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REVIEW

THERMOSENSITIVE MICROGELS OF POLY-N-ISOPROPYLACRYLAMIDE FOR DRUG CARRIERS – PRACTICAL APPROACH TO SYNTHESIS

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Abstract: The aim of the work is to present the main actual information on the preparation of polymers, derivatives of N-isopropylacrylamide, formed into microgels. The most often used comonomers, crosslinkers, and initiator systems are gathered herein. The known methods of emulsion polymerization and precipitation polymerization are also described, including the application of the surfactants, as well as the surfactant free emulsion polymerization. Finally, the procedures of lab-scale production of microgel were evaluated in the paper, with special intact on the thermosensitive N-isopropylacrylamide derivatives for application in biomedical field.

Keywords: microgel, N-isopropylacrylamide, emulsion polymerization, precipitation polymerization

Microgels are typically defined as crosslinked polymer particles dispersed in colloidal form in a suitable medium, which usually is water. Due to the presence of specific functional groups, depending on the type of dispersion medium, they may be subject of extensive swelling (1). Sometimes, the microgels are also referred to cross-linked latex particles that swell in water, and release water as a result of changes in thermodynamic conditions, such as the presence of different additional solvents, the change in environmental temperature, pH or ionic strength of the solution. In practice, the swelling leads to significant hydration of the microgel particles. In this case, the macroscopic picture can be virtually imperceptible with the naked eye. The particles classified in a number of scientific publications as microgels vary in the terms of diameter, although they are usually in the range from 1 nm to 10 μm, some authors accept a diameter range of 50-500 nm for dried microgels. Hydrodynamic diameter of microgels is the result of osmotic pressure on the one hand. On the other hand, the elastic forces and respective tension, present in the molecule, influence the diameter.

First study, confirming receipt of the microgel, in accordance with generally accepted definition, appeared more than 70 years ago in chemical journal printed in Berlin - Staudinger and Huseman presented the synthesis and properties of polystyrene particles (2). Less than 15 years later, Baker introduced the term “microgel” for cross-linked polymer of butadiene and styrene (3). According to his observation of the resulting polymer, in contrast to previously manufactured, he found that microgels are characterized by solubility of the corresponding linear polymers in the sol state (4). In the late eighties and in nineties, microgels based on N-isopropylacrylamide (NIPA) have become extremely attractive subject of study by developing a simple and efficient method for their preparation by Pelton and Chibante (5). Schematic course of the synthesis of poly-N-isopropylacrylamide (PNIPA) is shown in Figure 1.

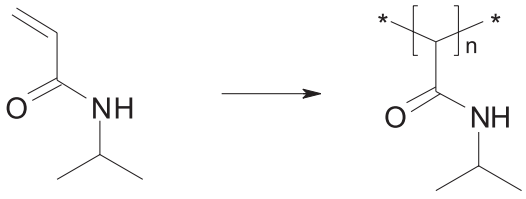

Figure 1. Scheme of PNIPA synthesis

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Poly-N-isopropylacrylamide is sometimes referred to various acronyms, such as PNIPA, PNIPAAm, PNIPAA, PNIPAm. It is a thermosensitive polymer and through the application of crosslinking agent forms a stable three-dimensional structures, known as microgels or macrogels - depending on the type of structures formed. Since the critical phase transition temperature is around 32^{o}C, in dilute solutions the expanded structure – so called coil structure - is collapsing. It transforms into a globular form when the temperature of the volume phase transition temperature is achieved, as illustrated in Figure 2. In the course of water removal from the area between the polymer chains, the loss of nearly 90% of the particles mass occurs (6). When the NIPA copolymer is applied intravenously, the elimination of circulating NIPA copolymers by the kidney is possible, if the molar mass of macromolecules does not exceed 32,000 g/mol (7).

Microgels synthesized using NIPA enjoy a growing interest among specialists in drug form technology, bioengineering and biocompatibile polymers (8-10). This is due to the above mentioned fact of removal large amounts of water from particles of PNIPA, around Volume Phase Transition Temperature (VPTT). Consequently, one can expect the release of drug substance from the microgels of PNIPA under the influence of the thermal factor. Importantly, the VPTT is in the range of known physiological temperatures, e.g., in the range of the temperature of human skin surface. By modifying the composition and structure of derivatives of NIPA it is possible to obtain a number of macromolecules with programmed VPTT in the water system. The high compatibility of this group of polymers with the tissues of the body is of extreme importance. It involves not only the chemical properties of PNIPA. The high water content in the macromolecule contributes to high biocampatibility of forming microgels. This fact enables the development of experimental methods in the field of tissue engineering, such as synthesis of resorbable implants and intervertebral discs (11, 12). In addition, the PNIPA microgels form very stable colloidal dispersions, are relatively simple to prepare, and their functionalization does not pose particular difficulties, assuming suitable manner of reaction processing. The size of the obtained particles can be well controlled, and the polydispersity index is usually maintained at a sufficiently low level. Through appropriate functionalization, i.e., through the introduction of fixed functional groups, the obtained microgels become sensitive to: the temperature factor, the factor of pH, or to changes in ionic strength of the solution. The area of potential applications of thermosensitive polymers is currently being developed intensively (13).

Initiation systems, copolymers, crosslinking agents

The first mention of the use of NIPA goes back to the fifties of the twentieth century, when it was tested due to potential repellent properties. Also the first approaches to the synthesis of NIPA polymers were done at that time. Preparation of PNIPA was carried out in different ways, although only in certain cases, suitable microgels were obtained. The straight chains of PNIPA were formed in the course of free radical polymerization. In this case, the organic solvents are used, such as methanol, benzene, tetrahydrofuran, *tert*-butanol, dioxane, and chloroform, with specific initiators: azo-bis-isobutyronitrile, benzyl peroxide or lauryl peroxide.

Another way to obtain chains of PNIPA is a process carried out using a redox initiator in aqueous

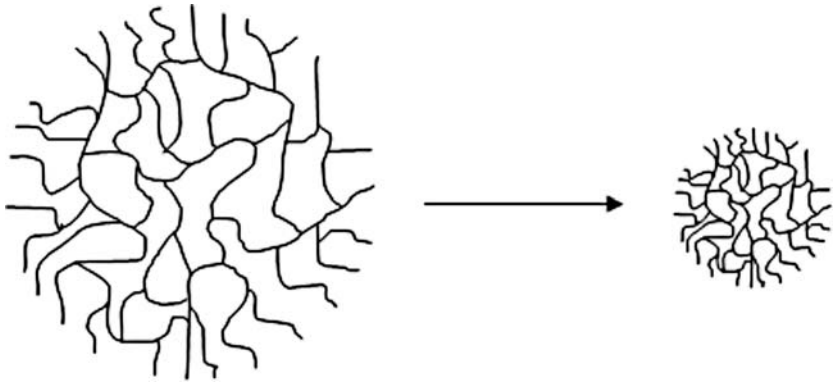

Figure 2. Depiction of volume phase transition of PNIPA microgel initiated by temperature increase in aqueous environment

Table 1. Exemplification of reactants composition in surfactant free precipitation polymerization of PNIPA.

Reactant type	Reactant	Acronyms
Initiator	Ammonium persulfate	APS
Accelerator	N,N,N',N'-tetramethylethane-1,2-diamine	TEMED, TMED
Monomer	N-isopropylacrylamide	NIPA, NIPAA, NIPAAc
Crosslinker	N,N'-methylene-bis-acrylamide	MBA, MBAm, BIS
Reaction environment	Aqueous	
Temperature	70^{o}C	

medium. The reaction is initiated using the appropriate initiator, usually: ammonium persulfate (APS), potassium persulfate (KPS) and sodium persulfate (NPS). As the initiator is sometimes also used azo-bis-isobutyronitrile (AIBN). Each of the initiator molecules, resulting in activation, may become the center of the polymerization. The spherical structures are formed around polymerization centers. They are treated in the bibliography as solid particles, dispersed in the aqueous phase. However, the resulting material is in varying degree filled with water, and connected in the areas of polymer where the hydrophilic functional groups exist. Increased temperature in the course of synthesis affects the isolation of the polymer phase with low water content from the external aqueous phase. This phenomenon manifests itself in turbidity or opalescence when the macroscopic view of the reaction mixture is observed.

The formation of hydrogels as nano- or microstructures was evaluated by numerous authors, whereas Dušek developed in details the problem of so called microsyneresis; in the case of NIPA increased temperature plays important role in microgel formation around the polymerization centers (14-16). The concentration of the initiator is also crucial for the properties of the resulting polymer. According to research of Xiao, with increasing concentration of initiator - APS in the reaction mixture, the ability of the polymer to swell increased (17). Table 1 presents the sample composition of the initial reaction mixture used to obtain microgels of PNIPA.

Free radicals derived from initiator are dissolved in water, and they remain in the solution, hence the initial reaction site is an aqueous solution, as presented in Figure 3 in stage I. As time passes, the oligomer chains with ionized groups are forming, coming from the initiator molecule. The resulting oligomers are amphiphilic, due to the presence of the hydrophilic ionic group and the lipophilic chain of the resulting polymer - Phase II. This leads over time to a micellar structures - Phase III, soon saturated with a solution of monomer (stage IV); at this point the reaction site is situated within the micelles, resulting in larger particles with time (stage V), which are stabilized by implementing an appropriate crosslinking agent. The hydrophilic groups are forming outer layer of the micelles. Due to the surface charge, the mutual repulsions stabilize the resulting colloid. Addition of electrolyte in the course of the reaction, such as sodium chloride, favors the formation of larger colloidal particles. It is caused by a reduction of repulsive forces between particles.

The main factor, used for initiation of the polymerization reaction is the temperature increase, but also visible light, ultraviolet radiation or X-ray irradiation are used (18). To ensure consistent molecular weight of macromolecules obtained in the course of the polymerization initiated in redox conditions, proper pH of the reaction mixture should be kept, using e.g., complex buffers in the pH range 6.5 or 7.4 (19). In order to accelerate the reaction it is necessary to supplement the accelerator, such as N,N,N',N'-tetramethylethane-1,2-diamine (tetramethylethylenediamine, TEMED), or sodium metabisulfite (sodium pyrosulfite). PNIPA, a linear polymer, was first comprehensively described by Howard G. Schild from Research Division of Polaroid Corporation in the early nineties of the last century (20).

In order to obtain a microgel it is necessary to ensure proper composition of the mixture of substrates, including the main monomer, comonomer, crosslinker, initiator system, and in some cases surfactant. Polymerization carried out in one reactor, the so called "batch-synthesis", requires the selection of such components, in which the individual components will react with each other with similar rate. The rate of reaction of individual comonomers affects the final composition of the resulting poly-

mer, according to the Mayo-Lewis equation (21). The compositions used for the receipt of the microgel are extremely diverse and include NIPA, the above mentioned ingredients and comonomers, e.g., acrylic acid (22), methacrylic acid and fumaric acid (23), acryl amide (24), maleic acid (25), hydroxyethyl methacrylate, N-vinylpyrrolidone (26), or N-*tert*-butylacrylamide (27, 28). The copolymers are usually used to obtain specific properties of the microgel. Introduction of anionic functional groups to the microgel can be achieved by applying suitable copolymers: unsaturated monocarboxylic or dicarboxylic acids, such as the above-mentioned acrylic acid, methacrylic acid and pentenoic acid (29). Table 2, prepared on the basis of Rzaev et al. work (30), summarizes selected comonomers used for the preparation of functionalized microgels.

Production of a stable microgel is essentially conditioned by a suitable crosslinking agent. The most commonly used crosslinking agents, and perhaps with the longest tradition of use in studies of microgels, is N,N'-methylene-bis-acrylamide (31-34). However, there are also used another crosslinkers. In previously conducted studies, special attention was paid to influence of the content of the crosslinking agent on physicochemical properties of the resulting polymer. We also evaluated the implementation of the crosslinking agent to the polymer particles (35). Among the crosslinking agents N,N'-bis-cystaminoacrylamide is applied in the synthesis (36, 37). It was observed that with increasing content of crosslinking agent increases the phase transition temperature, although the course of the hydrodynamic diameter changes as a function of increasing temperature is much milder and the phase transition point is less well visualized (38).

Between various known crosslinkers, bifunctional derivatives of polyethylene glycol are used, as factors affecting the morphology and thermosensitivity of PNIPA microgels (39). For controlled delivery of insulin, the PNIPA derivative microgels were synthesized with polyethylene glycol 400 - dimethacrylate derivative crosslinker (40, 41). Derivatives of NIPA in solution of chitosan were synthesized with the addition of ethylene glycol diacrylate which comprised four glycol units in the chain. It led to the formation of a thermosensitive gel (42). The use of ethylene glycol diacrylate with

Table 2. Choice of comonomers applied in the synthesis of copolymers of NIPA.

Group of comonomers	Examples of comonomers
Comonomers with acidic groups	Acrylic acid
	Methacrylic acid
	4-Pentenoic acid
	2-Acrylamido-2-methyl-1-propenesulfonic acid
	2-(Dimethylmaleimido)-N-ethylacrylamide
	Maleic anhydride
	Itaconic anhydride
	4-Vinylphenylboronic acid
	DNA
Acrylamide type comonomers	Acrylamide
	Acrylonitrile
	2-(Dimethylamine)propylmethacrylamide
	N-[3-(dimethylamine)propyl]methacrylamide
	2-(Dimethylmaleimido)-N-ethylacrylamide
	N-*tert*-butylacrylamide
	N-butylacrylamide
Heterocyclic comonomers	N-vinyl-2-pyrrolidone
	4-Acryloyl-morpholine
	1-Vinylimidazole
	2-Methacryloylamidohistidine
	N-acryloylpyrrolidine

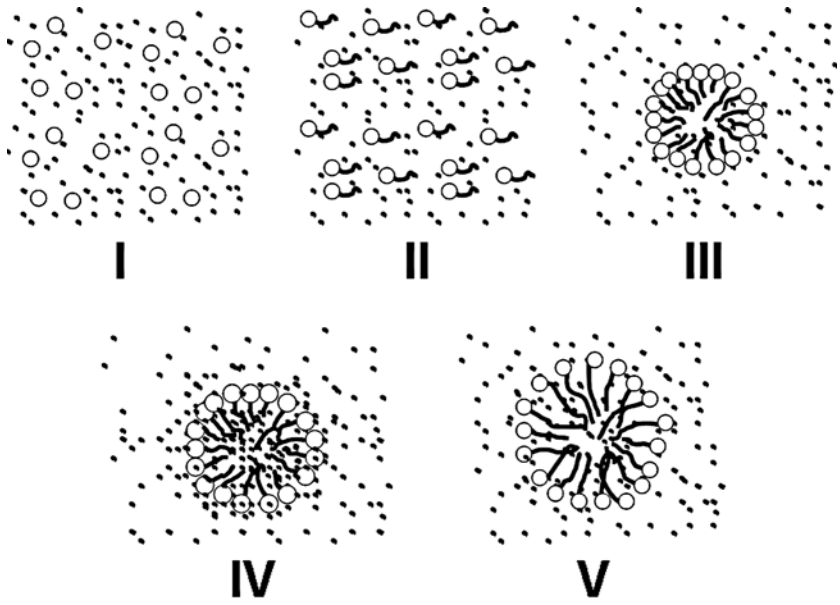

Figure 3. The changes of the locus of synthesis in the course of SFDP of NIPA derivatives, details in the text

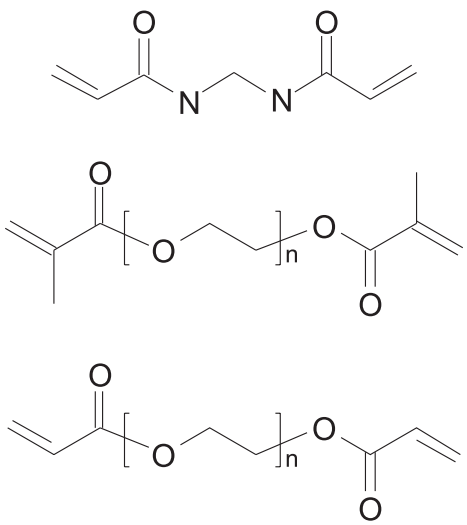

Figure 4. Crosslinking agents with varied chain length: N,N'-methylene-bis-acrylamide, glycol polyoxyethylene dimethacrylate, glycol polyoxyethylene diacrylate, respectively

one (ethylene glycol dimethacrylate, EGDMA), and three ethylene segments (triethylene glycol dimethacrylate, TEGDMA), resulted in microspheres with a diameter of 356 and 444 nm, respectively (43). In studies of crosslinking agents with different solubility in water, such as tetraethylene glycol dimethacrylate (TETGDMA), ethylene glycol dimethacrylate (EGDMA), dimethacrylate butanediol-1,3 (1,3-BDDMA), and 1,4-BDDMA the increase of solubility was accompanied by increased hydrodynamic diameter of the microgels obtained. However, the VPTT remained unchanged (44). To obtain submicron microgel some authors used glycerol dimethacrylate (GDMA), pentaerythritol triacrylate (PETA) and its propoxy derivative: pentaerythritol triacrylate propoxylate (PEPT) - in the case of crosslinking agents with the three acrylic functionals, microgels with low diameter were obtained, whereas in the case of crosslinkers with two acrylic functionals higher diameters were observed (45). Due to the possibility of handling crosslinking agents with diversified polyoxyethylene chain lengths within diacrylate and dimethacrylate derivatives, they are examined in the context of the controlled release of therapeutic substances (46). Figure 4 shows examples of crosslinking agents of different chain lengths.

Erbil et al. proposed the acrylated poly(dimethyl)siloxane (47). This resulted in a change in the VPTT, compared with NIPA polymer obtained from conventional crosslinking agent - N,N'-methylene-bis-acrylamide. More sophisticated methods include the use of biodegradable crosslinking agents, such as ACL - 3,9-divinyl-2,4,8,10-tetraoxaspiro[5.5]undekane (acid-degradable crosslinking agent) (48). Efforts are also attempted to synthesize microgel of PNIPA without the addition of crosslinking agent (49).

The course of the reaction

A key element in the course of the synthesis of microgels of PNIPA is that the NIPA is soluble in water. Along with changes in temperature also the changes in the solubility are observed, as demonstrated in studies using nuclear magnetic resonance. Concentrated solution of NIPA delaminates, at temperatures below 25°C, as the concentration excess the standard solubility of NIPA, while above 25°C the solubility is lower than the standard NIPA solubility value (50). In contrast to NIPA, its polymer is insoluble in water. However, it is true only at temperatures above the VPTT. Thus, the polymer particles are separating from the solution and in the course of polymerization the turbidity or iridescence of the reaction mixture is observed in the process of surfactant free precipitation polymerization (SFPP) or surfactant free emulsion polymerization (SFEP) (51).

Preparation of microgels is usually conducted by one of the three currently mostly available methods: by means of emulsion polymerization (EP), by precipitation polymerization (PP), and in the process of inverse emulsion polymerization (IEP). The monomer in the system remains in the form of drops, in the course of the EP reaction. In classical terms, during the EP three phases are distinguished: an aqueous phase, submicron particles of polymer, and monomer droplets. The polymerization process is supplied from the monomer droplets dispersed in the form of an emulsion in an aqueous medium, and an example of such a polymerization reaction is the formation of polystyrene. Simplified diagrams of EP and PP are shown in Figure 5A and 5B.

The aqueous phase (W) is constituted by dissolved initiator, while the monomer (M) is dispersed with the use of suitable surface active compound (surfactant-stabilized monomer droplets are shown as circles circled with dashed line). The arrow indicates the direction of migration of the monomer molecules to macromolecules of resulting polymer indicated in Figure 5A as a black dot. In the case of microemulsion polymerization, the droplets of

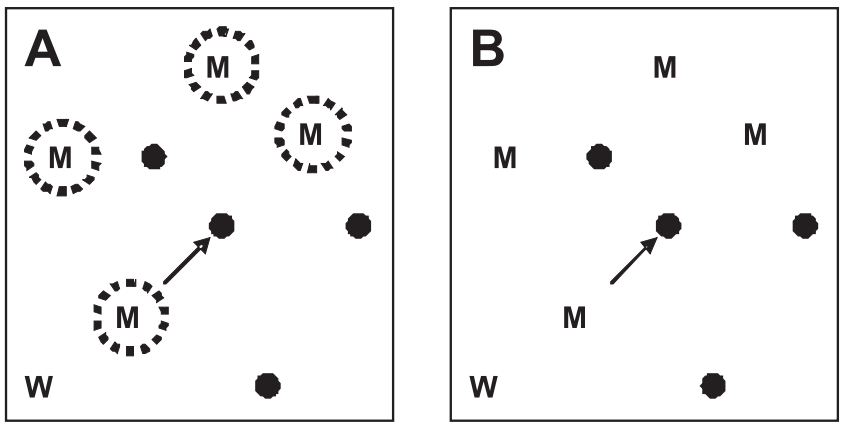

Figure 5. The scheme of EP (left panel – A) and PP (right panel – B), details in text

Table 3. Acronyms of methods evaluated for synthesis of PNIPA derivatives.

Method	Acronym
Emulsion polymerization	EP
Miniemulsion polymerization	MEP
Inverse emulsion polymerization	IEP
Surfactant free emulsion polymerization	SFEP
Precipitation polymerization	PP
Surfactant free precipitation polymerization	SFPP
Dispertion polymerization	DP
Surfactant free dispertion polymerization	SFDP

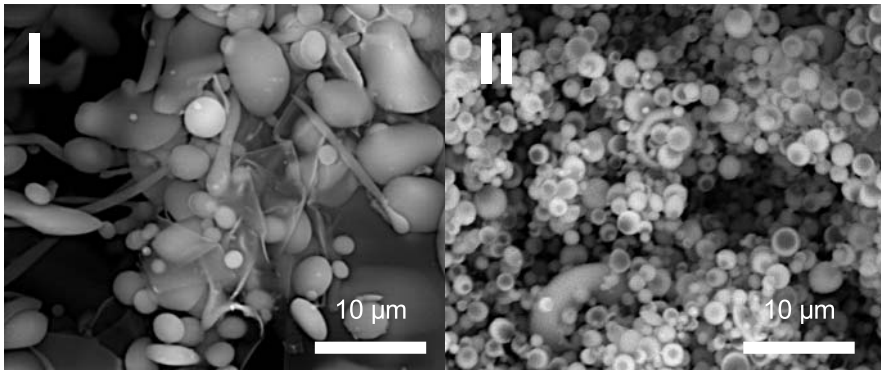

Figure 6. SEM images of NIPA derivatives, synthesized in similar conditions. The batch of microgels with higher diameter (left panel – I) was synthesized without comonomer, whereas the batch with lower diameter was synthesized with N-*tert*-butyl acrylamide as comonomer (right panel – II), details in text

monomer are characterized by very small radius of several nanometers, and this is the right place for polymerization. PP bases on the fact of precipitation of insoluble polymer obtained by polymerization of a monomer soluble in water - Figure 5B. Evaluated methods with its variations and respective acronyms are gathered in Table 3.

In the course of EP, suitable emulsifier can be used. But in some cases, the components of the reaction mixture may be maintained in the dispersed phase without adding any surfactant. In this case, we are talking about EP without surfactant i.e., SFEP. For the synthesis of PNIPA, the terms SFEP or SFPP are used in bibliography. This second definition better reflects the process, because as mentioned earlier, the monomer, NIPA, in contrast to styrene, is soluble in water and the resulting polymer is water insoluble. IEP bases on the phenomenon of polymer synthesis of monomer dissolved in the aqueous phase, which in turn is dispersed in a continuous oil phase. The advantage of this method is the possibility of incorporation of water-soluble therapeutic substances or bioactives dispersed in water, to the particles obtained in the polymerization process. Group of such methods is also referred to as the miniemulsion polymerization (MEP), although there are some differences here in relation to the IEP. Consequently, we can distinguish reversed suspension polymerization, in which monomer is suspended in the droplets of aqueous phase dispersed in the oil phase. Another way is to obtain microgels *via* microemulsion polymerization. Lin and colleagues conducted a comparison of methods for the polymerization of NIPA. According to the results of their work, they received the smallest diameter of the microgels using EP, the intermediate values were observed in the case of SFEP, while the relatively large microgels were obtained by polymerization at a temperature not exceeding 25^{o}C (52).

The use of surfactants usually affects the determined average diameter of the microgel. The diameters are in this case at a lower level than in the techniques without the use of surfactant. At the two opposite poles, in terms of size of particles obtained, are the microgels obtained by polymerization through the reverse microemulsion polymerization and through EP - in the case of the former the synthesized microgels are of 100 nm, and the monomer droplets are of diameter up to 10 nm. In the case of EP, the particle size is of about 10 µm. In the case of EP, drops can have a diameter between 10-100 microns, and in MEP the magnitude of initial monomer droplet is 30-500 nm (53). The emulsifiers used in the manufacture of microemulsions include: dodecyltrimethyl ammonium bromide and cetyltrimethyl ammonium bromide as examples of cationic emulsifiers, whereas the anionic emulsifiers are e.g.: 1,4-bis (2-ethylhexyl) sulfosuccinate sodium and sodium lauryl sulfate (54).

Emulsion polymerization without surfactant

Many authors classify methods SFPP and SFEP as polymerization by homogeneous nucleation. This means that the monomer is dissolved in a suitable solvent. As a result of the polymerization process, the polymer centers are formed, around which are growing polymer macromolecules. PNIPA, as insoluble in water, is precipitating in the course of the reaction, so the turbidity or opalescence is observed in the previously transparent system. Typically, in the reaction system all the reactants are present, except the initiator system. The introduction of the initiator initiates the sequence in which oligomers are formed, and then unstable pre-

cursor particles. Their aggregation leads to the formation of colloidally stable primary particles with unified sizes. The further course of the reaction affects the polydispersity. The polydispersity index increases as a result of aggregation of primary particles. This method may be developed to the, so called, seeding polymerization, which will play a role in synthesis in more sophisticated core polymeric structures, such as microgels with shell - core-shell microgels (55). SFEP method is successfully used for the preparation of microgels with positively charged surface - cationic microgels based on NIPA and 4-vinylpyridine (56). EP is subjected to numerous modifications, including the "semi-batch" process. In that case the reactants are added to the reaction mixture in portions - as demonstrated by Zhang et al. They obtained NIPA copolymer using the modified method, and resulting microgels were characterized by a lower diameter, while the polydispersity index was dependent on nucleation time (57). Fernandez and colleagues, in the course of polymerization of microemulsion particles, obtained PNIPA with a diameter of 30 nm using a system of benzyl peroxide initiator and TEMED (58). In Figure 6, there are images from scanning electron microscopy (SEM), of microgels from two batches of different PNIPA derivatives synthesized in the same SFEP conditions. The only difference includes the addition to the reactant mixture a lipophilic comonomer - N-*tert*-butylacrylamide. The resulting structures are smaller and more homogenous when additional lipophilic comonomer is applied (27).

Emulsion polymerization and precipitation polymerization using a surfactant

The use of an emulsifier, in concentrations exceeding the critical micellar concentration, allows the stabilization of primary particles. This mechanism enables generally to obtain particle sizes lower than in the SFEP. The surfactant is binding to the surface of primary particles and protects them against aggregation with other particles in the system. There are several interesting studies on the preparation of the polymers of NIPA by precipitation method (59). In one study, authors used nonionic surfactant Triton X-405, and in effect the microspheres were pH-sensitive and changed the structure due to the varied magnetic field – with possible application in the columns for the fractionation of DNA (60). In a similar manner the synthesis was carried out for copolymer of NIPA and acrylic acid with 2-acrylamide-deoxyglucose, in order to obtain microspheres of approximately 100 nm loaded with glucosamine (61).

Inverse emulsion polymerization

In the IEP particles are prepared from pre-gel, i.e., from droplets of a solution of monomer suitable for polymerization. The solution is dispersed in the oil phase. As a result, in the course of the reaction in aqueous phase, homogeneous microgels and aggregates of numerous microparticles smaller than the diameter of the emulsion droplets may be formed. An example of this type of polymerization is production of thermosensitive NIPA microgels, sensitive to an additional factor - the pH. Dowding et al. (62) applied here heptane as the continuous phase, while the NIPA was in the dispersed phase with MBA as a crosslinking agent. In IEP performed by Zhang et al., the microspheres were synthesized from NIPA, which enabled controlled release of ibuprofen (63). In the course of reverse MEP, the PNIPA was synthesized with the cobalt tetrafluoroborate as a soft template (64).

Dispersion polymerization

Dispersion polymerization (DP) may be considered as an interesting alternative to previously mentioned polymerization methods that affords micron-size monodisperse particles, using a single batch process. The DP is a type of PP in which the polymerization is performed from a monomer in the presence of a suitable polymeric stabilizer soluble in the reaction medium. Both the monomer and the polymeric stabilizer should be easily soluble in the applied solvent, whereas the formed polymer must be insoluble in the medium, usually an organic solvent. Initially, the system consists of a homogeneous solution of monomer with initiator and dispersant. The progression of the process leads to formation of sterically stabilized polymer particles by the precipitation of the resulting polymer. Due to the increase of monomer conversion rate, the properties of the solvent evolve. Finally, the obtained polymer particles can achieve diameter of 0.1–15 mm, and high monodispersity. Dispersant polymer may play a role as a reactive, polymerizable macromonomer. The course of the reaction may involve a block copolymer with specific affinity to the surface of the precipitated polymer, as efficient dispersant. Also application of a soluble polymer called "stabilizer precursor" with grafting feature is possible. In DP the dispersant polymer with hairy layer is a crucial factor in the process, due to the specific adsorption or incorporation onto the surface of the polymer particles obtained by PP. The type of dispersant polymer controls the stability of the colloidal system, and influences particle size of formed objects (65, 66). Lee et al. synthesized

crosslinked copolymer of NIPA and chitosan, using DP. They applied the anionic initiator APS and the cationic initiator AIBA. The homogeneous morphology was obtained in the case of APS, whereas the copolymer particles synthesized with AIBA as the initiator presented a core–shell morphology (67). Interesting structures were obtained by Akashi et al. (68), who applied as a dispersant polymer - PNIPA macromonomer 18 in ethanol, and synthesized thermosensitive microspheres 0.4–1.2 mm in diameter consisting of a polystyrene core, and PNIPA branches on the core surface. Also the magnetic microspheres of PNIPA were synthesized by the DP method; obtained particles were sensitive to the magnetic field and shrunk into an increasingly collapsed state at ca. 40^{o}C (69).

Suspension polymerization

Within the process of suspension polymerization the monomer, relatively insoluble in water, is dispersed in the form of liquid droplets, with addition of steric stabilizer. The vigorous stirring during the course of polymerization process enables production of polymer particles which are maintained in the liquid phase, however, the dispersed particles form a dispersed solid phase. The initiators must be soluble in the liquid monomer phase. Parallel terms: pearl and bead polymerization are simultaneously applied for the description of the suspension polymerization process, in the case of production of non-porous particles. The main target in suspension polymerization is to elicit uniform dispersion of monomer droplets in the liquid - aqueous phase, succeeded by precise coalescence of the droplets in the polymerization course. Several factors influence the uniformity and size of obtained polymeric forms: the interfacial tension, the agitation and the type of the reactor device. The synthesized polymeric forms usually reach the range of 10 μm to 5 mm in diameter. Application of suspending agents results in reduction of the coalescence of monomer droplets, and in the reduction of the adjacency of nascent particles. This leads to high uniformity of the dispersion of synthesized polymer. For some polar monomers, e.g., acrylic acid, the dispersing medium should be non-polar. The paraffin oils are applied in this case. The isolation of obtained particles is possible by filtration or sedimentation, especially when the beads diameter does not exceed 10 μm (70, 71). Within the process, a so called stable state is ultimately reached, in which individual drops maintain their size over prolonged periods of time. In some cases the initially low-viscous solution of liquid monomer is transformed progressively into a viscous dispersion of polymer in monomer solution; finally solid particles are observed (72).

Table 4. Choice of bibliography dealing with NIPA derivatives applied in research which aimed controlled or targeted drug delivery.

Pharmacological group	Bioactive substance	Application route	Ref.
ABA	Chlorhexidine	topical	(75)
	Lysozyme	topical	(76)
	Ofloxacin	parenteral	(77)
CVA	Fluvastatin	stent platform	(78)
	Nifedipine	topical	(79)
	Propranolol	topical	(80)
	Caffeine	topical	(81)
ACA	Doxorubicin	parenteral	(82)
	Doxorubicin	parenteral	(83)
	5-Fluorouracil	topical	(84)
	5-Fluorouracil	parenteral	(85)
LA	Procaine	topical	(86)
	Lidocaine	topical	(87)
	Bupivacaine	parenteral	(88)
NSAID's	Diclofenac	topical	(89)
	Naproxen	topical	(90)

ABA - antibacterial agents; CVA - cardiovascular agents; ACA - anticancer agents; LA - local anestetics; NSAID's - non-steroidal antiinflammatory drugs

Zhou et al. gave a detailed prescription, considering the suspension polymerization of PNIPAM microgel particles (73). The NIPAM, BIS, and sodium dodecyl sulfate (SDS) were dissolved in deionized water, heated to 70^{o}C and stirred at 200 rpm for 40 min with a nitrogen purge to remove oxygen. After nitrogen purge, the KPS dissolved in deionized water was added to initiate the polymerization. The reactant mixture was mixed at 1000 rpm for 4 h. The SDS was removed by centrifugation cycles with decantations and dispersions in deionized water, and concentrated with the use of centrifuge. Thermoresponsive macroporous poly(acrylamide-co-NIPA) microgels were synthesized by inversion suspension polymerization by Hao et al (74). The microgels possessed large surface area, which is promising factor for future applications in the area of drug delivery.

Approach to practical applications of NIPA derivatives in drug delivery

Numerous publications consider the topic of controlled or targeted drug delivery, by the use of thermosensitive polymeric carriers – nanospheres or microspheres synthesized from the main monomer NIPA, and respective comonomers. In Table 4, we present a choice of bibliography dealing with NIPA derivatives synthesized in the form of microgel, applied in research which aimed controlled or targeted drug delivery. The reviewed bibliography covers choice of original papers published in last two years in the considered area.

Due to the data presented in Table 4, the NIPA derivatives were synthesized to obtain parenteral or topical carriers for numerous drug moieties: antibacterial agents (ABA), cardiovascular agents (CVA), anticancer agents (ACA), local anestetics (LA), non-steroidal antiinflammatory drugs (NSAID). The simple and efficient method of surfactant-free DP was applied for synthesis of microgels loaded by chlorhexidine for topical drug delivery, where the temperature change was the factor influencing drug release (75). Similarly, topical application of lysozyme was proposed with the use of copolymer of carboxymethylcellulose (CMC) and NIPA – the synthesis based on decorating the backbone of CMC with linear chains of NIPA (76). Another antibacterial agent, ofloxacine, was incorported into NIPA crosslinked by acrylate terminated poly(L-lactic acid)-b-poly(ethylene glycol)-poly(L-lactic acid) (77).

In several cases CVA were loaded to the systems which consisted of NIPA derivatives. The process of DP was used to obtain matrix/microgel copolymer system for application of fluvastatin in the form of stent platform (78). Nifedipine, potent inhibitor of calcium channels was introduced into poly(2-acrylamido-2-methylpropanesulfonic acid) synthesized by single-spinneret electrospinning technique (79). Propranolol hydrochloride was combined with polyampholite N-isopropylacrylamide-based hydrogels copolymerized with acrylic acid and N-(3-aminopropyl)methacrylamide; the process of polymerization was performed *via* free radical polymerization using NIPA and cross-linker BIS in dimethyl sulfoxide (80) Caffeine, an agent applied both for cardiovascular system, as well as topical agent for skin imperfections was loaded to semitelechelic poly(*tert*-butyl methacrylate)-b-PNIPAAm brush-like polymers – the synthetic approach included complex method with chain transfer agent (81).

One of the rising branches in the field of drug micro- and nanoforms is development of potential carriers for targeted and controlled delivery of ACA. Doxorubicin with folic acid were implemented into the PNIPA-acrylamide-allylamine coated magnetic nanoparticles, which are presumed for parenteral application (82). In another approach, doxorubicin was loaded to thermo and pH dual responsive, polymer shell coated, magnetic mesoporous silica nanoparticles, obtained *via* PP (83). Nanogel of PNIPAM-co-chlorophyllin was synthesized by SFEP, and applied for the development of topically applied 5-fluorouracil (84). The targeted delivery of 5-fluorouracil was also studied in folate-targeted poly[(p-nitrophenyl acrylate)-co-(NIPA)] nanohydrogel, polymerized in the process of PP (85).

There is some information on the use of microgels synthesized *via* SFEP or PP for the controlled topical delivery of LA – procaine (86) and lidocaine (87). One study involved complex problem of parenteral application of acrylic acid-functionalized microgels loaded by bupivacaine (88). Behind the LA also the NSAID's are studied for topical applications. Diclofenac diethyl ammonium was embedded into system, which based on sodium methacrylate and NIPA, as hydrophilic/pH-sensitive and thermo-responsive monomers, and on methacrylate bovine serum albumin as cross-linker (89). Naproxen sodium release was studied from 4-vinylpyridine-based smart nanoparticles synthesized with NIPA, 2-hydroxyethyl methacrylate, and acrylic acid, obtained in the process of PP in the presence of shell-forming monomers (90). In one study, 5-aminolevulinic acid for topical application was implemented to PNIPA-co-acrylic acid microgel; the microgel was synthesized *via* microemulsion polymerization in oil-in-water system (91).

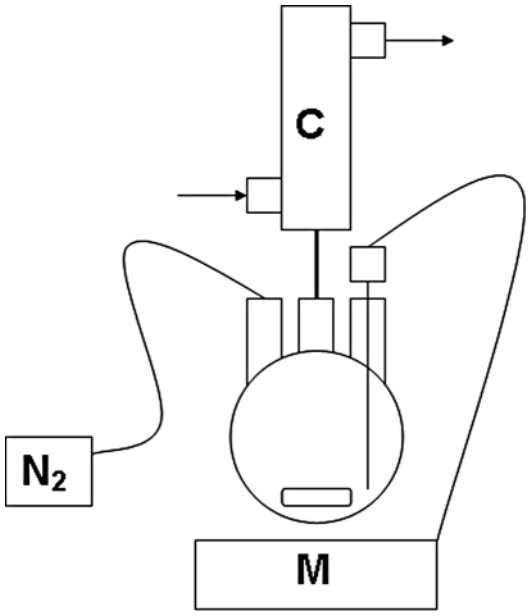

Figure 7. Synthesis set for preparation of NIPA microgels, with magnetic stirrer and feed-back control of temperature (M), cooler (C) and nitrogen inlet (N_2)

Several works dealt with the influence of newly synthesized NIPA derivatives on the release of model particles: dyes or aromatic model drugs; in some cases the dyes were introduced to reflect the structure of obtained polymer system. Interesting approach to the synthesis of drug carrier was presented by Wynter et al., who proposed microfluidic synthesis for production of copolymer in a form of monodisperse microgels, using so called co-flow glass capillary device for making single emulsion droplets of 25 – 100 μm in diameter (92). Detailed NMR study of influence of temperature on the release of five model drugs: salicylaldehyde, *m*-hydroxybenzaldehyde, ethylvanillin, 3,4-dimethoxybenzaldehyde and *p*-hydroxybenzaldehyde from beads synthesized *via* PP was described by Hofmann and Schönhoff (93). Fluorescein-labeled dextran was used in the study of magnetic composite with obtained by surfactant-free DP (94). Single fluorescein sodium salt was introduced to poly(acrylonitrile-co-NIPA) core–shell nanoparticles, obtained in a complex procedure with amidoximation and quaternization of the shell material (95). In another study, polyvinyl alcohol (PVA) matrix with nanosized pores was obtained by treatment with silica and glutaraldehyde; afterwards, the internal pores of the dry PVA matrix were filled with PNIPA, and the rhodamine B dye was used for visualization of the release process (96). Microfluidic generation of organic/aqueous/organic double emulsions and subsequent photopolymerization of the monomer residing in the aqueous phase of the droplets resulted in fabrication of PNIPA microgels containing hexadecane droplets: the system was investigated using hydrophobic dye 7-diethylamino-3,4-benzophenoxazine-2-one i.e., Nile Red, and hydrophilic dye 4',6-diamidino-2-phenylindole, to assess the characteristics of the obtained NIPA derivative system (97). Microgel beads of PNIPA-co-acrylic acid copolymer obtained by PP were studied by loading with fluorescent dye – FITC (98).

The synthesis of NIPA microgel is determined by the use or proper composition of reactants, and respective conditions of the reaction. The system usually consists of a glass reactor with a lid for the introduction of substrates to the reaction mixture and to control the course of synthesis. The controlled steps of the procedure include the fixed reaction temperature conditions, the appropriate sequence of the substrates, the isolation of the system from the influence of atmospheric oxygen, and the speed and method of stirring. Sample set for the synthesis of PNIPA microgels is shown in Figure 7.

New approaches to synthesis of NIPAM derivatives involve synthesis of cross-linked poly(N-isopropylacrylamide) microparticles in supercritical carbon dioxide (99).

SUMMARY

NIPA is a substrate for the synthesis of the corresponding copolymers with different properties and numerous possible applications in drug dosage form technology and in various branches of medicine. Synthesis of NIPA copolymers takes place mainly as a free radical process. In order to obtain microgels with the desired properties, the relevant systems, initiators and accelerators, as well as the specific comonomers and crosslinking agents must be arranged. This procedure allows one to transmit an appropriate charge to microgel surface or to maintain planned hydrophilic or lipophilic properties of the microgel. An important factor influencing the particle size obtained in the course of emulsion polymerization, is application of a surfactant. Also precipitation polymerization is often used, which therefore does not consume any surfactant. There are numerous modifications of the emulsion polymerization and they are still under development.

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