Polymers Exhibiting Lower Critical Solution Temperatures as a Route to Thermoreversible Gelators for Healthcare

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The ability to trigger changes to material properties with external stimuli, so-called “smart” behavior, has enabled novel technologies for a wide range of healthcare applications. Response to small changes in temperature is particularly attractive, where material transformations may be triggered by contact with the human body. Thermoreversible gelators are materials where warming triggers reversible phase change from low viscosity polymer solution to a gel state. These systems can be generated by the exploitation of macromolecules with lower critical solution temperatures included in their architectures. The resultant materials are attractive for topical and mucosal drug delivery, as well as for injectables. In addition, the materials are attractive for tissue engineering and 3D printing. The fundamental science underpinning these systems is described, along with progress in each class of material and their applications. Significant opportunities exist in the fundamental understanding of how polymer chemistry and nanoscience describe the performance of these systems and guide the rational design of novel systems. Furthermore, barriers to translating technologies must be addressed, for example, rigorous toxicological evaluation is rarely conducted. As such, applications remain tied to narrow fields, and advancements will be made where the existing knowledge in these areas may be applied to novel problems of science.

1. Introduction

“Thermoreversible gels” or “thermogelling materials” are polymer solutions that transition to a gel state upon an increase in temperature above a critical point (Tgel), undergoing the reverse transition upon cooling. Thermoreversible gels have also been termed “thermoreponsive” or “thermosensitive” gels,[1] but this terminology is more frequently applied to materials which exist in a gel state at all temperatures, altering other physical or chemical properties when warmed or cooled. Such materials include chemically cross-linked hydrogels which expel water when warmed[2] and recently reported physical gels which alter their optical properties with temperature.[3] In the latter example, poly(styrene-ran-benzyl methacrylate-ran-methyl methacrylate) in an ionic liquid was demonstrated to exist in a gel state at all temperatures, but thermally-induced changes to nanostructure allowed switching from transparency to opacity.[3] Thermoreversible gels have enabled ground-breaking discoveries in fields including protein and peptide delivery,[4] drug delivery,[5,6] gene delivery,[7] tissue engineering,[8] cell culture,[9] and 3D/bioprinting.[10] Thermoreversible gels have benefits in medicine as these materials are low viscosity fluids below a critical temperature, and so can pass through an applicator or syringe,[11] and subsequently form gels upon contact with an elevated temperature, such as the heat from the body’s core. This viscous gel state can offer enhanced retention and prolonged drug delivery at the target site.[12] This, in turn, would allow for reduced dosing frequency, enhanced efficacy through prolonged absorption, and localization of delivery. In bioprinting and cell-culture the switchable gelation process triggered by small changes in temperature gives external control of 3D microenvironment and macrostructure.

There are two broad classes of thermoreversible gel. The first class rely on a hydrophilic/hydrophobic balance and include poloxamers ((poly(ethylene oxide) (PEO)-b-polypropylene oxide)-b-PEO) and copolymers of PEO and poly(lactic-co-glycolic acid).[13] These materials exist as micelles over a broad range of temperatures. However, temperature enhances the hydrophobic effect driving a greater fraction of micelles to form, potentially altering micelle shape, and enhancing inter-micelle interactions which in turn induces gelation.[15] Poloxamers, in particular, have been widely studied and are appealing due to their history of use in human medicine.[16,17] However, there are several limitations of these materials, including a high dependence of Tgel on polymer concentration leading to a reverse gel–sol transition upon dilution,[18] weak gel strengths for some applications such as mucosal drug delivery and 3D printing,[17,19] and low long-term cell viability in their presence for tissue engineering and cell culture purposes.[20] Thus, novel materials are required to meet these limitations and address the requirements of modern biomedical technologies.

The second class of thermoreversible gel requires a temperature-responsive polymer component which triggers self-assembly during temperature changes. There are two types of thermoreversible temperature-responsive polymer, those which exhibit a “lower critical solution temperature” (LCST) and

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DOI: 10.1002/adfm.202008123
advancing science news
www.advancedsciencenews.com

Figure 1. LCST-exhibiting polymers discussed within this literature review.

2. Thermodynamics of the LCST

The LCST is the critical temperature above which components in a mixture are no longer miscible. In polymer solutions, the LCST typically results in a transition from a coil to a globule, reducing contact with solvent. The collapsed polymer globules may then aggregate and form “mesoglobules.” If these particles are sufficiently large to scatter ambient light strongly, this transition is accompanied by a “cloud point” (T_C). Polymer precipitation at the LCST is an entropically driven process and can be described in terms of the Gibbs free energy change accompanying mixing (Equation 1).

\[ \Delta G_{\text{mix}} = \Delta H_{\text{mix}} - T \Delta S_{\text{mix}} \]  

The spontaneous process of dissolution of a simple solute is characterized by a negative change in the Gibbs free energy change of mixing (\(\Delta G_{\text{mix}}\)). A negative value of \(\Delta G_{\text{mix}}\) may be achieved due to the enthalpic or entropic processes, the latter of which, using this theoretical approach, are dependent upon

those which exhibit an “upper critical solution temperature” (UCST). LCST-exhibiting blocks may impart sol–gel transition with warming, where UCST-exhibiting polymers typically trigger the reverse transition that is, gel to sol, and thus are excluded from this review. The UCST phenomenon may be used for in situ gelation where it has been demonstrated that poly(N-acryloyl glycaminide) exhibiting a UCST in water of \(\approx 44^\circ C\) may be warmed to just above this temperature and injected into mice where the body cools and sets the gel which delivers drug over 100–200 h. Another example of the application of UCST systems is where gold nanorods were incorporated into a gel exhibiting a UCST. Excitation of the nanorods with near-infrared heated the gel to above its UCST triggering a gel–sol transition which released a protein payload, allowing for externally triggered delivery. There are existing reviews which may be referred to for information related to UCST systems. The chemical structures of several polymers exhibiting LCSTs in water are shown in Figure 1. Poly(N-isopropyl acrylamide) (PNIPAM) is the most widely reported polymer with an LCST, which is particularly attractive due to its sharp, environmentally-insensitive, transition at \(\approx 32^\circ C\) however there is conflicting evidence regarding its biocompatibility. Poly(N,N-diethyl acrylamide) (PDEA) is an alternative polymer with controllable LCST between \(\approx 25^\circ C\) and \(36^\circ C\), but its biocompatibility has been poorly explored. Poly(2-(N-dimethylamino) ethyl methacrylate) (PDMAEMA) is known to be biocompatible, having been included in pharmaceutical excipients, and allows for dual pH- and thermo- response which is advantageous in tailoring LCST, but the material is sensitive to changes in the environment, such as mild variation in salt concentration or pH. Poly(N-vinyl caprolactam) is another biocompatible polymer used in pharmaceutical excipients and which exhibits an LCST as low as \(30^\circ C\). However, the monomer is difficult to polymerize in a controlled manner. Poly(oligoethylene glycol [methyl ether] [meth]acylates) (POEG(ME)(M)As) allow precision tailoring of LCST dependent upon pendant chain length and end group and are believed to be biocompatible due to their poly(ethylene glycol) (PEG) -like structures. Poly(2-oxazolines) are an emerging class of thermoresponsive polymer which also allow manipulation of LCST based on their pendant group, but which have not yet been used in approved medicines. This selection of LCST-exhibiting systems form the majority of this review and are described in more detail within.

This review focuses on literature describing the generation of thermoresversible gels using polymers which exhibit LCSTs. The thermodynamics of the LCST are explained to give the reader an introductory overview of these materials. Poloxamer 407 is discussed as the current “gold standard” material which any novel thermoresversible gels must improve upon. Thermoresversible gelators are then described separately based on the polymer component which exhibits an LCST. 2-hydroxypropyl methacrylate materials are excluded as they are weakly hydrophobic even at low temperatures and thus do not exhibit an LCST. In this review particular focus is placed on PNIPAM, which remains the most commonly studied polymer with an LCST, and PNIPAM materials in order to pose key questions and inform discussion regarding the potential for thermoresversible gelators to be translated into human healthcare products. Applications of thermoresversible gelators are given, highlighting risks and opportunities for developing novel technologies.
temperature. Using classical thermodynamics to describe the case of polymer dissolution in water, water-water, and intra- and inter-molecular polymer–polymer bonds are broken, and water–polymer intermolecular bonds are formed, resulting in a change in $\Delta H_{\text{mix}}$.\(^\text{(17)}\) When a polymer is dissolved there is also an $\Delta S_{\text{mix}}$, which in most ideal dissolution processes would be positive, due to the increased number of configurations in the system, showing an increase in disorder.\(^\text{(18)}\) However, polymers may exhibit a negative $\Delta S_{\text{mix}}$ due to the ordered layers of solvent molecules around the polymer chains in solution, which may be entropically unfavorable in cases where the undissolved polymer solid is inherently disordered.\(^\text{(19)}\) For a polymer to exhibit an LCST in an aqueous solution using this theoretical framework, the $\Delta S_{\text{mix}}$ of mixing must be negative, as well as the $\Delta H_{\text{mix}}$. Upon surpassing a critical temperature known as the “spinodal point”, the $\Delta G_{\text{mix}}$ changes from negative to positive, making the mixing of polymer and water disfavored resulting in demixing to a two-phase system.\(^\text{(20)}\) The temperature at which the two-phase system is formed is known as the LCST, resulting in precipitation.\(^\text{(21)}\) When a polymer exhibiting an LCST is covalently bonded to a hydrophilic moiety, this LCST transition may instead induce amphiphilicity upon heating, in turn favoring the formation of nanostructures and physical entanglements which induce gelation. LCSTs may be affected by degree of polymerization (DP), concentration, neither of the two, or both.\(^\text{(22)}\)

Whilst classical thermodynamics allows an intuitive interpretation of the LCST, the thermodynamics of dissolution for simple solutes in water does not hold true for high molecular weight polymer solutions. In polymeric solutions where there is a great difference between the molecular sizes of the solute and solvent, Flory–Huggins theory is applicable. Flory–Huggins theory is a lattice model which incorporates the number of polymer-solvent interactions when calculating the $\Delta G_{\text{mix}}$.\(^\text{(23)}\) This theory takes into account the great dissimilarity between the molecular sizes of polymers and the solvent in solution. Flory–Huggins calculates $\Delta G_{\text{mix}}$ from the gas constant ($R$), the number of moles of the solvent ($n_1$) and polymer ($n_2$), the volume fraction of the solvent ($v_1$) and polymer ($v_2$), and the chi parameter ($\chi$), also known as the Flory–Huggins interaction parameter (Equation 2). The chi parameter considers the energy required to intersperse the polymer and solvent molecules in the solution. LCST behavior is often accounted for in this theory by variation in chi with temperature:\(^\text{(24)}\)

$$\Delta G_{\text{mix}} = RT\left[n_1 \ln v_1 + n_2 \ln v_2 + n_1 n_2 \chi \right] \quad (2)$$

Classical thermodynamics and Flory–Huggins theory are intuitive for interpreting LCST behavior, however, they do not rigorously provide predictions of LCSTs for novel macromolecules. Increasingly sophisticated models such as the group contribution lattice-fluid equation of state and the Sanchez–Lacombe equation provide more rigorous predictions of LCSTs.\(^\text{(25)}\) Models also exist for charged species exhibiting LCSTs.\(^\text{(26)}\) Rational design of novel thermoresponsive polymers would greatly benefit from the generation of generalizable models capable of robustly predicting LCST behavior from the molecular structure in a range of solvent systems. This could be achievable using computational chemistry approaches, such as the generation of Quantitative Structure Property Relationships or Artificial Neural Networks linking polymer descriptors to LCST in water. Models of this type have been described in organic solvents, and success is likely when applying this approach to LCSTs in water.\(^\text{(27)}\)

3. Structure and Physical Properties of Thermoreversible gels

Thermoresponsive gelators composed of di-block copolymers typically possess an LCST-exhibiting component and a hydrophilic block (Figure 2A).\(^\text{(28)}\) Tri-block copolymers can have ABA, BAB, ABC, or BAC structures where the A block is temperature responsive and the B and C blocks can be either hydrophilic or hydrophobic (Figure 2B,C).\(^\text{(29)}\) Tetra-block copolymers contain four blocks of polymer, where all four blocks can be different, or up to two blocks can be present twice. Common architectures are ABCD, ABCB, or BACB, where any block can be either temperature responsive, hydrophobic, or hydrophilic.
in nature.[50] Star-shaped block copolymers have three or more temperature-responsive arms, and usually contain a central branched hydrophilic polymer, such as PEG (Figure 2D).[51] Both tetra- and star-shaped thermoreversible gel-forming block copolymers are less common within the literature compared to di- and tri-block copolymers, due to their complex structures and properties as well as the relatively high cost of star-shaped starting macroinitiators. Thermoresponsive gelators from graft copolymers have been reported,[52] and consist of a linear synthetic or natural polymer backbone, often hydrophilic, which is grafted with a temperature-responsive component (Figure 2E), or vice versa. Heating any form of thermoreversible gel past a critical temperature results in sol–gel transition, caused by an overall increase in hydrophobic character above the LCST triggering self-assembly processes and physical interactions which increases the overall viscosity of the system.[58] It is noteworthy that this review considers gelation to occur when the storage modulus of the material (G’) exceeds the loss modulus (G’’), in line with the majority of literature in this area. However, this designation has the limitation that it arises from chemically cross-linked hydrogels exhibiting G’ > G’’ at all frequencies. For many semisolids termed “gels” the designation arises from observation that the materials resist flow due to gravity, which may not occur over the duration of observation simply due to sufficient viscosity, for example. Thus, G’ > G’’ must be taken with this limitation in mind. It is the authors’ perspective that applying this rule (G’ > G’’) for defining a gel may limit the quality of discourse in this area. Novel systems may be highly appropriate for a given application without necessarily fulfilling this criterion. Conversely, predominantly elastic materials (G’ > G’’) may not have sufficient strength for a given application. The rheology of the materials must be considered holistically to critically evaluate the performance of thermoreversible gelators.

Thermoreversible gels require both a critical concentration and temperature to exhibit a sol–gel transition.[54] Several mechanisms are suggested for this gelation process (Figure 3). Block copolymers which contain an LCST-exhibiting polymer are proposed to form gels by self-assembly into micellar structures, which may pack or undergo a conformational change,[55] and/or by physical polymer entanglements between thermoresponsive components above the LCST.[56,57] In order to form a gel via micellar packing, the phase volume of micelles must surpass a critical value.[58] Packing may occur in a liquid crystalline manner, giving rise to a gel state, or the percolating gel network occurring without long-range order. For example, spherical micelles may pack into face-centered cubic mesophases, as occurring in poloxamer 407.[57] Block copolymers with more than one temperature-responsive block are reported to not only form micelles, but also form physical entanglements above the LCST. These physical entanglements may anchor micelles together as described by Semenov et al.[53] Semenov’s theory is that “telechelic” polymer chains with hydrophilic centers and hydrophobic
terminals form gels by the formation of “flower-like” micelles, within which the polymer acts as a “loop”, which are associated by unimer “bridges” (Figure 3i). In cases where LCST-exhibiting blocks flank a hydrophilic core, however, the LCST results in the copolymer behaving as a telechelic chain. Micelles formed from block copolymers have also been reported to undergo a conformational change upon from spherical to “worm-like” flexible cylinders, due to an increase in temperature. These worm-like micelles can become intertwined to form a percolating network triggering the formation of a mesophase gel.[59] A crucial step in enabling the next-generation of high-performance thermoreversible gelators is a comprehensive understanding of the interplay between these nanostructures and the bulk rheology of these systems, which will allow rational design of materials.

4. Thermoreversible Gelators

4.1. Poloxamer 407—The Material to Beat

Poloxamers, also known by the trade-names Synperonics (Croda), Pluronics (BASF) or Kolliphors (BASF), are ABA tri-block copolymers of poly(ethylene oxide) (PEO) (A) and poly(propylene oxide) (PPO) (B). Two poloxamers (poloxamer 188 and poloxamer 407) exhibit thermoreversible gelation due to their ability to form lyotropic liquid crystalline phases when heated. The gelation temperatures of poloxamer 188 and 407 in 20% w/v solution occur at >50 °C and ~25 °C, respectively.[60] Due to the physiologically relevant gelation temperature of poloxamer 407, it has been widely investigated as a thermoresponsive in situ gelator for drug delivery.[61,62] It is contained in medicines with regulatory approval by the FDA, and as such is listed in the inactive ingredients database.[17] Poloxamer 407 contains 101 and 56 repeat units of PEO and PPO, respectively.[63] The solution and interfacial behavior of poloxamer 407 has been characterized using techniques including tensiometry,[64] dynamic light scattering (DLS),[65] cryogenic transition electron microscopy (cryo-TEM),[66] and small angle neutron scattering (SANS).[67] Tensiometry reveals that poloxamer 407 exhibits a critical micelle concentration of 0.7% w/v at 15 °C, which is depressed to 0.005% w/v upon heating to 42 °C, indicating that an increase in temperature promotes the formation of micelles at lower concentrations (due to desolvation of the PPO core).[64] Whilst these thermoresponses of these is typically described as being due to their balance of hydrophilicity and hydrophobicity driving self-assembly, the equilibrium of which is temperature-dependent, in our view this may be considered an LCST phenomenon due to the desolvation of the PPO block. Prior studies of PPO indicate that low molecular weight polymers exhibit an LCST in water and that hydrophilic modification of PPO induces an LCST transition in higher molecular weight polymers.[68,69] Cryo-TEM studies have confirmed the structure of these aggregates formed by poloxamer 407 to be spherical in nature with a radius between 5 and 7 nm (Figure 4a).[70,71] Mortensen and Talmon performed SANS experiments of poloxamer 407 at 20% w/v in D2O.[70] Low concentrations of poloxamer 407 were successfully modeled as a dense spherical core (5 nm) consisting of PPO with a corona of PEO chains exhibiting Gaussian coil behavior. At higher concentrations, a structure factor describing hard sphere interactions was included to describe the scattering. This structure is consistent with subsequent SANS studies of poloxamer 407 gels, in which poloxamer micelles associate via hard sphere interactions to form a lyotropic liquid crystalline gel phase (Figure 4b).[72] A face-centered cubic structure was subsequently found to be present in poloxamer 407 gels by small-angle X-ray scattering (SAXS).[73]

The thermoreversible gelation of aqueous poloxamer 407 solutions is highly dependent upon polymer concentration and the presence of co-solutes or co-solvents. The minimum concentration required to form a gel (G’ > G″) has been reported as 12.6% w/v, however, the majority of studies use concentrations >15% w/v.[62,74] A difficulty in developing poloxamer 407 formulations is the relationship between gel properties and polymer concentration. Whilst 15% w/v poloxamer 407 solutions exhibit a Tgel of 45 °C, forming gels with an elastic modulus of ~200 Pa, increasing concentration to 20% w/v reduces Tgel to 25 °C, increasing the elastic modulus of the gel formed to ~13 kPa.[75] The decrease in gelation temperature is reported to be due to a greater overall number of polymer chains meaning that the critical phase volume of micelles required to form a gel (Φ > 0.53),[76] is reached at lower temperatures.[77] In addition to this, Alexandridis et al. found that increasing the polymer concentration (from 0.01 to 5.00% w/v) results in a reduced

Figure 4. a) Cryo-TEM micrograph of the micelles formed by poloxamer 407 in aqueous solution.[71] b) SANS curves of 3 w/v % solutions of poloxamer 407 in D2O at various temperatures: 5 (circle), 15 (triangle), 25 (square), 35 (inverted triangle), 50 (diamond), and 70 °C (filled square). Solid lines are fits to a Debye model (5 and 15 °C) and to a micellar model (25 °C and above).[72] a) Reproduced with permission.[55] Copyright 1995, PCCP Owner Societies. b) Reproduced with permission.[72] Copyright 2007, American Chemical Society.
micellization temperature from 35.5 to 19.5 °C, which may contribute to a decreased Tgel.\[26\] This inverse proportionality between concentration and Tgel leads to issues with dilution (e.g., in physiological fluids) triggering a gel–sol transition due to elevated Tgel.\[18\] Additionally, >20% w/v concentrations of poloxamer 407 are required to form viscous gels with G’ > 10 kPa which then typically exhibit Tgel at ≤25 °C, where the ideal in situ gelator will transition to a gel at close to a physiological temperature as possible, to avoid gelation in ambient conditions.

The thermoreversible gelation of poloxamer 407 may be modified using additives such as salts, polar organic solvents, polymers, and the inclusion of therapeutic agents.\[17\] The inclusion of salts in poloxamer 407 formulations typically results in a reduced micellization temperature and thus gelation temperature, where the magnitude of the salting-out effect follows the Hofmeister series.\[79–82\] It has been shown that the addition of co-solvents also affects the properties of poloxamer 407 thermogels. The addition of formamide and ethanol to water provides better solvent conditions for poloxamer 407 and therefore increases the micellization temperature and the gelation temperatures.\[83,84\] Additionally, ethanol negatively impacts on the viscosity of the poloxamer 407 gels formed.\[84\] In contrast, glycerol has been demonstrated to compete with poloxamer 407 for water, thereby reducing the micellization temperature.\[81\] Polymers such as poloxamer 188\[85,86\] poly(vinyl alcohol),\[87,88\] and Carbopol\[89–91\] have been included in poloxamer 407 formulations to attempt to modify the behavior of the thermoreversible gelators. The majority of studies generate relatively minor alterations in the gel strength of poloxamers (the most successful examples improve strength by ~30%), with knowledge gaps in how additive chemistry links to behavior of the formulation.\[17\] Future advances in this area may be supported by linking nanostructural characterization techniques to spectroscopic tools to probe interplay between changes in structure and intermacromolecular interactions and provide a rational basis for additive selection.

Incorporation of therapeutic agents has the potential to alter the properties of thermoreversible gelators. As such, there have been several studies which investigate the effect of active pharmaceutical ingredient (API) on the gelation temperature and gel strength of poloxamer formulations. In addition to this, the release kinetics of therapeutic compounds has also been investigated. Hydrophilic and hydrophobic small molecule APIs have been explored including lidocaine (clog P: 2.3, S: 20 m m),\[92\] fentanyl (log P: 4.05, S: 0.59 m m),\[93\] fluorouracil (clog P -0.89, S: 90 m m),\[94\] doxorubicin (clog P: 1.27, S: 4.8 m m)\[94\] tricam-cynolone acetone (clog P: 2.53, S: 0.048 m m),\[95,96\] capsaicin(clog P: 3.6, S: 95 m m),\[97\] progesterone (clog P: 3.9, S: 28 m m),\[97\] and tenofivir disoproxil fumarate (log P: 1.25, S: 20 m m),\[75\] as well as peptides (arginine vasopressin,\[98\] and insulin)\[99\] and glycopeptides (vancomycin).\[79,100\] When analyzing hydrophobic therapeutics, their solubility is typically enhanced in poloxamer 407 formulations when compared to pure water. The enhanced solubility is due to dissolution of the drug to the core of the poloxamer 407 micelles.\[100\] Incorporation of hydrophobic therapeutics may reduce the viscosity of poloxamer 407 gels. For example, the inclusion of 4% piroxicam reduced poloxamer 407 gel viscosity from ≈8000 mPas to ≈4000 mPas.\[102\] The therapeutic molecules may partition between the aqueous media/hydrophilic corona or the core of the micelle. Solubilization into the core of the micelle may increase micelle size and reduce their packing density while, solubilization of the therapeutics to the extra-micellar water may increase the distance between the micelles, resulting in fewer micellar–micellar interactions. Both of these are suggested to contribute to a decrease in the degree of hydrogen bonding between micelles and a reduction in gel strength.\[102\]

Overall, poloxamer 407 is an attractive thermoreversible gel for drug delivery as a result of its low viscosity at room temperature and sharp transition to viscous gel when reaching physiologically relevant temperatures (i.e., ≈37 °C). As a result of this property, poloxamer 407 can be applied via a syringe or an applicator to achieve in situ gelation at the target site. These formulations have also been found to allow incorporation of hydrophilic therapeutics and enhance the solubility of poorly-water soluble drugs. In addition to this, poloxamer 407 gels also offer prolonged release of both hydrophilic and hydrophobic therapeutics. Furthermore, poloxamer 407 has a history of use in pharmaceutical products as up to 15.1% w/v in intratympanic application.\[103\] As much as poloxamer formulations may be appealing, there are still significant drawbacks. For example, poloxamer 407 gels offer weak mechanical strength for applications under shear\[69\] as a result of the shear thinning character,\[94\] rapid dissolution,\[105\] and weak mucoadhesion.\[106\] Consequently, poloxamer 407 gels display poor residence times in vivo, with one study estimating that ~60% of a poloxamer gel was removed from the vagina of mice after 1 h.\[107,108\] Additionally, minor dilution may elevate Tgel above body temperature and reverse gelation, as seen in vivo after ocular administration.\[18\] There is a need for novel thermoreversible gelators, but crucially these materials must offer clear advantages over poloxamer 407 where the expense of translating technologies is substantial and risk of failure must be mitigated during material development. For example, an often-cited limitation of poloxamer 407 is that the strength of the gels formed limits certain applications under significant shear, however, the majority of novel materials reported have lower strengths than poloxamer.\[75,109,110\]

### 4.2. Poly(N-isopropyl acrylamide) and Related Poly(acrylamide)-Based Thermoreversible Gels

The most well-studied LCST-exhibiting polymer is PNIPAM which possesses an LCST at ≈32 °C in aqueous solution.\[111\] The LCST between room and body temperature makes PNIPAM ideal for the development of thermoreversible gels for many biomedical applications, particularly drug delivery. LCST behavior in PNIPAM is driven by the hydrophobic character of the isopropyl group which imparts a degree of amphiphilicity within the monomer subunit. Elevation above the LCST drives contact between the isopropyl group and the remaining polymer macromolecule. It is a common misperception that this state is “hydrophobic”, where it has been demonstrated that the PNIPAM globules contain ~50–60% bound water.\[112\] The LCST of PNIPAM is independent of changes in molecular weight but may be modulated by a few degrees by varying the
concentration of the polymer in solution.\[^{113}\] The LCST may be reduced using “salting out” agents from the Hofmeister series.\[^{114}\] Ions of the Hofmeister series compete with the polymer for water molecules, and as such fewer water molecules are free to solvate the polymer.\[^{115}\] This results in fewer polymer-water bonds which results in a lower energy requirement to induce polymer precipitation. Salts are permissible in pharmaceutical products at varying concentrations dependent upon the medicine and route of administration, with 0.9% w/v NaCl typical for injectables and up to 10% w/v in vaginal gels.\[^{103}\] Copolymerization of NIPAM with hydrophilic monomers is known to raise LCST in water.\[^{116}\] PNIPAM may also exhibit UCST behavior in mixtures of water and organic solvents, such as ethanol, propanol, and DMSO.\[^{117,118}\] This effect is due to the organic solvent preferentially interacting with water at low temperatures, reducing the solubility of PNIPAM.\[^{117}\] UCST behavior may also be seen in PNIPAM solutions in ionic liquids, attributed to enhanced H-bonding between PNIPAM chains at low temperatures.\[^{119,120}\] The LCST of PNIPAM has driven the design of many thermoreversible gelators, detailed hereafter.\[^{121}\]

A PNIPAM-PEG di-block copolymer, where both blocks have molecular weights of 2 kDa, was found to be a free-flowing clear solution at 25 °C and formed a turbid viscous gel at 37 °C (Tgel = 29 °C) with a G’ of 480 ± 70 Pa at 50 Pa and 1 Hz.\[^{122}\] This di-block copolymer was also found to have an oscillatory yield stress of 690 ± 90 Pa, above which the material shear-thins. Motokawa et al.\[^{123}\] investigated PEG-PNIPAM diblock copolymers with 7.3 kDa PEG and 25.8, 16.2, and 4.3 kDa PNIPAM by SANS, theorizing that gel phases form as a result of diffusion limited association of micelles at higher temperatures, driven by a thermodynamic requirement to minimize interfacial free energy. This leads to the formation of a percolating PEG/water phase incorporated in a PNIPAM-rich network. This structure continued to exist during synergesis observed in gels upon further heating, which the authors attributed to further aggregation and vitrification of PNIPAM chains. It is known that whilst chain collapse occurs over a narrow temperature range for PNIPAM homopolymer, the presence of PEG blocks drives this transition to occur over a broad temperature range.\[^{124}\] PNIPAM-b-poly(3-O-allyl-α-D-glucose) also exhibits a thermoreversible gelation, identified via tube inversion, in which the gel phase is stable between 20 and 40 °C.\[^{125}\] Above ~40 °C the gels underwent synergesis. The authors suggest that gelation is driven by the formation of core-shell spherical micelles with a PNIPAM core and poly(3-O-allyl-α-D-glucose) corona, which pack to form the mesophase. Syneresis was suggested to occur due to shrinkage of the micelles, but this was not directly studied.

There are reports in the literature of the synthesis of ABA tri-block copolymers where the A block is PNIPAM and the B block has been PEG,\[^{125,129-132}\] poly(vinyl pyrrolidone) (PVP),\[^{119}\] or poly[N,N-dimethylacrylamide] (PDMA).\[^{111,112}\] All of these block copolymers exist as free polymers in solution below the LCST of PNIPAM. Above the LCST of PNIPAM, these polymers transition to form aggregates which, in the case of PEG, have been determined to be either flower-like micelles\[^{113}\] or fractal-like structures.\[^{134}\] Lin and Cheng studied the thermoreversible gelation of a PNIPAM-b-PEG-b-PNIPAM block copolymer with 4.6 kDa PEG and 1.9 kDa PNIPAM blocks.\[^{122}\] At 37 °C, a 20% w/v aqueous solution formed a gel with a gel strength of 2000 ± 200 Pa and an oscillatory yield stress of 430 ± 50 Pa. The effect of molecular weight on gelation of PNIPAM-b-PEG-b-PNIPAM copolymers has been explored.\[^{126}\] Increasing the PEG molecular weight from 1 to 4 kDa resulted in an increase in transition temperature from ~34 to 40 °C and the gels formed exhibited fewer signs of syneresis. Conversely, increasing the PNIPAM molecular weight from 5 to 30 kDa decreased the transition temperature from ~42 to 34 °C while also increasing the gel strength from 0.01 to values approaching 10⁶ Pa (Figure 5). This remarkably high elastic modulus has not been reported in similar systems. Larger molecular-weight polymers were found to form gels at lower concentrations, which is in agreement with Cong (2014).\[^{126,130}\]

Aqueous solutions of PNIPAM₉₈-PEG₁₂₇-PNIPAM₉₈ (>20% w/v) undergo increases in viscosity at temperatures fractionally below body temperature (37 °C) which make the copolymer attractive as an in situ gelator. Both viscosity and Tgel may be tuned by increasing concentration, with 50% w/v solutions forming viscous gels (G’ = 13 kPa at 1 Pa and 1 Hz) with a Tgel of 36 °C, giving the material advantages over poloxamer 407, which exhibits Tgel near room temperature (Figure 6).\[^{27}\] ABA triblock copolymers of PNIPAM (A) and PDMA (B) with molecular weights of 6 and 10.5 kDa, respectively, also exhibit heat-triggered gelation. Increasing the polymer concentration from 10 to 20% w/v increased the gel strength from ~100 to 1000 Pa and the gelation temperature fell from ~60 to 50 °C. In the case of the PNIPAM-b-PVP-b-PNIPAM tri-block copolymers, increasing the molecular weight of the PNIPAM blocks from 11 to 26 kDa decreased the gelation temperature from ~27 to 23 °C for a 20% w/v polymer solution.\[^{130}\] The study also found the minimal concentration for gelation was lower for the polymer with the largest molecular weight (i.e., 48 kDa polymer formed a gel above 30% w/v while the 71 kDa polymer formed a gel above 20% w/v).

![Figure 5](https://www.afm-journal.de)
In addition to ABA containing tri-block copolymers, an ABC tri-block copolymer of poly(methyl methacrylate) (PMMA) (A), PDMAEMA (B), and PNIPAM (C) has been reported. In these systems, PMMA is hydrophobic, PDMAEMA is weakly basic (and thermoresponsive), and PNIPAM is thermoresponsive. 3% w/v aqueous solutions of these block copolymers were found to form gels at pH < 7.0 (with PDMAEMA in a majority cationic state) upon an increase in temperature, while in pH > 7.0 (with PDMAEMA in a majority unionized form) a precipitate was formed. Upon an increase in temperature, with pH < 7.0, the PNIPAM blocks aggregate and the polymer forms a network in the aqueous solution with two localized areas of PMMA and PNIPAM. These two localized areas are then bridged by protonated PDMAEMA chains, which results in the formation of a gel. In the case of pH > 7.0, continual increases in temperature cause micelle agglomeration due to desolvation of the PDMAEMA block, resulting in a precipitate rather than a gel. The viscosities of the materials formed were pH-dependent under pH 7, exhibiting a maximum at pH 4 (Figure 7). The authors attribute the increase in viscosity when reducing pH from 6 to 4 to the increased ionization of the PDMAEMA subunits increasing the rigidity of the PDMAEMA blocks within the gel, which acts as bridges between collapsed PNIPAM and PMMA domains (Figure 7c). Where salt content was uncontrolled, the authors attribute loss of viscosity at pH < 4 to screening of charge by the increased ionic strength of these solutions. Future studies must consider that salt content will dramatically affect the solubility of polyelectrolytes, as well as impact on LCST.

There are cases where PNIPAM has been used to synthesize thermoresversible gels with more complex architectures, such as star-shaped and graft copolymers. 4 and 8 arm block copolymers of PNIPAM and a central PEG are reported. Solutions of 20% w/v 4 arm and 8 arm star-shaped PNIPAM and PEG copolymers were found to have gel strengths of 2500 ± 200 and 1050 ± 150 Pa, respectively, at 37 °C. In addition to this, the star-shaped block copolymers were found to have oscillatory yield stresses of 860 ± 80 and 200 ± 30 Pa, respectively. This indicates that the increase in number of temperature-responsive arms resulted in a weaker gel that was less resistant to shear. The mechanisms behind this were not studied, and future investigations will benefit from complementing rheology with small-angle scattering to fully explore these systems. Another study by Teodorescu et al. prepared 4 arm PNIPAM-b-PEG copolymers with a central PEG and four PNIPAM arms. This study found as the PNIPAM molecular weight was increased from 5 to 10 kDa, for example, the gelation temperature decreased from 41 to 36 °C while the gel strength increased from ≈ 1000 to 10 000 Pa. Increasing the PEG molecular weight from 2 to 6 kDa increased the gelation temperature from 35 to 37 °C.
Graft copolymers of PNIPAM and a cationic monomer (CH₃I quaternized N,N-dimethylaminoethyl methacrylate, termed “PDMA⁺”) with thermoresponsive gelation have been reported. Whilst pure PNIPAM polymer undergoes temperature-induced precipitation, the PDMA-g-PNIPAM copolymers form a gel phase, as determined by vial-inversion. The authors attribute this to a mechanism of core-shell micelle formation accompanied by bridging. It is noteworthy that PNIPAM-PDMA⁺ copolymers exhibit $T_{CP}$ at the same temperature as the pure PNIPAM studied ($≈33$ °C), where copolymerization with hydrophilic moieties typically elevates LCST. This effect is presumable due to the graft structure which leads to the pendant PNIPAM chains being unaffected by the presence of comonomer. Chitosan grafted with PNIPAM has also been synthesized. This polymer was found to increase in viscosity at $≈29$ °C and reach a gel with a viscosity of $≈400$ Pas. The material was demonstrated to maintain viability of chondrocytes and meniscus cells with the aim of being used as a tissue scaffold. Whilst modification of polysaccharides is inherently complex, this approach will allow researchers to combine the functionality achievable by nature with the control over physical behavior which synthetic polymer chemistry imparts.

PNIPAM has been exploited to trigger colloidal gel phase formation using temperature, where PNIPAM microgels above $≈160$ nm are able to form a gel phase in saline above their volume phase transition. A mixture of PNIPAM or PNIPAM-co-allylamine microgels and solid drug nanoparticles has also been shown to aggregate upon increase in temperature to form

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**Figure 7.** a) Viscosity of 3% w/v PMMA-b-PDMAEMA-b-PNIPAM triblock copolymer solution at different temperatures with variation of pH between 1 and 6. b) Frequency sweeps at 0.5% strain for PMMA-b-PDMAEMA-b-PNIPAM copolymer at 25, 50, and 75 °C. c) Proposed mechanisms of thermal gelation in PMMA-b-PDMAEMA-b-PNIPAM solutions. Reproduced with permission. Copyright 2017, The Royal Society of Chemistry.
a solid nanocomposite material.\cite{139} This aggregation is driven by the volume phase transition of the PNIPAM microgels upon heating above the LCST (Figure 8i). The materials are explored as depot-forming injectable preparations which released Lopinavir over 120 days (Figure 8ii). Whilst highly promising, the release profile exhibited significant burst, and future technologies to alter the kinetics to deliver ideal zero-order delivery would be highly advantageous. Zero-order delivery is often achieved by reservoir-type systems and the development of soft matter which mimics the core and membrane structures of reservoir technologies may be an approach to achieving this.

Biodegradable PNIPAM gelators have been reported based on poly(polyethylene glycol citrate-co-N-isopropylacrylamide) (PPCN).\cite{140} This material had intrinsic antioxidant properties, being able to scavenge free radicals, chelate metal ions, and inhibit lipid peroxidation. PPCN gels were subcutaneously administered to rats, leading to tissue reformation with resorption of the material over a period of 30 days (Figure 9). It is the authors’ perspective that in order to translate these technologies, regulators are likely to expect additional identification of the degradation products and metabolites of biodegradable systems intended for parenteral administration.

There are other poly(acrylamides) exhibiting LCSTs in water, but these have been investigated to a lesser extent when compared to PNIPAM for the production of thermoreversible gelators. One such polymer is PDEA, which exhibits an LCST between 25 and 36 °C,\cite{25} which is dependent on both concentration and molecular weight.\cite{141} An ABA tri-block copolymer of PDEA (A) and poly(acrylic acid) (PAA) (B) has been prepared and the thermogelling properties are investigated.\cite{142} The PDEA-b-PAA-b-PDEA tri-block copolymer was found to form a gel above 60 °C in 3% w/v aqueous solution with a viscosity of 10 000 Pas. Whilst the LCST was very high in this system, the viscosity achieved even at low concentrations makes these DEA
Native tissue is indicated with a dotted white line; T = native tissue, macrophages (green) were conducted. The border of the polymer with nuclear counterstain (blue) and CD68 immunohistochemical staining of at 3 or 30 days. E–H) Immunofluorescence staining of the tissue. DAPI — the most likely route for PNIPAM to become used in pharmaceutical products. There are, however, several studies describing the cytotoxicity of PNIPAM is by Vihola et al. The majority of studies assessing the safety of PNIPAM describe in vitro cytotoxicity assessment against a cell line relevant to the proposed application. In a typical assay, cytotoxicity is determined by measurement of a cell’s mitochondrial activity (e.g., by an MTT assay) and membrane integrity (e.g., by LDH assay). It is important to acknowledge that cytotoxicity measurement is not a test which the FDA specifically require for gaining regulatory approval of a new excipient in a medicine — the most likely route for PNIPAM to be used in pharmaceutical products. There are, however, guidelines by the International Organization for Standardization (ISO) for cytotoxicity assessment for medical devices, such as catheters or stents. A selection of studies determining cytotoxicity of PNIPAM is shown in Table 1. So that results of these studies may be directly attributable to PNIPAM, only papers which report use of the PNIPAM homopolymer are shown.

It can be seen (Table 1) that many studies report that PNIPAM in a variety of forms (particles, grafted to a surface, and polymeric) does not induce reduction in mitochondrial activity or an increase in membrane permeability in a number of cell lines. The NIPAM monomer is cytotoxic, and it has been reported that commercially-available PNIPAM samples may exhibit cytotoxicity due to the presence of residual monomer. The major study describing the cytotoxicity of PNIPAM is by Vihola et al. This study demonstrates that 12 h exposure of 5 mg mL⁻¹ PNIPAM to caco-2 cells at 37 °C results in a reduction in mitochondrial activity. Whilst this may be due to the cytotoxicity of PNIPAM, the study has the limitation that the MTT assay used is not supported by a secondary technique, such as a membrane-permeability assay. Furthermore, cytotoxicity under these conditions is also seen for poly(N-vinyl caprolactam), which is known to be safe for human exposure, and is used in pharmaceutical excipients (e.g., Soluplus by BASF) and personal care (e.g., Luviskol by BASF). Thus, cytotoxicity measurements are not necessarily good surrogates for evaluating safety in vivo, particularly where different sites of administration (e.g., the skin) have significant barriers on their surface which will reduce the ability of polymers to infiltrate into the tissue, causing toxicity. The study has been highly-cited as a supporting case of the cytotoxicity of PNIPAM.

4.2.1. What Evidence is There for the Cytotoxicity of PNIPAM?

Despite the enormous body of research exploring their potential applications, PNIPAM-based materials have not been incorporated in any approved medicines to date. One often-cited reason for this lack of translation is a belief that PNIPAM is cytotoxic, which is often used as a justification to explore alternative polymers displaying LCSTs. There are, however, many studies demonstrating that a variety of cell lines remain viable in the presence of PNIPAM. Cytoxicity arising from PNIPAM has sometimes been attributed to its hydrolysis products. However, PNIPAM is reported to be relatively resistant to hydrolysis due to steric hinderance from the isopropyl moiety, and PNIPAM copolymers appear to be stable in solution at room temperature for months. Furthermore, some authors have already highlighted that the safety profile of PNIPAM may be misrepresented. For instance, Hoffman (2013) states: “...the potential toxicity of [PNIPAM has] often been assumed but to the author’s knowledge, not yet clearly elucidated.”

There is therefore a need to understand fully the safety profile of PNIPAM in standardized toxicity testing procedures recognized by regulators so that a well-considered case may be made for whether it is possible for products containing this polymer to ever find use in humans. Existing studies into the cytotoxicity of PNIPAM are considered below.

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Measurements of toxicity using cells in typical 2D culture assays are limited in its prediction to real in vivo adverse events. Toxicity testing programmes for excipients typically move through to more relevant models, such as those suggested by the International Pharmaceutical Excipients Council in their tiered toxicity testing programme for pharmaceutical excipients.
Table 1. Selected studies investigating the compatibility of PNIPAM-based materials in vitro on cell lines.

| Reference          | Sample details                                      | Cell Line                                      | Key findings                                                                 |
|--------------------|-----------------------------------------------------|------------------------------------------------|------------------------------------------------------------------------------|
| Wadajkar et al.[154]| PNIPAM nanoparticles (hydrodynamic diameter 100, 300, and 500 nm) | 3T3 fibroblast cells, human aortic smooth muscle cell (HASMC), and human micro-vascular endothelial cell (HMVEC) | -PNIPAM nanoparticles tolerated at concentrations up to 10 mg mL⁻¹ for 96 h   |
|                    |                                                     |                                                | -cells exposed to NIPAM monomer exhibited toxicity even at very low concentrations. |
|                    |                                                     |                                                | -Nanoparticle size seemingly unrelated to cytotoxicity                       |
| Naha et al.[159]   | PNIPAM nanoparticles (hydrodynamic diameter: 78 nm)  | Skin Keratinocytes (HaCaT) and human colon adenocarcinoma SW480 cells | -No cytotoxicity measured by Alamar blue assay for mitochondrial activity up to 1 mg mL⁻¹ for 96 h. |
|                    |                                                     |                                                | -No genotoxicity detected by comet assay over 72 h exposure.                 |
|                    |                                                     |                                                | -Uptake of nanoparticles into lysosomes detected in both cell types          |
| Cooperstein and Canavan[150] | PNIPAM grafted onto silicon wafers. | Bovine aortic endothelial cells (BAEcs), smooth muscle cells (CRL444, SMCs), fibroblasts (MC3T3-E1, 3T3s), and Vero cells (CCL-81). | -NIPAM monomer is cytotoxic to all cell types after 48 h exposure using ISO 10993-5 testing procedures. |
|                    |                                                     |                                                | -All cell types were viable on PNIPAM after 96 h using a direct contact test. |
|                    |                                                     |                                                | -Cell-culture media which had been exposed to PNIPAM-coated surfaces for 24 h were not classified as cytotoxic until cells had been exposed to the extract for 48 h. |
|                    |                                                     |                                                | -Unbound commercially-available PNIPAM caused cytotoxicity at concentrations above 5 mg mL⁻¹ in BAEcs, whereas a synthetic PNIPAM did not. The authors attribute this to NIPAM impurity in the commercial sample. |
| Nguyen et al.[154] | A commercially-available PNIPAM, explored in a previous study[159] | BAECs, Fibroblasts (MC3T3-E1, 3T3s), and Vero cells (CCL-81) | -Commercially prepared PNIPAM is cytotoxic, which the authors attribute to residual NIPAM monomer, detectable by NMR. |
| Deptula et al.[155] | PNIPAM nanoparticles (hydrodynamic diameter: 61–94 nm) | HeLa, human embryonic kidney cells (HeK293) | -No cytotoxicity determined by MTT assay                                      |
| Vihola et al.[156] | PNIPAM (156 kDa, PDI/1.4)                          | Human epithelial colorectal adenocarcinoma cells (caco-2) | -PNIPAM does not reduce metabolic activity (MTT) or lead to membrane leakage (LDH) after 3 h exposure at concentrations up to 10 mg mL⁻¹ at 23 °C. |
|                    |                                                     |                                                | -At 3 h incubation, PNIPAM induces a small reduction in mitochondrial activity at 37 °C relative to cells at 23 °C, but activity remains greater than control cells. |
|                    |                                                     |                                                | -After 12 h incubation in 5 mg mL⁻¹ PNIPAM at 37 °C, caco-2 cells showed a ~50% reduction in mitochondrial activity. No LDH test results reported. |
|                    |                                                     |                                                | -Cytotoxicity profiles similar between poly(N-vinyl caprolactam) and PNIPAM at 37 °C |

There are several studies on the safety of PNIPAM outside of simple cell culture described in the literature.[158–162]

Inflammation potential may be determined by COX-2 expression using ex vivo tissue. Samah et al.[158] report an inflammation assay conducted on porcine ear skin to investigate COX-2 expression following exposure to NIPAM and PNIPAM. PNIPAM and NIPAM monomer did not appear to elicit a statistically significant pro-inflammatory effect using this model with an in-tact stratum corneum, an important barrier to protect the underlying tissue from xenobiotics which is not present in simple 2D culture.

The oral toxicity profile of PNIPAM has been reported in mice,[159] dosing at 2 g kg⁻¹ for a 28 days long subacute study. The PNIPAM was synthesized by the group, with a Mn of 4.5 kDa and a PDI of 12.3. No significant changes in clinical signs, weight, food consumption, hematology, clinical chemistry, or absolute organ weight were seen. Histological examination of the major organs showed no marked differences between a saline control and the PNIPAM-dosed mice. No cumulative toxicity was noted.

The toxicity of PNIPAM has also been investigated following intravitreal administration to rabbits.[160] The PNIPAM was synthesized in-house and purified by precipitation. No characterization information was reported. Histological and electoretinography studies of eyes injected with 0.1 mL of 50% PNIPAM in saline demonstrated absence of retinal toxicity. In a separate study, rabbits were implanted with PNIPAM at the retina. No toxicity was reported, though the sample size was small (n = 3).[161]

Blood compatibility of PNIPAM has also been studied.[162,163] Whilst in vitro experimentation did not reveal any effect on blood coagulation, animal studies led to the identification of anti-coagulant activity which may lead to side-effects such as excess bleeding if PNIPAM is administered into the system. PNIPAM also lead to the dose-dependent development of
protrusions in red blood cells, which the authors attribute to interaction with the cell membrane (Figure 11). However, levels of haemolysis induced by the polymer remained at <1%.[163]

Almost 15 years ago in their seminal paper on oligo(ethylene oxide) methacrylates Lutz and co-workers asked “is the age of PNIPAM over?”[28]—it appears the answer is “not yet”. A plethora of alternative thermoresponsive polymers continues to emerge, but PNIPAM persists due to its sharp LCST, the independence of LCST on many environmental factors, and ease of polymerization. Despite a possibly undeserved reputation for cytotoxicity, it is clear that there are numerous studies that support the biocompatibility of PNIPAM. However, the evaluation of PNIPAM in standardized toxicity testing programmes recognized by regulators is a crucial step in the entry of PNIPAM materials into medicine. Furthermore, consideration of distribution, metabolism, and elimination in vivo will be of importance for parenteral applications in particular, where the ultimate fate of the systems must be known where the polymer backbone is not intrinsically susceptible to hydrolysis.

4.3. Poly(N-vinyl caprolactam)-Based Thermoreversible Gels

Poly(N-vinyl caprolactam) (PNVCl) is a potential alternative to PNIPAM, with an LCST in water between 30 and 32 °C—promising for biomedical applications.[27] The addition of small quantities of ethanol and isopropanol to water has been shown to lower the LCST of PNVCL to ≈29 and 17 °C whilst n-propanol and t-butanol decreases the LCST further to ≈10–12 °C.[27] In addition to this, PNVCl is a component of Soluplus, a pharmaceutical excipient, and as such has reduced risk for translation where the manufacturer has conducted substantial toxicity testing programmes.[49,164,165,166] PNVCL exhibits type I demixing, where the LCST is dependent on polymer molecular weight and decreases as molecular weight is increased.[167] Its LCST in water may be increased by the presence of ionic liquids, which are hypothesized to act as a bridge between PNVCL and water, improving solvation.[168] A drawback of this polymer arises due to difficulty experienced when attempting to polymerize the N-vinyl caprolactam monomer in a controlled manner.[169] This is as a consequence of the poor radical stability formed by the monomer during polymerization, leading to N-vinylcaprolactam being considered a “less activated monomer”.[170]

Triblock copolymers of PNVCl-b-PEG-b-PNVCl have been reported with the effect of block molecular weight on the gelation properties studied.[171] For a 20% w/v triblock copolymer with a PEG molecular weight of 4 kDa, increasing the molecular weight of PNVCl block from 3.6 to 22.5 kDa decreased the gelation temperature from 47 to 37 °C while simultaneously increasing the gel strength from 1 to 1000 kPa (at 1 rad s⁻¹). Increasing the PEG molecular weight from 2 to 10 kDa increased the gelation temperature from 38 to 41 °C without affecting the strength of the gel (Figure 12). The polymers produced in this work were polydisperses (PDI up to 3.55), demonstrating GPC traces with substantial shoulders, and the
rheograms often show two transitions. Thus, it is possible that this data are confounded by multiple species in solution.

Soluplus is a pharmaceutical grade excipient which is a copolymer of PNVCi-poly(vinyl acetate)-PEG. The thermogelling properties of Soluplus in water, ethanol, and water, a range of phosphate buffered saline solutions and sodium chloride solutions have been studied.\cite{145} The Soluplus solutions with concentrations greater than 20% were found to form gels with $G'$ maxima greater than $\approx$500 Pa. Increasing the concentration of Soluplus from 20 to 40% resulted in a decrease in gelation temperature from 40 to 37.5 °C. Preparing the same Soluplus formulations in 25% ethanol:water mixtures led to a decrease in $G'$ maxima to $\approx$10 Pa and prevented the formation of a gel ($G' > G''$). This finding demonstrates that the presence of cosolvents, often required in topical medicines, may adversely affect the ability of thermoreversible gelators to function. The gelation properties were unaffected by changes in pH from 7 to 4 but the gelation temperature was found to be decreased to 28.9 from 40 °C upon dissolution in 1 M NaCl. Sodium chloride is a member of the Hofmeister series and can induce a “salting out” effect upon the polymer, which presents itself as a reduction in gelation temperature.\cite{145} Soluplus is an excipient in oral “Febuxostat Zentiva”, which received a marketing authorization by the European Medicines Agency in 2018. This prior history of use in humans de-risks the excipient for future use for other routes of administration.\cite{172} The rheology of Soluplus solutions demonstrates a balance of viscous and elastic behavior, behaving as a Maxwell material at low frequencies, exhibiting behavior more analogous to a viscous polymer solution than to a highly elastic gel.\cite{173,174} Future research efforts should seek to enhance the functionality of Soluplus as a thermoreversible gelator by improving this rheological profile, informed by the successful strategies employed for poloxamer 407.\cite{17}

4.4. Poly(2-((N-dimethylamino) ethyl methacrylate), Poly(oligoethylene glycol (meth)acrylates), and Related Poly((meth)acrylate)-Based Thermoreversible Gels

PDMAEMA responds to both pH and temperature.\cite{175} PDMAEMA exhibits an LCST in aqueous solution which is highly dependent on molecular weight, concentration, salt, and pH.\cite{26,176} The polymer is weakly basic with apparent pK$_{a}$H reported to be in the range 6.2–8.0, dependent upon polymer architecture.\cite{135,177,178} The LCST is linked to the degree of ionization of the polymer with a 1 mg mL$^{-1}$ solution of a PDMAEMA 108-mer having a $T_{CP}$ of 79 °C at pH 7, which is depressed to 39 °C at pH 10. In another example, pH alters the LCST of PDMAEMA (DP:100) from $\approx$35 to 47 when reducing pH from 10 to 7, with the polymer no longer exhibiting an LCST at pH 4.\cite{179} Thus, the temperature at which gelation occurs for PDMAEMA-containing thermoreversible gels may be tuned to a required temperature, however, this sensitivity provides a challenge where factors such as polydispersity, the presence of physiological salts and dilution will affect $T_{gel}$. PDMAEMA is a component of Eudragit E100, a pharmaceutical grade excipient found on the FDA inactive ingredients database\cite{180} which has been used in oral drug delivery.\cite{181} PDMAEMA may also exhibit UCST behavior in the presence of trivalent metal hexacyano anions which electrostatically interact with the tertiary amine moiety.\cite{182}

Thermoresponsive gelators containing PDMAEMA have been widely studied by Georgiou and co-workers.\cite{50,109,178,183} Six PDMAEMA—poly(ethylene glycol methacrylate) (PEGA)—butyl methacrylate (BuMA) terpolymer architectures were reported, as well as a PEGMA$_{9}$-PDMAEMA$_{42}$ di-block copolymer. The rheology of the copolymers was highly dependent on architecture, with the increased quantity of the hydrophobic BuMA promoting gel formation, and statistical and diblock copolymers being poor gel-formers.\cite{109} This study was expanded to a further 12 terpolymers, which reinforced a strong dependence of thermoreversible gelation upon BuMA content, and revealed a further dependence upon molecular weight, requiring optimization of both for this behavior to be seen.\cite{110} The effect of hydrophobic component, ethyl-, butyl-, and hexyl-methacrylate (EtMA, BuMA, and HMA), on an ABC, BAC, or ACB terpolymer architecture was then evaluated.\cite{184} It was found that an increased length of alkyl group in the hydrophobic monomer reduced $T_{CP}$. The thermoreversible gelators formed gels of $<100$ Pa with $T_{gel} > 45$ °C. The authors also suggest that a PEGMA-b-(EtMA/BuMA/HMA)-b-PDMAEMA architecture gives the sharpest $T_{gel}$. The length of PEG has also been varied in PEGMA-b-PBuMA-b-PDMAEMA terpolymers,
studying methoxy di-, penta-, and nona (ethylene glycol) methacrylate as PEGMAs.[185] The study found that PEG length was proportional to $T_{CP}$ and that methoxy (diethylene glycol) methacrylate copolymers had $T_{gel}$ at a physiologically relevant temperature of $\approx 35 \, ^\circ C$. Interestingly, the above studies discuss mechanistic details from the paradigm that PEGMA exists as a non-ionic hydrophilic monomer without thermoresponse, where LCST transitions arise from PDMAEMA. It is known that the PEGMAs studied do exhibit LCST transitions between $\approx 25$ and $70 \, ^\circ C,[186]$ which may also contribute to temperature-dependent solution behavior observed. The group also studied BuMA-PDMAEMA-BuMA triblock copolymers, investigating the effect of symmetry and asymmetry.[178] The study found once again that block copolymers are more likely to exhibit thermoresponsive gelation than statistical copolymers. Additionally, the authors found that symmetrical macromolecules were more effective gelators than asymmetric ones. This was rationalized through the Semenov theory of associating polymers, where symmetrical copolymers were hypothesized to form more effective bridges, but the precise mechanisms of gelation were not specifically studied. Tetrablock copolymers of PEGMA ($M_n \approx 300 \, \text{g mol}^{-1}$, A block), n-butyl methacrylate (BuMA, B block), and DMAEMA (C block) have also been explored by the group, but only two specific architectures were identified as injectable gelators. These in situ gel-forming materials had the composition BABC and ACBC with monomer percentages of 20–35–45 w/w % and 20–30–45 w/w % (PEGMA–BuMA–DMAEMA), respectively.[90] The studies above typically have the perspective that these block copolymers form spherical micelles, and attempts are made to rationalize behavior based on this paradigm. Future work probing whether the materials conform to this hypothesis will be useful in supporting this model.

POEG(ME)(M)As have gained interest in the development of thermoresponsive gels due to their range of LCSTs in water and the relative environmental insensitivity of this transition.[28] POEG(M)As have LCSTs ranging from 17 to 90 °C, which is dependent on the length of the OEG chain on the monomer structure (Figure 13).[187,188] This increase in LCST is as a result of the increased hydrophilicity when increasing length of PEG.[188] POEG(M)As are also advantageous as copolymerization of mixed-length POEG(M)As allows further tuning of transition temperatures.[186] The LCSTs of these three polymers are known to be independent of both molecular weight and concentration.[189,190] The POEG(M)As are also believed to be biocompatible, but, to our knowledge, are not currently used as pharmaceutical excipients.[188] UCST behavior is observed in these materials when dissolved in a wide range of alcohols.[191] A di-block copolymer of poly-(methoxytri(ethylene glycol) acrylate-co-acrylic acid)-b-poly-(ethoxydi(ethylene glycol) acrylate) (P(TEGMA-co-AA)-b-PDEGEA) has been demonstrated to form thermoreversible gels when dissolved in water. A 25% w/v aqueous solution formed gels with control of $T_{gel}$ using pH. An increase from pH 3.2 to 5.8 elevated $T_{gel}$ from 17 to 30 °C and decreased the maximum value of $G'$ from 2.9 to 2.1 kPa (at 1 Hz).[192] This process was linked to an increased degree of ionization of the carboxylic groups which elevated the CMT determined by DLS, accounting for increased $T_{gel}$ and which led to inter-micelle repulsion detrimental to $G'$. Whilst control of LCST with pH may be considered positive in tuning properties, the inter- and intra-subject variability of in vivo pH for some sites, such as the vagina or GI tract, may lead to this being a limitation in those applications. DLS and SAXS supported a gel structure consisting of micelles in a body-centered cubic arrangement. Zhao and co-workers have studied dual pH- and temperature-responsive poly(ethoxydi(ethylene glycol) acrylate-co-acrylic acid)-b-poly(ethylene oxide)-b-poly(ethoxydi(ethylene glycol) acrylate-co-acrylic acid) (P(DEGEA-co-AA)-b-PEO-b-P(DEGEA-co-AA)) and poly(methoxydi(ethylene glycol) methacrylate-co-methacryl acid)-b-PEO-b-P(DEGMMA-co-MAA) as gelators which allow for tuning of gel properties with pH.[193,194] Elevated pH reduced gel
strength in P(DEGEA-co-AA)-b-PEO-b-P(DEGEA-co-AA) solutions, however rather than attributing this effect directly to micelle repulsion (due to increased ionization), the authors predict that there are a reduced number of unimers capable of bridge formation as pH is increased in telechelic system above the LCST. The number of active bridging units was calculated from the plateau in $G''(G'_N)$ taken from frequency sweeps using the relation $G'_N = \nu k_B T$, where $\nu$ is the number of elastically active bridging chains per unit volume, $k_B$ is the Boltzmann constant, and $T$ is the absolute temperature. It was found that increasing from pH 3.0 to pH 5.5 lead to a reduction from $\approx 95$% to $20$% of the polymer chains being active in the bridging.

(P(DEGMMA-co-MAA)-b-PEO-b-P(DEGMMA-co-MAA) demonstrated thermoreversible sol–gel transition, which could be reversed by changes in pH.[194] When pH was 3.3 $T_{gel}$ had a value of $\approx 39$ °C, which was greatly elevated to 47 °C when pH was raised to 5.4. The authors attribute this to the increased hydrophilicity of the MAA as pH approaches pKa (5.59), thereby increasing ionization degree of the MAA units.

ABA tri-block copolymers of PDEGEEA-co-POEGMA (A) and PEG (B), where the POEGMA monomer contained 9 PEG repeat units have been reported by Negru et al.[196] The study found that increasing the PDEGEEA-co-POEGMA DP from 50 to 200, while maintaining 5 mol% POEGMA, decreased the gelation temperature from 42 to 27 °C, while the gel strength remained constant at $\approx 100$ Pa. In addition to this, increasing the percent of POEGMA from 0% to 10% increased the gelation temperature from 19 to 56 °C without altering the strength of the gel. Another key finding from this study was the influence of the PEG central block which, when increased from 4 to 10 kDa, reduced $T_{gel}$ from 38 to 28 °C, while increasing the gel strength from $\approx 10$ to 1000. Whilst the reduction in $T_{gel}$ with putative hydrophilicity of the copolymers is counterintuitive, it is possible that the increased length of the PEG chains favors gel formation.

Thermoresponsive PEOGA-containing star-shaped block copolymer with a central PEG block and arms composed of random copolymers of PDEGMEMA and POEGMA (dp 8) have been explored by the Lutz group.[197,198] This star-shaped block copolymer formed a gel with a viscosity of $\approx 40$ Pa at 38 °C in 25% w/v aqueous solution. The study found that the gelation temperature fell by $\approx 5$ °C when using phosphate-buffered saline as a solvent, which is as a result of a salting out effect, but the viscosity was not compromised.

Poly(penta(ethylene glycol) methyl ether methacrylate) (PENTEGMA)-b-poly(BuMA)-b-poly(2-(diethylamino)ethyl methacrylate) (DEAEMA) has been explored as a thermoresversible gelator.[199] Monomer ratios affected the ability to form gels.
Increasing BuMA and (2-(diethylamino)ethyl methacrylate) content promoted gel formation, with $T_{gel} > 48^\circ$C. Gels were stable over a relatively small temperature range, and future research on these systems could seek to optimize and extend this plateau so that minor changes in environment do not lead the systems to undergo a gel–sol transition.

Saunders and co-workers have explored the stabilization of poly(caprolactone) (PCL) microspheres with a copolymer based on P(DEGMEMA)-grafted to poly(2-dimethylamino)ethyl methacrylate) methyl iodide quaternary salt (PMA).[200] Transition to a colloid gel state was triggered by the LCST transition of PDEGMEMA, leading to a mixed gel formed of PCL–PCL and PMA–PMA networks connected by PCL–PMA contacts. Rheology of the colloid gel could be tailored depending on the phase volume of the thermoresponsive PMA component. Below a critical value ($\Phi_{PMA} = 0.057$) the colloid gel was dominated by PCL–PCL contact, having relatively high values of $G'$, which were proportional to the phase volume of PCL, and a brittle character, exhibiting low yield stresses. Above this critical value the rheology of the gel was dominated by the PMA phase and became more ductile, exhibiting an increase in yield stress. This two-network model was proposed to explain rheological behavior in similar studies on thermoreversible colloid gels. The consideration of multiple components giving complex

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**Figure 15.** Phase diagrams showing temperature-dependent solution properties of PENTECMA-b-PBuMA-b-PDEAEMA (represented as blue, red, and yellow spheres, respectively). Approximate ratios of each monomer shown above phase diagrams, with full details available in the original publication.[199] Reproduced with permission.[199] Copyright 2020, Elsevier.
rheological features is underexploited in thermoreversible gelators. It would be of interest to exploit double-network phenomena from mixtures of polymers chemically cross-linked in situ with thermoreversible gelators. This could generate highly functional materials with a combination of rigidity and ductile behavior which may be exploited in applications under high payload, such as joints.\textsuperscript{[203]}

4.5. Poly(2-oxazolines) in Thermoreversible Gelators

Poly(2-oxazolines) may be accessed from 2-oxazolines via cationic ring-opening polymerization to give polymers that have a tertiary amide where the nitrogen is incorporated in the polymer backbone.\textsuperscript{[202]} LCST behavior may be observed in water for these systems when the poly(2-oxazoline) possesses small hydrophobic side chains.\textsuperscript{[202]} For example, poly(2-ethyl-2-oxazoline) (EtOx) and poly(2-n-propyl-2-oxazoline) (nPrOx) exhibit LCSTs between 60–100 °C and 20–40 °C dependent upon molar mass.\textsuperscript{[203]} Many of these poly(2-oxazolines) conversely exhibit UCST behavior in ethanol and water:ethanol mixtures.\textsuperscript{[204]} Poly(2-oxazolines) have been reported to exhibit pH sensitivity, with poly(EtOx)—grafted polyesters having depressed LCST in basic conditions.\textsuperscript{[205]} This was attributed to a reduction in solvophilicity at higher pH. This effect should be considered in future applications of poly(2-oxazolines), however this report does not control overall ionic strength of the solutions which may confound effects. LCST may be controlled by copolymerization, for example, Park and Kataoka demonstrated that the LCST of poly(nPrOx-ran-EtOx) may be controlled between 24 and 75 °C dependent upon monomer ratio.\textsuperscript{[206]} Di-block copolymers of 2-isopropyl-2-oxazoline (iPrOx) and EtOx and have also been reported, where it was found that the two thermoresponsive components exhibited distinct LCSTs.\textsuperscript{[207]} Heating above the LCST of poly(iPrOx) triggered an amphiphilic state inducing formation of star micelles, subsequent further heating above the LCST of EtOx lead to phase separation. The first thermoresponsive gelator composed of poly(2-oxazolines) was recently reported by Monnery and Hoogenboom.\textsuperscript{[203]} ABA copolymers nPrOx\textsubscript{100}-EtOx\textsubscript{700}-nPrOx\textsubscript{100} and nPrOx\textsubscript{100}-EtOx\textsubscript{500}-nPrOx\textsubscript{500} were found to exhibit thermoresponsive gelation at 20% w/v in water. The materials exhibited T\textsubscript{g} values of 35 and 43 °C, respectively, with the former material allowing in situ gelation of medicines. nPrOx\textsubscript{1000}-EtOx\textsubscript{7000}-nPrOx\textsubscript{1000} exhibited unusual responses upon repeated heating-cooling cycles, where an initial “up” ramp from 25 to 50 °C resulted in sol-gel transition, but the “down” ramp from 50 to 25 °C simply lead to an increase in viscosity. A second cycle increased viscosity further up to an unusually tough 600 kPa. The authors rationalize this process using a paradigm derived from the Semenov mechanism discussed previously.\textsuperscript{[225]} The repeated cycling under stress was believed to promote loop formation between flower-like polymer micelles, consolidating elasticity within the system. Further exploration of this phenomenon would be of interest in developing tough thermoreversible gels, but will have the limitation that removal of the material from the body may be difficult and not achievable through a needle, for instance. “Poloxamer-like” ABA poly(2-oxazolines) have also been reported, termed as such due to hydrophilic A blocks and relatively hydrophobic B blocks. An ABA triblock copolymer based on a hydrophilic poly(2-methyl-2-oxazoline) (MeOx) block (A) and a relatively hydrophobic nPrOx block (B) exhibits thermally-induced increases in viscosity, however these materials were predominantly dissipating energy with G'' > G'.\textsuperscript{[208]} It was later reported that minor alteration to this structure, using poly(2-iso-butyl-2-oxazoline) instead of nPrOx gave turbid thermoreversible gelators (G’ > G’’) with G’ up to 600 Pa at 37 °C.\textsuperscript{[209]} The hydrogels formed were highly shear-thinning but recoverable. Furthermore, cell culture studies indicated low cytotoxicity. Poly(MeOx)poly(2-n-propyl-2-oxazine) has recently been reported as a thermoreversible gelator for 3D printing.\textsuperscript{[210]} Laponite clays were used to improve the rheology of these materials to allow the printing of high-resolution structures with good shape fidelity. The mixing of additives to modify rheology of thermoreversible gelators is underexploited outside of poloxamer 407 and is a promising way of enhancing these systems without the need for additional synthetic steps.

5. Applications of Thermoreversible Gels

Thermoreversible gels have been explored for a variety of uses such as depots for tissue regeneration and drug delivery,\textsuperscript{[211,212]} drug delivery to topical sites,\textsuperscript{[213,214]} and 3D printing.\textsuperscript{[215–217]} Cells encapsulated in thermoreversible gels may be administered parenterally to induce in situ tissue regeneration or cell delivery.\textsuperscript{[218]} Thermoreversible gels are attractive in this instance where they may be administered through a needle, rather than a more invasive procedure to implant a hydrogel which is already cross-linked.\textsuperscript{[219]} These gels require long residence times to allow for cell proliferation and tissue growth.\textsuperscript{[220]} Injected poloxamer gels are known to remain at the injected site for \approx 24 h which is too short for tissue regeneration or long term drug delivery, where residence may ideally be required for months or even years to allow tissue maturation, which is currently achieved primarily with chemically cross-linked hydrogels.\textsuperscript{[219,221]} Some LCST-exhibiting in situ gelators for tissue regeneration and drug delivery are covalently cross-linked polymers.\textsuperscript{[222]} These polymers are believed to offer greater retention times due to their cross-linked nature but may be difficult to inject due to their greater viscosity even at lower temperatures when compared to non-covalently bonded polymers.\textsuperscript{[223]} The biodegradation pathways of these materials would also need to be investigated prior to use as injectables. A series of papers by the Matsuda group discuss the preparation of a series of PNIPAM-grafted gelatin copolymers and their tissue regeneration properties investigated.\textsuperscript{[224–226]} This group found that cells cultured in the PNIPAM-gelatin gel were able to form smooth muscle tissue after 14 days at 37 °C.\textsuperscript{[225,226]} PNIPAM-gelatin has also been explored as an injectable scaffold for bone meniscal stem cells, regenerating bone in vivo over a 12 week study.\textsuperscript{[227]} Colloid gels have also been investigated for cell delivery/culture where thermoresponsive copolymers are able to induce gelation of microparticles upon which cells could be cultured, allowing for an injectable delivery system for cell therapies.\textsuperscript{[228]} Magnetic-response has been added to allow for rapid extraction of cultured cells after culture in the colloid gel (Figure 16).\textsuperscript{[229]} Cell culture, rather than tissue engineering
Figure 16. a) Human mesenchymal stem cells (hMSCs) may be seeded onto Fe₃O₄-doped polystyrene microparticles stabilized with a PDEGMEMA surfactant. A colloid gel may be formed by warming to 37 °C and cells cultured. After culture, b) the colloid and cells may be separated by passing the cooled culture mixture past a magnet, c) leading to high recovery of cells. Reproduced under the terms of the CC BY 4.0 license.[229] Copyright 2015, Wiley-VCH.

or cell delivery, is an attractive application for these materials where regulatory hurdles are significantly reduced.

The release of therapeutics from parenteral thermoreversible drug delivery systems has been investigated to a greater extent than the topical route. The release of relatively lipophilic molecules from novel thermogelling block copolymers has been reported. These include paclitaxel,[230] methylene blue,[125] and rhodamineB[130] which exhibit LogPs of 3.96,[233] 0.75,[232] and 1.95,[233] respectively. The release of paclitaxel from what is described as a “micelle shedding” in situ gelling formulation containing a PNIPAM-PEG-PNIPAM triblock copolymer has also been investigated.[230] It was found that this formulation approximately doubled the half-life of paclitaxel in a mouse model. The release of methylene blue from a PNIPAM-b-poly(3-O-allyl-α-D-glucose) copolymer was found to reach 80% release after 120 h.[125] This release, however, exhibits an initial burst of 50% after 20 h, and the final 20% of methylene blue was not liberated from the gel. The release of methylene blue from thermoreversible gel formulations of PtBA-b-PDMA-b-PNIPAM is dependent upon both pH and temperature.[234] Higher temperatures and lower pHs retarded release of methylene blue, where both changes also increased the viscosity of the system. Rhodamine B release from PNIPAM-b-PVP-b-PNIPAM, was found to be slowed approximately threefold by increasing temperature from 25 to 37 °C, delivering 100 and 33% of rhodamine after 18 h in PBS.[110] The reduced release at elevated temperature is suggested to be as a result of an increase in viscosity which hinders the diffusion of drug from the gel matrix. The release of bovine serum albumin (BSA), as a model protein drug, from a poly(2-(N-diethylamino)ethyl methacrylate)-co-PDEGMEMA-co-OEGMA)-b-PEG-b-poly(2-(N-diethylamino)ethyl methacrylate)-co-PDEGMEMA-co-OEGMA) thermogel has also been investigated.[235] BSA is an water-soluble protein,[236] which is commonly used in cell culture experiments, and may be used to promote tissue regeneration.[237] The release was found to be hindered upon an increase in temperature from 32 to 43 °C, where 20% release was achieved after 10 and 60 min, respectively.[235] At lower temperatures, a micellar gel is not formed, and the copolymer chains are separated in solution, allowing for a rapid BSA release. Upon an increase in temperature a micellar gel is reported to form, and this creates a tortuous path for the release of BSA, resulting in a slower release. Whilst the ability of thermoreversible gelators to flow through needles makes them attractive for parenteral administration, the application is high risk from a translational point-of-view. Parenteral administration by nature results in systemic exposure, for which the risk of severe adverse reactions is greater than via the topical route. Furthermore, the ultimate fate of the gelators must be determined. Whilst glomerular filtration is a potential elimination route for macromolecules under ≈4.2 nm diameter,[238] efforts are typically focused on designing biodegradable materials,[140] however the resultant products of this process must also be known to be safe.

Thermoreversible gelation is an appealing method of improving retention of topically-applied medicines, enhancing duration of action for local effects, and allowing a sustained period for absorption to occur. Furthermore, the topical route can avoid systemic exposure, reducing the potential for adverse effects. Chitosan grafted with PNIPAM has been explored as an in situ gel-forming ocular formulation containing timolol maleate.[239] The in vivo studies found the chitosan-g-PNIPAM copolymer delivered ≈25 μg mL⁻¹ into the aqueous humor after 10 min, while a conventional eyedrop released ≈20 μg mL⁻¹. This was attributed to the increase in viscosity of the thermogelling formulation which allows for enhanced retention. Thermoreversible gelators based on PNIPAM₉₈–PEG₁₂₂–PNIPAM₉₈ and poloxamer 407 were found to be effective solubility enhancers for progesterone, a relatively lipophilic molecule with poor water solubility.[79] PNIPAM₉₈–PEG₁₂₂–PNIPAM₉₈ offered a temperature-induced retardation in progesterone delivery over 144 h, significantly longer than a poloxamer 407 formulation (100% release over 100 h). Progesterone
delivery from PNIPAM\textsubscript{98}–PEG\textsubscript{122}–PNIPAM\textsubscript{98} and poloxamer 407 exhibited a significant lag time, which should be reduced in future iterations of the medicines. Tenofovir disoproxil fumarate, a relatively hydrophilic and water-soluble drug, release was also investigated from the two materials. Tenofovir disoproxil fumarate solubility was not improved by the presence of either poloxamer 407 or PNIPAM\textsubscript{98}–PEG\textsubscript{122}–PNIPAM\textsubscript{98}, and its release from the gels relatively rapid compared to progesterone due to it largely being excluded from the polymer micelles. Within all drug delivery applications an underexplored phenomenon is the influence of drug on the nanostructure and properties of thermoreversible gels. The presence of drug has been shown to induce changes in nanostructure, for example, inducing particle formation below the LCST\textsuperscript{[73]} or leading to growth of spherical micelles.\textsuperscript{[240]} Unusual release kinetics seen in thermoreversible gels are likely a result of alteration in nanostructure as the material is depleted of drug. Future research should seek to understand this phenomenon to guide the development of desired release kinetics, such as zero-order release.

3D printing of thermoreversible gels is attractive where the temperature-induced sol–gel transition may be used to induce solidification during the printing process without harsh conditions such as chemical cross-linkers and UV irradiation. Poloxamer 407 has previously been 3D printed and dried to produce vaginal disks which can carry paclitaxel and rapamycin for the treatment of ovarian cancer in a mouse model.\textsuperscript{[205]} These were found to be therapeutically effective and preventative of postsurgical peritoneal adhesion. Both the in situ gelling formulation and 3D printed disk were found to exhibit prolonged release, where 80% and 60% release of the loaded rapamycin and paclitaxel was achieved after 24 h, respectively. To improve the mechanical strength of printed poloxamer 407 scaffolds, terminal acrylate functionality has been introduced via a nucleophilic substitution with acryloyl chloride. This allows for post-printing cross-linking via UV irradiation.\textsuperscript{[241]} In addition to this, PNIPAM grafted hyaluronan with methacrylated hyaluronan have been 3D printed to produce scaffolds for the encapsulation and growth of chondrocytes.\textsuperscript{[242]} These cells were found to be viable and as such this polymer may be used to culture a wide range of cell types. In order to maintain the solidity of this scaffold, cross-linking by UV irradiation is required. Novel thermoreversible gels should aim to achieve this prolonged fidelity without the need for harsh cross-linking conditions which adversely affect sensitive cell types or molecules.

6. Concluding Remarks and Future Perspectives

Thermoreversible gels have untapped potential to generate novel advanced technologies for healthcare. Poloxamer 407 has been extensively investigated as a thermoreversible gel to deliver hydrophobic and hydrophilic therapeutics, or to act as a scaffold for cells. The literature has identified that the gelation properties of poloxamer 407 may be tuned by varying the polymer concentration, including additives or by preparing co-polymer solutions. These materials can achieve sustained drug release over the course of a few hours, but this is dependent on the residence time of the gel. This residence time is typically short, due to shear thinning character, weak mucoadhesion, and prompt dissolution. Furthermore, the high dependence of gelation temperature on concentration means that any uptake of water in vivo may result in a rapid gel-to-solution transition, reducing retention.\textsuperscript{[18]} The compatibility of cells with poloxamer 407 gels is also limited, with long term viability poor. Thus, novel thermoreversible gels are required to generate next-generation materials with enhanced performance.

PNIPAM remains a popular choice of thermoresponsive material due to its attractive LCST, relative environmental insensitivity, and ease of polymerization, whilst POEG(ME)(M) As are attractive alternatives for the same reasons. Both materials also typically exhibit a low dependence of LCST on molecular weight. PDMAEMA materials are attractive due to their history of use in pharmaceutical excipients, but their LCSTS are sensitive to changes in molecular weight, pH, and salt content.\textsuperscript{[76]} This may be particularly disadvantageous in drug delivery applications where physiological fluids may alter T\textsubscript{gel} and gel strengths, and the local pH and salt content must be considered during formulation. PNVCl has also been used in existing pharmaceutical excipients but suffers from a dependence of LCST on molecular weight and difficulty in polymerization, being considered a “less-activated” monomer. Poly(2-oxazoline)s are underexplored and offer opportunities to develop novel materials with potential biodegradation pathways.

Factors including architecture, block molecular weight, additives, and polymer concentration, may be used to tune the gelation properties of LCST-exhibiting thermoreversible gels. In terms of block copolymer architecture, ABA copolymers bearing LCST-exhibiting A blocks with a hydrophilic B block consistently produce viscous gels through the ability of unimers to bridge micelles, improving cohesion. Star-shaped systems are promising but underexploited. The molecular weight of each block can have a profound effect on the gelation, where increasing both blocks can lead to an increase in gel strength. However, increasing the LCST block molecular weight decreases the gelation temperature, while if the remaining block is hydrophilic, the gelation temperature increases. Thus, balancing the requirements of producing strong gels with an appropriate T\textsubscript{gel} requires careful optimization. Lastly, increasing the polymer concentration and including additives such as sodium chloride can reduce the gelation temperature without compromising the gel strength. Whilst this simple approach of using additives to modulate thermoreversible gel behavior has been well-explored for poloxamer 407, it has been underexploited in novel systems, where it can yield further benefits.

A critical limitation of the literature is that the performance of novel thermoreversible gels is rarely demonstrated to be superior to existing excipients. Of the literature reviewed, only two tri-block polymers, PNIPAM-b-PEG-b-PNIPAM and PNVCl-b-PEG-b-PNVCl, have been found to form gels which are as strong as those formed by poloxamer 407, to our knowledge.\textsuperscript{[77,243]} However, even these studies do not directly compare the thermoreversible gels rheologically, and this comparison was made between publications. The comparison is also only made at a single frequency, which does not describe the true rheological behavior. Furthermore, comparison to poloxamer 407 in pharmaceutical performance tests was not conducted. Gelation characteristics such as gelation time, resistance to shear stress, and mucoadhesion are important features to
evaluate when preparing formulations for topical drug delivery, for example.\cite{244} These features may be used to determine the possible target sites that these formulations may be appropriate for. In order to translate these materials into pharmaceutical applications, toxicity, stability and in vivo performance testing must also be completed.\cite{245} Thus, there is a need to develop novel thermoreversible gels which show beneficial properties in comparison to poloxamer 407 to justify this cost of translating new technologies.

Great advances in this field will arise from a more in-depth understanding of the fundamentals of these systems which in turn will facilitate novel applications. These fundamental advances require comprehensive mechanistic understanding of systems to allow rational design of thermoreversible gelators, and will require a battery of techniques including nanoscale characterization through small-angle scattering, spectroscopic investigation of macromolecular interactions, and computational approaches such as atomic molecular dynamics. Whilst these gelators are well-explored for drug delivery, novel applications will emerge through alignment with emerging areas such as soft-robotics and tissue engineering. To further break ground in pharmaceuticals, the rational development of high-performance gelators which are driven through to translation employing appropriate risk-mitigation strategies, following recognized toxicity testing regimen, is required. Importantly, information from failures must be shared so that the scientific community can learn, adapt, and innovate.

Acknowledgements
M.T.C. acknowledges support from the EPSRC (EP/T00813X/1). The University of Hertfordshire are thanked for funding the research project of P.H.

Conflict of Interest
The authors declare no conflict of interest.

Keywords
bioprinting, drug delivery, hydrogels, stimuli-responsive polymers, temperature-responsive polymers, thermogelling materials

Received: September 23, 2020
Revised: October 29, 2020
Published online: November 20, 2020

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