

Hydrogels in Miniemulsions

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Abstract In the last decade, the synthesis of polymeric materials that respond to specific environment stimuli by changing their size has attracted widespread interest in both fundamental and applied areas of research. Hydrogels in dispersions are composed of randomly oriented, physically or chemically crosslinked hydrophilic or amphiphilic polymer chains. The synthesis of these gels at the nanoscale (nanogels or microgels) is especially of great importance for their application in drug delivery and controlled release systems, and in biomimetics, biosensing, tissue regeneration, heterogeneous catalysis, etc. The focus of this review is to present the versatility of the miniemulsion process for the formation of monodisperse nanogels from synthetic and natural polymers. Several applications of the obtained microgels are briefly described.

Keywords Confined reaction environment · Heterophase polymerization · Microgels · Miniemulsion · Nanocapsules · Nanoparticles

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1 Miniemulsion Polymerization

“Miniemulsion” generally implies a method that allows one to create small stable droplets in a continuous phase by applying high shear stress [1]. Under high shear, e.g. ultrasonication or high-pressure homogenization, broadly distributed (macro)droplets are broken into narrowly distributed, defined small nanodroplets in the size range between 50 and 500 nm. The size of the droplets mainly depends on the type and the amount of the emulsifier used in the particular system. The idea of miniemulsion polymerization is to initiate the polymerization in each of the small stabilized droplets, meaning that polymerization takes place in small nanodroplets. To prevent the degradation of miniemulsion through coalescence or Ostwald ripening, an efficient surfactant and a costabilizer that is highly soluble in the dispersed phase, but insoluble in the continuous phase, should be employed in order to prevent Ostwald ripening. Typically, long-chain alkanes and alcohols as (ultra)hydrophobes have been used as costabilizers for direct (oil-in-water) miniemulsion. In Fig. 1 the process of direct (oil-in-water) miniemulsion polymerization is presented.

The principle of miniemulsion may also be extended to the inverse (water-in-oil) case, in which the Ostwald ripening is suppressed by an agent that is insoluble in the continuous oily phase, a so-called lipophobe. Ionic compounds (e.g., salts or sugars) show a low solubility in organic solvents and can be used as lipophobes,

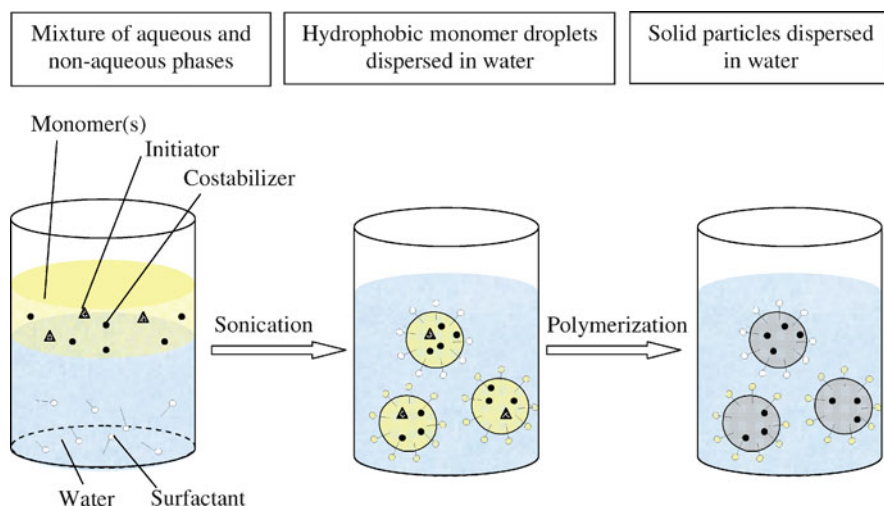


Fig. 1 Synthesis of polymeric nanoparticles from hydrophobic monomers(s) via the direct miniemulsion process

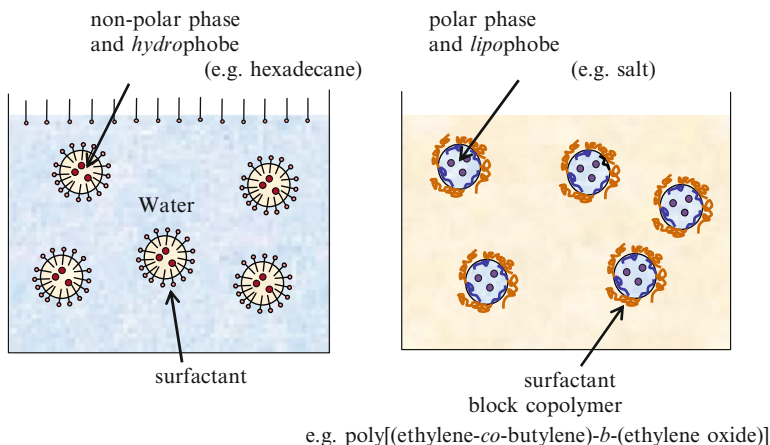


Fig. 2 Comparison between direct (*left*) and inverse (*right*) miniemulsion

and therefore act as costabilizers in water-in-oil miniemulsions [2]. Due to the change of the continuous phase from hydrophilic to hydrophobic, surfactants with low hydrophilic–lipophilic balance (HLB) values are required. Owing to the polymeric and steric-demanding nature, the nonionic block copolymer stabilizers, like poly(ethylene-*co*-butylene)-*b*-poly(ethylene oxide) [P(E/B-*b*-EO)], (which consists of hydrophilic and hydrophobic blocks), are the most efficient [2]. They provided maximal steric stabilization, which is the predominant mechanism in inverse emulsions. In the case of CO₂ miniemulsions, block copolymers with a hydrophilic block and a fluoro- or silicon-containing block like poly(1,1-dihydroperfluorooctyl methacrylate)-*b*-poly(ethylene oxide) (PFOMA-*b*-PEO) have to be utilized [3]. Figure 2 compares direct and inverse miniemulsion systems.

2 Polymerization of Hydrophilic Monomers in Inverse Miniemulsion

In inverse (water-in-oil) miniemulsions, hydrophilic monomers can easily be polymerized. Here, the monomer or the monomer solution is miniemulsified in a continuous hydrophobic phase. The polymerization can be initiated either from the droplet or from the continuous phase. Using crosslinking agent(s) in the monomer mixture leads to the formation of microgel nanoparticles after polymerization, keeping their identity after transferring them into water as continuous phase. This way, nanoparticles based on acrylic acid, acrylamide (AAM) and HEMA could successfully be obtained (see Fig. 3) [2].

As a new approach to the preparation of water-soluble polymers in inverse miniemulsions, a redox initiation system consisting of ceric ions and carbohydrate-based surfactant Span60 as a reducing agent has been successfully used for the

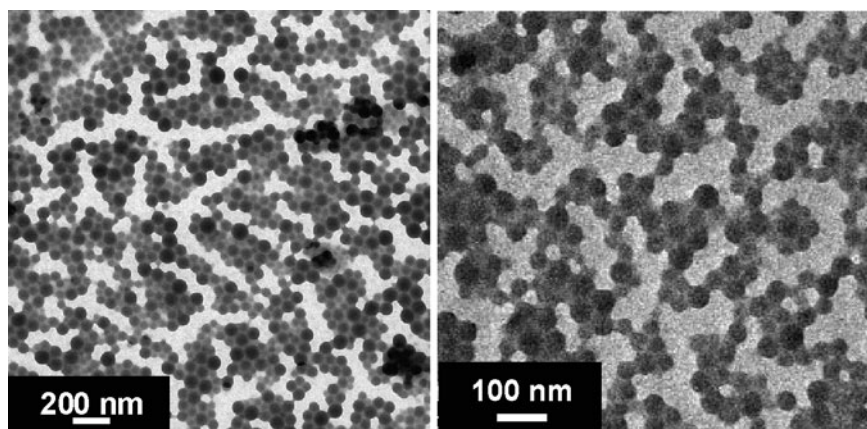


Fig. 3 TEM micrograph of polyacrylamide (*left*) and poly(acrylic acid) (*right*) nanoparticles as obtained by the inverse miniemulsion process

polymerization of AAm. Such an initiation system provides chain nucleation and growth near to interfacial boundaries. As a result, stable uniform polyacrylamide (PAAm) latexes with particle radius of about 50–70 nm are obtained. The prepared PAAm has rather high M_w of up to about 2×10^6 Da. A homogeneous crosslinking, and therefore the formation of microgel nanoparticles, can be obtained by using 2 mol% of *N,N'*-methylene-bis-acrylamide as a crosslinking agent.

3 Gelatin Nanoparticles

As a different approach, preformed hydrophilic polymers in aqueous solution can also be used for the miniemulsification process. In this case, the formulation process should be carried out in an inverse miniemulsion with a hydrophobic continuous phase. In order to obtain microgel nanoparticles, the polymer chains have to be crosslinked in the inverse miniemulsion prior to the transfer to an aqueous continuous phase. As a nice example, gelatin has been used for the formation of microgel nanoparticles [4].

Gelatin is a proteinaceous polyampholytic gel obtained by the partial hydrolysis (acidic or basic) of collagen. Depending on the process used, gelatin is produced as type-A or type-B gelatin. Whereas the acidic treatment yields type-A gelatin with an isoelectric point (*pI*) around 9 and a broad molecular weight profile, the alkaline hydrolysis yields type-B gelatin with a *pI* of around 5 [5, 6]. Due to its chemical and physical nature, gelatin is often used as drug carrier system. By changing the pH or the temperature, the phase behavior of gelatin in solution changes significantly. At low temperatures (around 20°C) and pH 7, the gelatin chains are physically crosslinked, leading to a gel. At high temperature (above 40°C) and pH above 7, the

physical crosslinks break and the gelatin becomes soluble in water. However, chemical crosslinkage results in the formation of a temperature- and pH-independent material, and therefore enables the formation of a microgel.

The different functional groups in the chain allow the incorporation of more functionalities and the introduction of modifications by chemical derivatization. Poly(ethylene glycol)-modified gelatin [7, 8], thiolated derivatives of gelatin [9, 10], chitosan-conjugated gelatin [11], and poly(D,L-lactide)-grafted gelatin [12], have been reported as gelatin-based derivatives for potential pharmaceutical applications. Gelatin-DNA nanospheres as gene delivery vehicles have also been reported [13]. Gelatin can also be used as a matrix for mineralization processes in tissue engineering [14]. The use of gelatin nanoparticles as microgel reactors allows potential application for the release of a variety of different components. However, for this purpose, the gelatin has to be crosslinked chemically in order to keep the nanoparticulate structure at elevated temperature or at low pH.

Several techniques have been used to synthesize gelatin nanoparticles from gelatin and gelatin derivatives. Well-established techniques are desolvation [15–19], coacervation [20–22], and water-in-oil emulsion techniques [23–25]. The flexibility offered by these techniques in tailoring the properties of the nanoparticles is limited. With increasing gelatin concentration and by using a gelatin having a broad molecular weight distribution, it is not possible to effectively and uniformly achieve high gelatin content within the particles. In the desolvation technique, the final particle yield constitutes about only 70–75% of the original amount of gelatin used. As much as 40% of free gelatin chains were still present, irrespective of the amount of crosslinker used [16]. The coacervation and desolvation techniques are both based on phase separation during the preparation step; the crosslinking step is performed after phase separation, when the nanoparticles are already formed and gelatin chains are no longer in the dissolved state. Due to problems with diffusion of the crosslinker molecules into the interior of the nanoparticles, crosslinking only occurs at the surface, and the chains in the interior are not crosslinked. This results in inhomogeneous crosslinking of the nanoparticles with free polymer chains. Among the water-in-oil emulsion techniques, the emulsifier-free water-in-oil approach leads to relatively large particles with an average size of 840 nm [23]. The use of the water-in-oil microemulsion approach in order to obtain smaller particles, however, demands a large excess of surfactant – the ratio of gelatin to surfactant can be as high as 1:1600 [25]. The degree of crosslinking with increasing gelatin concentration and scalability to industrial production are often limited.

In order to overcome the aforementioned problems in producing uniformly glutaraldehyde-crosslinked gelatin nanoparticles with high degrees of crosslinking, a convenient synthetic route based on the concept of nanoreactors (individual nanosized homogeneous entities) using the miniemulsion technique has been used [1, 26].

The miniemulsion technique is here a straightforward approach because it does not rely on phase separation and offers the flexibility of easily varying the gelatin content and the degree of crosslinking using small amounts of surfactant [4]. For the preparation of homogeneously crosslinked nanoparticles, a gelatin solution is

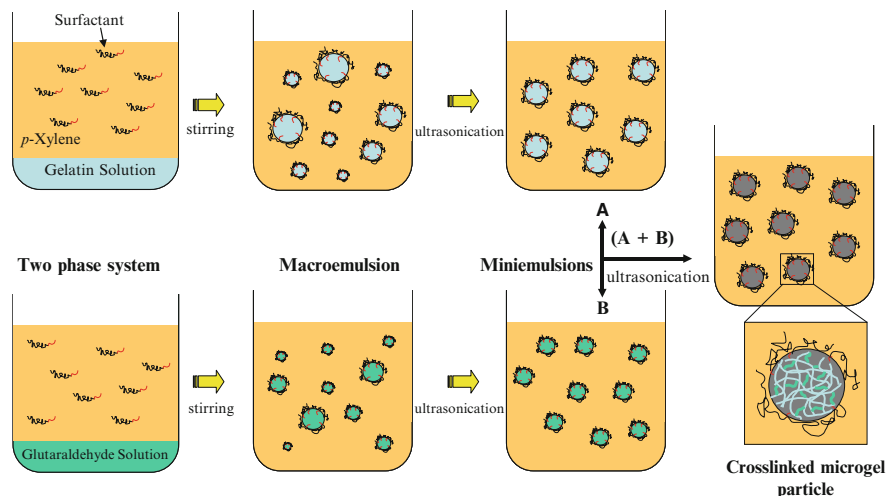


Fig. 4 The inverse miniemulsion process for synthesis of crosslinked gelatin nanoparticles (for details see text) [4]

miniemulsified at elevated temperature in a hydrophobic continuous phase, resulting in a stable inverse miniemulsion [4]. By adding a second inverse miniemulsion consisting of droplets with the crosslinking agent glutaraldehyde, the crosslinking reaction can be performed by a fission and fusion process between the different droplet species (see Fig. 4). The technique allows one to use different types of gelatin without purification or fractionation, and the amount of the gelatin in the droplet and the degree of crosslinking can be varied over a wide range. However, it is of interest to use a minimum concentration of crosslinking agent, at which all chains are crosslinked and no free chains are left behind within the particles. It has been demonstrated that, independent of the molecular weight distribution of the gelatin used, stable nanoparticles can be produced with a small amount of surfactant (see Fig. 4 left). After the synthesis, the organic solvent can be removed and the particles transferred to an aqueous continuous phase. The stability of the dispersion, particle size, and the efficiency of crosslinking (by analyzing the non-crosslinked free chains) have been studied in detail. As gelatin can undergo the volume transition induced by temperature, the swelling behavior of the gelatin particles can be seen when the nanoparticles in water are subjected to a thermal cycle in which the temperature changes between 20°C and 45°C. At 20°C, swollen hydrogel with water present in the channels/pores between the networks is detected. Increasing the temperature to 45°C leads to a loss of the physical crosslinks, resulting in an increase in particle size.

Such microgel nanoparticles with varying gelatin concentration and crosslinking density have a high potential for use in drug delivery applications. The gelatin nanoparticles can also be used as template particles for the formulation of apatite

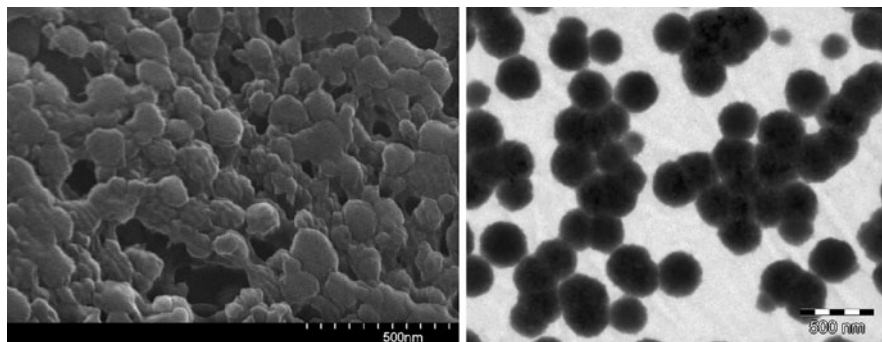


Fig. 5 Air-dried gelatin particles using HRSEM (*left*) [4]. Bright field TEM image of hybrid gelatin nanoparticles (*right*) [27]

nanocrystals [27]. Here, it could be shown that the nano-environment promotes a different growth environment for the crystal due to the confinement inside the hydrogel particle. The formation of hydroxyapatite (HAP) inside the particles follows Ostwald's rule of stages. At first an amorphous phase is formed, which itself has a great potential to be used as a resorbable bone substitute. This further transforms into single crystalline HAP via an octacalcium phosphate intermediate. The solution-mediated transformation into the HAP phase without any calcination step has been studied in detail using transmission electron microscopy (TEM) (see Fig. 5 right) and X-ray diffraction (XRD) measurements.

4 Gels for Release

Smart or stimulus-responsive polymer gels undergo sharp, often jump-wise, volumetric transitions under small changes of external conditions, such as ionic strength, pH, temperature, the action of electric current, mechanical force, etc. [28–30]. These features of smart gels make them increasingly attractive for biotechnology and medicine, as well as for the development of new devices, for example, artificial muscles [31]. The incorporation of diverse particles in the gels often imparts new properties. Gels with entrapped magnetic particles are responsive to a magnetic field [32], and gels containing clays have improved mechanical and adsorption properties [33, 34]. Gels having isolated pores filled with water effectively trap linear macromolecules [35] and low molecular weight multicharged ions [36].

A series of gels with incorporated emulsions have been obtained recently [37]. These gels were used for the preparation of polyelectrolyte gels containing voids [36], in particular, gels with voids having charged walls [38]. It was demonstrated that composite gels that contain entrapped emulsions are effective absorbers of oil-soluble organic substances [39]. Such gels are expected to be of significant practical interest as effective drug delivery systems. The polymer matrix of

similar composite gels can be formed either by the network of crosslinked synthetic polymers (e.g., PAAm [36]) or by physical gels (e.g., agarose-based gels [39]). It was shown that such composites containing entrapped emulsions of water-insoluble hydrocarbons like tetradecane (TD) are very stable and do not release the “oily phase” for at least several months. However, PAAm- and agarose-based gels are not “smart” in the sense that they do not markedly change their swelling degree under changed external conditions, as do poly(*N*-isopropylacrylamide) (PNIPAM) gels. “Smart” thermo-responsive composite PNIPAM gels and cryogels, both containing entrapped TD emulsions, were prepared by three-dimensional free-radical copolymerization of NIPAM and *N,N'*-methylene-bis-acrylamide or bis(acryloyl)cystamine, respectively, in the presence of a dispersion of TD stabilized with sodium dodecyl sulfate (SDS). The polymerization of PNIPAM gels was carried out at room temperature, and cryogels were fabricated in a moderately frozen reaction system below the water crystallization temperature. The latter technique is known to produce macroporous gels with labyrinth-like interconnected gross pores [40]. Both composite gels and cryogels thus obtained were capable of heat-induced collapse at certain critical temperatures. It was found that the extent of the collapse of the composite gel prepared at room temperature was much smaller and without visible squeezing of the liquid lipophilic phase out of the shrunk gel.

5 Hyperbranched Polymers in Miniemulsion

As a basis for hydrogels, hyperbranched polymers [41] can also be used. These polymers can be connected by “click” chemistry in miniemulsion droplets in order to obtain hyperbranched polyglycerol (HPG)-based particles. Such materials are of great interest for drug release because they are nontoxic [42, 43] and show similar behavior to poly(ethylene glycol)s.

Due to the inherent dendritic topology, hyperbranched polymers are of interest for a wide range of optical [44], medical [45–47], materials, and reagent immobilization applications [48]. Compared to perfect dendrimers, the synthesis of hyperbranched polymer is much easier to achieve. The first HPGs were reported by Vandenberg in the 1980s. He used the anionic polymerization of glycidol, resulting in relatively monodisperse, highly branched products with a controlled molecular weight in the range of a few thousand Daltons [49, 50]. Recently, Brooks extended this approach to HPGs with up to 1 MDa molecular weight by conducting the polymerization in bulk heterophase using a nonsolvent (dioxane) as an emulsifying agent [51]. The resulting HPGs are very compact and have spherical conformations in water with a diameter in the order of about 10 nm.

By utilizing polymerization in the miniemulsion system, larger HPG analogues can be created by linking several HPG units to a nanoparticle in order to obtain an optimum diameter of 50 nm [116]. This size range is considered to be ideal for drug delivery carriers that may accumulate in tumors or inflamed tissue by the enhanced permeation and retention effect (EPR) [52].

The majority of reported miniemulsion reactions are between low molecular weight monomers, giving rise to linear polymers via radical or polyaddition processes. The crosslinking of the hyperbranched polymer represents an approach to the crosslinking of existing (2–3 nm diameter, $M_n \approx 5$ KDa) HPG dendritic macromonomers to higher homologues, using the “nanoreactor” template to control size. For this, as facile crosslinking “click” reaction, the Huisgen alkyne/azide cycloaddition was used [53–56]. A similar approach has been reported recently with miniemulsion atom transfer radical polymerization (ATRP) crosslinking of macromonomers by Oh et al. [57]. The Huisgen reaction has been used in macroemulsion to crosslink dextran macromonomers, but this approach has not yet been realized in miniemulsion [58]. Therefore, the “click” chemistry concept has been utilized in order to polymerize highly branched polyvalent macromonomers in miniemulsion, resulting in dendritic nanoparticle covalent aggregates. Dynamic light scattering (DLS) and TEM characterize the formation of HPG-based particles with a narrow size distribution that is tunable between 25 and 85 nm diameter.

For the formulation of hydrophobic nanoparticles, hydrophobic HPG was dissolved in chloroform and then this solution was miniemulsified in water using SDS as surfactant (see Fig. 6a). The crosslinking reaction was performed by CuSO_4 and sodium ascorbate. Hydrophilic nanoparticles of the hyperbranched polymer were obtained in an inverse miniemulsion system by dissolving the polymer in DMF and miniemulsifying the solution in cyclohexane using the block copolymer P(E/B-*b*-EO) as surfactant (see Fig. 6b). After the crosslinking reaction performed at 80°C, the obtained particles could be transferred into an aqueous phase.

Due to the presence of the “click” substrates on the particle surface it is possible to perform further functionalization directly on the surface.

6 Amphiphilic Copolymers

The formation of amphiphilic copolymers consisting of hydrophilic and hydrophobic monomer units leads to hydrogel structures. Amphiphilic copolymers consist of polar and nonpolar monomeric units and are able to stabilize diverse interfaces in aqueous systems. Due to the different polarities, such systems are, however, not easy to make and it is still a great challenge to control the relative incorporation of each monomer in the resulting polymer chain, as well as the homogeneity of the obtained structures. Radical copolymerization reactions in solvents like dimethyl formamide or butanone allows the preparation of some amphiphilic copolymers that can also be suitable for the formation of hydrogels [59, 60]. However, due to solubility problems, the limited range of accessible polymers and control of the monomer sequence are major problems.

Therefore, another idea is the synthesis of amphiphilic systems via radical polymerization using a two-phase or heterophase starting situation. In principle, these techniques allow the kinetic control of the copolymer structures or monomer sequence of the polymer chain. The final polymer chain is not only defined by the

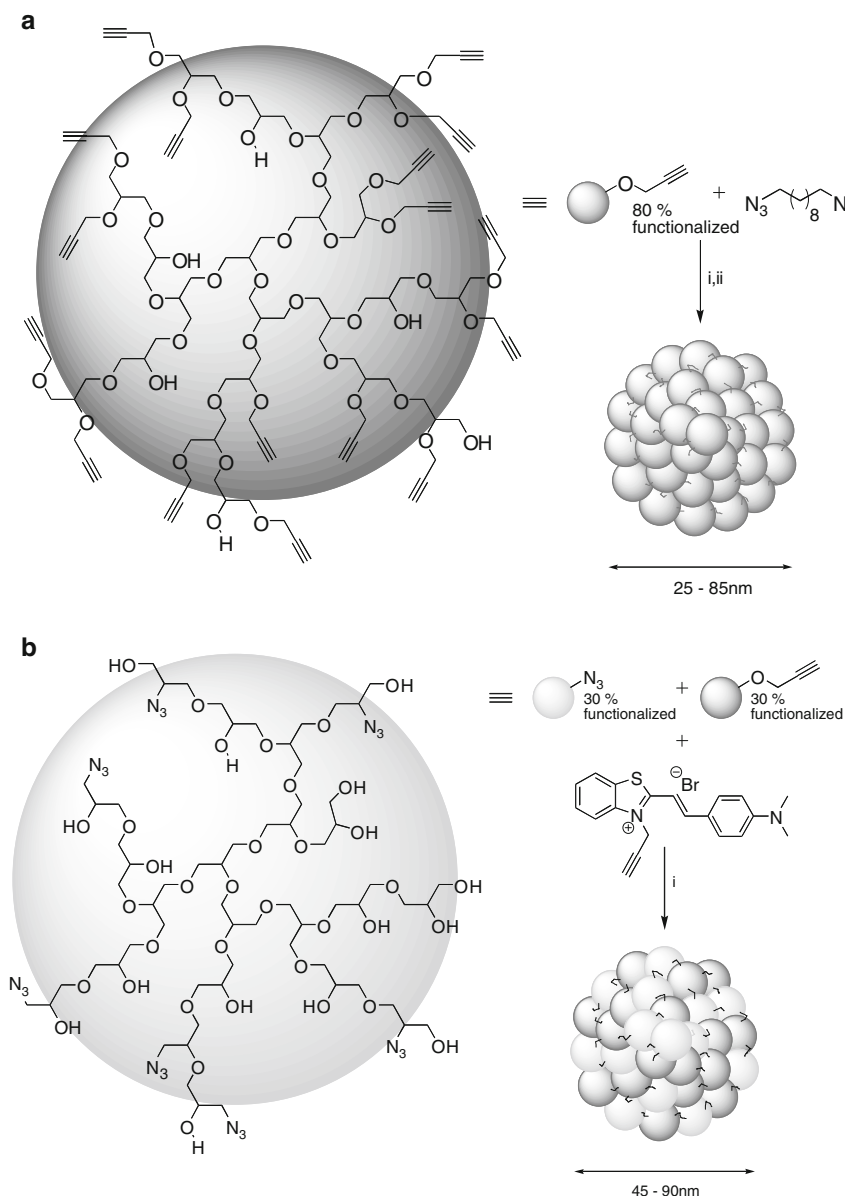


Fig. 6 (a) Procedure employed for the formation of hydrophobic nanoparticles carried out in direct miniemulsion: (i) the hyperbranched polymer dissolved in CHCl_3 is miniemulsified in continuous phase consisting of H_2O and SDS, (ii) catalytic amounts of CuSO_4 or sodium ascorbate are added, and the reaction mixture stirred for 3 h at 60°C , followed by reaction for 16 h at room temperature. (b) Procedure employed for the formation of hydrophilic nanoparticles carried out in inverse miniemulsion: (i) the hyperbranched polymer dissolved in DMF is miniemulsified in a continuous phase consisting of cyclohexane and P(E/B-*b*-EO) as surfactant, followed by reaction at 80°C for 16 h [116]

copolymerization parameters, but also by the locus of polymerization and its change throughout the reaction. Using the techniques of microemulsion and micellar polymerization, one creates a large interface area between the hydrophobic and the hydrophilic sites where polymerization between two polarities can occur. The most remarkable effect of copolymerizations in microemulsions is the improvement of structural homogeneity, even for monomers that are not suitable for copolymerization in solution or emulsion. Monomers of very different hydrophilicities can now be successfully copolymerized. With higher amounts of the hydrophilic monomer, hydrogel materials could be obtained. In microemulsion, the comonomers are preferably oriented towards the water–oil interface, and charge effects are shielded that way. Kaler et al. [61] studied the copolymerization of styrene and acrylic acid in a cationic microemulsion. In this case, isolated acrylic acid units were randomly distributed among polystyrene blocks.

To synthesize water-soluble or swellable copolymers, inverse heterophase polymerization processes are of special interest. The inverse macroemulsion polymerization is only reported for the copolymerization of two hydrophilic monomers. Hernandez-Barajas and Hunkeler [62] investigated the copolymerization of AAm with quaternary ammonium cationic monomers in the presence of block copolymeric surfactants by batch and semi-batch inverse emulsion copolymerization. Glukhikh et al. [63] reported the copolymerization of AAm and methacrylic acid using an inverse emulsion system. Amphiphilic copolymers from inverse systems are also successfully obtained in microemulsion polymerization. For example, Vaskova et al. [64–66] copolymerized the hydrophilic AAm with more hydrophobic methyl methacrylate (MMA) or styrene in a water-in-oil microemulsion initiated by radical initiators with different solubilities in water. However, not only copolymer, but also homopolymer was formed. The total conversion of MMA was rather limited (<10%) and the composition of the copolymer was almost independent of the comonomer ratio. This was probably due to a constant molar ratio of the monomers in the water phase or at the interface as the possible locus of polymerization. Also, in the case of styrene copolymerizing with AAm, the molar fraction of AAm in homopolymer compared to copolymer is about 45–55 wt% [67], which is still too high for a meaningful technical application.

Corpart and Candau [68, 69] described the formulation of polyampholytes containing both positive and negative charges in inverse microemulsions. The copolymers can show very different behaviors in the aqueous solution, ranging from insoluble, water-swollen hydrogels to water-soluble compounds, depending on the monomer composition. For polyampholytes with balanced stoichiometry, the polymer behavior is controlled by attractive electrostatic forces. The compound is usually insoluble in water, but becomes soluble upon the addition of salt.

It could be shown that miniemulsion polymerization is a very suitable method for synthesis of amphiphilic copolymer of high homogeneity and for control of the primary sequence [117]. Both direct and inverse miniemulsions can be formed by placing the same monomers in the dispersed or continuous phase, reciprocally. The high interfacial area of miniemulsions is expected to stimulate the change of the growing radical from one phase to the other and, therefore, the formation of copoly-

mers is possible. Finally, in comparison to microemulsion, miniemulsion can be formed with much less surfactant, which is of high interest for the direct application of the resulting copolymers. This is what also makes miniemulsions very promising for the generation of amphiphilic copolymers.

The polymerization process of two monomers with different polarities was carried out in direct or inverse miniemulsions using the monomer systems AAm/MMA and acrylamide/styrene (AAm/Sty). The monomer, which is insoluble in the continuous phase, is miniemulsified in the continuous phase water or cyclohexane in order to form stable and small droplets with a low amount of surfactant. The monomer with the opposite hydrophilicity dissolves in the continuous phase (and not in the droplets). Starting from those two dispersion situations, the locus of initiation (in one of the two phases or at the interface) was found to have a great influence on the reaction products and on the quality of the obtained copolymers, which can act as hydrogels.

In the AAm/MMA system, the best copolymer with respect to low content of homopolymers, low blockiness, and good redispersibility in polar and nonpolar solvents was obtained in inverse miniemulsion with initiation in the continuous phase cyclohexane (see Fig. 7). In this case, the MMA chains grow in the continuous phase until they become insoluble and precipitate onto the AAm droplets, which enable the radicals to cross the interface. AAm units can then be added to the polymer chain.

In the AAm/Sty system, the best copolymer was also obtained in the inverse miniemulsion process, but using interfacial initiation. This leads to almost homopolymer-free copolymer samples with low blockiness, indicating a fast change of the growing radical between the phases in order to add Sty and AAm units. A copolymerization in the direct miniemulsion with water as continuous phase, using the interfacial initiator PEGA200, results in a higher homo-AAm content. This can be attributed to the fact that the initiator, due to its hydrophilicity, has a slightly higher tendency to be in the water phase, where AAm units can be captured.

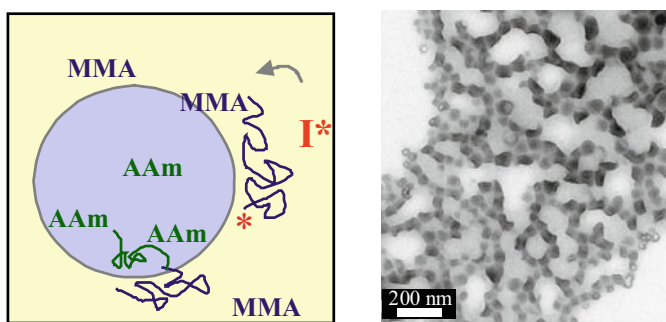


Fig. 7 Copolymerization of hydrophobic (MMA) and hydrophilic (AAm) monomers in inverse miniemulsions with MMA dissolved in the continuous phase in order to obtain a copolymer with low content of homopolymers, low blockiness, and good redispersibility in polar and nonpolar solvents (*left*). I^* and * signifies a free radical at the initiator molecule and in the end of the polymeric chain, respectively. TEM image of the copolymer nanoparticles (*right*) [117]

7 Hydrogel Formation at the Particle Surface

7.1 Copolymers with Hydrophilic Functional Monomers

As shown above, the miniemulsion is a very efficient system for production of copolymer particles from hydrophobic and hydrophilic monomers. In the case of direct (oil-in water) miniemulsion, if the hydrophilic monomer is used in smaller quantities, there is a possibility to form an amphiphilic copolymers close to the interface of the nanoparticles. This shell region of the polymeric particle can be considered as a hydrogel shell. The structure of the hydrogel shell mainly depends on the monomer(s) concentration, reactivity ratios of the monomers, their solubility in water, and the type of surfactant used.

Recently, carboxyl- and amino-functionalized polystyrene latex particles were synthesized by the miniemulsion copolymerization of styrene and acrylic acid or 2-aminoethyl methacrylate hydrochloride (AEMH) [70, 71]. The reaction was started by using an oil-soluble initiator, 2,2'-azobis(2-methylbutyronitrile) (V-59). Two types of surfactant, i.e., ionic negatively charged SDS or positively charged CTMA-Cl, and nonionic Lutensol AT50 (which is a PEO hexadecyl ether with an EO block length of about 50 units) were used to stabilize the initial droplets and final particles.

From the particle size measurements it was found that, in the case of carboxyl-functionalized samples stabilized with SDS, the particle size is relatively constant (around 100 nm) until 10 wt% of added acrylic acid. At higher amounts of acrylic acid, the diameter sharply increased, reaching an average value of 140 nm. The increase in particle size with increased amount of acrylic acid was explained by the formation of a “hairy” layer around the particle, which is mainly composed of the hydrophilic poly(acrylic acid) units. In contrast, the size of the amino-functionalized particles is not strongly dependent on the initial amount of functional monomer and was in the range 110–130 nm. This was expected because, in contrast to acrylic acid, the AEMH ($pK_a = 8.5$) is completely water-soluble at the experimental pH below 3.5. Moreover, AEMH is very reactive and shows strong chain-transfer behavior [72, 73], and therefore the surface layer mainly consists of short chains.

Determining the amount of surface carboxyl groups as a function of the surfactant, it was shown that the dense monolayer of carboxylic groups (0.68 nm^2 per COOH; 1.47 groups per nm^2) on the particles prepared with nonionic surfactant was almost achieved with 3 wt% of acrylic acid. More than 10 wt% of acrylic acid was required in the case of SDS-stabilized particles. TEM images of carboxyl-functionalized polystyrene particles stabilized with nonionic (Lutensol AT50) and ionic (SDS) surfactant are presented in Fig. 8.

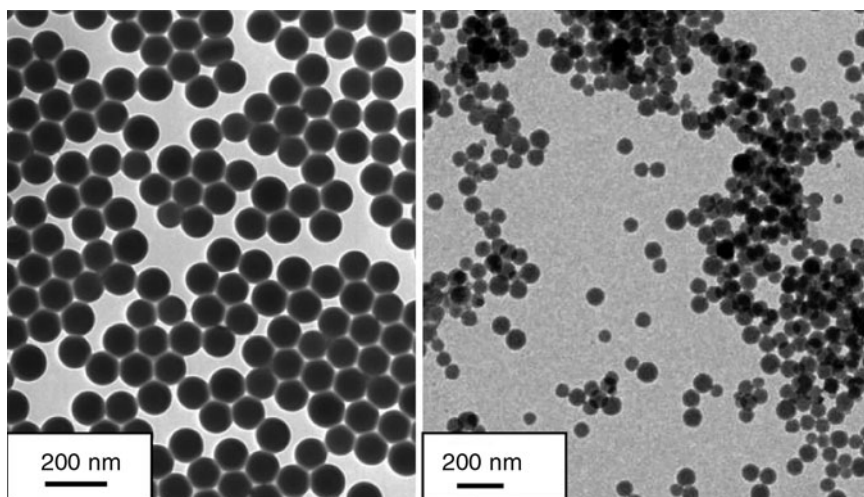


Fig. 8 TEM images of carboxyl-functionalized polystyrene particles: with 3 wt% of acrylic acid and stabilized with Lutensol AT50 (*left*); or with 15 wt% of acrylic acid and stabilized with SDS (*right*)

7.2 Nanoparticles Surface as Templates

The use of polymeric nanoparticles as templates for the crystallization of inorganic materials is an intriguing approach because it offers the feasibility of synthesizing organic/inorganic hybrid materials for a broad spectrum of applications. Besides surfactant assemblies [74–76], hydrogels [77, 78], block copolymers [79, 80], polyelectrolytes [81], self-associated nanogels [82], emulsions, and microemulsions [83–85], there have also been reports of biomimetic routes for the mineralization of calcium phosphates (apatites) employing biodegradable synthetic polymers [86, 87], collagen [88, 89], and gelatin [90, 91]. The synthesis of hybrid particles by the encapsulation of preformed inorganic materials within a polymeric matrix has also been reported [92]. However, the in-situ formation of inorganic materials allows varying several parameters and offers better organic–inorganic interface integrity. Because the compatibility of the inorganic component with the organic matrix plays a crucial role in the encapsulation efficiency, very often an additional coating of the inorganic material with a polymer or a surfactant (in this case as a surface modifier as well as to separate individual entities to ease encapsulation) is a prerequisite. Moreover, the inorganic component is encapsulated very often within the matrix. For applications involving the inorganic materials outside of the nanoparticle matrix, the synthesizing possibilities are very much limited.

Surface-functionalized nanoparticles are very good candidates as templates for crystallization on the outside surface of the particles owing to their monodisperse size and large surface area. The use of such nanoparticles for biomimetic HAP

deposition is interesting for applications involving bioimplants. For example, implants made of titanium and its alloys can be coated with HAP in order to eliminate the failure of the implant owing to poor osteoconductive properties, and to impart better osteointegration and bonding between implant and bone [93–95]. In order to take advantage of the biocompatibility of HAP as well as the mechanical properties of the underlying metallic substrate, it is necessary to optimize the coating possibilities of implants with complex shapes. One of the excellent options for enhancing the surface-coating possibilities is to use HAP-coated nanoparticles. The presence of HAP on the surface of the nanoparticles offers several advantages: it allows the implant material to adapt to the surrounding tissues; it can act as a scaffold for nucleation and growth of new bone materials; and it can impart nanoscaled features on the surface, thereby modifying the surface topography and influencing the physicochemical properties as well as the biochemistry at the surface [96]. Above all, a valuable aspect is that these nanoparticles can also act as carriers of biomolecules and drugs.

Surface-functionalized nanoparticles can be very well exploited as templates for the growth of HAP crystals on the polymeric nanoparticle surface. Tamai and Yasuda [97] have reported HAP-coated polymer particles by employing Pd⁰-immobilized poly(styrene-*co*-acrylic acid) copolymer particles synthesized using emulsifier-free emulsion polymerization. Later, the formation of HAP nanocrystals on the surface of β -diketone-functionalized polymeric nanoparticles employing styrene and acetoacetoxyethyl methacrylate (AAEM) obtained by emulsifier-free emulsion polymerization was reported [98]. The carboxylated polystyrene latex particles have also been used previously for the preparation of other inorganic materials like Ag/AgO [99]. However, in these studies the amount of functional groups was always fixed and the amount of inorganic material precipitated was controlled by controlling either the reaction parameters [97, 99] or the amount of added salts [98]. The synthesis of functionalized polystyrene particles by miniemulsion polymerization has been well studied and documented [71]. The miniemulsion technique allows the synthesis of amino- or carboxyl-functionalized polystyrene particles with different amounts of bound surface charge groups by varying the amount of functional comonomer such as amine ethyl methacrylate or acrylic acid, respectively [70, 71].

In the previous studies using self-assembled monolayers (SAMs) with several functional groups ($-\text{OH}$, $-\text{SO}_3\text{H}$, $-\text{PO}_4\text{H}_2$, $-\text{COOH}$) on Ti wafers, the $-\text{COOH}$ functional group has been proven to be an optimal endgroup for producing highly crystalline and thick layers of HAP [100]. Thus, the use of carboxyl-functionalized polystyrene particles synthesized via the miniemulsion polymerization as templates seems to be a practical way to synthesize hybrid colloids with highly crystalline HAP. These hybrid particles could be used for the coating of implants in order to make them more osteoconductive as well as for the preparation of scaffolds for tissue engineering.

Recently, surface-functionalized particles with covalently bound carboxyl groups were prepared using an ionic as well as a nonionic surfactant as templates to perform crystallization on the surface of the particles [101]. This approach of crystallization outside of the particle (Fig. 9a) is in contrast to a previous report [27], where the

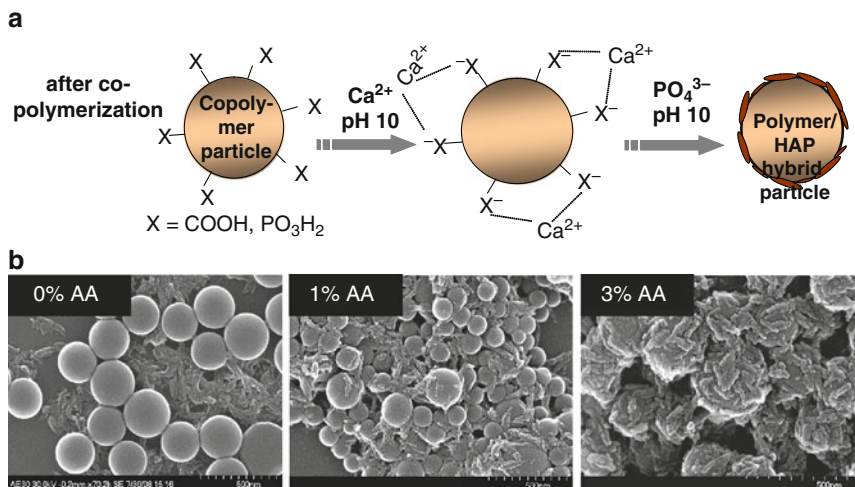


Fig. 9 (a) Surface-functionalized nanoparticles as templates for biomineralization. (b) TEM micrographs of carboxylated nanoparticles with hydroxyapatite nanocrystals [101]

crystallization was performed inside gelatin nanoparticles that served as a confined reaction environment. The influence of the type of surfactant with respect to particle size, density of functional surface groups, and HAP formation were studied in detail using high-resolution scanning electron microscopy (HRSEM), TEM, and XRD. TEM micrographs of carboxylated nanoparticles with HAP nanocrystals as a function of the initial amount of acrylic acid are presented in Fig. 9b.

It was shown that for a fixed concentration of Ca²⁺ and PO₄³⁻ ions added, the amount of HAP formed on the particle surface increases with the increase in the concentration of acrylic acid added during synthesis of nanoparticles. The absence of HAP on latexes prepared with 0 wt% acrylic acid for both ionic and nonionic surfactant types confirms that the amount of HAP formed depends only on the amount of carboxyl groups present on the surface. HAP formation was pronounced for particles prepared with nonionic surfactant, which is in agreement with the high amount of –COOH groups as compared to the latexes prepared with SDS as anionic surfactant. However, to obtain high HAP nanocrystal formation it was also found that, in addition to high carboxyl functionalization on the surface of the particles, it is absolutely necessary to have an optimum surfactant concentration for particles prepared with nonionic surfactant [101].

8 Microgel Nanocapsules

Not only solid particles can be built up by using hydrogel as shell material, but also capsules. Over the past two decades, the encapsulation of materials has become an extensive area of research activity owing to their utilization as submicrome-

ter containers for the encapsulation of biologically active substances. The main advantage of nanocapsules for drug delivery is the efficient protection of therapeutic agents against degradation or oxidation. It is possible to achieve durability, compatibility, and controlled release of the ingredients. Weidenheimer et al. altered the formulation for the synthesis of soft gelatin capsules by adding plasticizing agents such as formamide, acetamide, lactamide, mannitol, or glycerine [102]. Starch as a native polymer and modified starches are now established for the microencapsulation of, e.g., fragrances [103, 104] or drugs [105].

The suspension polymerization process allowed the formation of capsules of 1–30 μm consisting of migrin oil as core and polyurea as wall material. The latter was formed by interfacial polycondensation reactions between different diisocyanates and emulsified ethylenediamine [106].

The synthesis of nanocapsules can best be obtained in miniemulsion using different approaches [107]. One possibility is based on the phase separation process within a droplet during the polymerization [108]. Here, vinyl monomers were polymerized in the presence of a hydrophobic oil. During the polymerization, the polymer becomes insoluble in the oil, leading to a phase separation. With properly chosen physicochemical properties of monomer and encapsulated material, a polymeric shell surrounding the liquid core can be formed.

In another approach, the polymer is precipitated from the continuous phase onto stable nanodroplets in an inverse miniemulsion [109]. In this case, a miniemulsion with the liquid core material is formed in a continuous phase that consists of a mixture of a solvent and a nonsolvent for the polymer. That way, PMMA nanocapsules encapsulating an antiseptic agent could be produced.

As a third possibility, nanocapsules in a miniemulsion system could be achieved using different interfacial reactions in inverse miniemulsions. The formation of polyurea, polythiourea, and polyurethane nanocapsules synthesized through the polyaddition reaction has been described in detail [110–112]. The size of the nanocapsules could be controlled by the amount of surfactant used and the addition time of the diisocyanate. The wall thickness was adjusted by the amount of employed monomers. dsDNA molecules were successfully encapsulated into polybutylcyanoacrylate (PBCA) nanocapsules by anionic polymerization, which took place at the interface between the miniemulsion droplets and the continuous phase [113].

The crosslinking of starch at the droplet interface in inverse miniemulsion leads to the formation of hydrogels. The formulation process for the preparation of crosslinked starch capsules in inverse miniemulsion is schematically shown in Fig. 10. The influence of different parameters such as the amount of starch, surfactant P(E/B-*b*-EO), and crosslinker (2,4-toluene diisocyanate, TDI) on the capsule size and stability of the system were studied. The obtained capsules were in a size range of 320–920 nm. Higher amounts of starch and surfactant result in a smaller capsule size. The TEM images of crosslinked starch capsules prepared with different amount of crosslinker (TDI) are presented in Fig. 11. The nanocapsules can be employed as nanocontainers for the encapsulation of dsDNA molecules with different lengths [114] and for the encapsulation of magnetite nanoparticles.

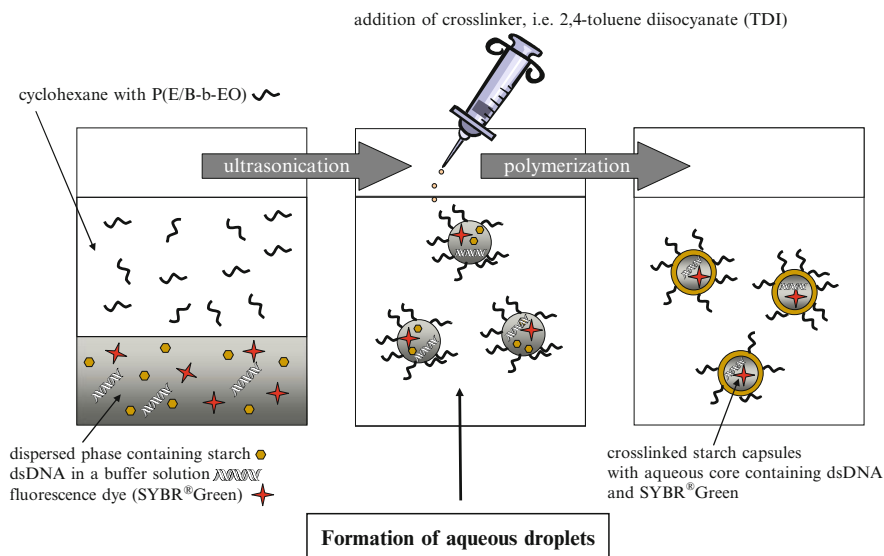


Fig. 10 Preparation of crosslinked starch capsules in an inverse miniemulsion

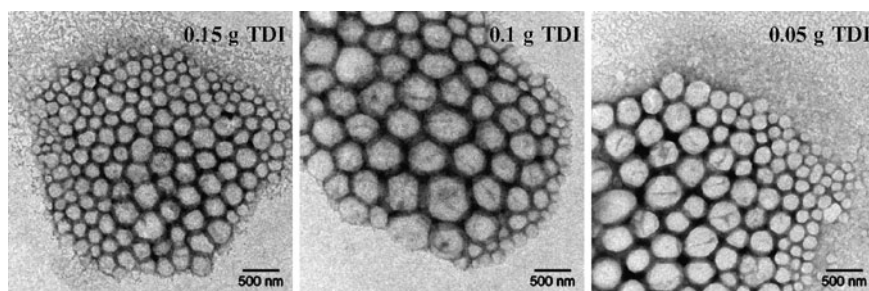


Fig. 11 TEM images of starch-based hydrogels in cyclohexane using different amounts of TDI [114]

9 Molecular Imprinting

The preparation of noncovalent molecularly imprinted particles consisting of EGDMA as crosslinker and methacrylic acid as functional monomer was described using the process of miniemulsion polymerization [115]. The obtained particles were in a size range of 185–220 nm. A high amount of crosslinking agents leads to the formation of microgel particles and therefore enables the polymer network to be maintained during dissolving the template. In this specific case, hydrogel nanoparticles were not prepared, but microgel nanoparticles that can swell in organic solvents. The preparation of molecularly imprinted particles and their utilization for molecular recognition are schematically presented in Fig. 12. Different kinds of particles

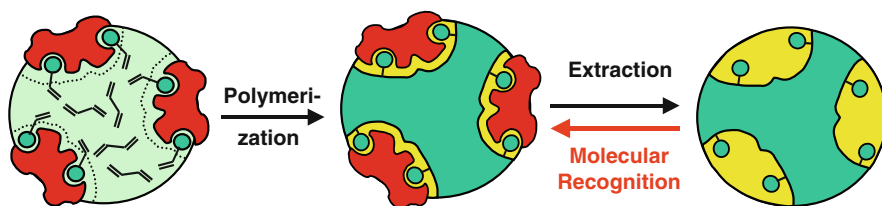


Fig. 12 Preparation of molecularly imprinted particles and their use for molecular recognition: Template molecules induce the formation of binding sites during the miniemulsion polymerization. The templates are extracted from the highly crosslinked particles and are molecularly recognized by the selective binding sites on the nanospheres [114, 115]

were synthesized by varying the molar ratio of monomer to crosslinker and in the presence or absence of a chiral template, i.e., L- or D-Boc-phenylalanine anilid. A protected amino acid, L- and D-Boc-phenylalanine anilid (BFA), were chosen as model templates SDS was used as surfactant and hexadecane as hydrophobic agent to prevent Ostwald ripening of the miniemulsion. The efficiency of the molecular imprinting effect was examined by binding experiments and quantified by UV. It was found that the best enantioselective molecular recognition properties were for particles with a molar ratio of the monomer to crosslinker (MAA:EGDMA) of 0.25:1. The preparation of molecularly imprinted polymers using the miniemulsion polymerization approach is highly efficient in regard to the yield of useful particles as well as in recycling of the templates [115].

10 Concluding Remarks

In summary, the formation of various gel-type materials using different synthetic routes has been described in this contribution. The advantages of the miniemulsion technique include its versatility in terms of the materials used and the reaction conditions, simplicity of formulation, and high reproducibility. The miniemulsion technique allows one to produce nanoparticles with controlled size and surface properties, which are very important parameters for further application in the area of nanotechnology.

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