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Thermo-responsive polymers as surface active compounds: A review

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ABSTRACT

The great versatility and controllable properties that characterize polymeric materials allowed their spreading to many different areas. In the last years, this outstanding adaptability was even amplified by the introduction of smart polymers, *i.e.* materials able to sharply and often reversibly change their physico-chemical properties in response to external stimuli. In particular, the possibility of applying thermal stimuli in a controlled and simple way, coupled with the natural occurrence of thermal gradients, made thermo-responsive polymers particularly appealing, as they allowed to conceive applications that were not even imaginable for traditional materials. In this review we discuss the great potentialities of thermo-responsive polymers when used to functionalize a target surface or interface. The discussion will cover significant areas of interest where this class of materials has been employed, including cell culture, chromatography, colloidal stabilization and enhanced oil recovery. Many examples from the literature are reported in order to present the state of the art, the main advantages of this technology over conventional materials and the expected future developments. Moreover, some successful examples highlighting the innovative functionalities achievable by these active surfaces are presented.

1. Introduction

The great versatility and the unique properties achievable by polymeric materials allowed their massive diffusion in many areas, including biomedicine [1–3], oil&gas[4], coating[5], cosmetic [6], concrete industry [7], paint [8] and textile [9]. This wide affirmation of the polymer industry was mainly driven by the possibility of finely controlling the polymer molecular weight, (micro)structure and composition, allowing an *ad hoc* optimization of the material depending on the final application.

In the last decades, the advent of the so-called smart polymers, *i.e.* materials able to change their physico-chemical properties in response to an external stimulus, elevated this concept of adaptability. In fact, this peculiar feature provides smart polymers with a dynamic behavior in the local environment that can be advantageously exploited for advanced applications. In the literature, the list of stimuli that polymers can be responsive to includes pH [10], temperature [11], light [12], and redox potential [13]. Among these, the most studied and investigated are the thermo-responsive polymers. In fact, the frequent presence of thermal gradients and the relative easiness in artificially providing a controlled and reproducible thermal stimulus encouraged the interest towards this class of materials.

In particular, two different thermo-responsive behaviors are reported, namely the Lower Critical Solution Temperature (LCST) and the Upper Critical Solution Temperature (UCST), depending on whether the phase separation in a polymer rich and a polymer poor phases occurs by increasing (LCST) or decreasing (UCST) the external temperature [11,14–16] (Fig. 1).

Specifically, the LCST transition is usually referred to be an entropically driven process [17]. As a matter of fact, the energetically favorable bonds between the polymer chains and the solvent molecules are counter-balanced by the higher order in the solvent molecule organization and the consequent decrease in the mixing entropy. Increasing the temperature, the entropic term becomes predominant bringing to a positive free energy of mixing [17]. This makes polymer–polymer interactions more thermodynamically stable than polymer–solvent interactions, leading to the release of the solvent molecules in the bulk and the formation of a separate polymer-rich phase. This is the well-known behavior of poly(N-isopropylacrylamide) (PNIPAAm) [18], so far the most studied thermo-responsive polymer, with a LCST in water of 32 °C.

On the other hand, in the UCST-based systems, the phase separation is driven by the mixing enthalpy [17,19]. The cohesive polymer–polymer interactions responsible for the chain self-association are in fact destabilized above a critical temperature, leading to the formation of one single phase. These interactions can arise from the hydrogen bonding between the polymer side groups, as in poly(N-acryloyl

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glycinamide) (PNAGA) and its derivatives [20], or from coulombic forces between polymer chains combining cationic and anionic groups, or polyzwitterions, like poly(betaines) [21].

In both cases, the phase separation is accomplished by a coil-to-globule conformational transition of the polymer chains, which results in the formation of a cloudy suspension. For this reason, the temperature at which this transition occurs is commonly called cloud point (T_{cp}), which is any point on the binodal curve at which the phase separation occurs [22]. As such, the T_{cp} has a variance of 2, while the LCST and UCST, being the minimum and the maximum of the binodal curve, respectively, are univocally defined for a polymer–solvent mixture at a given pressure.

The peculiar change in the optical properties of the mixture during the phase separation is exploited to easily determine the polymer T_{cp} through turbidimetry techniques [14]. This parameter, in turn, determines the specific application the polymer is more effective for. For instance, the LCST of 32 °C for PNIPAAm makes it particularly appealing for biomedical applications [23–24].

These critical temperatures are properties of the monomer composition and functional groups present within the molecule [25–26]. For this reason, any modification to the chemical structure or incorporation of a different monomer in the polymer would result in a translation of the binodal curve in the phase diagram. Specifically, the incorporation of a solvophobic monomer during the synthesis of a LCST-type polymer would result in a lower T_{cp}, due to the more favorable polymer–polymer interactions. Vice versa, the addition of a solvophilic monomer will cause a raise in the T_{cp} due to the increased solubility of the polymer in the solvent [24,27–28]. The opposite effect is achieved in UCST polymers, where the addition of a solvophobic (or solvophilic) monomer will cause an increase (or decrease) in the T_{cp} [25–26,29–30]. The situation is different when the two monomers, *i.e.* the one providing the thermo-responsive behavior and the solvophobic or solvophilic one, are well compartmentalized in two distinct segments. In fact, it was demonstrated that in block copolymers the same T_{cp} can be maintained after the addition of a chemically different monomer in a separate segment [22]. This high control in the polymer microstructure and properties can be achieved for example by the employment of controlled radical polymerizations [31–33]. These techniques, in fact, allow for the realization of well-defined block copolymers with tunable properties and poor inter-chain compositional drift [34], essential for a sharp and controlled phase separation.

It is also worth noticing that the polymer thermal response may be influenced by environmental factors. It is the case, for example, of those polymers showing an UCST in aqueous solutions driven by electrostatic interactions between the side groups (*e.g.* zwitterionic polymers). For these polymers, the T_{cp} is strongly influenced by the ionic strength, polymer concentration or pH of the medium. For this reason, a precise control over the T_{cp} is more difficult, causing a reduced interest towards this class of materials in favor of their LCST counterparts [19,35].

Regardless of the nature of the phase separation, thermo-responsive polymers, with their interesting behavior, can be conveniently adopted to confer a dynamic response to a surface or an interface, when they are adsorbed or grafted to the target substrate. This allows to modulate the interaction of the functionalized system with the external environment in response to a thermal stimulus. A plethora of surfaces, with dimensions spacing from the nanometers to the micro- and even millimeters scale, can be provided with this smart behavior, gaining specific functionalities that cannot be even imagined with traditional surfaces. This dynamic behavior conferred by thermo-responsive polymers to the target surface found great interest and intensive investigation in different areas, from the biomedical one to the advanced separations applied in the oil and gas field.

Given the importance and the intensive research that thermo-responsive polymers as surface active agents are attracting, in this review we provide an updated and comprehensive insight into the state of the art of the production and application of functionalized thermo-responsive surfaces and interfaces. The focus is specifically on cell culture, chromatography, colloidal stabilization and enhanced oil recovery (EOR). To facilitate the reading of the text, the review is divided by application, which is also connected to a specific scale of the surfaces, from millimeters (cell culture applications) to micrometers (chromatography columns packing) down to nanometers (nanoparticles for colloidal stabilization and oil&gas applications).

2. Thermo-responsive polymers for cell culture applications

One of the most flourishing fields where thermo-responsive polymers have been applied to provide a dynamic behavior to a surface is that of cell culturing. Indeed, the possibility of using thermo-responsive polymers to favor the spontaneous detachment of anchorage-dependent cells from their *in vitro* culture without using any exogenous compound was first demonstrated at the beginning of the 90s [36–37]. It is known that cells adhere and proliferate preferentially on hydrophobic substrates, while the adhesion is prevented on hydrated surfaces. This is the idea at the basis of using thermo-responsive polymers for controlling cell adhesion and detachment from culture devices. By coating their surface

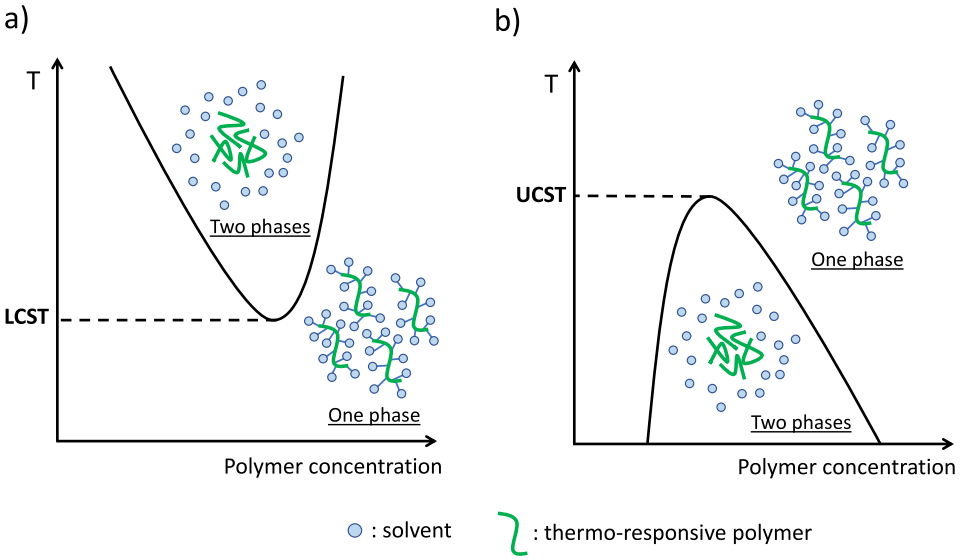


Fig. 1. Phase diagram of a binary polymer/solvent mixture in the case of LCST (a) and UCST (b) behavior.

with thermo-responsive polymers displaying a LCST below the typical cultivation temperature, *i.e.* 37 °C, it is possible to induce the cell adhesion and proliferation with traditional protocols (Fig. 2). On the other hand, by lowering the temperature below the cloud point, the incipient hydration of the surface induces the self-detachment of the grown cells (Fig. 2) [38]. The advantage of using thermo-responsive surfaces to detach the cells from the substrate lies in the preservation of membrane proteins fundamental for cell survival and cell–cell interactions. In fact, in the common routine, proteolytic enzymes (*e.g.* trypsin) are used to collect the cells, following the lysis of the membrane proteins that mediate the cell-substrate interaction. However, in this way, the protein-mediated cell–cell interactions are damaged as well, leading to the death of a significant percentage of the cells and resulting in the impossibility of growing continuous cell sheets, which were demonstrated more effective than single cell transplantation for regenerative medicine [39–42].

In the literature, two main strategies are described for the functionalization of tissue culture polystyrene (TCPS) dishes with thermo-responsive polymers, namely the "grafting to" and "grafting from" strategies (Fig. 3).

While the former can be achieved either via chemical or physical modification of the surface, the latter necessarily needs a chemical functionalization. In fact, in the "grafting to" approach, a preformed polymer is deposited to the surface thus providing the thermo-responsive features. This deposition can be performed in different ways, including electron beam irradiation [43], plasma irradiation [44], UV irradiation [45] and physical adsorption [5,34]. It is worth mentioning that these techniques (with the exception of the physical adsorption) can be used in the TCPS functionalization also through the "grafting from" strategy, consisting in the anchorage to the surface of suitable molecules enabling the initiation of the polymerization [46–47]. The latter strategy has the main advantage of allowing a more homogeneous spatial distribution of the chains on the surface compared to the former. This is due to the reduced steric hindrance experienced in fixing small molecules (*e.g.* initiator, transfer agents, etc.) to the surface instead of a preformed polymer chain. On the other hand, particular attention needs to be paid to the reaction kinetic, which can be modified by the presence of the surface. This issue is less critical in the "grafting to" approach since the polymerization is performed in controlled conditions before any contact with the surface. However, this strategy suffers from a poorer control over the functionalization step, which can lead to polymer stratification. In this situation, owing to the shielding effect of the lower layers, some polymer would be weakly bound to the surface, with the risk of desorption [48–49]. This detachment can have negative effects on the coating thickness homogeneity, with consequences on the cellular viability and on the surface thermo-responsiveness.

In fact, the coating thickness is extremely important to guarantee a correct thermo-responsive-mediated attachment-detachment process. Specifically, in the case of a low coating thickness, the mobility of the chain anchored to the surface is restricted, thus limiting the water penetration rate and preventing a correct cellular detachment. On the other hand, a thick coating could result in high chain mobility also above the T_{cp}, with consequent reduced cellular adhesion. Nagase et al. investigated this critical coating thickness as the maximum extension of the polymer in the medium allowing cell spreading and adhesion. The authors found that for TCPS, cell-adhesive conditions could be maintained with a coating up to 25 nm thick. This distance was significantly compressed on glass surfaces, for which a critical thickness of 5 nm was found. [50] It is then clear that an optimal thickness of the thermo-responsive coating must be aimed at, also with respect to the selected material, to maximize the adhesion and self-detachment of the cells. In addition, particular attention needs to be paid to the washing step, in order to eliminate any unreacted monomer or contaminant which can have a detrimental effect once the cells are seeded. [51].

Another point to consider during the design of the thermo-responsive coating for this application, independently from the strategy followed, is the proper balance between the portion deputed to anchoring the polymer to the surface and the one providing the thermo-responsive behavior. The former is required to ensure an effective surface functionalization without the risk of desorption, with the consequent contamination of the cell culture [5,49]. At the same time, the limited mobility that affects the first layer needs to be considered in the design of the material since it can have an effect on the T_{cp}, thus risking to compromise the thermo-responsive behaviour [52].

Once all these difficulties are overcome and fully functionalized surfaces are obtained, the advantages compared to the traditional cell culture techniques are meaningful and pave the way to innovative concepts such as cell sheet engineering, and regenerative medicine.

Over the years, different solutions have been proposed in the literature, showing nice examples of grafting of thermo-responsive polymers to or from TCPS surfaces for the controlled growth and detachment of different cell lines. A summary of the most relevant examples is provided in Table 1. For example, Sponchioni et al. [49], following a "grafting to" approach based on the physical adsorption of pre-synthesized polymers, were able to recover intact sheets of Chinese Hamster Ovary (CHO) cells upon cooling. The authors developed a rapid adsorption method to simplify the surface functionalization step, thus allowing the achievement of homogenous surfaces with a protocol that can be easily applied in traditional biological laboratories.

This approach can be elaborated even further, enabling the fabrication of multicell sheets, preserving the functionalities and phenotypes of the seeded cells, thus moving a step forward the structure of human tissues. In particular, Tsuda et al. [53] were able to cultivate and harvest a co-culture of hepatocytes and endothelial cells. To achieve this result, the authors exploited the possibility of reducing the T_{cp} of PNIPAAm by incorporating butyl methacrylate (BMA) in a statistical copolymer. Islands of this P(NIPAAm-*co*-BMA) copolymer were then introduced on

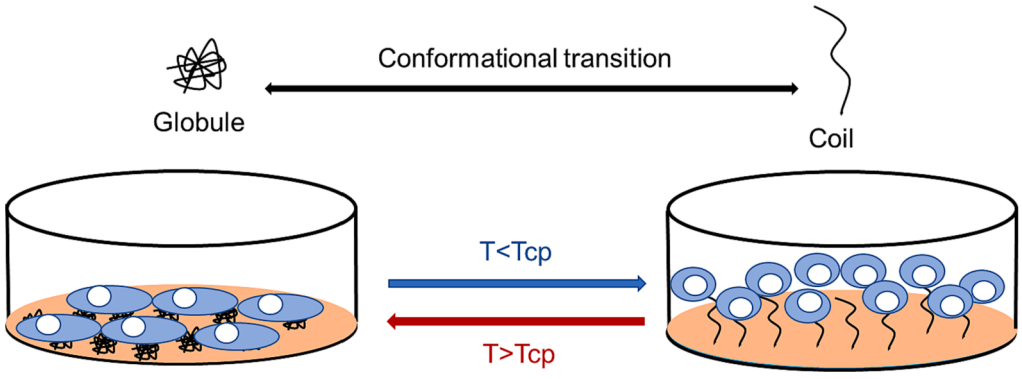


Fig. 2. Schematic representation of the mechanism that regulates the thermo-responsive cell attachment-detachment process.

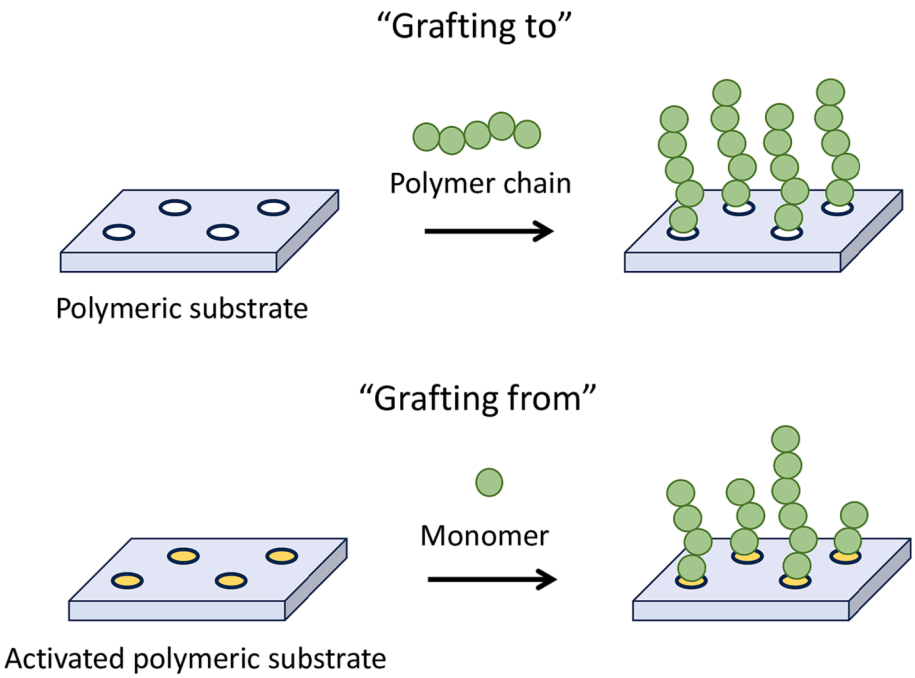


Fig. 3. Visual representation of the "grafting to" and "grafting from" techniques.

the substrate with a suitable masking tape on a layer of PNIPAAm, previously deposited on the culture surface. At temperatures higher than the T_{cp} of P(NIPAAm-*co*-BMA) and lower than the T_{cp} of PNIPAAm, such islands are lipophilic and adhesion friendly, while the surrounding PNIPAAm is hydrophilic, and hence cell-repellent. This allowed the selective adhesion of hepatocytes only on the P(NIPAAm-*co*-BMA) islands (Fig. 4). After the complete adhesion and washing, the temperature was increased above the T_{cp} of PNIPAAm, which allowed the adhesion of the endothelial cells on the empty portions of the surface. Finally, by lowering the temperature below the lowest T_{cp}, the detachment of a single sheet comprising both cellular lines was achieved.

LCST-active surfaces have been successfully implemented also in the harvesting of cells grown in a serum-free culture [49,57]. This, due to the batch-to-batch variability of the fetal bovine serum (FBS) commonly adopted in cell cultivation, its high costs and ethical concerns related to the production, is becoming a compelling topic and strategies based on serum-free formulations are being pursued. In serum-free conditions, proteins of the extracellular matrix (ECM) mediating the adhesion of cells to the TCPS surface need to be introduced separately. A nice strategy to guarantee cell adhesion without the introduction of any additional component and at the same time preserve the thermo-responsive feature of the surface is reported by Sponchioni et al. [49]. In this work, thermo-responsive polymers have been functionalized with an arginine-glycine-aspartic acid tripeptide (RGD), known to be the sequence within the fibronectin of the ECM responsible for the interaction with the integrins located on the cell membrane. The phase separation of the polymer brings the advantage of reversibly shielding and exposing this RGD sequence in response to external thermal stimuli, as shown in Fig. 5. In fact, for temperatures above the T_{cp}, the dehydrated chains collapse on the surface, exposing the hydrophilic RGD to water and favoring cell adhesion (upper part in Fig. 5). On the contrary, when the temperature is lower than the T_{cp}, the chains start to hydrate and extend to the bulk thus hiding the RGD motif, which causes the cellular sheet detachment (lower part of Fig. 5).

These superior performances achievable using thermo-responsive TCPS surfaces allowed Nishida et al. [54] to favor the reepithelization of four different corneas by detaching continuous cell sheets composed of autologous cells. Similarly, Shimizu et al. [55] were able to reconstruct continuous myocardial sheets which were implanted *in vivo*. In particular, the preservation of the cellular interactions allowed the cells to jointly respond to the external electric stimulus applied *in vitro*. Moreover, the effective capacity to maintain the electrical activity also once implanted *in vivo* was demonstrated. These great results, obtained thanks to the preservation of the cell–cell interactions, underline once more the tremendous potentiality of this technology.

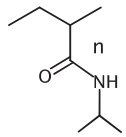
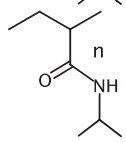
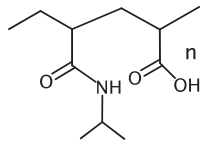
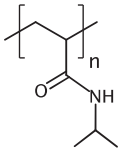
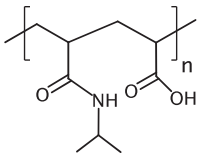
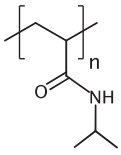
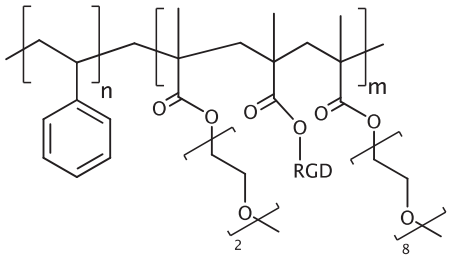
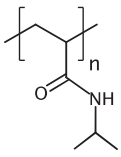
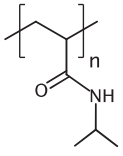
Despite LCST-type polymers are the most used in the tissue engineering field, there are few studies reporting the UCST-type polymers as surface modifiers for cell culture.

In particular, with these materials, the behavior is usually opposite to the one described for the LCST-type polymers, with the cell adhesion being favored at lower temperature. This mechanism can be exploited to obtain a controlled detachment of the cells once incubated at the culture temperature [58]. However, by properly designing these materials, it is possible to achieve the same effect as for LCST polymers (*i.e.* detachment at lower temperature). For example, Shimada et al. using poly(allylamine-*co*-allyl urea) were able to induce a monolayer-to-spheroid transition once the cells are removed from the incubator, favoring the formation of 3D structures usually obtained through the employment of non-fouling surfaces [56] (Fig. 6). In particular, the polymer was designed to phase separate at low temperature forming coacervates able to suppress the interaction between the cells and the surface of the culture dish. In this way, the cells were free to move and started to aggregate forming 3D structures in order to minimize the contact with the coacervates. On the contrary, once the TCPS was placed in the incubator, the polymer chains started to dissolve allowing the cell to anchor to the surface in a monolayer configuration.

UCST polymers, and in particular polyzwitterions, are more extensively investigated to obtain reversible non-fouling surfaces [34,59–61]. However, the sensitivity of their T_{cp} to the pH and ionic strength of the cell medium, makes a conscious design of these polymers particularly challenging [62–63]. For this reason, the UCST coatings have been less investigated than the LCST ones.

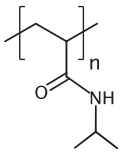
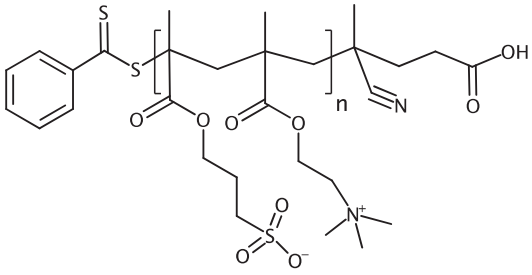
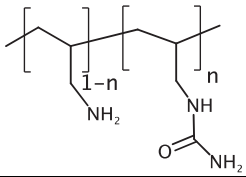
In the same field, thermo-responsive polymers are recently encountering attention in the realization of organs-on-a-chip. These *in vitro* 3D models of a living tissue or organ provide an excellent platform to study the absorption, distribution, metabolism and excretion (ADME) of a drug or the pathophysiological features of a disease [64–65]. The historical application of thermo-responsive polymers in this area was as

Table 1
List of the thermo-responsive polymers, grafting technique and cells used in the functionalization of TCPS surfaces.

Polymer	Polymer structure	Thermo-responsive behavior	Grafting technique	Cell line	Reference
PNIPAAm		LCST	Grafting from	Bovine hepatocytes	[36]
PNIPAAm		LCST	Grafting to	Fibroblasts	[37]
PNIPAAm-*co*-Acrylic Acid		LCST	Grafting to	Bovine aortic endothelial	[43]
PNIPAAm		LCST	Grafting from	Endothelial	[46]
PNIPAAm-*co*-Acrylic Acid		LCST	Grafting to	Mouse fibroblasts	[45]
PNIPAAm		LCST	Grafting from	Bovine endothelial	[48]
Poly(ethylene glycol methyl ether methacrylate-*co*-styrene)		LCST	Grafting to	Chinese hamster ovary	[49]
PNIPAAm & P (NIPAAm-BMA)		LCST	Grafting from	Rat primary hepatocytes-*co-cultured*-Bovine carotid artery endothelial	[53]
PNIPAAm		LCST	Grafting to	Autologous Oral Mucosal Epithelium	[54]

(*continued on next page*)

Table 1 (*continued*)

Polymer	Polymer structure	Thermo-responsive behavior	Grafting technique	Cell line	Reference
PNIPAAm		LCST	Grafting to	Cardiomyocyte	[55]
Poly(SPMAK-*co*-MADQUAT)		UCST	Grafting to	A375-P	[34]
Poly(allylamine-*co*-allyl urea)		UCST	Grafting to	NIH-3 T3	[56]

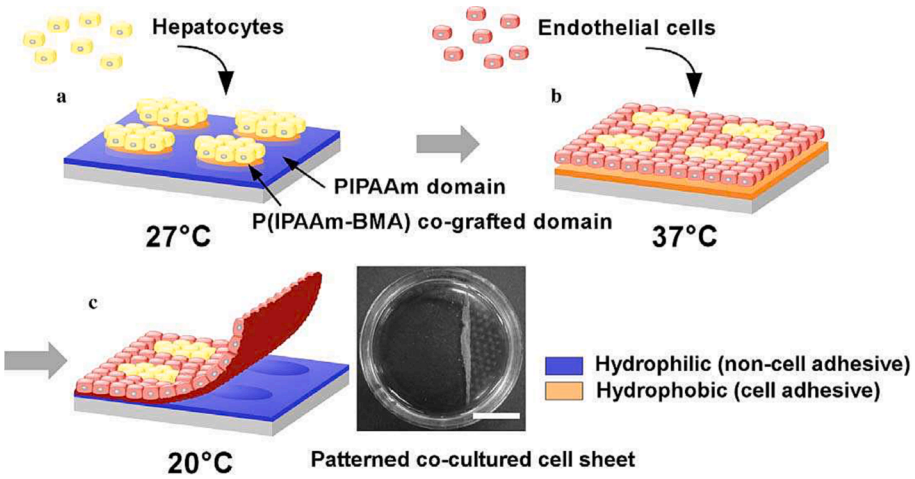


Fig. 4. Co-culture of hepatocytes and endothelial cells by means of two thermo-responsive polymers. Reprinted with permission from [53] Tsuda et al. Copyright © 2006 Elsevier Inc.

microfluidic actuators. In fact, the capability of smart hydrogels to change their swelling, and hence volume, in response to temperature changes can be advantageously exploited to realize valves and flow controllers responding to temperature stimuli. [66–70].

More recently, the phase separation of thermo-responsive polymers was exploited in the realization of sacrificial templates, allowing to include specific geometrical features in the microfluidic chip. As an example, Wan et al. adopted a thermo-responsive polymer to generate a microfluidic channel embedded in myoblast C2C12 cells. The dissolution of the polymer following an increase in the temperature allowed to seed endothelial cells in the emptied channel, thus simulating the vascularization and providing a model of the skeletal muscle-on-a-chip that can be potentially grown for longer time compared to the traditional systems. [71] This approach of using sacrificial thermo-responsive templates is indeed paving the way to the so-called 4D bio-printing, an additive manufacturing process that, in addition to the precise control of the biological geometry (3D), includes the responsivity to temperature as the 4th dimension. [72–74] Thanks to its precise control over the construct features and geometry, this bio fabrication approach has the potential to revolutionize not only the drug screening and development stages, but also tissue engineering, providing patient-specific tissues and organs grown *in vitro*.

3. Thermo-responsive polymers in chromatography

The possibility of using thermo-responsive polymers to functionalize

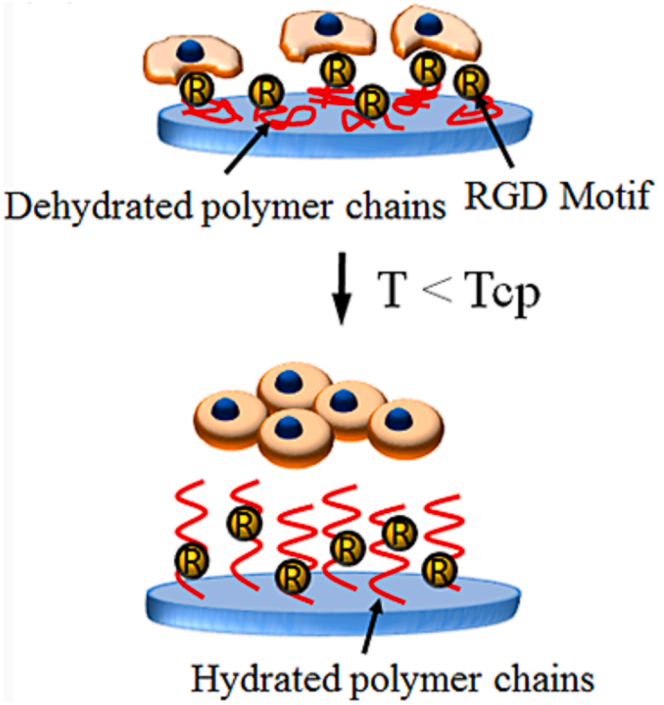


Fig. 5. Thermo-responsive cell detachment in serum-free cell cultures. Reprinted with permission from [49]. Copyright 2020 American Chemical Society.

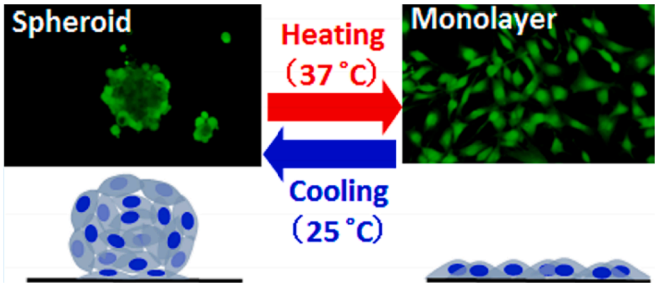


Fig. 6. Temperature-mediated spheroid formation. Reprinted with permission from [56]. Copyright 2016 American Chemical Society.

the stationary phase used in chromatographic separations was first reported by Gewehr et al. at the beginning of the 90s [75]. In this work, functionalized PNIPAAm was covalently attached to porous glass beads and used to separate dextran of different molecular weight *via* thermo-responsive gel permeation chromatography. The authors demonstrated that the elution time of dextran was a function of the conformational state of the polymer chains decorating the stationary phase, with longer elution times once the temperature was raised above the T_{cp}. Indeed, the application of a temperature gradient is a valuable strategy to increase the resolution of thermo-responsive chromatographic systems, thus enabling the separation of molecularly similar species without the necessity of chemical compounds (*e.g.* salts, acids, bases and solvents) traditionally adopted to access these separations.

Kanazawa et al. [76] demonstrated this concept in high-performance liquid chromatography (HPLC), reporting the possibility of separating molecules via reversed-phase chromatography (RPC) avoiding the presence of any organic solvent. In fact, RPC is commonly exploited in the biopharmaceutical industry to separate biologically active molecules. However, in order to improve the efficiency of the separation and reduce the elution time, a gradient of an organic modifier is required, with potentially detrimental effects on the target molecules. On the contrary, functionalizing the stationary phase with PNIPAAm allowed the authors to achieve good separation of hydrophobic steroids by simply changing the external temperature. In fact, an increase in the temperature above the T_{cp} causes a collapse of the polymer chains and in turn a better interaction between the hydrophobic compound and the stationary phase. On the other hand, a reduction in the external temperature favors the conformational transition of the PNIPAAm chains to extended coils, which triggers the molecule desorption from the resin [76] (Fig. 7). This strategy has been extensively investigated for bio-separations in the last years and has been reviewed in detail by Nagase et al. [47].

In all these applications, the efficacy of the separation is directly related to the ability of the thermo-responsive material to sharply and promptly change its conformational state following the temperature variation. This possibility is dependent on both the polymer chemical composition and coating properties, like density and thickness. In particular, the polymer composition allows the tuning of the T_{cp} as well as the sharpness of the phase separation, in analogy to what has been described in **Section 2** for the cell-culture applications. On the other hand, the grafting density and thickness are of paramount importance in guarantying a good temperature-dependent separation [47,77]. In fact, a dense coating results in a limited molecular adhesion even above the T_{cp}. This effect is usually attributed to a limited chain mobility and poor coil-to-globule transition that prevents the correct polymer dehydration, limiting the access to the functional sites present on the stationary phase. On the contrary, a low grafting density could result in an incomplete and inhomogeneous coating of the stationary phase, which loses the thermo-responsive feature, thus leading to a broad elution peak. A similar effect is obtained by changing the thickness of the grafted polymer. With a thick coating, the surface is more hydrophilic even above the T_{cp}, leading to a poorer molecule adhesion. On the other hand, thin coatings could be unable to properly shield the stationary phase once the temperature is decreased below the T_{cp}, thus preventing molecule desorption. This situation was observed by Nagase et al. in the temperature-driven separation of steroids using PNIPAAm-grafted silica beads. For polymer coatings at low molecular weight (*i.e.* number-average molecular weight, Mn = 5600 g/mol), poor variation in the retention time was experienced at different temperatures. On the other hand, for high molecular weight (*i.e.* Mn = 42′900 g/mol), despite a shift in the retention time was properly observed above the LCST, the dispersion of the steroids in the thick polymer layer led to broad elution peaks. [78].

It results that an optimization of the material and coating strategy is extremely important in order to guarantee an effective surface functionalization.

Specifically, the grafting properties (*i.e.* thickness and density) can be modulated with the coating strategy. Again, the “grafting to” and the “grafting from” are the strategies of choice for the preparation of thermo-responsive resins. The meaning of these two terminologies is the same as described in **Section 2** for the coating of the cell-culture petri dishes.

Initially, the “grafting to” strategy was mainly followed due to its higher simplicity and improved control over the polymer properties [76]. Indeed, the possibility of producing the polymer in a preliminary stage allows a stricter control over its T_{cp} and final chain length, which influences the coating thickness. However, such an approach limits the maximum density reachable due to the steric hindrance and limited mobility of already formed chains compared to the monomer units. For these reasons, the researchers started to fix the initiator molecules on the stationary phase and perform the polymerization reaction in situ (“grafting from” strategy) [79]. In this way, the control over the grafting density was improved at the expense of the thickness. This intrinsic trade-off between density and thickness was alleviated by the introduction of controlled radical polymerizations, such as the atom transfer radical polymerization (ATRP). This technique allowed to exploit the “grafting from” techniques without losing control over the coating

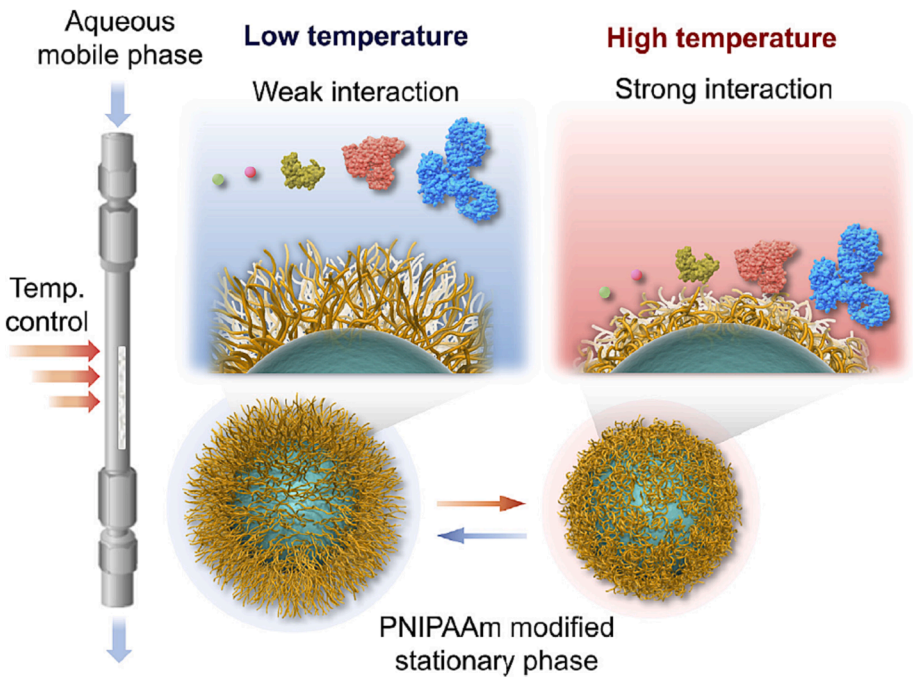


Fig. 7. Thermo-responsive separation achieved using a thermo-responsive stationary phase. Reprinted with permission from[47] Nagase et al. Copyright 2020 Elsevier B.V.

thickness [78]. In fact, the controllable and living nature of this polymerization allows the homogenous and simultaneous growth of all the polymer chains, avoiding the premature formation of long polymer chains able to sterically prevent the monomer diffusion [80].

Moreover, to improve the interaction between the smart polymer and the stationary phase, a lipophilic monomer is often added to the thermo-responsive one with the aim of obtaining block copolymers with a greater affinity to the resin [81].

The great majority of applications reported in the literature include the functionalization of the stationary phases with PNIPAAm modified with a specific chemical group or another monomer in order to provide adhesion to the surface [82–84]. The reasons beyond the large use of this polymer relies on its convenient T_{cp} that is comprised between 25 and 37 °C, two key temperatures for bio-compounds. However, some other materials have been investigated so far, including poly(2-dimethylaminoethyl methacrylate)-block-poly(acrylic acid) [85], Poly (N-vinyl caprolactam) [86], poly(acryloyl-l-proline methyl ester) [87], poly(2-(2-methoxyethoxy)ethyl methacrylate (MEO2MA)–*co*-oligo (ethylene glycol) methacrylate [88–89] (Table 2).

An interesting application of thermo-responsive stationary phases was presented by Ayano et al. [90] In this work, triblock copolymers comprising a PNIPAAm, a PBMA and a poly(N,N-dimethylaminopropyl acrylamide) (DMAPAA) portion, were used to efficiently separate oligonucleotides *via* a combination of thermo- and pH-responsive effect. In fact, these species are commonly separated through a multi-step approach comprising a combination of hydrophobic interaction and ion-exchange chromatography [92]. On the contrary, the functionalization proposed by Ayano et al. allows to speed up the separation process since a single stationary phase is required. More in detail, the variation in the temperature was leveraged to separate the species based on their hydrophobicity/hydrophilicity by changing the hydration of the copolymers. The further separation between the charged and uncharged compounds was obtained by varying the pH of the mobile phase and in turn the surface charges, allowing for an efficient separation.

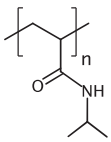
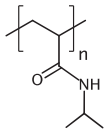
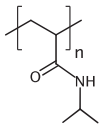
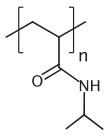
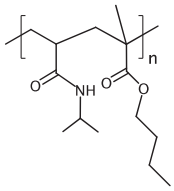
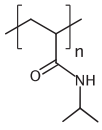
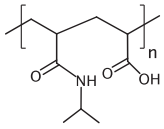
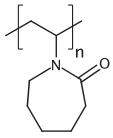
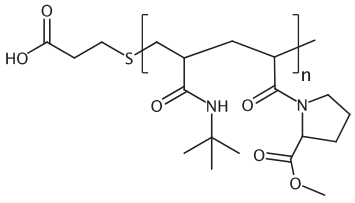
Another interesting application of these intelligent surfaces is in antibody purification. Usually, this separation is achieved using expensive techniques relying on affinity chromatography. On the contrary, Nagase et al. [91] developed a class of anionic thermo-responsive polymers able to selectively separate antibodies by modulating the hydrophobic and electrostatic interactions *via* a thermal stimulus. In particular, the authors synthesized triblock copolymers comprising a thermo-responsive, a lipophilic and an anionic monomer. First, the authors investigated the hydrophobic properties of the stationary phase by studying the adsorption of hydrocortisone and dexamethasone, while the ion-exchange behavior by separating dopamine and adrenalin. Finally, the capacity of the column to separate the antibody rituximab from its contaminants was tested. In particular, the drug was successfully retained at high temperature due to the electrostatic interaction between the weak positive charges present in the antibody and the negative ones in the polymer chains. On the contrary, once the temperature is reduced below the LCST, the antibody was eluted due to the extension of the thermo-responsive moieties in the mobile phase and the consequent shielding of the negative charges.

It is worth mentioning that all the materials listed above show a LCST-type behavior, mainly due to the higher control and reproducibility of their thermo-responsive behavior. There are few cases in which the stationary phases have been grafted with polyzwitterionic materials showing an UCST behaviour [93–98]. However, in general, in these applications the goal is to separate positively and negatively charged compounds *via* ion-exchanged zwitterions. This functionality is achieved by adjusting the pH of the aqueous solution in order to leave the isoelectric point of the material and provide the stationary phase with either a positive or negative net charge.

4. Thermo-responsive polymers as colloidal stabilizers

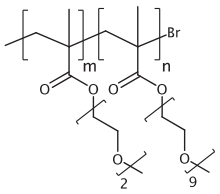
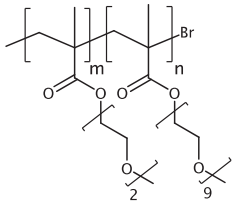
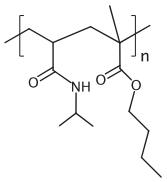
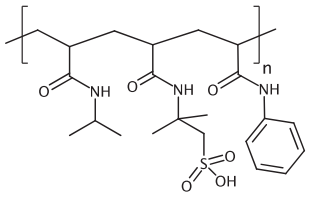
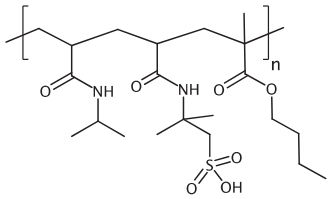
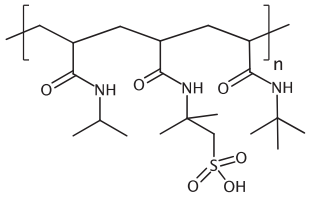
Surface active agents, or surfactants, are notoriously important in the stabilization of polymer particles at the nanoscale. Due to their lower diffusivity and possibility of tuning the steric and electrostatic interactions, polymeric stabilizers are more and more used in replacement of the traditional surfactants for the formulation of paints, adhesives, cosmetic products and medicinal products [99–100]. In this direction, thermo-responsive polymers have been investigated for their potential of a reversible stabilization/destabilization effect guided by temperature.

Table 2
List of the polymer, grafting technique and molecule separated via chromatography based on thermo-responsive stationary phases.

Polymer	Polymer structure	Thermo-responsive behavior	Grafting technique	Molecule separated	Reference
PNIPAAm		LCST	Grafting to	Dextran	[75]
PNIPAAm		LCST	Grafting to	Hydrophobic steroids	[76]
PNIPAAm		LCST	Grafting from	Hydrophobic steroids	[79]
PNIPAAm		LCST	Grafting from	Steroids and peptides	[78]
PNIPAAm-*co*-Butyl Methacrylate		LCST	Grafting to	Amino acid phenyl thiohydantoins	[81]
PNIPAAm		LCST	Grafting from	Protein complexes (tobacco mosaic virus)	[82]
PDMAEMA-*co*-Acrylic Acid		LCST	Grafting to	Low molecular weight acids, bases and small proteins	[85]
Poly(N-vinyl caprolactam)		LCST	Grafting from	Steroids	[86]
poly(NIPAAm-*co*-acryloyl-l-proline methyl ester)		LCST	Grafting to	Steroids	[87]

(*continued on next page*)

Table 2 (*continued*)

Polymer	Polymer structure	Thermo-responsive behavior	Grafting technique	Molecule separated	Reference
poly(2-(2-methoxyethoxy)ethyl methacrylate (MEO2MA)–co-oligo(ethylene glycol) methacrylate		LCST	Grafting to	Steroids	[88]
poly(2-(2-methoxyethoxy)ethyl methacrylate (MEO2MA)–co-oligo(ethylene glycol) methacrylate		LCST	Grafting from	Steroids	[89]
PNIPAAm-*co*-BMA		LCST	Grafting to	Nucleotides	[90]
PNIPAAm-*co*-2-acrylamido-2-methylpropanesulfonic acid (AMPS)-*co*-N-phenyl acrylamide (PhAAm)		LCST	Grafting from	antibody drug	[91]
PNIPAAm-*co*-AMPS-*co*-BMA		LCST	Grafting from	antibody drug	[91]
PNIPAAm-*co*-AMPS-*co*-*tert*-butyl acrylamide (tBAAm)		LCST	Grafting from	antibody drug	[91]

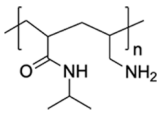
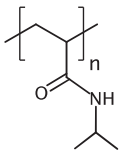
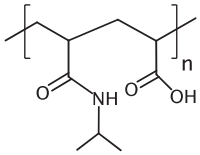
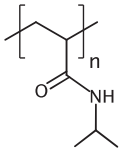
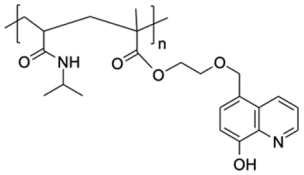
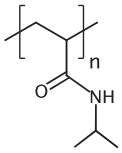
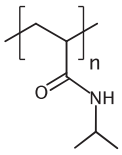
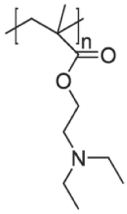
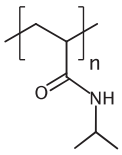
A list of thermo-responsive polymers with some interesting examples of colloidal stabilization is provided in Table 3.

The possibility of using thermo-responsive polymers in the stabilization of colloidal suspensions was initially introduced for cancer therapies. The idea was to exploit the higher temperature of the tumor site (generally 1–2 °C higher than the normal body temperature) to selectively accumulate the drug through a destabilization/dissolution of the carrier, thus maximizing the therapeutic index of the formulation [113]. However, the small difference in temperature compared to normal tissues requires a careful design of the carrier as well as a fine tuning of the T_{cp}. For these reasons, different colloidal structures and approaches for a temperature-induced release of antitumor drugs have been developed [113].

Thermo-responsive colloids can be obtained by combining, in a block copolymer, either a hydrophilic or hydrophobic segment with a thermo-responsive one. In the first case, colloids able to self-assemble once the T_{cp} is overcome are obtained (Fig. 8**a**). The thermo-responsive portions, becoming insoluble, phase-separate forming the core of these colloids, with the hydrophilic segments mainly locating at the polymer–solvent interface. Evidently, this situation does not include the formation of a thermo-responsive surface and, being out of the scope of this review, will not be further investigated.

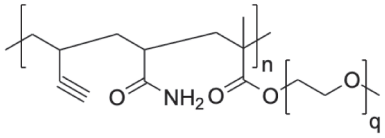
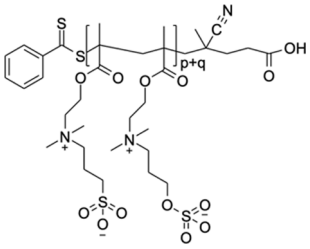
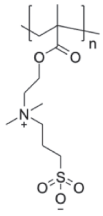
More interesting for this work is the second approach, represented by colloids obtained from the self-assembly of copolymers combining a thermo-responsive block and a hydrophobic one. The thermo-responsive shell provides colloidal stabilization as long as the temperature is below

Table 3
List of thermo-responsive polymers and their application as colloidal stabilizers.

Polymer	Polymer structure	Thermo-responsive behavior	Application	Reference
PNIPAAm-*co*-Allylamine		LCST	Drug delivery + imaging	[101]
PNIPAAm		LCST	Drug delivery + imaging	[102]
PNIPAAm-*co*-poly(sodium styrene-4-sulfonate)		LCST	Seawater desalination	[103]
PNIPAAm		LCST	H_2O capture and release	[104]
PNIPAAm-*co*- 5-(2-Methacryloylethoxymethyl)-8-hydroxyquinoline (MQ)		LCST	Antimicrobial agent	[105]
PNIPAAm		LCST	Protein identification	[106]
PNIPAAm		LCST	Protein-NP conjugate activity regulation	[107]
Poly(2-(dimethylamino)ethyl methacrylate)		LCST	Pickering stabilizer	[108]
PNIPAAm		LCST	Pickering stabilizer	[109]

(*continued on next page*)

Table 3 (*continued*)

Polymer	Polymer structure	Thermo-responsive behavior	Application	Reference
poly(acrylamide-*co*-acrylonitrile)–*co*- poly (oligoethylene glycol methyl ether methacrylate)		UCST	Drug delivery	[110]
Poly(sulfobetaine-*co*-sulfabetaine)		UCST	Drug delivery	[111]
Poly(sulfobetaine)		UCST	Pickering stabilizer	[112]

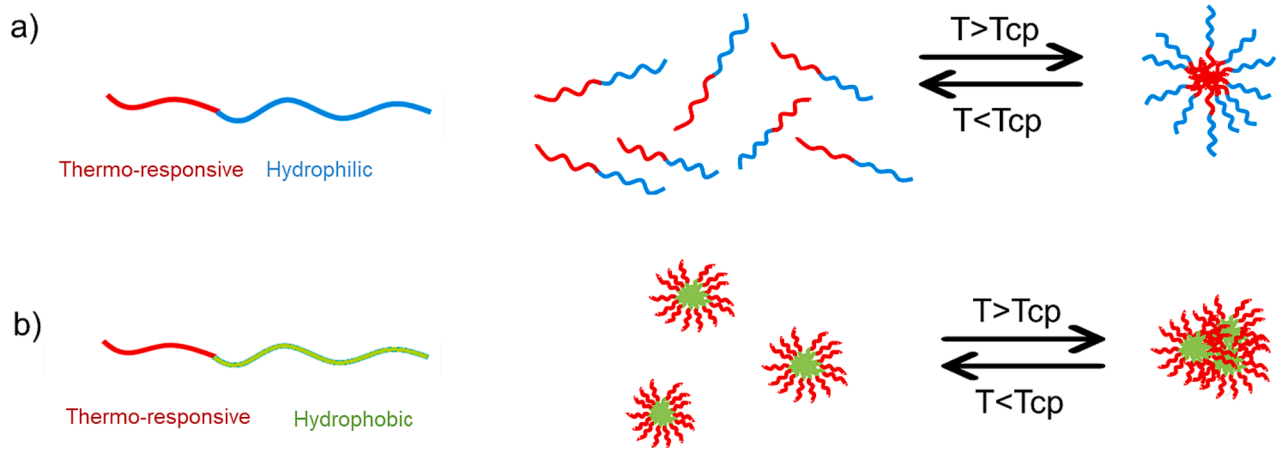


Fig. 8. Schematic representation of the thermal response of block copolymers formed by a thermo-responsive portion and a hydrophilic (a) or a hydrophobic (b) one.

(for LCST polymers) or above (for UCST polymers) the T_{cp}. This stability vanishes upon phase separation, leading to an extensive aggregation (Fig. 8**b**).

By loading a lipophilic drug in the core, an increased release rate can be induced by triggering this aggregation. The formation of lipophilic aggregates, in fact, was demonstrated to increase the diffusion rate of the drug, likely due to the faster transport from the bulk of the polymer to the surface, where the solubilization in the aqueous phase takes place [114–117].

This strategy is particularly suitable for the delivery of hydrophobic therapeutics. When hydrophilic compounds need to be delivered, thermo-responsive liposomes are often the material of choice. In fact, this structure allows the formation of a double emulsion (water in oil in water) with the oily phase being constituted by the lipidic membrane. In this way, it is possible to encapsulate both a lipophilic (in the liposome constituting portion) and a hydrophilic (in the inner aqueous phase) drug. However, traditional liposomes are often characterized by a cytotoxic release temperature [118]. For this reason, thermo-responsive polymers have been introduced in the stabilization of these colloids in order to favor their destabilization and in turn the release of the drug at more “cell-friendly” conditions [119].

One of the main advantages of thermo-responsive colloids is the great control in the carrier design in terms of T_{cp}, degradation time, size, and polydispersity. In particular, it has been demonstrated that the size of the colloid as well as the radius of curvature have an impact on the T_{cp} and must be carefully designed in order to assure the correct drug release [120].

In addition, the drug release rate is influenced by the nanoparticle (NP) core-forming polymer. In fact, it has been demonstrated that a soft core (*i.e.* glass transition temperature, Tg, lower than body temperature)

favors the surface destabilization and drug diffusion. However, when a low Tg is used, particular attention needs to be paid to the length of the block as well as to the hydrophilic-lipophilic balance of the final polymer. In fact, depending on these parameters, the aggregation of the NPs can lead to the formation of an irreversible precipitate and/or a gel, which can have detrimental effects in terms of formulation safety [121]. It is worth mentioning that this feature can be leveraged to selectively achieve injectable hydrogels, with a sol–gel transition in situ.

These enhanced performances in terms of controlled drug release achieved with thermo-responsive polymers were exploited by Pandey et al. to design an innovative theranostic system. In particular, the authors synthesized iron oxide magnetic nanoparticles and then decorated their surface with a thermo-responsive shell in order to selectively promote NP aggregation following temperature stimuli [101]. In this way, an efficient release of doxorubicin in response to thermal stimulation was combined with *in vivo* monitoring accessed through the magnetic core, unifying the therapeutic effect with a real-time imaging (Fig. 9).

The heat required to destabilize the polymer shell and enhance the doxorubicin release can also be obtained through hyperthermia by exploiting the superparamagnetism of the magnetite core, as demonstrated by Purushotham et al. [102]. More in detail, core–shell colloids formed by magnetic NPs covered with a thermo-responsive polymer were synthesized and loaded with doxorubicin. Then, an external alternating magnetic field was applied to induce the rapid flipping of the magnetic moments of the magnetite core. This caused an increase in the local temperature, which overcame the T_{cp} of the thermo-responsive polymer, causing its dehydration and favoring the doxorubicin release.

The possibility of functionalizing metal-based NPs with thermo-responsive polymers has been widely reported in the literature also for applications that exceed the biomedical field such as sensors [122], nano-catalysis systems [123] and water treatment applications [101,103–104,124–126].

As an example, PNIPAAm shells have been used by Guo et al. to selectively vary the optical and electrochemical properties of silver NPs [127], by changing the thickness of the stabilizing shell and in turn modulating the NPs aggregation process when the temperature was increased above the T_{cp}. Moreover, Ji et al. showed that small (1–4 nm) silver NPs have efficient antimicrobial effects which depend on the outer stabilizing shell. For this reason, by varying the external temperature, it is possible to change the efficacy of the system with the highest performances achieved for temperature lower than the Tcp [105].

The possibility of selectively switching on and off metal NPs via thermal stimuli was successfully implemented also in protein identification [106] and protein-NP conjugate activity regulation [128]. In particular, PNIPAAm-stabilized gold NPs can be used as competitive binders to fluorophores in order to identify proteins [106]. More in detail, the NP affinity to the fluorophore can be regulated by varying the extension of the polymer shell. This could be achieved either acting on the molecular weight or on the phase separation of the shell following an increase in the local temperature. The fluorophore minimum distance from the gold core allowing the protein analyte to appreciably compete for the binding to the particles allows to quantify the protein affinity and, in turn, its identification. The same strategy was successfully employed by Kumar et al. [107] in the separation of histidine-tagged fluorescein *via* metal-chelate affinity precipitation. In particular, poly (PNIPAAm-*co*-1-vinylimidazole (VI)) polymers were synthesized and complexed with Cu(II). In this way, polymers able to selectively expose Cu(II) binding sites depending on the external temperature were obtained. This allowed an efficient separation of the proteins at high temperature, as a result of the interaction between the histidine residues in the protein and the metal exposed on the surface of the globules formed above the T_{cp}. In turn, once the temperature was decreased below the T_{cp}, the shielding of the metal allowed an efficient release of the protein.

Moreover, functionalizing gold NPs with both protein and PNIPAAm it is possible to obtain a protein-NP conjugate with tunable bioactivity [128]. In fact, at low temperature the thermo-responsive chains are hydrophilic and extend to the bulk solution stabilizing the NP and hiding the protein active sites. On the contrary, once the temperature is raised above the T_{cp}, the PNIPAAm chains collapse on the surface of the NPs allowing the exposure of the protein site and thus increasing the conjugate activity up to 270% (Fig. 10).

Similarly to what has been described for TCPS surfaces and chromatography stationary phases, also metal NP functionalization can be achieved *via* a "grafting to" or "grafting from" [129] strategy. As described in the previous sections, the main difference between the two strategies is the final grafting density achieved [130]. This parameter has an effect on both stability and thermo-responsiveness of the functionalized NPs. As a matter of fact, it has been shown that high grafting densities are usually associated with a greater NP stability. However, they also restrict the polymer chain mobility and their temperature-mediated conformational change, resulting in a loss of thermo-responsiveness [131–132]. This aspect is not only a function of the grafting density but also of the functionalization technique. In fact, thermo-responsive NPs with comparable size and grafting density obtained respectively *via* "grafting from" and "grafting to" approach can present opposite behaviours [133–134].

Another interesting application of thermo-responsive NPs is their implementation as stabilizers in the so-called "Pickering emulsions". In these emulsions, droplets of the dispersed phase are stabilized by the physical adsorption of NPs at the interface with the continuous phse [135–136].

Consequently, greater and longer stability are achieved with respect to the conventional surfactant-stabilized emulsions. However, this improved stability can prevent the release of the dispersed phase for on-demand applications. It is the case of drugs or other oily compounds, such as fragrances and dyes, which typically require a fast release after a certain activation. For this reason, several studies on the possibility of stabilizing an oil phase in water and of selectively releasing it through

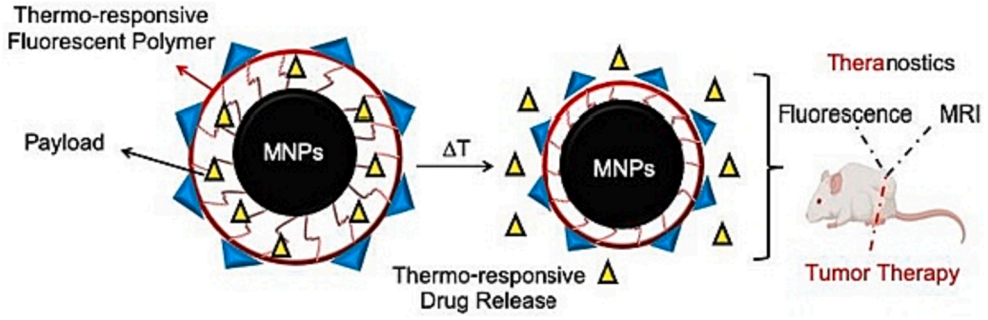


Fig. 9. Innovative theranostic system able to combine thermo-responsive drug release with an *in vivo* monitoring. Reprinted with permission from [101] Pandey et al. Copyright 2020 Nanotheranostics.

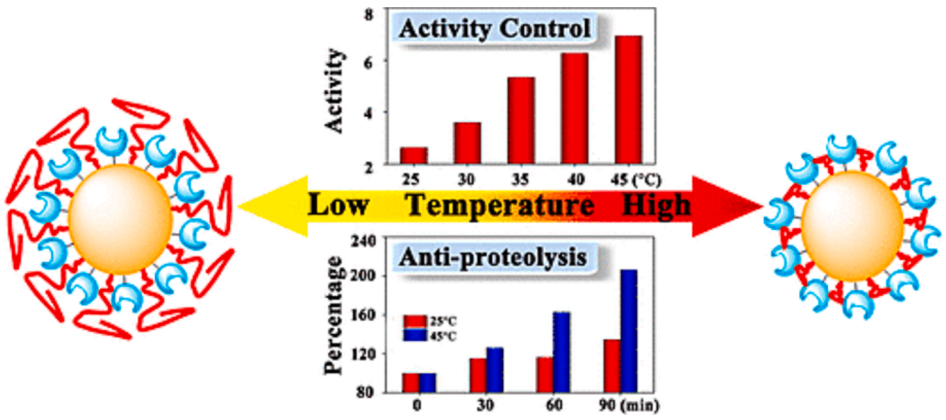


Fig. 10. Gold NPs coated with proteins and thermo-responsive polymers in order to obtain tunable bioactivity according to the external temperature. Reprinted with permission from [128] Yuan et al. Copyright 2015 American Chemical Society.

emulsion destabilization once the temperature is raised above the T_{cp} are reported in literature [137–139]. For example, Saigal et al. [108] grafted poly(2-dimethylaminoethyl methacrylate) (PDMAEMA) brushes from the surface of silica NPs and exploited the potentiality of this thermo-responsive colloid in stabilizing and selectively releasing xylene and cyclohexane in water. In particular, they showed that a critical grafting density is required in order to obtain proper Pickering emulsions at low temperature. As a matter of fact, a dense coating was proven to be less stable than a sparse one. Moreover, these stable emulsions were efficiently broken by simply increasing the temperature above the T_{cp} of the polymer shell. This, in fact, is associated to the aggregation of the colloidal silica, making it no longer effective as Pickering stabilizer.

Tsuji et al. [109] studied the Pickering emulsification and thermal coalescence of several oils employing PNIPAAm-decorated NPs. In particular, the authors showed that depending on the work of adhesion, either oil-in-water or water-in-oil emulsions can be formed. Moreover, the authors demonstrated the long stability of the Pickering emulsion as long as the temperature is kept below the T_{cp}. Conversely, as soon as the external temperature is raised above this threshold, a fast precipitation of the PNIPAAm NPs and consequent coagulation of the oil is achieved with a yield that is dependent on the oil/NP ratio used.

This concept of thermally-induced emulsion destabilization was exploited by Manfredini et al. [140] for the on-demand release of fragrances. The authors developed a new class of thermo-responsive NPs controllable in terms of size, degradation time and wettability and used them to emulsify limonene in water. This high tunability of the colloids have been demonstrated to affect the control over the properties of the final Pickering emulsions, allowing to achieve droplets of the dispersed phase of different and controllable size. Despite the high values of emulsion efficiency reached with all the NPs tested, a different fragrance release rate was exhibited by the different colloidal stabilizers once the temperature was raised above the T_{cp}, following the destabilization of the emulsion (Fig. 11). These differences were correlated to the physico-chemical properties of the NPs forming the Pickering emulsion, thus providing guidelines for the design of these emulsions.

Less literature is reported on UCST-based polymers for colloidal stabilization [141–147], mainly due to the high sensitivity of this class of materials to the environmental conditions. Furthermore, particular attention must be paid to the surface functionalization process, since the presence of some free UCST polymer can force NP aggregation [148].

Some effort has been put in producing UCST stabilized NPs for drug delivery applications, either by favoring a rapid micelle disassembly [149] or by leading to a quick NP aggregation [111,150] by increasing the temperature. In particular, Wu et al. [149] developed a class of magnetic NPs showing an UCST functionality thanks to the polymer coating made by a statistical copolymer of acrylonitrile (AN), acrylamide (AAm) and methoxy polyethylene glycolsuccinimidyl carbonate (mPEG-SC). These systems were able to release doxorubicin once the temperature was raised above the T_{cp}, where the polymers became completely soluble. More in detail, a near-infrared laser irradiation was

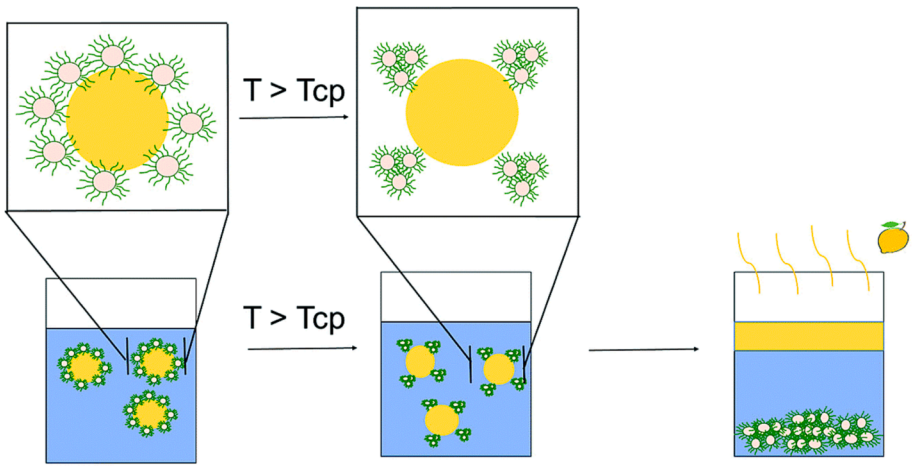


Fig. 11. Pickering emulsion of limonene in water stabilized by thermo-responsive nanoparticles. The temperature dependent behavior is useful to tune the release of the fragrance at will by simply changing the external temperature. Reproduced from [140] Manfredini et al. with permission from the Royal Society of Chemistry. Copyright 2019.

used to induce NP vibration causing a photothermal heating. This increase in the local temperature induced the micelle disassembly and in turn the drug release. In this way, a combined photothermal-chemothermal effect was achieved.

A similar result was obtained by Bordat et al. [151] through polymeric nanoparticles obtained from di-block copolymers made of poly (acrylamide-*co*-acrylonitrile) and poly(oligoethylene glycol methyl ether methacrylate) (POEGMA), showing, respectively, a thermo-responsive and stabilizing behavior. The copolymers were then mixed with a doxorubicin solution and nanoprecipitated in water to afford nanoparticles with tunable T_{cp} and size. In particular, the copolymer T_{cp} was set to 43 ℃, and the cytotoxicity of the drug-loaded nanoparticles was studied in mild hyperthermia conditions, typical of cancer. The results showed a decrease in the half maximal inhibitory concentration (IC_{50}) between heated and unheated conditions, which testifies the thermo-responsive behavior of the polymer and its direct connection to the availability of doxorubicin in the system.

The same improved drug bioavailability upon heating was achieved by Sponchioni et al. [111] by following a different approach. In fact, the authors developed a class of zwitterionic biodegradable NPs with tunable UCST. This control over the transition temperature was obtained by modulating the composition of the thermo-responsive portion of the copolymer, composed of sulfobetaine (SB) and sulfabetaine (ZB) methacrylate. In this way, the authors were able to set a priori the T_{cp} slightly below the body temperature by acting on the stoichiometry of the reaction mixture. As a result, as soon as the NPs were incubated above the T_{cp} (45 °C), their incipient destabilization determined an acceleration of the release process (Fig. 12) [111,152]. Finally, the biodegradable nature of these NPs prevented any polymer accumulation in the human body.

The use of UCST polymers in stabilizing NPs has beneficial effects also in terms of colloidal stability in biological media [93,153]. In fact, the high hydrophilicity of these materials (above the T_{cp}) allows for an efficient water molecule adsorption on the surface, which in turn prevents non-specific protein interactions. Moreover, the selective activation/deactivation of the outer shell can be used to efficiently separate cells and proteins [154] or to selectively form gels [147,155] by simply changing the external temperature.

UCST polymers, similarly to their LCST counterparts, have also been investigated in the preparation of colloids to be used as oil-in-water Pickering emulsifiers.

In a work from Vasantha et al. [112], nanoparticles with thermo-responsive zwitterion moieties were synthesized through surfactant-free emulsion polymerization to afford a stable and monodispersed latex. The introduction of poly(sulfobetaine) (PSB) in the copolymer allowed the formation of a system with a dual responsive behavior, which could be tuned as a function of both temperature and salt concentration. In the end, the dual responsive latex was successfully employed for the formation of stable O/W Pickering emulsions in a wide variety of conditions, typically encountered in the oil&gas applications. Interestingly, the emulsion could be reversibly broken at will by modifying either the temperature or the ionic strength of the medium.

The same purpose was achieved by Douyère et al. [156], who prepared polyethyleneimine (LPEI) hydrogels with a highly controllable self-assembling nature. Indeed, by acting on the temperature and pH of the system, the authors were able to induce a reversible sol–gel transition and control the rheological properties of the copolymers. These properties were then exploited for the formation of stable Pickering emulsions with typical gel-like and shear thinning behavior, which could be destructured/restructured at will through a simple variation of the environmental conditions.

5. Thermo-responsive polymers for enhanced oil recovery

Enhanced oil recovery (EOR) is the third phase of the oil extraction process, allowing to recover up to 75% of the original oil in place [157–158]. Given the large fraction of oil that cannot be recovered with the secondary process, much research effort has been devoted to the development of efficient EOR methods. Among these, particularly relevant are the chemical methods, which rely on the injection inside the reservoir of a fluid containing specific additives with the aim of modifying the oil viscosity and/or the oil/water interfacial tension and hence improve the recovery of the organic phase. [159] Among the additives currently investigated, surfactants are particularly efficient, thanks to their ability to lower the oil/water interface tension [160] and change the rock wettability [161–162], thus favoring the emulsification of the residual oil. However, one of the drawbacks of this technology is the difficult recovery of the organic fraction from the extracted emulsion, which increases the process costs [160].

In order to overcome these limitations, thermo-responsive polymers are raising increasing interest in this field. In fact, the possibility of selectively stabilizing and destabilizing oil-in-water emulsions by simply changing the environmental temperature has been considered immediately promising. Table 4 summarizes some examples where thermo-

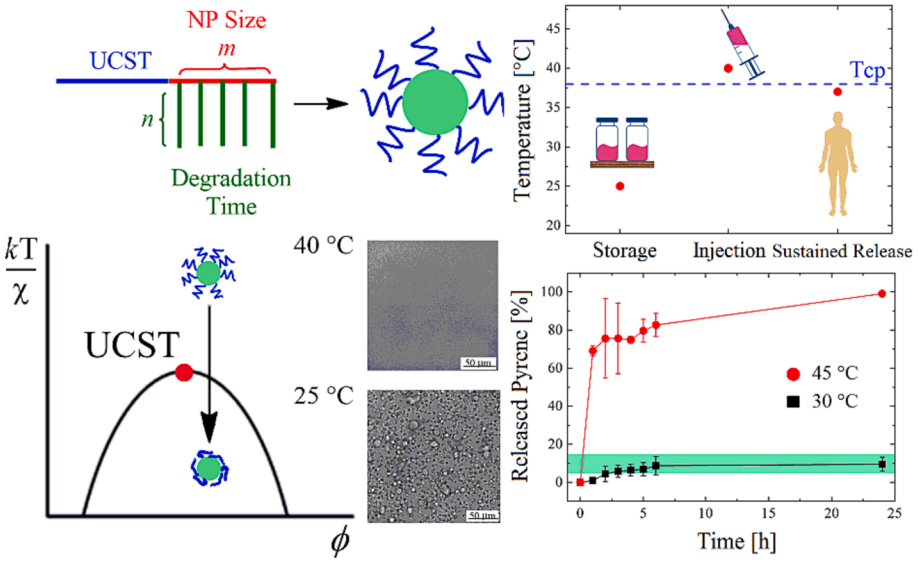
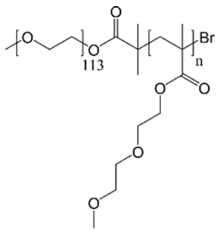
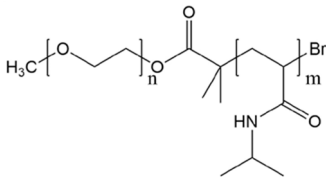
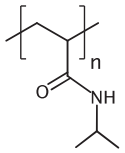
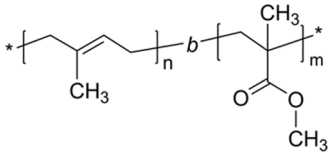
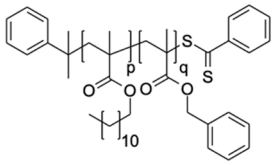
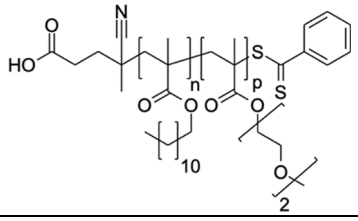


Fig. 12. Degradable UCST thermo-responsive polymers able to efficiently release highly lipophilic molecules (with a behavior similar to drugs) below their T_{cp}. Reproduced from [111] Sponchioni et al. with permission from the Royal Society of Chemistry. Copyright 2019.

Table 4
List of thermo-responsive polymers and their application for EOR purposes.

Polymer	Polymer structure	Thermo-responsive behavior	Solvent	Application	Reference
PEG-b-Poly(2-(2-methoxyethoxy) ethyl methacrylate)		LCST	Water	Oil recovery from oil sands	[163]
PEG-b-PNIPAAm		LCST	Water	O/W stabilizers	[162]
PNIPAAm		LCST	Water	O/W separation	[164]
Poly(isoprene-b-methyl methacrylate)		LCST	Aliphatic carbon	Oil viscosifier	[165]
Poly(lauryl methacrylate-*b*-benzyl methacrylate)		UCST	n-dodecane	Oil viscosifier	[166]
Poly(lauryl methacrylate-*b*- EG_2MA)		UCST	Decane-toluene mixture	Oil viscosifier	

responsive polymers have been successfully applied for EOR purposes.

In this sense, Yang et al. [163] developed an innovative class of thermo-responsive materials for oil recovery. These polymers, produced *via* ATRP of poly(2-(2-methoxyethoxy) ethyl methacrylate) (PMEO2MA) using PEG as macroinitiator, were successfully implemented in the recovery of oil from oil sands. In particular, oil sands were deposited at the bottom of a vial. Afterwards, the polymeric solution was added and the mixture was placed in a shaker for 24 h at a temperature higher than T_{cp}. The polymer phase separation allowed to form NPs in water, that favored the emulsification of the oil from the sand surface. These oil-in-water emulsions formed after shaking could be broken by simple incubation of the samples at room temperature. The lowering of the temperature below the polymer T_{cp}, in fact, re-dissolved the polymer chains in water as unimers, allowing to recover the oil by creaming with an efficiency of around 80%wt. This result is actually very appealing, not only for the high separation efficiency but even more for the possibility of recycling the polymer solution for further separations (Fig. 13).

A similar approach was followed by Ni et al. [162], who developed a class of PEG-PNIPAAm copolymers able to stabilize oil in water either *via* classical emulsion or *via* Pickering emulsion depending on the thermo-responsive surfactant concentration. Also in this case, an efficient oil separation and surfactant recovery was achieved once the temperature was lowered below the T_{cp}. Here, in order to reduce the induction time, *i.e.* the time required to interact with the oily phase, thermo-responsive surfactants with low molecular weight are preferred [167].

Thermo-responsive surfactants have been successfully used also for the flocculation of oil sands from mature fine tailings [168–169]. In particular, Zheng et al. reported the superior performances of cationic thermo-responsive surfactants with respect to the simple cationic ones [170]. This wider spectrum of action and better ability to flocculate fine sand particles were attributed to the decreased solubility of the thermo-responsive moieties.

Furthermore, good performances in the separation are achieved both when the thermo-responsive monomer is incorporated in the cationic surfactant and when it is added as a separate polymer in addition to conventional cationic surfactants [171].

The temperature-dependent change in polymer wettability and consequent ability to separate oil from water has been exploited successfully also through the realization of thermo-responsive membranes [164,172–173]. With this aim, Li et al. [172] investigated the selective oil/water separation through membranes based on PMMA-*b*-PNIPAAm

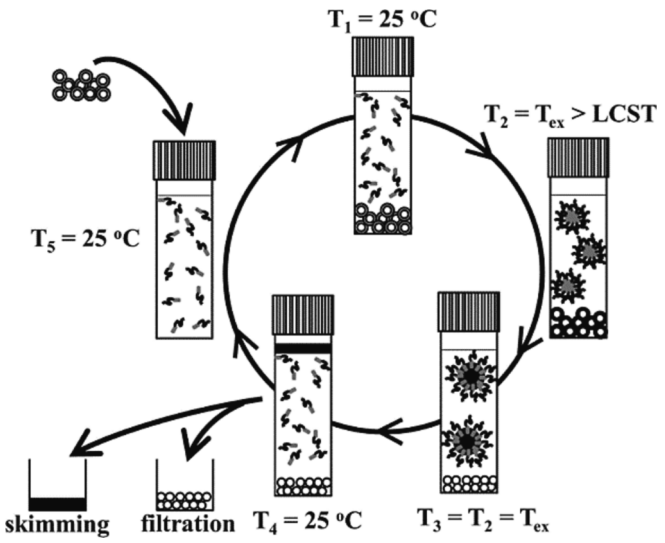


Fig. 13. Schematic representation of the oil extraction process. Reprinted with permission from [163] Yang et al. Copyright © 2015, American Chemical Society.

block copolymers. PMMA was used to improve the stability of the membrane while PNIPAAm was chosen due to its well-known thermo-responsiveness. In this way, by changing the temperature, it was possible to selectively retain on the membrane either the oil ($T < T_{cp}$) or the water ($T > T_{cp}$) phase with an efficiency as high as 98%. Alternatively, Ngang et al. [173] developed silica NPs functionalized with PNIPAAm and used them as modifiers of polyvinylidene fluoride (PVDF) membranes, typically used in wastewater treatments, in order to overcome the fouling problems that usually characterize these materials. In particular, the functionalization allowed to selectively change the membrane wettability properties favoring an efficient oil separation.

Another example is reported by Wang et al. [164], who grafted PNIPAAm on a regenerated cellulose nanofibrous membrane to create a smart material for oil/water separation. Indeed, the thermo-responsive polymer gives the surface super-lyophilic/super-lyophobic properties according to the external temperature, opening up to a controllable change in the wettability. In particular, the membranes could be used for water or oil uptakes by simply adjusting the operating temperature respectively below or above the T_{cp} and showed excellent reusability, up to 5 cycles.

Finally, thermo-responsive polymers can be used as thermo-thickeners to reversibly increase water viscosity when increasing the environment temperature, which is naturally obtained in underground oil wells. This peculiar behavior in turn can be leveraged to reduce the water permeability and favor oil displacement without compromising the injectability of the fluid [174–175]. In fact, a polymer showing a LCST comparable to the temperature in the oil reservoir would be soluble in water at room temperature and thus easily injectable inside the well together with the aqueous phase. Then, once the temperature increases and overcomes the T_{cp}, the polymer phase separates inside the water phase, thus causing an increase in the viscosity. For this application, particular attention must be paid to the design of the polymer that usually presents a comb-graft structure. In fact, in these systems, the T_{cp} is influenced not only by the thermo-responsive monomer but also by the grafting density and polymer molecular weight [174,176–177].

An interesting outcome is reported by Li et al. [178], who compared the EOR performances of commonly used partially hydrolyzed polyacrylamide (HPAM) with two newly developed smart thermo-viscosifying polymers, constituted by sulfonated acrylamides with the same amount of thermo-responsive moieties and different molecular weight. While aqueous HPAM solutions showed a rapid decrease in their viscosity at high temperature and salinity typical of oil reservoirs, the two thermo-responsive polymers were able to withstand harsh environmental conditions and offered an efficient and long-term viscosifying effect, more pronounced in the case of the material with higher molecular weight. The application of these polymers in three different types of reservoirs gave an enhanced oil recovery efficiency comparable to the commonly used materials, with the additional advantage of good injectability at low temperature due to their complete solubility in water below the LCST.

Another interesting application of thermo-responsive polymers in the oil&gas field is as drag reducing agents, as successfully shown by Bhambri et al. In this application, the main problem is the polymer adsorption on the surface of the well. However, by designing a material able to reversibly precipitate once the T_{cp} is overcome, this issue can be solved and the waste of material prevented [179].

Following the demonstration of the potential of thermo-responsive polymers in the oil&gas field, an expansion of their scope to all the phases of the oil extraction and refinery processes has been pursued in the very recent years. This led in particular to a blossoming of thermo-responsive materials directly synthesized in non-polar solvents, due to their high compatibility with the fluids encountered in the oil&gas field. [180–181] Indeed, these materials show a high affinity with the oil phase and allow to change its properties (*i.e.* viscosity, interfacial tension with water, etc.) according to the needs.

For example, Hutchings et al. [165] synthesized a series of polyisoprene-*b*-poly(methyl methacrylate) in aliphatic hydrocarbon solvents, carefully varying the ratio between the two blocks through controlled radical polymerization techniques. The self-assembly was then achieved by dispersing the polymers in a selective solvent, able to solvate only one of the blocks and trigger the rearrangement of the copolymers into a wide range of morphologies (spherical micelles, vesicles and worm-like particles) according to the molecular weight of the core. The possibility of reversibly switching between macrostructures with widely different viscosities by simply changing the temperature paves the way to their employment as on-demand viscosifiers.

Similarly, diblock copolymers composed by di(ethylene glycol) methyl ether methacrylate (EG_2MA) and an aliphatic monomer have been recently investigated for their peculiar UCST behavior in a mixture of decane and toluene, representative of both the paraffinic and aromatic fractions of crude oil. This behavior can be exploited to reversible go from molecularly dissolved polymers to nano-objects by reducing the temperature below the T_{cp}. [29–30] In turn, this parameter as well as the nano-object morphology can be precisely tuned by acting on the copolymer microstructure, which is made possible through controlled radical polymerizations, such as the reversible addition-fragmentation chain transfer (RAFT) polymerization. [30] These findings open up to the rational synthesis of oil-compatible additives able to change their affinity and macroscopic behavior in the outer medium. In fact, the controlled formation of either spherical, worm-like or vesicular particles by decreasing the temperature produces specific and important effects on the medium viscosity and surface tension, which can be advantageously exploited.

Despite the decisive steps forward, it is worth highlighting that this field is fairly new and still open to possible discoveries both on the synthesis and on the application side, which can boost even more the impact of thermo-responsive polymers.

6. Conclusion

In this work, we exhibited the potentialities and advantages that the functionalization of a target surface or interface with thermo-responsive polymers can provide. In particular, we showed the great versatility of these materials by focusing on different areas of interest, namely cell culture, chromatography, colloidal stabilization and enhanced oil recovery. In all the different sections, we reported the main functionalization strategies adopted nowadays with a critical comparison aimed at highlighting the benefits and drawbacks of one over the others. Then, we

reported examples that in our vision are particularly relevant to highlight the superior performances achievable by these activated surfaces, pointing out the main results achieved in the state of the art.

As a general conclusion, LCST-based polymers are still preferred to the UCST ones, in particular for applications in aqueous media, mainly due to the high sensitivity of the latter to the environmental conditions that significantly limits their applications. This disparity is mainly evident in the field of cell culture and chromatography where the media and eluent compositions may change during the process and hence strongly impact the control of the phase separation for UCST polymers.

Another relevant theme is how to ensure a sharp phase separation, in order to react promptly to the change in the environment temperature. This is of major interest since, as highlighted in this work, for many applications, such as controlled drug delivery, the thermal gradient that can be safely applied is often modest. At the same time, a broad molecular weight distribution and compositional drift in the case of copolymers make the phase separation to extend over a wide temperature range. In this scenario, the introduction of controlled radical polymerizations allowed the obtainment of polymers with well-defined microstructure and reduced interchain composition differences, thus favoring a sharp and homogeneous phase transition. This for example allowed the affirmation of thermo-responsive cell sheet engineering for *in vivo* applications. As a matter of fact, thermo-responsive TCPS surfaces already reached market maturity (see Nunc® UpCell ™ technology). On the contrary, smart chromatography stationary phases are still on a research level. This is mainly due to the high cost of production compared to the traditional strategies. In fact, contrarily to the cell culture applications, in this field the functionalities achieved by these smart polymers are similar to the conventional one with the exception of avoiding an organic solvent. However, this improvement is still not enough to justify the industrial spreading of this technology. Similarly, thermo-responsive polymers showed great potentialities in the colloidal stabilization in many research areas such as drug delivery, magnetic treatments, protein identification and Pickering emulsions. Despite few examples of thermo-responsive-based drug delivery systems reached the clinical stages, the majority of these applications is still at a research level, underlining the necessity of introducing additional improvements to reach the clinical maturity. Finally, thermo-responsive polymers are interestingly studied to increase the oil recovery during EOR. In this application the economic advantages of an increased recovery overcome the price of the smart material production, which nowadays is high due to the employment of controlled radical polymerization techniques requiring expensive controlling agents. However, an important issue that needs to be addressed is the recovery of the polymeric material, in order to prevent any plastic accumulation and ensure their reutilization. Until these limitations are not overcome, the use of these materials will remain mainly on a lab scale. Nonetheless, the potentialities and the progressive affirmation of controlled radical polymerizations in the industries are slowly but steadily reducing the gap with the traditional technologies and as soon as also the manufacturing costs will be competitive, the thermo-responsive polymers could eventually breakdown the market.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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