techniques: redox initiation, free radical initiation, ionic initiation, and also by using radiation.[41] Various functional groups have also been added to the polymer by copolymerization and post-polymerization modification, thereby giving multiresponsive and multifunctional polymers.

The behavior of any polymer in a solvent is related to the balance between solvent–solvent, solvent–polymer, and polymer–polymer interactions. For stimuli-sensitive polymers, the polymer solvation can be "switched" by strengthening one of these interactions or by weakening another. Figure 2 illustrates this concept schematically. For the case of pNIPAm in water, the polymer hydrogen bonds to water through the amide side chains. However, the isopropyl group on the side chain induces hydrophobic structuring of the water. This structured water leads to entropically driven polymer–polymer interactions by the hydrophobic effect.[41] Under the conditions where pNIPAm has a random-coil structure, the solvent–polymer interactions are stronger than the polymer–polymer interactions. At higher temperatures, the hydrogen bonds to the water molecules break and there is an entropi-

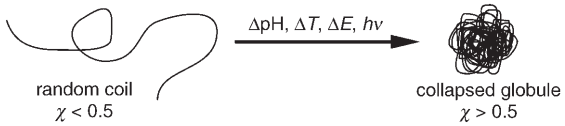


Figure 2. A stimuli-sensitive polymer undergoing phase transition as a result of a change in the solvent properties. Factors such as pH value, temperature, electric field, and light can cause these phase transitions depending on the polymer composition.

cally favored release of bound and structured water, leading to the formation of a globular polymer conformation. In this case the polymer–polymer hydrophobic interactions become stronger than the polymer–solvent interactions, and the polymer phase separates. The temperature at which this phase separation occurs is called the lower critical solution temperature (LCST). It is this behavior that makes pNIPAm a very attractive candidate for the fabrication of stimuli-responsive hydrogels. Note, however, that more than just the hydrophilic and hydrophobic side-chain contributions to polymer solvation must be considered when describing LCST behavior. For example, the polymer formed from *N*-isopropylmethacrylamide ($H_2C{=}C(CH_3)CONHCH(CH_3)_2$),[43–48] which differs from NIPAm by only a single methyl group, has a higher LCST in water, which suggests that it is more hydrophilic despite having a greater organic content. Apparently, this "increased hydrophilicity" does not arise from an increase in polymer polarity, but instead comes from a decrease in chain flexibility. This feature changes the entropic contribution to the free energy of mixing, and thus increases the LCST.

The LCST behavior of pNIPAm has been studied by a variety of techniques including UV-Vis spectroscopy, differential scanning calorimetry (DSC), light scattering, viscometry and fluorescence spectroscopy.[49–57] Wu et al. have extensively studied the phase transition of pNIPAm, where they observed that the transition is not first order, that is, the polymer does not directly go from a random coil state to a globular state, but there are other intermediate thermodynamically stable conformations (Figure 3).[52,54,58]

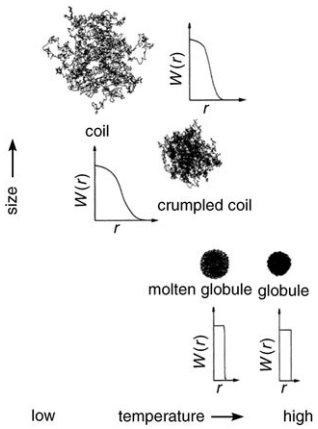


Figure 3. The thermodynamically stable states present during the phase transition of pNIPAm and the corresponding chain-density distribution along the radius for each state. Reprinted with permission from ref. [58].

There are other classes of thermoresponsive polymers that show an upper critical solution temperature (UCST). In this case the polymer is phase separated at low temperatures owing to inter- or intramolecular interactions, while at high temperatures these interactions break and the polymer dissolves or swells. Most often these interactions are hydrogen bonding or electrostatic. In one example Laschewsky and co-workers have synthesized a block copolymer of pNIPAm and 3-[*N*-(3-methacrylamidopropyl)-*N*,*N*-dimethyl]ammoniopropane sulfonate (SPP), which exhibits both LCST and UCST behavior.[59] The pNIPAm segment confers temperature sensitivity by the mechanism described above. On the other hand, SPP is a polyampholytic polymer, which at low temperatures displays strong electrostatic interactions between the oppositely charged regions of the polymers, whereas these interactions are broken at high temperatures.

Using this understanding of polymer solvation, Irie and co-workers have synthesized photoresponsive polymers by incorporating azobenzene side chains in pNIPAm.[60] Azobenzenes undergo *trans* to *cis* isomerization on UV irradiation; the transition is reversed by irradiation with visible light or by thermal relaxation. Furthermore, the dipole moment and hence the hydrophilicity of azobenzene is dependent on the isomerization state. In this fashion, the investigators were able to change the phase-transition temperature of pNIPAm by inducing isomerization of the azobenzene side chains, which in turn changed the free energy of mixing. Figure 4 shows the change in transmittance of poly-[*N*-isopropylacrylamide-*co*-*N*-(4-phenylazo-phenyl)-acrylamide] as a function of temperature before and during irradiation with UV light.

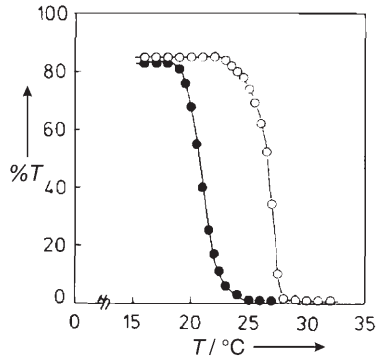


Figure 4. Temperature-dependent transmittance of the photoresponsive polymer before (●) and during (○) UV irradiation. Reprinted with permission from ref. [60]

1.4. Swelling Properties of Hydrogels

The nature of hydrogels is dependent on the type of the monomers used to synthesize them. In case of hydrogels synthesized from pNIPAm, the thermoresponsivity of the parent polymer is inherited by gels made from it. These hydrogels exhibit volume phase transition temperature (VPTT)[61] at around the LCST of pNIPAm. At temperatures higher than VPTT the hydrogel goes from a swollen (hydrophilic) state to a compact (relatively hydrophobic) state. The VPTT of the gels is dependent on several factors including cross-link density, hydrophobic-hydrophilic balance, ionic strength, and solvent composition. Correspondingly, hydrogels fabricated from titratable ionic monomers show pH-dependent swelling. The most common ionic monomers used are acrylic acid and methacrylic acid. At $pH > pK_a$ of the acid comonomer, the gel swells because of Coulombic repulsion

between the charged monomer units and the more favorable solvation of the deprotonated monomer by the solvent. The equilibrium gel-swelling volume is a balance between the osmotic pressure of the polymer network, which is governed by polymer–solvent interactions, and the elasticity of the network.[5]

In ground-breaking work, the group of Tanaka showed that ionic thermoresponsive gels, when heated, display a discontinuous transition, while non-ionic gels undergo a continuous transition. They have also shown that the shrinking rate of the gel is inversely proportional to the square of the smallest dimension of the gel.[20,35,62–64] Taking advantage of the diffusion length scale, Yan and Hoffman have shown that polymerizing pNIPAm gels at temperatures higher than the LCST of the polymer results in gels that have large pore sizes, which in turn have faster swelling rates.[65] Reporting all the properties of hydrogels is beyond the scope of this Review; hence we have mentioned just a few important properties, and some others will be discussed in the later Sections.

The swelling behavior of hydrogels has made them useful in some interesting applications. Thermosensitive pNIPAm gels have been used in controlling the activity of enzymes. Park and Hoffman demonstrated that by immobilizing an enzyme in the gel, the diffusion of the substrate to the enzyme could be controlled by changing the pore size of the gel, which in turn depends on the temperature of the system. In this way they were able to switch the enzyme on or off by controlling the temperature.[66] Kim and Healy have synthesized pNIPAm gels with peptide cross-links. These gels can be used as an extracellular matrix mimic, where the peptide can be cleaved by a metalloproteinase, which subsequently leads to gel erosion. Figure 5 shows a schematic depiction of this process.[67]

1.5. Microgels and Nanogels

Colloidally stable particles made from hydrogels, also referred to as micro- or nanogels, have properties similar to those of their macrogel counterparts, that is, a pNIPAm microgel, just like the bulk gel, will also undergo a VPTT near the LCST of the polymer.[68,69] In addition to these properties, microgels have other characteristics of colloidal dispersions, such as zeta potentials,[69–71] and can also form ordered colloidal phases.[72–75]

Some very important studies have focused on the differences between macro- and microgels with respect to their phase behavior. For example, Wu et al. have shown that the VPTT of the microgels is slightly higher than the LCST of pNIPAm (Figure 6), and also that the transition region is less

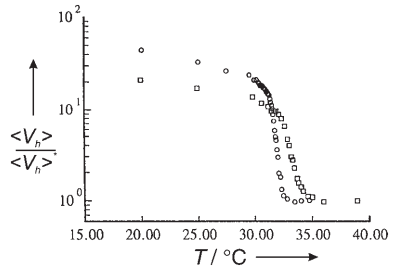


Figure 6. Deswelling ratio of linear pNIPAm chains (○) and cross-linked microgels (□) in water. Owing to the heterogeneity of the cross-links, microgels have a broader phase-transition region than the linear pNIPAm chains. Reprinted with permission ref. [76].

sharp than that of bulk gels.[76] The reason for this continuous transition is due to a greater heterogeneity in the subchain lengths of the microgels than in the traditionally prepared macrogels. When the microgels are subjected to $T >$ VPTT, the regions of the particle with longer subchain lengths collapse at a lower temperature than the regions with shorter subchains. Thus the observed phase transition for a microgel can be thought of as being the summation of the phase transitions of the different sub-networks in the particle. We have also observed this behavior in core–shell structured microgels by using fluorescent probes to interrogate cross-linker gradients.[77]

PNIPAm microgels show interesting behavior in mixed solvents owing to the cononsolvency of pNIPAm (i.e., the non-linear dependence of solvation on solvent volume fraction).[78] For example, the LCST of linear pNIPAm decreases with increasing methanol concentration until 55% methanol, beyond which the LCST increases sharply.[79] The same effect has been observed by McPhee et al. for microgels.[80] The mechanism for cononsolvency is explained by clathrate formation. Cosolvents such as water and methanol form clathrate structures, which compete with pNIPAm for the water molecules that hydrate the polymer. Hence the deswelling of the polymer is observed as the water molecules are removed from the interior of the gel by the other solvents. In related work, Daly and Saunders have studied the

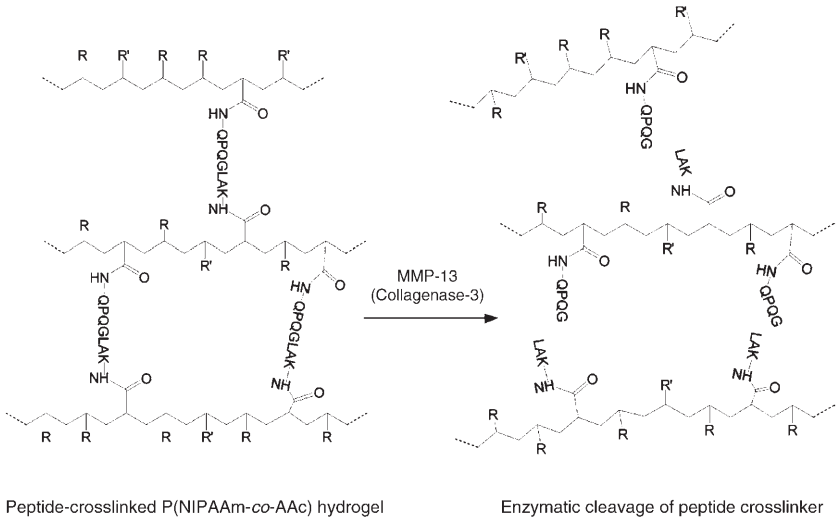


Figure 5. Peptide-cross-linked hydrogel degradation by MMP-13 (collagenase-3). The letters indicate the single-letter amino acid designations. Reprinted with permission from ref. [67].

effect of added electrolyte on the stability of the microgels. It was observed that at a particular temperature particles flocculate at higher ionic strength. The swelling ability of the particles also depends on the ionic strength of the salt and its position in the Hoffmeister series, which relates to the effect of ions on the activity of water.[70] An interesting study by Greinert and Richtering involved the modulation of polyelectrolytic pNIPAm microgels by the adsorption of oppositely charged polyelectrolytes in solution.[81] The charge compensation and bridging effect of the polyelectrolyte adsorption have a profound effect on the LCST behavior of the microgels. These studies may offer insight into the swelling phenomena observed in microgel-based polyelectrolyte multilayers,[82–85] which are discussed in Section 3.1.

Many researchers have carried out electrophoretic mobility measurements for charged particles. One of the first reports was from Pelton et al., they studied the electrokinetic behavior of pNIPAm microgels as a function of temperature and salt concentration.[86] They observed that at $T < \text{VPTT}$, mobility was nearly zero owing to a low charge density on the particles, but at $T > \text{VPTT}$ a sharp increase in mobility was observed. This change was attributed to the increase in charge density on the smaller deswollen particle. Since the charge per particle is constant, smaller (deswollen) particles will have a higher charge density than swollen particles. Daly and Saunders carried out a similar study and found that the onset of the increase in electrophoretic mobility was about 5–6 K higher than the VPTT of the particles. This behavior was proposed to result from a three-stage phase transition in the microgels (Figure 7).[70]

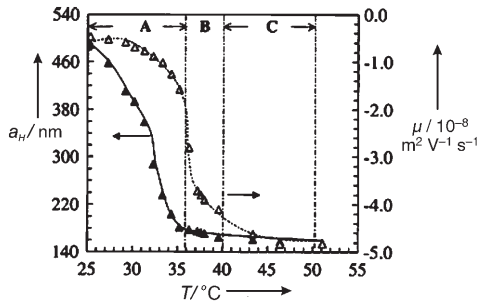


Figure 7. Electrophoretic mobility (△) and hydrodynamic radius (▲) as a function of temperature for pNIPAm microgels. The regions A, B, and C represent the three stages in the collapse of the particle. Reproduced with permission from ref. [70].

Surfactants can have a strange effect on the swelling of pNIPAm microgels. Tam et al. have observed that the microgels swell in presence of anionic surfactant.[87] They attributed this property to the interaction of the hydrophobic tail of the surfactant with the polymer network. However, in the presence of a cationic surfactant of the same chain length, no such effect was observed. Wu et al. carried out similar studies and observed by NMR spectroscopy that at concentrations less than the critical micelle concentrations (CMC), the anionic surfactants form micelles in the polymer network owing to a higher effective concentration in the network.[88] The cationic surfactants failed to form micelles in the polymer network even at very high concentration, and hence no microgel swelling was observed.

Understanding the structure of hydrogel nanoparticles is important for explaining their detailed phase-transition behavior, mass-transport through the network, and colloidal stability. Light scattering is one of the most common techniques used for structural analysis of hydrogel nanoparticles. Both dynamic and static light scattering have been used extensively and remain the standard techniques for particle characterization.[69,76,89–91] However, other techniques have allowed for even greater insight into the structure of microgels. Guillermo et al. used NMR spectroscopy to determine the cross-linker distribution in microgel particles and confirmed that these particles have a heterogeneous structure, wherein the periphery of the microgel is more loosely cross-linked than the interior.[92] Similar studies were carried out by Saunders, in which small-angle neutron scattering (SANS) was used to investigate the structural details of pNIPAm microgels synthesized by surfactant-free polymerization.[93] This study again revealed that the microgels prepared by precipitation polymerization have a heterogeneous structure, in this case it was also determined that a thin layer of loosely cross-linked chains was present at the particle periphery. Woodward et al. used DSC to determine the influence of cross-linking density on the phase transition of the particles. They observed an overall decrease in phase-transition enthalpy with increase in cross-linker density.[94] In addition to DSC they also used pulsed-gradient spin echo (PGSE) NMR spectroscopy to study the mobility of the solvent in the particle as a function of cross-linker density and observed that the diffusion of solvent is reduced with the increase in the cross-linker density, hence the particles are more rigid at high cross-linker density. Figure 8 depicts the change in relative diffusion coefficient of water in the microgels, measured by PGSE-NMR spectroscopy, for samples with different cross-linker densities.

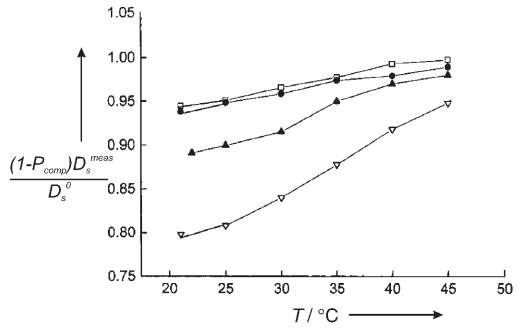


Figure 8. Relative diffusion coefficient of water inside the polymer network for 0.25% (□), 0.5% (●), 5% (▲), and 30% (△) cross-linked pNIPAm microgels as a function of temperature. The diffusion coefficient of water within the particle decreases with an increase in the cross-link density. Reprinted with permission from ref. [94].

Senff et al. have used rheometry to understand the phase behavior of microgels and to determine the effective "softness" of microgel particles.[73,95,96] This group has also carried out studies aimed at determining the internal structure of

microgels.[97,98] Again, it was determined that the cross-link density is highest at the center of the particle and decreases gradually to the surface. They also found that the internal structure of the microgels depends on the manner in which they are synthesized. A semibatch reaction (reactants are added gradually over a certain time) gives more homogeneous morphology than a simple batch reaction (all the reactants are added at the start) This effect is attributed to the faster reactivity of the cross-linker, which gets incorporated at a faster rate during the early stages of a batch reaction. Similar behavior has also been reported by Pelton.[68,99]

2. Synthesis of Hydrogel Nanoparticles

Hydrogel Nanoparticles have been synthesized by numerous approaches. In this Section we will discuss some of the commonly used methods, where the synthetic technique being employed is typically dictated by the desired application or the type of study to be carried out.

2.1. Emulsion and Precipitation Polymerization

Surfactant-free emulsion polymerization (SFEP), or precipitation polymerization, is the most common technique to synthesize thermosensitive hydrogel particles. Although pNIPAm microgel synthesis by this method was not reported until 1986, the technique was apparently first utilized by Philip Chibante in 1978.[68,100] If carried out carefully, this method can afford particles with very narrow size distribution. In this method all the monomers, NIPAm, and the cross-linker (typically *N*,*N*′-methylenebisacrylamide (BIS)), are dissolved in water. The solution is purged with N_2 and heated to a temperature above the pNIPAm LCST (usually around 70 °C), then an initiator such as ammonium persulfate (APS) or potassium persulfate (KPS) is added.

Microgels synthesized by this method are formed by homogenous nucleation. There are good reasons for carrying out the polymerization at high temperature: the sulfate radicals, which initiate the polymerization need to be formed. After initiation the NIPAm monomer is attacked by the sulfate radical, there then follows radical propagation and chain growth. Once the chain reaches a critical length, it collapses upon itself producing precursor particles. The chain collapses because the polymerization temperature is higher than the LCST of the polymer, hence the name precipitation polymerization. The precursor particles grow by aggregation with the other precursor particles, by being captured by existing particles, by capturing growing oligoradicals, and by monomer addition. The charge imparted by the initiator stabilizes the microgels once they have reached a critical size. Figure 9 shows the proposed mechanism for precipitation polymerization. This method is extremely versatile from the standpoint of particle size control. For example, to synthesize smaller microgels, the precursor particles must be stabilized earlier in the reaction. Since there is not enough charge available from initiator fragments to stabilize small precursor particles, an ionic surfactant can be added to impart colloidal stability earlier in the reaction. Similarly, larger particles can be obtained by decreasing the surfactant concentration. Precipitation polymerization can also be used to incorporate comonomers in the microgel. We and others have copolymerized ionic monomers with pNIPAm to create pH-responsive microgels.[22,29,101–105] We have also copolymerized a hydrophobic comonomer *N*-*tert*-butylacrylamide (TBA, $H_2C{=}CHCONHC(CH_3)_3$) to give pH and temperature responsive microgels; these particles exhibit phase transitions at lower temperatures owing to the increase in hydrophobicity of the gel while retaining a pH-tunable VPTT.[105] Similarly, other functionalities can also be copolymerized into these particles by this method.

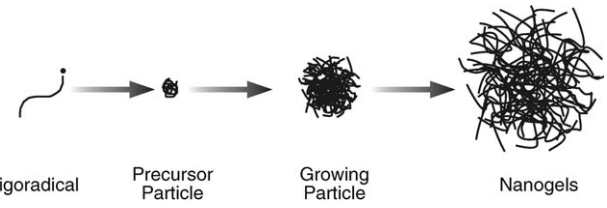


Figure 9. Precipitation polymerization of colloidal micro or nanogels from phase-separating polymers.

The presence of a cross-linker is critical in preventing the dissolution of the polymer particle as it is cooled below the LCST. However, Gao and Friskin have reported that it is possible to make pNIPAm microgels by precipitation polymerization, without using a cross-linker.[106,107] They attributed this effect to "self cross-linking" of pNIPAm chains by a chain-transfer reaction occurring at either or both of two possible sites: the hydrogen atom attached to the tertiary carbon atom on the pendant isopropyl group and the hydrogen atom on the tertiary carbon atom of the main-chain backbone. These results indicate that in precipitation polymerization the cross-link density might be higher than expected on the basis of the cross-linker concentration used in the reaction.

Precipitation polymerization has a few drawbacks. First, the method is useful only for materials that are stable at high temperatures and hence cannot be used to incorporate biological macromolecules. Also the method is best for materials which are hydrophobic so that they can attach to the collapsed precursor particle easily. Hence, if a hydrophilic comonomer is to be copolymerized, only a certain wt % can be incorporated. Beyond that wt % the growing oligomer will be too hydrophilic to undergo efficient chain collapse, thereby resulting in a very polydisperse microgel dispersion, or the complete lack of particle formation. To overcome these hurdles many researchers have used inverse microemulsion polymerization. In this method an aqueous solution of all the monomers is added to an appropriate amount of oil and surfactant and is stirred to form thermodynamically stable microemulsions. Polymerization can be initiated by having the initiator in the aqueous or in the oil phase. This method has been used to synthesize hydrogel particles with high wt % of ionic monomers; Neyret and Vincent used microemulsion polymerization to prepare zwitterionic pNIPAm microgels.[108] In this case they used a UV-activated photoinitiator

dissolved in the organic phase. McAllister et al. used the same technique to synthesize a highly cationically charged hydrogel. These particles were able to bind to DNA and can potentially be used in gene delivery.[109] One other method for preparing microgels, which is similar to microemulsion polymerization, utilizes lipids to form liposomes inside which the particle is formed. Kazakov et al. have demonstrated that liposomes can form a "nanoreactor" inside which microgels can be polymerized.[110] Figure 10 illustrates the general procedure.

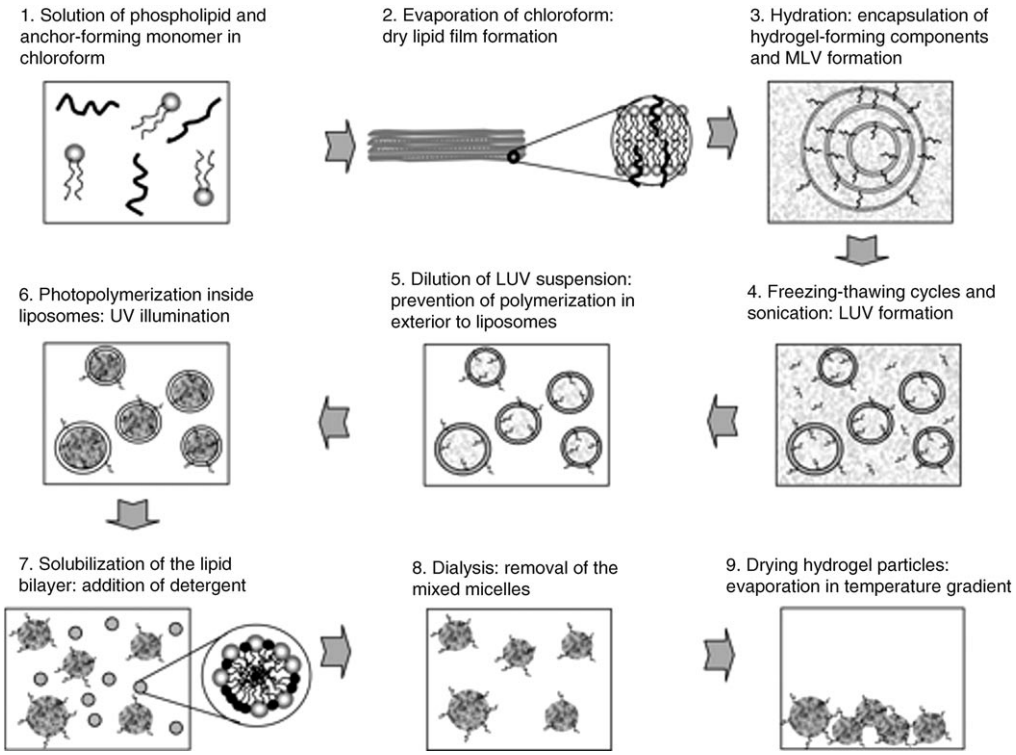


Figure 10. Hydrogel particle synthesis in liposomes. Reprinted with permission from ref. [110].

2.2. Core–Shell Structured Materials

Core–shell hydrogel particles can broadly be divided in two classes: 1) where the core is made from non-hydrogel material and the shell is made from hydrogel and 2) where both the core and the shell are made of a hydrogel-like material. In the first class of materials the core is usually made of solid material, such as polystyrene, silica or gold nanoparticles. Dingenouts et al. synthesized a polystyrene core with small amount of NIPAm as a co-monomer by SFEP.[111] The polystyrene-*co*-pNIPAm particles were stabilized by the sulfate groups from the initiator. These cores were then used as seeds for polymerizing a cross-linked shell of pNIPAm. For the shell synthesis the reaction was carried out at 80 °C, which provided a core particle with a deswollen pNIPAm-rich periphery, onto which pNIPAm polymerizing in solution aggregated by a precipitation polymerization mechanism. Xiao et al. synthesized similar particles in which pNIPAm chains were grafted onto the polystyrene core resulting in a "hairy" particle.[112]

In another example, Zha et al. have used silica particles prepared by the Stöber method[113] as a core material.[114] Vinyl groups were introduced to the surface of the particles by a silane coupling reaction. These particles were then used as seeds for precipitation polymerization of pNIPAm, which resulted in the formation of silica-core pNIPAm-shell particles. This approach allowed the synthesis of hollow pNIPAm capsules by dissolution of the silica with HF. Similarly, Kim and Lee have synthesized gold-core pNIPAm-shell particles by SFEP. The size of these citrate-stabilized gold nanoparticles was approximately 60 nm. The gold nanoparticles were then treated with oleic acid, which helps in attaching the pNIPAm hydrogel shell to the gold core during SFEP.[115]

The second type of core–shell materials is the one which has hydrogel in both the core and the shell. Our group first reported the synthesis of this type of hydrogel particles by two-stage precipitation polymerization.[22] In this method a polymer shell with the same or different structure or functionality as the core, is added onto preformed core particles thereby allowing control over the radial distribution of the functional groups in the particle. In a typical synthesis, preformed pNIPAm core particles are heated to approximately 70 °C, then the shell monomer solution is added, and polymerization is initiated. The reaction is carried out for around 4 h and then the mixture is cooled and separated by filtration. This method gives core–shell particles with no increase in polydispersity, as all the oligomers formed in solution precipitate onto the preformed core particles. To prevent heteronucleation there are several important parameters that should be controlled, including the concentration of the core, initiator, surfactant, and the shell monomer. The mechanism by which this reaction takes place is somewhat similar to that for the core microgels. Since the reaction temperature is well above the VPTT of the core particles, the particles are in a collapsed state. The collapsed particles are hydrophobic and hence they tend to capture the growing oligomers, which results in the formation of the shell.

Core–shell particles prepared in this fashion can exhibit very interesting properties.[21,43,44,77,116–119] Since the shell can be synthesized using different comonomers to the core, the particles can show multiple phase-transition behavior with

temperature.[22,43,44] Furthermore, depending upon the cross-linker density of the shell, compression or "shrink-wrapping" of the core can be observed resulting from a cross-link gradient in the shell.[116,117,119] We have also used this synthetic method to make hollow hydrogel capsules, Figure 11.[120] To

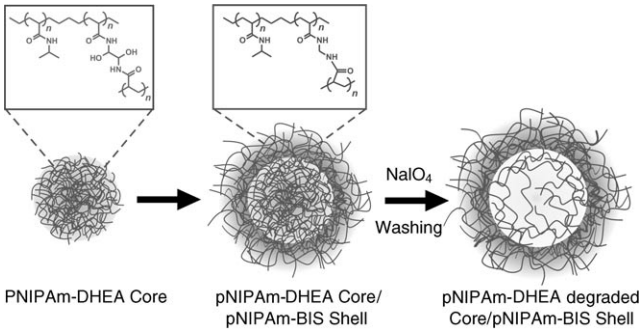


Figure 11. Preparation of thermosensitive hollow microgels by oxidation of the core cross-links. Reprinted with permission from ref. [120].

accomplish this, the core is fabricated with a degradable cross-linker and the shell with a non-degradable one. The degradable cross-linker that we have used contains a vicinal diol, which can be degraded by stoichiometric addition of periodate. After core degradation, the particles were cleaned extensively by centrifugation, after which DLS and fluorescence were used to confirm the hollow structure.

Berndt and Richtering have also synthesized core–shell particles which have two different polymers in the core and the shell.[43] In their demonstration, the core was made of pNIPAm and the shell consisted of poly(*N*-isopropylmethacrylamide) (pNIPMAm), which has a LCST of 45 °C. They studied the thermoresponsivity of these particles and found, in a similar fashion to previous work from our group, that the particles had two transitions corresponding to the LCSTs of the two polymers (Figure 12).

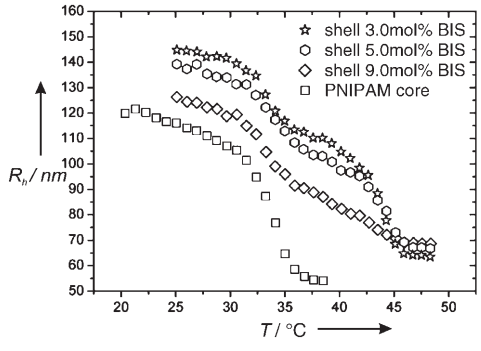


Figure 12. Hydrodynamic radius as a function of temperature for pNIPAm-core/pNIPAMAm-shell microgels with different shell cross-linker concentrations. The sample containing 3.0 mol% BIS clearly shows that the particle is undergoing two phase transitions, the first transition corresponding to the LCST of pNIPAm and the second to that of pNIPMAm. Reprinted with permission from ref. [43].

2.3. Block-Copolymer Micelles

Hydrogel nanoparticles can also be formed by using the ability of block copolymers to self-assemble into micelles.[121] Most often block copolymers are like surfactants in that they have a hydrophobic and a hydrophilic block. Depending on the polarity of the solvent and the concentration of the polymers, block copolymers can form spherical micelles. In hydrophilic solvents the hydrophobic block forms the core and the hydrophilic block is exposed to the solvent. In some cases these micelles can be cross-linked to form stable nanoparticles. For example, Zhu and Napper used pNIPAm-*b*-PEO (PEO = poly(ethylene oxide)) to form microgels.[122,123] Initially the block copolymer was synthesized using the ceric ion redox system in nitric acid at 50 °C, then addition of the cross-linker BIS formed cross-linked microgels. It was observed that the size of the microgels, as measured by DLS, was dependent on the concentration of NIPAm and PEO, and also on the rate of heating during polymerization.

The Wooley group has extensively studied cross-linked block-copolymer micelles, which they refer to as shell cross-linked knedels (SCK).[121,124–137] The size range for SCKs is around 5–200 nm. They are prepared from amphiphilic block copolymers, which self-assemble into polymeric micelles. The micelles are further stabilized by cross-linking of the side-chain functional groups in the shell of the micelles (Figure 13). The first SCK reported was fabricated from a

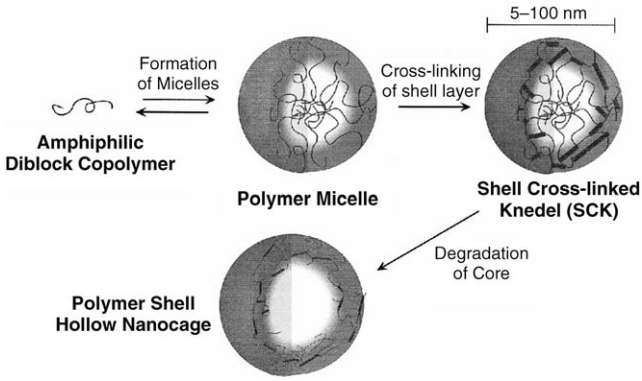


Figure 13. General synthetic approach for synthesizing SCK nanoparticles. The degradation step is used for the preparation of hollow particles. Reprinted with permission from ref. [131]

polystyrene and poly(4-vinylpyridine) block copolymer.[124] Before self-assembling the polymer into micelles, the pyridyl nitrogen was quaternized by reaction with *p*-chloromethylstyrene to impart hydrophilicity to the polymer. Once the polymer formed micelles the styrene moiety in the shell was polymerized to give the cross-linked structure. The dimensions and topologies of the particles can be controlled by varying the length of the hydrophilic and hydrophobic blocks.

The Wooley group has also prepared hydrogel-coated particles by using a polystyrene-*b*-poly(acrylic acid) copolymer.[137] The polymer self-assembled into a polystyrene core and acrylic acid shell micelle. The acrylic acid groups were

then cross-linked by using variety of bifunctional amine cross-linkers. They have also prepared SCKs with degradable cores. In one example they used poly(ε-caprolactone)-*b*-poly(acrylic acid) copolymers. This polymer forms micelles with the caprolactone core and acrylic acid shell. The shell is cross-linked by the methods mentioned above to form SCKs, the core is then dissolved by acid or base hydrolysis.[130] In a second demonstration, they have used poly(isoprene-*b*-acrylic acid) to form hollow SCKs.[126] The micelles formed by this polymer have isoprene groups in the core and the acid groups on the periphery. The acid groups in the shell were cross-linked by amidation and the core was dissolved by isoprene oxidation, resulting in hollow capsules. The Wooley group has also synthesized an entirely hydrophilic SCK, which was pH responsive.[134] The polymer was poly(acrylic acid)-*b*-poly(methyl acrylate), which forms an acrylic acid shell and a methyl methacrylate core micelle. The shell was cross-linked, subsequent hydrolysis of the core using LiOH, which converted acrylate into acid groups, made the entire particle hydrophilic. The acid groups in the core and the shell made the SCK pH responsive.

Akiyoshi et al. have found that polysaccharides partly modified by hydrophobic groups, such as cholesterol, can form nanoparticles in water.[138] The sizes of these particles typically range from 20–30 nm with excellent monodispersity, according to size-exclusion chromatography, DLS, and TEM. These particles are not true block-copolymer micelles but are closely related to this class of particles, since the association forces are similar. These particles were first prepared in 1993 by using hydrophobized pullulan having 1.6 cholesterol groups per 100 glucose units,[138] which aggregated intermolecularly to form nanoparticles. These nanoparticles are considered to be hydrogels in which the cross-links are provided by the associated hydrophobic groups. The size of the particles can be controlled by the number of the hydrophobic groups and also by the structure of the polymers. The Akiyoshi group has also made particles in which pNIPAm was incorporated to create thermoresponsive pullulan particles. These particles can capture and entrap macromolecules, such as proteins, in the gel network.[139–142] The self-assembly of these polymers can be controlled from the molecular level (association of hydrophobic groups) to the nanoscale level (association of hydrophobized polymers) and macroscopic level (association of nanoparticles). The hierarchy of pullulan assembly is shown in Figure 14.

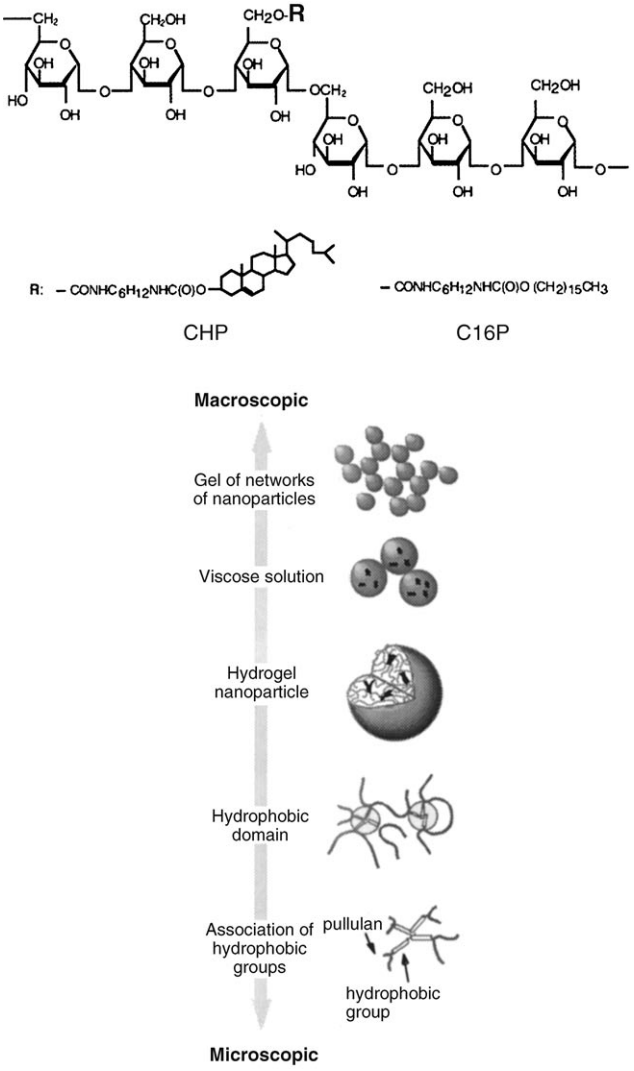


Figure 14. Chemical structure and the hierarchical self-assembly of hydrophobically modified pullulan. Reprinted with permission from ref. [142].

2.4. Post-Polymerization Modification of Hydrogels

For certain applications not all of the desired hydrogel functional groups can be added during the polymerization step. There are several reasons for this: the desired functional groups may not be stable during the polymerization step or the molecules are simply not polymerizable. This situation is true for most biomolecular structures from which hybrid gels would be prepared. To allow further functionalization of hydrogels, most often a small amount of comonomer with acidic or basic functional groups is copolymerized during the polymerization step. These functional groups are then used for the attachment of molecules that could not be directly incorporated by polymerization. This particular class of syntheses encompasses a large variety of particles, of which only a few will be mentioned in this Review.

We have created photoresponsive microgels by post-polymerization modification.[143] An amine comonomer was copolymerized in the pNIPAm microgels. This amine group was then used for attaching malachite green isothiocyanate to the microgels. Malachite green is a temperature-jump dye molecule that converts light ($\lambda_{max}^{abs} = 620$ nm) to thermal energy by nonradiative relaxation. Since the polymer is thermoresponsive, the microgels undergo deswelling when irradiated with a HeNe laser. Photoresponsive microgels exhibit dye-concentration dependent deswelling, that is, for the same laser intensity, microgels having higher concentration of malachite green deswell to a larger extent than microgels with lower concentration of the dye.

The Kawaguchi group has used post-polymerization modifications extensively for making particles with variety

of functions.[144] For example, to synthesize a tetra-functional particle, they first synthesized standard microgels using acrylamide, BIS, methacrylic acid, and *p*-nitrophenyl acrylate as the co-monomers. The thus introduced ester side chain can be hydrolyzed to give acidic particles, or after hydrolysis can be treated with ethylene diamine to give amphoteric particles. The acid groups were also coupled with a long-chain alkyl amine to form hydrophobic particles. Finally the acid groups were coupled to an immunoglobulin G (IgG) to form bio-functionalized microgels. This particular example illustrates the tremendous versatility in microgel structure and function that is offered by post-polymerization modification.

Another interesting system involved the synthesis of pNIPAm chains bearing terminal carboxyl groups, which were then grafted to microgels. Some of the carboxy termini were then used to attach the enzyme trypsin, resulting in a particle having two different kinds of pNIPAm chains on the surface; one with trypsin and the other without it. Surprisingly, it was found that the two chains had different transition temperatures. The free chains collapsed at a lower temperature than the trypsin-conjugated chains, thereby exposing the enzyme for substrate binding. Hence by this simple construct it was possible to control the enzyme activity by controlling the temperature (Figure 15).[145]

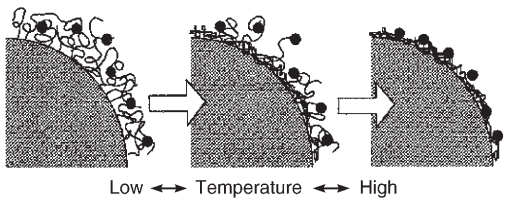


Figure 15. Changes in the surface of the particle conjugated with two different types of pNIPAm chains (filled circles depict the enzyme trypsin) with temperature. These types of phase transitions enable thermally modulated enzyme activity. Reprinted with permission from ref. [145].

Delair, et al. described the immobilization of DNA on microgels by post-polymerization modification. PNIPAm microgels with an amine comonomer were synthesized. Single-stranded DNA with an amine group at the 5′-end was treated with 1,4-phenylene diisocyanate in 1:2 ratio so that one of the isocyanates was coupled to the DNA, while the other one remained free. After purification, the DNA was coupled to the pNIPAm microgels by allowing the free isocyanate to react with the amines on the surface of the particles. The DNA particles were then used for detection of viral DNA and also for formation of two-dimensional arrays on planar substrates.[146]

Post-polymerization modification can not only be used for coupling biomolecules but also for grafting synthetic polymer chains. Hu et al. synthesized pNIPAm microgels and grafted pNIPAm chains on these particles by reversible addition fragmentation chain transfer (RAFT).[147] They then used these particles to study the thermal behavior of the grafted polymer. In another example Hu and Wu grafted PEO chains onto the microgel particles. The hydrophilic PEO chains were observed to stretch from the particle surface as the particles size decreased with increasing temperature.[148]

3. Hydrogel Particles in Nanotechnology

The synthetic methods described in the previous Sections have enabled the field to advance towards the application of hydrogel nano- and microparticles in more complex biotechnology and nanotechnology applications. In this Section, we will describe some of these applications, highlighting systems where the ability to create synthetically and topologically complex hydrogels has led to successful incorporation into advanced nanosystems.

3.1. Drug and Gene Delivery

Recently, significant efforts have been put into devising colloidal drug carriers. It has been hypothesized that an actively targeted particulate drug carrier will increase the therapeutic efficacy of a drug by delivering that drug to the diseased site, while also reducing systemic side-effects of the drug. An ideal drug carrier should be able to target and deliver only to the diseased sites, it should not induce immune response, and it should be degradable and produce nontoxic degradation products.[149]

The particulate carriers that have been most widely studied are liposomes and polymer nanoparticles. Liposomal drug carriers have been studied extensively, and a few liposomal formulations are currently available in the market, while many others are in the development "pipeline". One important drawback of liposomes is payload leakage. Since the boundary of the liposomes is a simple lipid bilayer, performance can be hampered by passive diffusion of drugs across that boundary.[150]

Among polymer particles, the most widely studied are poly(lactic acid-*co*-glycolic acid) (PLGA) particles.[151] The popularity of this material largely stems from its degradation into nontoxic by-products, which can be removed from the body by the renal system. However, this construct suffers from numerous drawbacks, as it is a very hydrophobic, immunogenic polymer with acidic degradation products. The increase in acidity associated with polymer degradation can induce nonspecific inflammatory responses, which can be very detrimental in targeted delivery applications. Non-viral gene-delivery systems have been proposed as a safer alternative to viral vectors, since they will induce host immune response to a lesser extent than viral vectors. Several cationic polymers, such as polyethyleneimine, polyamidoamine, and polylysine, have been used for non-viral gene delivery, but they all lack the biocompatibility needed for in vivo use.[152] Conversely, hydrogel nanoparticles, are a potentially useful class of materials in drug-/gene-carrier systems, but have been studied much less extensively. Herein we report a few examples of recent efforts involving nanoparticulate hydrogel delivery vehicles.

In an effort to employ naturally occurring polymers as delivery vehicles, Wang and Wu used agarose gel particles for

protein delivery.[153] A model protein, ovalbumin was encapsulated in approximately 500-nm diameter, spherical agarose gel particles. After protein loading, it was demonstrated that the particles released ovalbumin in a temperature-dependent fashion, where the rate of protein release was higher at elevated temperatures. This effect is due to the higher swellability of agarose at higher temperature, which facilitates mass transport.

Another example of protein delivery was reported by Li et al.,[154] who demonstrated that poly(vinyl alcohol) hydrogel nanoparticles could be used for this application. These particles were prepared by an emulsion technique without incorporating any cross-linker. The model protein, bovine serum albumin (BSA) was incorporated during the particle formation. As with the previous example, the rate of release of BSA increased with temperature. The authors attribute this behavior to the decrease in the number of cross-links in the polymer network at higher temperature, thereby making the gel more "open" for the transport of BSA. Using this methodology, they were able to demonstrate release of BSA over 30 h.

Biodegradable hydrogel nanoparticles have been prepared by Kim et al. using glycidyl methacrylate dextran as the major comonomer and dimethacrylate poly(ethylene glycol) as a covalent cross-linker.[155] In this case, the particles were prepared by free-radical polymerization and a hydrophobic drug, clonazepam, was then loaded in the particles. It was found that the release rate was dependent on the pH value as well as the concentration of the enzyme dextranase, which degraded the dextran and eroded the particles.

Na and Bae have used self-assembled hydrogel particles of pullulan acetate and sulfonamide conjugates to study the release of the drug adriamycin.[156] In this case the pullulans had a pH-responsive polymer incorporated into their structure, which caused the particles to shrink and aggregate at $pH < 7$. The shrinking of the particles in turn caused the expulsion of the drug into the surrounding medium.

An interesting example of the controlled release of a drug from a microgel comes from Needham et al.[157] They used approximately 6.5-μm diameter methacrylic acid microgels as a matrix for drug encapsulation and release. While these particles are by no means in the nanoscale-range, they illustrate an interesting concept that could be extended to the nanoscale. Under conditions where the particles are deprotonated, and hence swollen (as a result of Coulombic repulsion between the negatively charged regions), the particles could be loaded with the anticancer drug doxorubicin. Upon reducing the pH value of the medium below the acid pK_a, the particles condensed. To prevent leakage of the drug from the polymer, the particles were then coated with a lipid bilayer. These lipid-coated particles were then suspended in a medium of $pH > pK_a$. Surprisingly, no gel swelling was observed and hence no drug was observed to leak from the construct. To release the drug, a series of voltage pulses was applied to electromechanically disrupt the bilayer and cause the swelling of the particle. Thus, it was illustrated that the drug could be protected and then be released on demand using a subtle stimulus. The concept is illustrated in Figure 16.

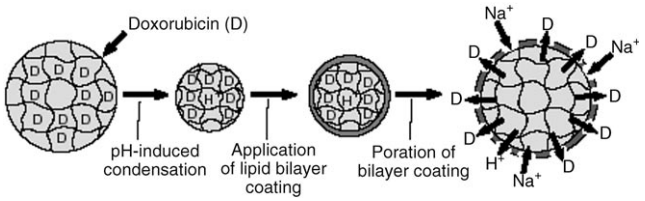


Figure 16. Loading and release of doxorubicin from lipid-coated microgels. Reprinted with permission from ref. [157], Copyright 1998 Macmillan Magazines Limited.

A similar example was published by Moore and coworkers. Again, in this example the polymer constructs are not at the nanoscale level but nonetheless provide an interesting concept.[158] A pH-sensitive gel was photopolymerized in a cylindrical shape. The gel was fabricated from 2-hydroxyethyl methacrylate, acrylic acid, and ethyleneglycol dimethacrylate. Palmitoyl chloride was then covalently bound to the surface of the gel. This modification of the gel created an ion barrier that enabled the pH-sensitive gel to remain in a condensed state even in media with a high pH value. Only when this barrier was disrupted by a surfactant did the gel swell (Figure 17). The ability of the fatty acid layer to maintain a sharp chemical gradient is analogous to the function of a cell membrane and shows the promise of creating highly non-equilibrium systems from well-designed nanomaterials.

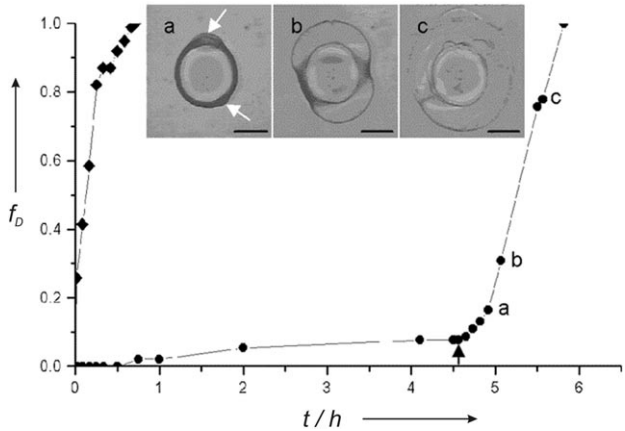


Figure 17. Lipid-modified pH-responsive gels. When the lipid-modified gel (●) is exposed to a buffer solution at pH 12, it resists swelling for a significant time, whereas the unmodified gel swells immediately (◆). The photographs show the effect of SDS on the lipid–modified gel in pH 12 buffer. The arrows indicate the points at which instabilities are first observed; swelling propagates from these points. The scale bar is 250 μm. Reprinted with permission from ref. [158].

While the previous examples were simple demonstrations of ex vivo controlled release from hydrogel particles, others have applied nanoparticulate hydrogels to in vivo delivery. For example, Hsiue et al. have used pNIPAm nanoparticles for ocular delivery.[159] Two formulations were used, one was composed of a solution of linear pNIPAm, the other was a mixture of linear pNIPAm and pNIPAm particles. The drug-

release and cytotoxicity studies were carried out on rabbits. The drug epinephrine, which reduces intraocular pressure, was then delivered from each of the two formulations. It was observed that the intraocular pressure was decreased for around 24 h when the linear pNIPAm system was used, while the mixed system extended the therapeutic effect to about 32 h. Systems such as these are therefore potentially interesting for the clinical treatment of glaucoma.

As mentioned above, an ideal drug carrier should not induce an immune response in the host. This property is commonly achieved by making the surface of the particle hydrophilic, which can prevent opsonization (i.e. adhesion enhanced phagocytosis) by macrophages.[160] For example, Gaur et al. synthesized cross-linked poly(vinylpyrrolidine) hydrogel nanoparticles ($\approx$100 nm diameter).[161] The surface of these particles was then made hydrophilic by attaching poloxamers and poloxamines, which are PEG/poly(propylene glycol) block copolymers. In vivo studies in mice indicated that less than 1% of the dose was retained by the macrophages in the liver, and even after 8 h of injection around 5–10% of these particles were still circulating in the vasculature. This enhanced circulation time, and the lack of liver accumulation, could enable the use of such particles in drug delivery. They also reported that an increase in size and hydrophobicity of the particles increased the uptake by reticulo-endothelial system, suggesting that both factors may play a role in the ability of the body's defense mechanisms to recognize the particles as foreign invaders.

Targeting is an important property for a drug carrier, as potentially, the uptake and retention of the nano-carrier at the site of disease can be enhanced by active targeting. We have synthesized a folic acid labeled pNIPAm core–shell microgel that can target cancer cells.[162] Folic acid is a well known ligand for targeting cancer cells because most tumors overexpress folate receptors. In this demonstration, pNIPAm core–shell hydrogel particles were synthesized, in which the pNIPAm core was fluorescently labeled and the pNIPAm shell contained a small amount of a comonomer containing a primary amine. We then covalently coupled folic acid to the amine-containing hydrogel shell to localize the targeting ligand on the shell surface. When these particles were incubated with cancer cells that were overexpressing the folate receptor, the hydrogel nanoparticles were taken up by receptor-mediated endocytosis (Figure 18). It was also observed that the particles exhibited thermal cytotoxicity above the phase-transition temperature. The exact reason for this effect is not known but is suspected to be intracellular aggregation and protein adsorption onto the deswollen, hydrophobic pNIPAm particles. Since these particles apparently retain their thermoresponsivity in the cytosol, it was hypothesized that they could enable thermally triggered delivery of chemotherapeutic payloads, thereby enabling both active targeting and triggered delivery in one vehicle.

In another example of active targeting, Choi et al. used pNIPAm microgels for targeting liver cells.[163] They used pNIPAm-*co*-acrylic acid microgels that were tagged with fluorescein, while the targeting moiety in this case was galactose, which is a ligand for asialoglycoproteins. Through this ligand–receptor interaction the galactosylated microgels

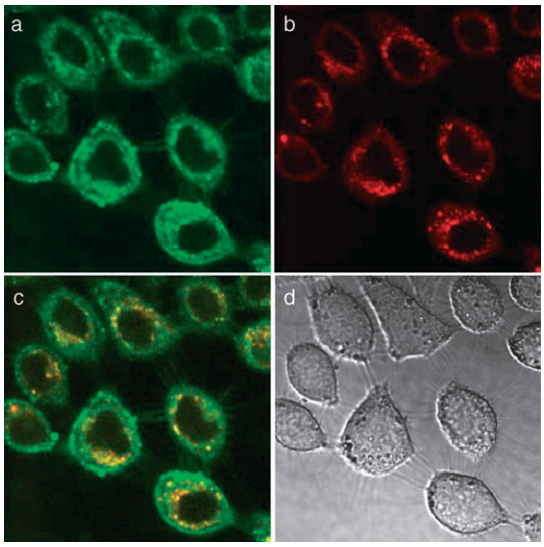


Figure 18. Confocal images of HeLa cells incubated with folate-conjugated nanoparticles. a) Green-fluorescent particle channel, b) lysotracker red-dye channel, c) overlap of both the channels and d) transmittance image of the cells. Experiments performed in collaboration with Jean Chmielewski at Purdue University.

were internalized in the cells. Furthermore, because these particles are thermoresponsive, their temperature-dependent uptake was studied. The uptake efficiency increased with the increase in temperature, which was suggested to arise from the enhanced uptake efficiency of smaller particles, although the increase in particle hydrophobicity could also enhance uptake. The Wooley group has investigated SCKs with targeting ligands such as folic acid, integrins, and peptides.[164–166] They have also demonstrated that nanoparticles coupled with a short peptide belonging to the protein transduction domain of HIV exhibited targeting ability for CHO and HeLa cell lines.

Synthetic–viral composite systems have also been explored. For example, Jana et al. prepared poly(vinylpyrrolidone) nanoparticles and encapsulated them in a reconstituted Sendai viral envelope containing only the fusion proteins.[167] These particles were incubated with human hepatoblastoma cell lines, which resulted in internalization of the polymer particles, as confirmed by fluorescence spectroscopy. Na et al. have used self-assembled polysaccharide (curdlan) particles for targeting.[168] Curdlan was hydrophobically modified with a carboxylated sulfonylurea derivative. The targeting ligand was lactobionic acid, which targets HepG2 cells. As expected, the degree of nontargeted uptake was significantly diminished relative to that for particles targeted to HepG2 cells.

Many groups have used cationic polymers, such as chitosan, for gene delivery. Chitosan is a natural cationic polysaccharide consisting of D-glucosamine and *N*-acetyl-D-glucosamine. This polymer has been shown to be biocompatible, non-immunogenic, and degradable, thereby making it potentially suitable as a delivery vehicle. In the presence of polyanions, chitosan can form hydrogel nanoparticles by complex coacervation. For example, chitosan–DNA nano-

particles have been widely studied for their application in gene delivery.[152] Targeting agents, such as transferrin, have also been conjugated to these particles to increase the internalization, while drugs such as chloroquine have been encapsulated within these particles to investigate controlled release. Mitra et al. have used chitosan to encapsulate doxorubicin, a highly toxic chemotherapeutic drug.[169] For encapsulation they first conjugated doxorubicin with dextran. This drug–dextran conjugate readily formed particles when mixed with chitosan. In vivo studies then showed that the chitosan–drug conjugate circulated in the blood longer and decreased the size of tumor to a larger extent than the free drug.

We have developed a technique to fabricate microgel thin films, which can potentially be used as drug-delivery implant devices.[85] The films are made by assembling charged microgels on a surface by a traditional layer-by-layer approach.[170] The microgels incorporated in these films retain their pH- and thermoresponsive properties.[83,85] In one example, we have loaded the microgels with insulin before assembling them into films.[82] The films then release insulin when subjected to a temperature above the microgel VPTT. The data shown in Figure 19 illustrate that this release can be thermally modu-

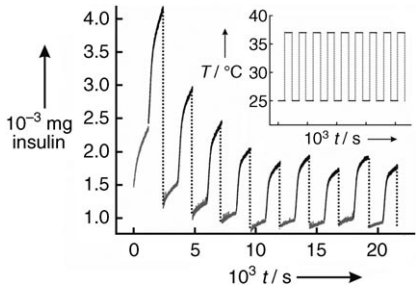


Figure 19. Insulin release during each thermal pulsing of 30-layer microgel film. Inset: the time–temperature wave form used for temperature modulation. The *x*-axis of the inset is identical to that of the main panel.

lated by alternating between heating and cooling cycles. Such films were extremely stable over extended periods of continuous thermal cycling. In addition to the release of macromolecules, these films have also been used for releasing small drug molecules such as doxorubicin.[84] Unlike in the insulin case, these films were first fabricated and then loaded with doxorubicin by thermal cycling. Similar results were obtained for the doxorubicin loaded films, where film deswelling resulted in the expulsion of the drug over many thermal cycles. These initial experiments provide a basis for creating constructs that can release therapeutic agents in a pulsed fashion when prodded by an external signal.

3.2. Encapsulation and Microreactors

The large internal free volume and hydrophilicity of hydrogels makes them useful in encapsulation of various species, such as DNA, RNA, small molecules, and proteins. When encapsulated, the hydrogel network can protect these species or release them in a controlled manner, as discussed in section 3.1. In this Section we will discuss the encapsulating property of microgels for applications other than drug delivery.

It has been observed that mass-transport rates of small molecules in hydrogels depend on the pore size, where larger pore sizes make it easier for the molecules to diffuse in the gel. In case of thermoresponsive microgels, a temperature-dependent diffusion is observed. At $T < \mathrm{VPTT}$ higher diffusion rates are observed than at $T > \mathrm{VPTT}$, owing to a decrease in the pore size of the polymer network at higher temperatures. Also, the charge on the polymer network is an important parameter, because it is easier to encapsulate molecules that are oppositely charged with respect to the polymer. In addition, a hydrophobic polymer network will enhance the encapsulation of a hydrophobic moiety.

Akiyoshi et al. have used cholesterol-bearing pullulans to encapsulate various proteins and peptides, for example, insulin, for which they observed the conformational stability of the macromolecule using circular dichroism (CD) spectroscopy.[139,140] No spontaneous release of insulin was observed from the nanoparticle, suggesting a very strong complexation in the macromolecular assembly. They observed that free insulin aggregated when subjected to heating but the encapsulated protein resisted such aggregation. They further studied the insulin stability against α-chymotrypsin activity, which degrades insulin. The pullulan-insulin complex resisted degradation to a much larger extent than the free insulin. Hence this system can potentially be used for the protection of the species of interest. Similarly, pullulans have been used as molecular chaperones for an enzyme.[141] Carbonic anhydrase B is an enzyme that denatures and aggregates at high temperature. The enzyme, in its denatured state, was complexed with the pullulans. The complex was cooled and the enzyme was released from the particles. The enzyme refolded in its native state and again became active.

SCKs have also been used for encapsulation and release of various entities. Murthy et al. have synthesized a polystyrene-*b*-poly(acrylic acid) SCK which has a thermally labile linkage at the core–shell interface.[136] After self-assembly and cross-linking of the shell, the particles were heated to 125 °C, which cleaved the bond between the polystyrene block and the poly(acrylic acid) block. The resulting release of the hydrophobic polystyrene chains occurred as a function of the cross-link density in the shell, with more heavily cross-linked shells releasing a smaller amount of hydrophobic polymer. In a further study, SCK particles were fabricated with poly(ε-caprolactone)-*b*-poly(acrylic acid).[129] Poly(ε-caprolactone) is a semicrystalline polymer with a lamellar lattice structure. In this case it was observed that at room temperature the core crystallizes and forces the particle to adopt a disc-shaped structure, which was confirmed by AFM. When the particles were heated above the melting point of the crystal, the particles adopted a spherical structure. Hence by using this novel construct it was possible to study the crystallization of poly(ε-caprolactone) in a confined system. SCKs have also been used to package DNA and thus offer protection against enzymatic degradation.[171]

Soft nanomaterials have potential for being used as reaction vessels, which can then control the volume in which a specific reaction can take place. Liu et al. reported the use of self-assembled triblock copolymers, which they refer as shell cross-linked micelles, for preparation of gold nanoparticles.[172] The block copolymer that they used was composed of poly(ethylene oxide)-*b*-poly(glycerol monomethacrylate)-*b*-poly(diethylamino)ethyl methacrylate (PEO-GMA-DEA) as well as a second version in which the middle block was replaced by hydroxyethyl methacrylate (PEO-HEMA-DEA). These triblock copolymers form "onion like" micelles, where the core is made of DEA, the middle shell is of HEMA/GMA, and the outer shell is of PEO. After micellization, the middle shell was cross-linked with divinyl sulfone to make the structure stable. The DEA in the core was protonated by $HAuCl_4$ and subsequent in situ reduction of Au^{III} to Au^0 using $NaBH_4$. The mean size of the resultant gold nanoparticles was around 1–5 nm. The gold particles formed were not very monodisperse since each "nanoreactor" contained several gold particles and also the size was not controllable. This technique has one main advantage, that the dimensions of the nanoreactors are preserved during the reduction step as a result of the cross-linked shell. Zhang et al. have also synthesized inorganic nanoparticles in microgels.[173] They used pNIPAm-*co*-acrylic acid-*co*-2-hydroxyethyl acrylate microgels cross-linked with BIS. First the acid groups were deprotonated at high pH value and then, depending upon the type of nanoparticles desired, different precursor cations were introduced into the microgels. Three different types of nanoparticles were synthesized within the microgel matrix: CdS (a solution of Na_2S was added to a Cd^{2+}/microgel solution), Ag (an Ag^+/microgel solution was reduced by $NaBH_4$), and Fe_3O_4 (an aqueous Fe^{2+}/microgel solution was oxidized by $NaNO_2$). Most importantly, the size and polydispersity of the nanoparticles could be controlled by controlling the composition and the structure of the microgels.

3.3. Analytical Applications

Given the ability to synthesize a variety of responsive hydrogel structures, chemical and biological sensing applications remains an intriguing application. One of the earliest types of hydrogel nanoparticle employed in "sensing" was a pH-responsive particle. The simplest method of fabrication involves the use of a pH-responsive moiety, such as a weak acid, which can be copolymerized into the polymer network. At low pH value, the acid groups are protonated and the particles will be in a somewhat condensed form, while at a higher pH values at which the acid groups are deprotonated, the particles adopt a swollen structure because of Coulombic repulsion among the negatively charged regions and a change in the free energy of mixing with water. Similarly, charged microgels are responsive to ionic strength, where an oppositely charged ion neutralizes the charge and causes the gel to shrink. Similar approaches have enabled the fabrication of cross-linked block copolymer micelles that are pH-responsive. Although this approach appears to be a generalizable motif by which it is possible to imagine designing hydrogel nanoparticles that "sense" their surroundings, very little else has been done on creating hydrogel nanoparticles for real chemical-sensing applications. However, a few examples are beginning to emerge. For example, we have demonstrated that hydrogel microstructures can be rendered sensitive to protein binding, provided the interaction is multivalent and therefore results in an increase in the microgel cross-link density.[40] This approach has the potential to be extended to reversible biosensors based on antibody–antigen displacement or competitive binding. New methods for glucose sensing based on shell-restricted swelling in core–shell microgels are also currently under development.[174] It is clear that numerous opportunities exist for the design of new bioresponsive structures, and given the current focus on extending the complexity of hydrogel-nanoparticle architectures, more studies of this type can be expected in the near future.

In addition to these sensing applications, microgels have been used for the separation of proteins from complex media. Kawaguchi et al. reported that proteins could be separated using thermoresponsive microgels.[145] They used regular pNIPAm microgels and observed that at $T >$ VPTT larger amounts of protein bound to the particles than at $T <$ VPTT. The higher degree of protein adsorption at $T >$ VPTT was attributed to the hydrophobic interaction between the protein and dehydrated polymer. In an approach that utilized Coulombic interactions, Elaissari et al. used cationically charged pNIPAm microgels for the extraction of RNA. It was observed that the interaction between the cationic particles and negatively charged RNA decreased with an increase in pH value, ionic strength, or temperature, thereby indicating that adsorption was mainly governed by electrostatics.[175] In an immunoseparation study, Kondo et al. synthesized poly(styrene/NIPAm/glycidyl methacrylate) microgels. These particles were designed so that they flocculated at high temperature and at high ionic strength. Using the glycidyl methacrylate comonomer as a chemical handle for chemoligation, BSA was coupled to the particles, which were then used for the immunoseparation of anti-BSA from serum. After incubation with the serum, the particles were separated by flocculation.[176] Similarly, particles that contained magnetite were used for separation and purification, the particles could be collected by using a magnetic field.[177]

Stimuli-sensitive microgels can also be envisioned for applications in which the partition coefficient of a solute can be tuned over a wide range. For example, Kanzawa and coworkers have used the thermoresponsive properties of pNIPAm in chromatography for the separation of variety of compounds, such as steroids, peptides and proteins. Their construct uses silica particles, to which pNIPAm is grafted. These particles are an interesting stationary phase in chromatography, as the partition coefficient of a solute between the mobile and stationary phase should be strongly temperature dependent. Below the pNIPAm LCST, the particles are considered to behave as a normal, hydrophilic chromatography phase, while above the LCST, the particles should act as a hydrophobic reversed phase. The relative hydrophobicity of the polymer and the LCST can be further changed by incorporating other copolymers. Figure 20 shows chromato-

grams for separation of steroids with respect to increasing the hydrophobic content of the stationary phase. Using these methods, it has been possible to increase the number of theoretical plates in separations of a wide range of analytes by running temperature gradients as opposed to the standard solvent-composition gradients that are the norm in most HPLC methods.[178–180] Doherty et al. have used nanogels in capillary electrophoresis (CE) based separation of DNA.[181,182] Currently CE is widely used for high-throughput DNA sequencing, in which polyacrylamide solutions are the most common sieving matrix. In the case of nanogel-based matrices, inverse emulsion polymerization was used to synthesize materials of the desired size and cross-linker concentration. These particles were synthesized from acrylamide and a small amount of BIS to obtain sparsely cross-linked networks. These nanogels were used for both conventional capillary electrophoresis and chip-based electrophoresis. In both cases they gave better selectivity and longer read lengths for single-stranded DNA than that obtained with conventional matrices.

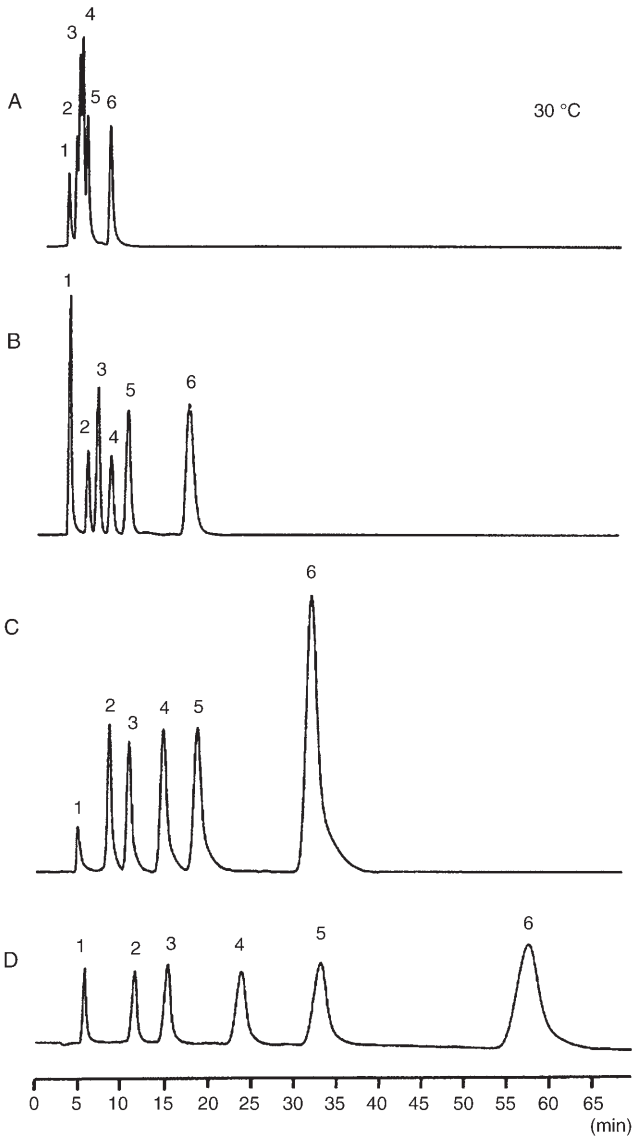


Figure 20. HPLC separations of steroid mixtures using a column packed with particles having thermally tunable hydrophobicities. The hydrogel hydrophobicity increases from A to D. The chromatograms indicate that a higher hydrophobicity results in a better separation under these mobile-phase conditions. Reprinted with permission from ref. [178].

Hydrogel nanoparticles have also been employed in a molecularly imprinted polymer (MIP) scheme. The principle behind MIP is based on both shape and molecular-recognition templating. When the polymerization is carried out in the presence of "template" molecules, it is envisioned that the polymer will rigidify around that template, forming a cavity that is optimized for binding that molecule. After the templates are removed, it is hoped that the cavity retains its shape and is able to bind and detect that particular molecule or similar molecules in a complex mixture. Ye et al. have synthesized hydrogel nanoparticles in the presence of theophylline and 17-β-estradiol. The sensing molecules were dissolved in the mixture of methacrylic acid and trimethylolpropane trimethacrylate ($[H_2C{=}C(CH_3)CO_2CH_2]_3CC_2H_5$) and then polymerized either thermally or by UV irradiation. In these studies radio ligand binding analysis was used to determine the sensitivity and selectivity of analyte binding.[183] Competition binding experiments showed high selectivity for the analyte.

In an attempt to couple simple optical interrogation of microgel behavior with responsive structures, we have developed a method to fabricate dynamically tunable microlenses that respond to light, temperature, pH value, and protein binding.[40,184–186] For this application, large (>1 micron) pNIPAm-*co*-acrylic acid particles were synthesized. These particles were then attached to a glass surface by electrostatic assembly. Since these microgels are soft, they deform upon attachment to the surface into planoconvex or hemispherical shapes. Because of the dual responsive nature of this simple polymer, the refractive index of the microgels can be controlled by temperature and pH value, where the more swollen form has a lower refractive index, with the index increasing as the gel becomes more condensed. Because of the specific shape of the microgels, the particles behave as planoconvex lenses, and the lens performance can be interrogated on a simple optical microscope (Figure 21).

In addition to these constructs we have also fabricated microlenses that respond to light.[184] In this experiment gold nanoparticles are assembled on the surface prior to attaching of the microgels. Gold nanoparticles can cause local heating when the surface plasmon modes are excited by a frequency-doubled Nd:YAG laser. We observed that only the region interrogated by the laser underwent phase transition (as a result of the local heating caused by the underlying gold-nanoparticle layer) and projected sharper images. The image quality was also controlled by the power of the laser, ambient temperature, and the pH value of the solution (Figure 22). Finally, microlenses with surface-localized ligands display dramatic changes in focal length upon protein binding, provided the protein–ligand interaction is a multivalent one.[40] This technique of fabricating microlens arrays is inexpensive, simple, and provides a method to interrogate

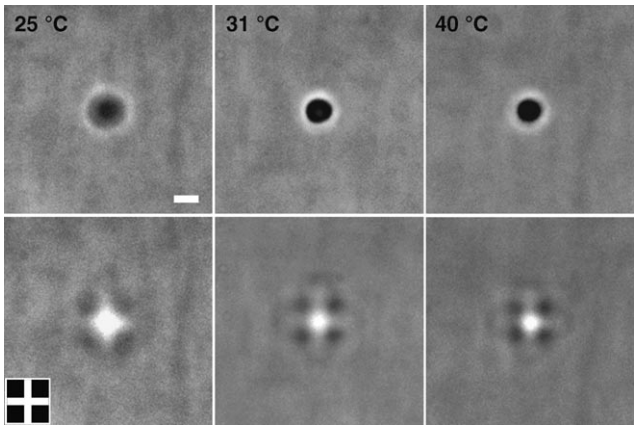


Figure 21. DIC microscopy images (top) of microgels on a glass substrate. The microgel acts as a microlens by projecting a cross pattern (bottom). The quality of the image improves with the increase in the temperature and concomitant gel deswelling. Reprinted with permission from ref. [186]

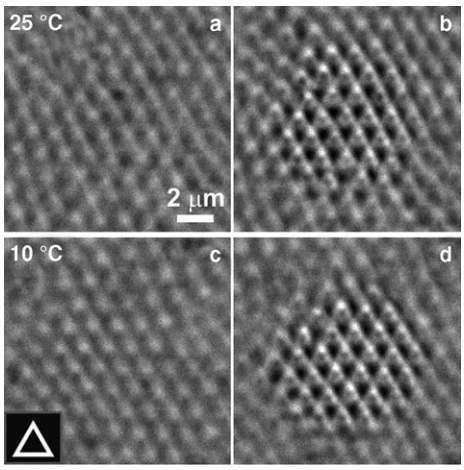


Figure 22. Photoswitching of a microlens array at 25 °C (a and b) and at 10 °C (c and d) as a function of laser power, where in the case of (b) and (d) the laser power is high enough to collapse the particles and thereby increase the microlens focusing power.[184]

single transducing elements, which makes it an attractive construct for various optical and sensing applications.

3.4. Biomaterials

Perhaps the broadest definition of biomaterials comes from the National Institutes of Health Consensus Development Conference: *"[a biomaterial is] any substance (other than a drug) or combination of substances, synthetic or natural in origin, which can be used for any period of time, as a whole or part of a system which treats, augments, or replaces any tissue, organ, or function of the body"*.[187] Research in the field of biomaterials is often linked to developing a more biocompatible system, such as making an implant or surgical device less damaging to the body. In case of hydrogels, much of the effort has been put in to coating the surface of the implants or devices with polymers or bulk gels. With the advent of responsive hydrogels, many researchers began working on making "smarter biomaterials", materials which can sense a change in the environment and respond in a programmed fashion to it. In this Section we describe a few of those examples pertaining specifically to hydrogel nanoparticles.

As mentioned previously, Kawaguchi and co-workers have studied the interaction of proteins with microgels extensively.[145,188,189] For example, the group has studied the effect of temperature on the nonspecific adsorption of proteins to thermoresponsive microgels. They have also investigated the activity of enzymes that are covalently bound to the microgels. In one example, trypsin peroxidase was attached to pNIPAm microgels and its activity was studied as a function of temperature.[190] The enzyme activity decreased with increasing temperature. This change was attributed to the decrease in the pore size which caused the decrease in the rate of diffusion of substrate to the enzyme. In a similar system with a small molecule (ubiquinone) attached to the particle, similar temperature-dependent results were obtained.

Duracher et al. have studied the adsorption of HIV-1 capsid protein p24 on polystyrene-core-pNIPAm-shell particles.[191] As predicted by numerous studies, at $T >$ VPTT of pNIPAm a higher adsorption was observed which was due to hydrophobic interactions. Similarly Urakami et al. studied the phagocytosis of polystyrene-*co*-polyacrylamide gel particles as a function of hydrophobicity. They observed that phagocytosis by granulocytes increased with the increase in polystyrene content of the particles, again presumably arising from an increase in hydrophobic association with the granulocyte.[192] In similar studies, Kimhi and Bianco-Peled used isothermal titration calorimetry (ITC) to study the adsorption of small molecules (aspartic acid and valine) to pNIPAm microgels as a function of temperature.[193] At 25 °C aspartic acid binds strongly to the polymer particles owing to the formation of hydrogen bonds, at 37 °C valine binds strongly because of the hydrophobic effect.

More advanced architectures can be prepared that take advantage of biocatalytic systems. For example, Ogawa et al. have synthesized pNIPAm microgels containing a pendant vinyl imidazole side-chain, which again allows for pH-tunable gel swelling.[38] The enzyme urease, which catalyses the hydrolysis of urea into ammonia, was then physically entrapped in the particles. As the enzyme produced ammonia, the pH value of the medium increased. Hence, in the presence of urea the particles shrank owing to the increase in pH value and subsequent deprotonation of the imidazole unit. When the substrate was removed, the particles swelled to their original size as the local pH value equilibrated with the pH value of the surrounding bath. To demonstrate the potential utility of such a biomechanical system, these particles were incorporated into a membrane. Upon introduction of urea to one of the solvent reservoirs, the permeability of the membrane increased as the particles shrank.

An important aspect of many implanted biomaterials relates to the ability of cells to adsorb and proliferate on the material surface. Even materials with low surface energies

and hence low degrees of non-specific protein adsorption can tend to foul over time in cell culture or after implantation. Hence, it is important to evaluate fouling of biomaterials as well as to arrive at new strategies for mediating cellular recruitment at synthetic surfaces. Thus, in addition to the aforementioned protein adsorption studies, Kawaguchi and co-workers have also studied the effect of cell binding to thermoresponsive particles on a solid surface.[145] pNIPAm microspheres were deposited on a plate to produce a 2D array upon which the cell culture medium was seeded. It was observed that the cells produced more reactive oxygen species at 37 °C than at 25 °C, indicating that the cells are under more mechanical stress at the higher temperature. This stress is presumably due to stronger attachment at $T > \mathrm{VPTT}$. It was also observed that the amount of reactive oxygen species produced when the system was heated from 25 °C to 37 °C was much higher than when just incubated at 37 °C. This result was attributed to the stimulus inflicted by the dynamic deswelling process. In addition to this system a ligand–receptor system was also used a to study the mechanical stress on the cells.[145]

Our group has fabricated particles in which the adsorption of the proteins to the particles is reduced by using PEG grafting.[194] We used pNIPAm-core/pNIPAm-shell particles and attached PEG either to the core or to the shell by copolymerization of PEG-monomethacrylate. Reduced protein adsorption was observed for both the core- and shell-grafted PEG particles. NMR spectroscopy and protein adsorption measurements, showed that at high temperature the PEG chains phase separate to the particle surface, and because of the polymer's hydrophilicity reduce protein adsorption. For particles in which the PEG is attached to the core, the PEG chains are able to penetrate the shell and phase separate to the surface, thus reducing the surface energy of the deswollen particles.

In another example of core–shell particles that may have utility in biomaterials applications, we have prepared a system in which the shell acts a barrier between the protein in the solution and a core-localized ligand buried under the shell.[195] In this case we have synthesized a core to which biotin is attached, then added a shell containing a degradable cross-linker. Initially the cross-linker density is high enough that the pore sizes are smaller than the size of the protein avidin. As the cross-linker is degraded, the average pore size increases and allows permeation of avidin to the core, where it can bind to biotin (Figure 23). We have also observed protein-size-dependent permeation, that is, for larger proteins, more cross-links have to be degraded to allow for binding. These systems are interesting from the view of the topological complexity and also because they may be a model system for a particle that can "express" a particular functionality at the surface in response to a biological or chemical signal which disrupts shell-localized cross-links.

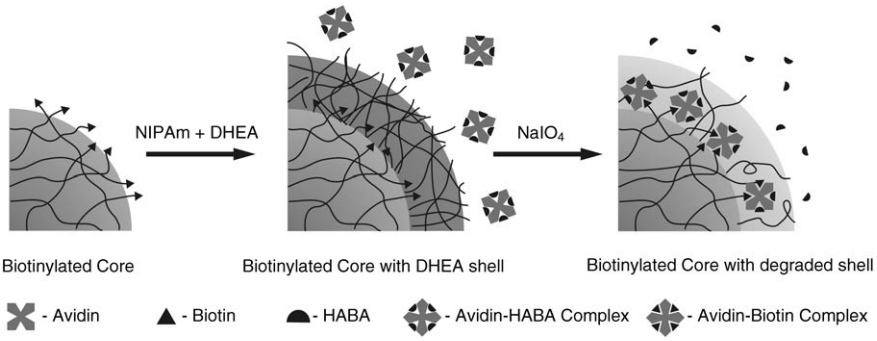


Figure 23. Permeation-selective core-shell microgels. The particles exhibit shell cross-linker-density dependent permeation of the protein.[195]

3.5. Colloidal Assemblies

Perhaps one of the most intriguing properties of monodispersed colloidal systems is their ability to form thermodynamically stable, ordered, periodic phases.[196] The most common of these phases is the cubic crystalline lattice observed in natural opal.[197,198] Synthetic colloidal crystals have potential applications in the field of diffractive optics, especially in the context of photonic band-gap structures.[199] Traditionally, hard sphere particles, such as silica or polystyrene beads, are used to form synthetic crystals. Recently, however a great deal of effort has been put into assembling crystals from soft colloidal precursors owing to their interesting phase behavior and the potential for creating dynamically tunable assemblies.[72–75,200–202]

Of particular interest is the potential for using microgel particles as colloidal model systems.[69,203–205] The Richtering group has extensively investigated the microgel phase diagram and has shown that deviations from hard-sphere behavior can be observed at high particle concentrations.[73,98,203,206,207] Furthermore, neutron-scattering studies have enabled the elucidation of the microgel network structure, and how that structure dictates the observed phase behavior.[207] Others have used computational methods to investigate how particle "softness" can change the effective interaction potential and the resultant phase diagram of both microgels[205,208,209] and star polymers.[210–214] Importantly, these calculations have revealed a potentially very rich phase behavior for soft colloids,[205,209,211,212] in contrast to the structures observed for hard spheres.[215]

Our group has worked intensively in the field of soft-sphere assembly, wherein we have developed methods for preparing colloidal crystals from thermoresponsive hydrogel particles. For an in-depth discussion of our work, see a recent review article on this subject.[200] One of the particular advantages of using thermoresponsive particles is that the volume phase transition allows assemblies to be annealed that have been formed by harsh methods, such as centrifugation.[72] In this fashion, it is possible to form crystals much more rapidly than those prepared by slow sedimentation of hard-sphere colloids. The thermoresponsivity of the particles has also been used to tune the Bragg peak of the crystals, both in a

dynamic sense, and also by controlling the temperature at which the particles are centrifuged.[216]

We have also studied the phase behavior of particles that display unusual, weak attractive interactions.[217] pNIPAm-*co*-acrylic acid particles were synthesized and assembled at various volume fractions. Owing to the soft nature of the particles it was possible to overpack the system so that the effective volume fraction was greater than 100%. It was also observed that, in comparison to the hard spheres, the crystals did not melt until very low volume fraction. This property arises from multibody attractive interactions that result from dipole–dipole and ion–dipole interactions. Images corresponding to such crystals at various stages of growth are shown in Figure 24.

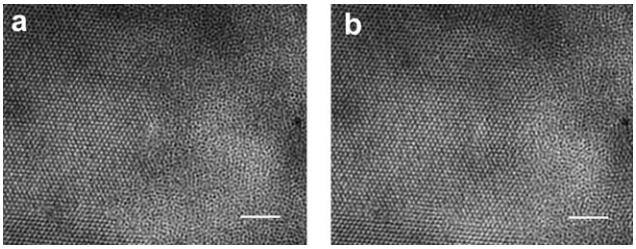


Figure 24. Growth process of weakly attractive hydrogel colloidal crystals as observed by differential interference contrast microscopy. Note that the effective particle volume fraction for this sample is approximately 40%. Panels (a) and (b) were acquired 5 minu apart; the progression of the crystal growth can be observed as the crystalline region extends further into the sample in panel (b). The scale bar is 20 μm. The unique phase behavior of these crystals is described in reference [217]. We thank Saet Byul Debord for supplying these images.

Composite colloidal crystals have also been made by incorporating gold nanoparticles, for photopatterning, into the system.[218] A frequency doubled Nd:YAG laser ($\lambda =$ 532 nm) was used to optically heat the system. In this fashion, it was possible to convert crystalline regions into glassy state (non-ordered state) and vice versa, by controlling the laser power and illumination times. Using this approach it was also possible to write patterns in the crystal assembly by a laser direct-writing approach.[219]

Asher and co-workers have fabricated polymerized colloidal crystal arrays from hydrogels for a number of applications. In one example they crystallized polystyrene beads and then polymerized the system using pNIPAm.[201] This system allows thermal tuning of Bragg diffraction, as the thermoresponsivity of the polymer chains is used to control the interparticle distance and hence the Bragg peak. These systems have also been used for detecting various analytes, where the polymer that connects the particles is responsive to the analyte and, in the presence of that analyte, causes a change in lattice spacing.[220] In one example boronic acid groups were incorporated into the crystalline array for glucose-sensing applications.[221]####Another example of polymerized colloidal crystal assembly was reported by Hu et al.[74] They synthesized two types of pNIPAm microgels, one had acrylic acid units and the other had 2-hydroxyethyl acrylate units. These nanoparticles were self-assembled to form crystals and then bonded together covalently using small linking molecules. For the acrylic acid particles epichlorohydrin was used for covalent bonding of the particles and for 2-hydroxyethyl acrylate particles divinylsulfone was used. These assemblies exhibited iridescent color patterns indicating that the particles assembled in a crystal phase. The covalent nature of the assembly provided stability to the crystal structure, which then enabled the color of the crystal to be tuned by temperature or an electric field.

4. Conclusions and Outlook

Towards the end of the last century, it was commonly thought that the term "nanotechnology" was coined exclusively for "hard materials" but this has changed as polymeric materials became more common ingredients in nanometric systems. Herein we have discussed various types of hydrogel nanoparticles and their applications in nanotechnology. As more and more research is carried out in this field, it becomes clearer that these materials hold great promise on their own, and as a bridge between more traditional nanostructures and biological systems. For example, gels that respond to a change in their environment are potentially useful in the context of truly bioresponsive structures that may enable natural systems to be manipulated in a rational fashion.

Although a great deal of research has been carried out in this field, we believe that most of the potential resources are untapped. There are opportunities for new efforts in advanced synthetic approaches to complex hydrogel nanomaterials, both in colloid synthesis (e.g. size and shape control) and in new chemoligation methods for controlled bioconjugate synthesis. The rational design of multifunctional architectures will be enabled by such efforts, thereby allowing the improvement of current applications, as well as the implementation of hydrogels in new arenas. In the case of core–shell particles reported by our group and others, the range of potential applications in which such materials can be used is just beginning to be uncovered. Early studies of the detailed structure–function relationships in hydrogel particles are now leading to the design of primitive applications-oriented nanomaterials. These proof-of-concept studies can then provide guidance for the synthesis of the second-generation of materials. In parallel, it will become increasingly important to perform detailed studies of cytotoxicity, immunogenicity, and pharamcokinetics, if these materials are to be employed in biotechnological applications. Finally, ground-level cooperation between chemists, biochemists, engineers, and clinicians is desired to enable the design, synthesis, and testing of structures that are truly applicable in clinical applications, such as drug-delivery devices, implantable biomaterials, biosensors/assays, and targeted chemotherapeutic formulations.

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