Studies on thermoresponsive polymers: Phase behaviour, drug delivery and biomedical applications

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Abstract

The present review aims to highlight the applications of thermoresponsive polymers. Thermo-responsive polymers show a sharp change in properties upon a small or modest change in temperature. This behaviour can be utilized for the preparation of so-called ‘smart’ drug delivery systems, which mimic biological response behaviour to a certain extent. Such materials are used in the development of several applications, such as drug delivery systems, tissue engineering scaffolds and gene delivery. Advances in this field are particularly relevant to applications in the areas of regenerative medicine and drug delivery. This review addresses summary of the main applications of thermoresponsive polymers which are categorized based on their 3-dimensional structure; hydrogels, interpenetrating networks, micelles, films and particles. The physico-chemical behaviour underlying the phase transition is also discussed in brief.

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1. Introduction

Thermoresponsive polymers are a class of “smart” materials that have the ability to respond to a change in temperature; a property that makes them useful materials in a wide range of applications and consequently attracts much scientific interest. Thermoresponsive polymers are used for biomedical applications including drug delivery, tissue engineering and...
gene delivery [1–5]. Temperature-responsive polymers exhibit a volume phase transition at a certain temperature, which causes a sudden change in the solvation state. Polymers, which become insoluble upon heating, have a so-called LCST. Systems, which become soluble upon heating, have an UCST. LCST and UCST systems are not restricted to an aqueous solvent environment, but only the aqueous systems are of interest for biomedical applications. The change in the hydration state, which causes the volume phase transition, reflects competing hydrogen bonding properties, where intramolecular hydrogen bonding of the polymer molecules are favoured compared to a solubilisation by water [6–12].

Typical LCST polymers are based on NIPAM [13,14], DEAM [15], MVE [16,17], and NVCl [18,19] as monomers. A typical UCST system is based on a combination of AAm and AAc [20]. The combination of a thermoresponsive monomer like NIPAM with one of a pH-responsive monomer yields double-responsive copolymers [21]. Most applications use the change from e.g. room temperature to body temperature in order to induce a change in the physical properties for e.g. gelation, especially in topical applications and in injectable biodegradable scaffolds. In vitro applications in cell culture are also using the stimulated swelling and collapsing of hydrogels with their change in surface properties.

2. LCST and UCST behaviour

The solubility of a polymer in aqueous solution is dependent on various factors such as molecular weight, temperature or addition of a co-solvent or additive. If the phase diagram of a polymer/solvent mixture vs. temperature shows both a one-phase and a two-phase region, one can identify the critical solution temperature: the UCST or LCST (Fig. 1). Often the terms UCST and LCST are used in a misleading fashion, therefore, it has to be noted that they should only be used, if the phase diagram has been determined. Then it is the maximum (UCST) or the minimum (LCST), respectively, of the phase diagram. Any other transition from soluble to insoluble or vice versa (at a given concentration) should be denoted as transition temperature (Tt). However, some polymers like PNPAM exhibit a phase transition, which is almost independent of the concentration or molecular weight. Then the Tt at any given concentration is almost identical to the LCST.

Table 1 gives a selection of polymers with either LCST or UCST behaviour in aqueous solution. These polymers have the transition temperature in the temperature region, which is interesting for biomedical applications (~20–40 °C). It has to be noted that the transition temperature can be strongly dependent on factors such as solvent quality, salt concentration, etc. (besides molecular weight and concentration). Obviously, the transition temperature has to be determined for the setting of the intended application [22].

3. Influence of the salt concentration, surfactants or co-solvents on the transition temperature

Since the thermoresponsive behaviour depends on the solvent interaction with the polymer and the hydrophilic/hydrophobic balance within the polymer molecules, it is not surprising that additives to polymer/solvent system can influence the position of the volume phase transition. Three interesting “additives” are salts, surfactants and a co-solvent, because all of them relate to the biomedical applications, either as additive in a potential drug formulation or as molecules present in an in vivo environment. All additives can alter the solvent quality and therefore can alter the polymer–solvent (+additive) interactions. Surfactants are as amphiphiles of particular interest, because as soon as a surfactant absorbs to a polymer molecule it substantially alters the hydrophilic/hydrophobic balance. Therefore, the transition temperature can be shifted to a large extent or it can even disappear. Other aggregation forms such as micellisation can also occur (in contrast to a coil-to-globule transition) [19]. PNPAM and PVC differ in their response to addition of a surfactant. Where PNPAM shows a monotonous increase in the hydrodynamic radius (rH) upon addition of an ionic surfactant like SDS, rH of PVC is initially decreasing when adding SDS. In both cases the transition temperature increases with
increasing surfactant concentration until it levels out at a certain surfactant concentration [19,23].

4. Selected thermoresponsive polymer classes

4.1. Poly(N-alkylacrylamide)

PNIPAM is the most prominent candidate as thermo-responsive polymer even though a second polymer in this class has a nearly identical transition temperature: PDEAM [13]. However, the transition temperature of PDEAM depends on the tacticity of the polymer, which is in contrast to PNIPAM. Its biocompatibility and the position of the LCST at 32–33 °C makes PNIPAM a very interesting material, e.g. for controlled release application. The LCST of PNIPAM is independent of the molecular weight and the concentration [24], but it can be changed upon shifting the hydrophilic/hydrophobic balance. PNIPAM copolymers have been mainly studied for the oral delivery of calcitonin and insulin. The peptide or hormone is immobilised in polymeric beads, which stay stable while passing through the stomach. Then in the alkaline intestine the beads disintegrate and the drug is released.

4.2. Poly(N-vinyl caprolactam) [PVC]

PVC has not been studied as intensively as e.g. PNIPAM, but it also possesses very interesting properties for medical and biotechnological applications, e.g. solubility in water and organic solvents, biocompatibility, high absorption ability and a transition temperature within the settings of these applications (33 °C) [19].

4.3. Poly(N-ethyl oxazoline) [PEtOx]

Poly(N-ethyl oxazoline)s have a transition temperature around 62 °C, which is too high for any drug delivery application. Recently a double thermo-responsive system was prepared by graft polymerisation of EIOx onto a modified PNIPAM backbone [25]. Currently these systems are explored for their potential in drug delivery, because they tend to aggregate micellise above the LCST.

4.4. Poly(methyl vinyl ether) [PMVE]

Poly(methyl vinyl ether) has a transition temperature exactly at 37 °C, which makes it very interesting for biomedical application. The polymer exhibits a typical type III demixing behaviour, which is in contrast to the thermal behaviour of PNIPAM [26]. PMVE has to be synthesized by cationic polymerisation using inert condition. Nucleophiles like alcohol or amino groups cannot be tolerated during the synthesis, which limits the potential of PMVE.

4.5. Poly(acrylic acid-co-acrylamide)

An interpenetrating network of poly(acrylic acid) and polyacrylamide is one of the few examples of a system with UCST behaviour within the biomedical setting. The transition temperature is at 25 °C [20]. The UCST behaviour is caused by the cooperative effects coming from the hydrogen bonding between AAc and AAm units [27].

4.6. Elastin-like oligo- and polypeptides

Polypeptides can also show LCST behaviour, when hydrophilic and hydrophobic residues are balanced well. A polymer made out of the pentapeptide GVGVP as repeating unit exhibits a volume phase transition at 30 °C, which is the hydrophobic folding and assembling transition. Below the phase transition, water molecules are structured around the polymer molecule; the attractive forces weaken upon heating and they finally go into the bulk phase. Above their phase transition temperature, there is the stabilization of secondary supramolecular structure, i.e. a twisted filament structure of β-spirals, which have type II β-turns [28]. It occurs due to hydrophobic folding and assembly. Chilkoti et al. have designed a double-responsive doxorubicin-polypeptide conjugate for cancer therapy [29,30]. The LCST behaviour of these polymers is tailored in a way that the slightly higher temperature of the tumour is enough to undergo a phase transition, which means that the conjugate becomes insoluble once it reached the targeted tumour.

5. Applications

5.1. Delivery of therapeutic molecules

5.1.1. Drug delivery

Drug delivery, as the name suggests, is the method or process of administering a pharmaceutical compound (drug) to achieve a therapeutic effect in humans or animals. Key factors are to deliver the drug to the right area, at the right time and at the right concentration. The “smart” polymeric carriers are used to deliver drugs. These carriers allow delivery of the drug at the right time and concentration by only releasing the drug in response to an external stimulus. For example the polymer chains of a carrier may expand as a result of the temperature increasing, thus enabling the drug to diffuse out and be released from the carrier [31].
5.1.2. Gene delivery
Gene therapy aims at the treatment of many genetic diseases as it is a technique for correcting defective genes that are responsible for these genetic diseases. Specifically, the delivery of the appropriate, therapeutic gene (DNA) into the cells that will replace, repair or regulate the defective gene that causes the disease is a vital step for gene therapy. DNA, however, is a negatively charged, hydrophilic molecule; thus its delivery into the nucleus of the cell which requires it to pass through the also negatively charged and hydrophobic cell membrane is not feasible. In gene delivery studies where thermoresponsive polymers were used the temperature at which one or two of the aforementioned steps were performed was changed. In particular, in studies where PEI with grafted PNIPAM [32], chitosan grafted with PNIPAM [33], linear and branched NIPAM, DMAEMA and PEI polymers [2] and PEG polymers with grafted PEI chains [34] were used, the complexation and transfection temperature were changed to enhance the transfection efficiency. In other studies only the incubation or complexation temperature were varied using random terpolymers of PNIPAM-co-DMAEMA-co-BuMA) [35,36] or PNIPAM copolymers [37], while both complexation and incubation temperature were varied using a polyarginine polymer conjugated with PNIPAM [38]. In an interesting study by Zhou et al. using poly(N,N-dimethylamino propyl acrylamide)-b-PNIPAM-star polymers, they used the polymers thermoresponsive ability in a different manner from the above studies [39]. The complexation was undertaken at room temperature which was below the polymer LCST and then the complex was deposited on a surface above the LCST. On that surface, the cells were allowed to incubate at 37 °C. The result of this was increased transfection to cells cultured on the surface compared to cells grown on a surface where DNA with no polymer was deposited [39]. When using a polymeric carrier, the main steps of gene delivery are given in Fig. 2.

5.1.3. Tissue engineering
Tissue engineering as an interdisciplinary field that applies the principles of engineering and the life sciences towards the development of biological substitutes that restore or improve tissue function [40]. Thermoresponsive polymers in tissue engineering are commonly used in two situations: as substrates that enable the cell growth and proliferation and as injectable gels, for in situ of the scaffold. In the first application, the thermoresponsive ability of the polymers is used to regulate the cells’ attachment and detachment from a surface [41–44]. In fact, in one study, the polymer surface was even reusable for repeated cell culture [45]. The second application involves the encapsulation of cells in a 3D structures in the body [46]. The in situ formation of cell/scaffold contrast compared to the in vitro formation of the construct allows the delivery of encapsulated cells, nutrients and growth factors to defects of any shape using minimally invasive techniques. The basic idea of the in situ formation is shown in Fig. 3. Specifically, the thermoresponsive polymer is mixed at room temperature with the cells and then injected into the body. Upon injection due to the temperature increase (to 37 °C) that is above the polymer’s LCST, the polymer forms a physical gel. The cells are encapsulated within the 3D structure of the gel.

5.2. Applications of polymers based on their architecture/structure

5.2.1. Hydrogels
Hydrogels are polymer networks dispersed in water which form semi solid states containing upwards of 99% water w/w to polymer. These gels can be either covalently linked polymer networks or physical gels mentioned above. With reference to thermoresponsive polymers, covalently linked networks exhibit a change in their degree of swelling in response to temperature. PNIPAM is one of the most intensely studied polymers in reference to biomedical applications due to its LCST being very close to body temperature and it’s fast on off switching. When crosslinked into hydrogels, the coil-to-globule transition causes a rapid decrease in the volume of the gel resulting in a fast release of entrapped drug and solvent followed by a more linear, diffusion controlled release [47–49]. Okuyama et al. reported on the swelling kinetics of co-networks of NIPAM with BuMA, P(NIPAAm-co-BuMA), commenting on the need for zero order drug release profiles and found that after a burst release of drug from the outer part of the hydrogel a sustained release can be obtained [50]. Coughlan et al. showed the importance of understanding the nature of a loaded drug in a polymer network with the crosslinker concentration and drug interaction with the polymer having a large effect on the rate of release of drugs from PNIPAM gels [51,52]. Jhon et al. studied this effect with PNIPAM brushes and found salt to lower the LCST due to the Hofmeister effect of salt on the

![Fig. 2](image-url) – The main steps of gene delivery using a cationic polymer: (1) DNA complexation (2) complex traversing the cell membrane to the cytoplasm (3) DNA release into the cytoplasm and (4) DNA transfer into nucleus.

![Fig. 3](image-url) – In situ formation of a scaffold in tissue engineering.
structure of water molecules [53]. Several PNIPAM conetworks were synthesized by Jones et al. for the delivery of antimicrobial agents in conjunction with medical devices [48]. Numerous other thermoresponsive monomers have been utilized for the preparation of hydrogels including PDMAAm [54], PEG [55]. Martellini et al. synthesized a PDMAAm-co-Poly(methoxyethyl acrylate) and showed that at body temperature this hydrogel releases drug following a Fickian diffusion process with a linear relationship in respect to the square root of time [54]. Yoshida et al. produced PNIPAM hydrogels with polyamino acid crosslinked chains to produce thermoresponsive degradable hydrogels [56]. Rincon et al. and Bessa et al. synthesized elastin-like polymers with polypeptide repeat units [57,58]. A biodegradable hydrogel was prepared by Xiao et al. comprising thermoresponsive PNIPAM with cleavable lactic acid and dextran groups [59]. Similar work was done by Zhuo et al. Grafting PNIPAM and PVC-HEMA onto a dextran chain to produce injectable and biodegradable hydrogels. This gel was capable of delivering drugs over several days with negligible cytotoxicity [60]. Merten et al. produced hydrogels from modified xyloglucan polymers and showed that the LCST can be altered by the removal of galactose rendering the polymer more hydrophobic [61]. Hydrogels of PEGMA containing iron oxide were synthesized by Meenach et al. for possible drug delivery applications [62]. These hydrogels showed thermoresponsive ability with a deswelling of the gels upon increasing the temperature. Papaphilippou et al. prepared PEGMA hydrogels with superparamagnetic properties by incorporation of magnetite nanoparticles during the polymerization [63]. Polymers of different architectures have also been used as injectable gels for tissue engineering. Kwon et al. produced physical gels of PNIPAM-b-PEG with linear and star architectures [64] while Kirklan et al. used triblock copolymers of PNIPAM (block A) and PDMAAm (block B) [65]. Co-networks of PNIPAM, poly(HEMA) and a lactic acid monomer were synthesized by Ma et al. and found to exhibit LCSTs of 10–20 °C with PNIPAM contents of 80% or more. The gels had high tensile strength and degraded over several months with no cytotoxic byproducts when used in tissue engineering [66]. A thermoresponsive methylcellulose based hydrogel was prepared by Stabenfeldt et al. Methylcellulose was conjugated to thermoresponsive methylcellulose based hydrogel was pre-
cytotoxic byproducts when used in tissue engineering [66]. A block copolymer consisting of PNIPAM and PVPON was prepared [62]. These polymers formed micelles above a critical micelle temperature dependent on the polymer LCST. Wei et al. synthesized a thermoresponsive star block copolymer based on l-Lactide and NIPAM. These star polymers were found to self assemble into large micelle structures in water which showed a fast on/off drug switching with temperature [79].

5.2.3. Micelles
Combining hydrophilic and hydrophobic monomers into block copolymers allows the formation of ordered structures in solution, the most common of these being the micelle. Micelles are useful for encapsulating hydrophobic drugs and delivering them into an aqueous environment. Several studies have focused on using PNIPAM as the thermoresponsive block in the formation of thermoresponsive micelles [71–75]. Aki-moto et al. produced micelles of (NIPAM-co-DMAAm)-b-PLA, where PLA was poly(lactic acid), and showed that these micelles were able to internalize into cells above their LCST, specifically due to the increased interaction between the hydrated NIPAM outer sphere and the cells [76]. Degradable copolymers of poly(NIPAM-co-HPAM-lactate)-b-PEG [77] and PEG-b (HEMA-lactate) [78] were shown by Hennink et al. to form micelles above a critical micelle temperature dependant on the polymer LCST. Wei et al. synthesized a thermoresponsive star block copolymer based on l-Lactide and NIPAM. These star polymers were found to self assemble into large micelle structures in water which showed a fast on/off drug switching with temperature [79].

5.2.4. Films
Copolymer films of PNIPAM and poly (N-butylacrylamide) were shown by Wilson et al. and Doorty et al. to give a sustained release of drugs from the film over a considerable time period [3,80]. They showed the released amounts of drug loaded at room temperature to be inversely proportional to the hydrophobic monomer content once heated to 37 °C. Dinarvand et al. investigated the possibility of using a copolymer of PNIPAM with PAAm as a stimuli responsive membrane for the control of permeation of molecules for numerous applications like drug delivery [81]. A block copolymer consisting of PNIPAM and PVPON was prepared [82]. These polymers formed micelles above the LCST of PNIPAM with PAAm cores. Previously, the conjugation of insulin to PNIPAM was studied by Chen et al. and it was found that grafting of the polymer to a surface allows cell growth at insuln levels 10 times lower than free insulin and allows for easy detachment when polymer is cooled below its LCST [83,84]. Ito et al. prepare plates of polystyrene grafted with PNIPAM copolymers using a mask to create a micropatterned surface [85,86]. Cell growth and selected cell detachment was shown to be achievable with this approach. Cheng et al. showed the production of plasma polymerized PNIPAM films onto microheater arrays produced using photolithography. This method allows for localized heating and specific area detachment of cells with many possible applications [87]. An interesting 3D cell culture method was envisaged by Poon et al. They synthesized chitosan-graft-PEG-graft-methacrylate copolymers which gelled at 37 °C but were also UV curable. Cells were encapsulated layer by layer by heating each layer above the LCST and the final construct was cured with UV to
enable cooling below the LCST without hydrogel breakdown [88].

5.2.5. Particles
Li et al. synthesized nanoparticles of thermoresponsive polymers by fast heating of a solution of the thermoresponsive polymer P(PEGMA-co-Boc-Cyst-MMAm) [89]. These particles possessed sensitivity to a reducing environment, such as the intracellular cytoplasm, by reduction of the disulfide bonds in the polymer chain resulting in breakdown of the nanoparticles. Zhang et al. coated insoluble nanoparticles with PNIPAM rendering them stable in aqueous solutions with temperature dependant solution properties and suggested uses in drug delivery and biological sensing [90]. Pitch et al. demonstrated the stabilization of magnetite nanoparticles by a thermoresponsive polymer. In particular, microgels containing thermoresponsive PVC and a hydrophobic monomer, acetoxy ethyl methacrylate, were prepared and loaded with the nanoparticles. These microgels showed thermoresponsive swelling/deswelling whilst stabilizing the suspension of nanoparticles [91]. Nanoparticles of crosslinked P(NIPAM-co-AAm) were prepared by Fundueanue et al. by dispersion of the polymer in mineral oil and crosslinking with gluteraldehyde [92,93]. Jun Wang et al. have produced a series of thermoresponsive micellar nanoparticles from PVC and polyphosphoester and examined their cell change in size with temperature. They found that as the temperature was increased above the LCST the micelles became more hydrophobic and formed aggregates in a reversible way [94,95]. Vihola et al. synthesized PVC and PVC-graft-PEG microgels were formed by heating the polymer above its LCST and using salicylic acid as a crosslinker. The salicylic acid formed hydrogen bonds between the polymer chains forming a physical hydrogel. By adding a solution of polymer and drug to a solution containing the crosslinker at temperatures greater than the LCST, hydrogel particles were formed which showed sustained release. Interestingly, the PEG graft copolymers showed a slower drug release due to an increase in hydrogen bonding and hence increase packing from the PEG chains [7].

Overview of applications of thermoresponsive polymers are given in Fig. 4.

Fig. 4 – Overview of applications of thermoresponsive polymers.

6. Conclusion

Thermoresponsive polymers offer great advantages in drug delivery. Instead of acting passively as pure drug carriers, they will interact and respond to the environmental setting. This allows us to aim further for tailor-made drug delivery with superior pharmacokinetics while having all safety questions addressed. Unfortunately, we often do not know the basic parameters in order to establish where, how and when our drug delivery system reaches a particular tissue or cellular compartment. The many open questions e.g. around gene delivery indicate that much more need to be understood to synthesize the most suitable vector or polymer therapeutic. All of the recent advances in thermoresponsive polymers point to an area of chemistry that both is growing in popularity, but also vast in the scope to be covered and will be an area to watch in the future. Thermoresponsive polymers and their use in biomedical applications will have a definite impact in the medical field.

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