CHAPTER II

THEORY AND LITERARURE REVIEW

2.1 Stimuli-responsive polymers

Stimuli-responsive polymers are polymers that respond with large property changes to small physical or chemical changes in their environment. They are usually classified according to the stimuli they respond to as temperature-, pH-, ionic strength-, light-, electric-, and magnetic field-sensitive. Some polymers respond to a combination of two or more stimuli.

Poly(*N*-isopropylacrylamide), PNIPAAm, is a well-known thermo-responsive polymer which contains a hydrophilic amide and a hydrophobic isopropyl group in the polymer chains. PNIPAAm in an aqueous solution demonstrates lower critical solution temperature (LCST) at 32°C because of hydrogen bonding interactions between the amide group and water as shown in Figure 2.1. Hydrogen bonds are bound to the hydrophilic moieties and the polymer is in a swollen conformation below LCST and in a shrinkage state while the temperature is higher than LCST [1].

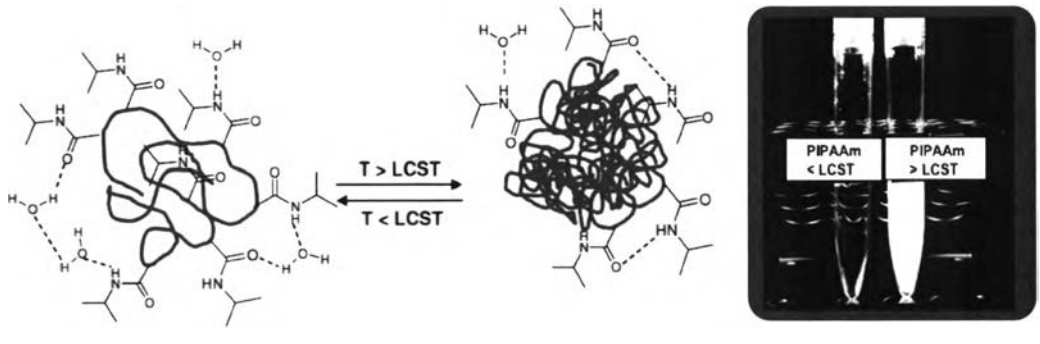

Figure 2.1. Reversible conformational changes in response to variations of the temperature of the PNIPAAm [1].

2.2 Polymeric activated esters

Polymers having multiple reactive units along their backbone are useful precursors for multifunctional polymer preparation. Ringsdorf and coworkers [2] and Ferruti *et al.*[3] performed pioneering work on reactive polymers based on activated esters in 1972.

The monomers *N*-acryloxysuccinimide (NAS) or *N*-methacryloxysuccinimide (NMAS) have been used extensively for the synthesis of macromolecular precursors [4] (Figure 2.2). These polymers can be converted with amines in a post polymerization modification under very mild conditions, yielding functionalized polyacrylamide or polymethacrylamide derivatives. However, a notable disadvantage of these two polymers is their limited solubility. They are only soluble in DMF and DMSO.

Activated esters in the form of pentafluorophenyl (meth-)acrylate, PFP(M)A [5] are very attractive choices in that their resulting polymers are hydrolytically stable in air and soluble in many common organic solvents such as dioxane, diethyl ether, chloroform, benzene, methanol, and n-hexane. Additional advantage lies on the fact that the reaction of the pentafluorophenyl ester groups can be conveniently monitored by ^{19}F NMR spectroscopy [5].

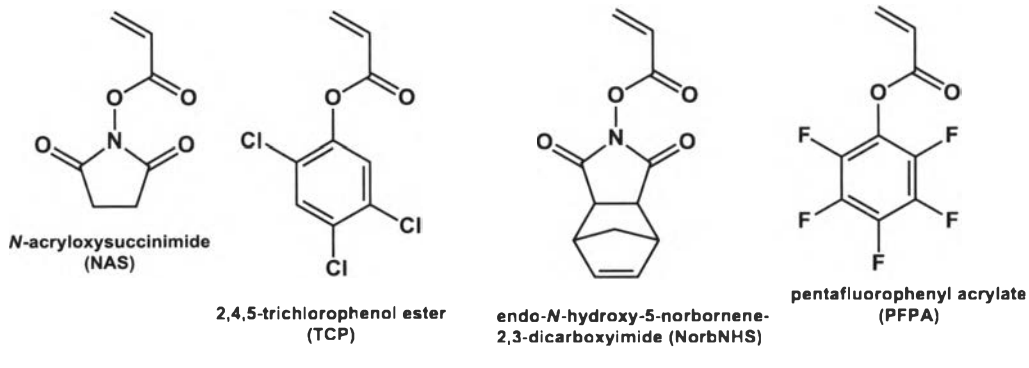

Figure 2.2. Chemical structures of various activated ester monomers [4].

2.3 Thermo-responsive polymeric micelles

Amphiphilic block copolymers, containing both hydrophobic and hydrophilic blocks, are well established as building blocks for the preparation of micellar structures. When amphiphilic block copolymers are dispersed in water, micellization occurs, with hydrophobic blocks forming the core and hydrophilic blocks forming the shell. The water solubility of these self-assembled structures is derived from their hydrophilic shell. The hydrophobic core makes them perfect carriers for hydrophobic compounds. It is believed that the hydrophobic core creates an adequate reservoir for poorly water-soluble molecules such as drugs or genes. The hydrophilic shell not only increases the solubility of the complex, but also protects the poorly water-soluble molecules from the external environment.

Thermo-responsive micelles assembled from amphiphilic block copolymers have been extensively studied. When the thermo-responsive component is combined with a hydrophobic block, the polymer will form micelles with a thermo-responsive shell. Upon heating above the LCST the shell collapses resulting precipitation/gelation of the micelles. The form of colloidal materials has been utilized to encapsulate hydrophobic molecules within the core and release them upon heating above the LCST as following reports.

In 1997, Okano *et al.*[6] prepared thermo-responsive polymeric micelles from amphiphilic block copolymers composed of *N*-isopropylacrylamide (NIPAAm) (a thermo-responsive outer shell) and styrene (St) (a hydrophobic inner core) as shown in Figure 2.3. The micelles have a unimodal size distribution (24 nm). These micelles have a small diameter with a LCST, providing a carrier that may have long blood circulation times. Later, in 1999, Okano *et al.*[7] developed poly(*N*-isopropyl-acrylamide)-*block*-poly(butyl methacrylate) micelles and encapsulated adriamycin within the core. At temperatures below the LCST of the *N*-isopropylacrylamide (NIPAAm) block (ca. 32°C), adriamycin release was slow with less than 20% release after 48 h. When the temperature was raised to 37 or 40°C release was rapid with 80% after 5 h and 90% after 48 h. Similar results have been reported by Yang and coworkers [8] for doxorubicin (DOX) release from poly[NIPAAm-*co*-(*N,N'*-dimethylacrylamide)]-*block*-poly[(*D,L*-lactide)-*co*-glycolide] micelles and by

Zhu *et al.*[9] for the release of paclitaxel from poly(NIPAAm)-*block*-poly[(hydroxyethyl methacrylate)-*graft*-polycaprolactone)] micelles.

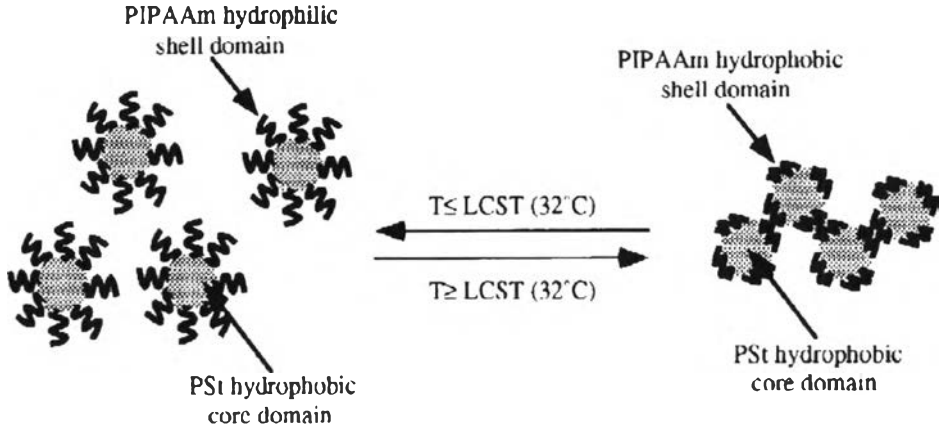

Figure 2.3. Structural changes occurring when the solution temperature is increased above the LCST of PNIPAAm/PSt micelles [7].

Much less work has been dedicated to the preparation of thermo-responsive micelles containing activated moieties. Recently in 2012, Zhuang *et al.* have developed water-dispersible nanogels based on pentafluorophenyl acrylate and poly(ethylene glycol) methacrylate random copolymer having diamine as a crosslinker (Figure 2.4). Isopropylamine and *N,N'*-dimethylethylenediamine were incorporated onto the cross-linked nanogel to demonstrate the possibility of surface engineering. These nanogels can encapsulate lipophilic guest molecules during the cross-linking step of the nanogel synthesis [10].

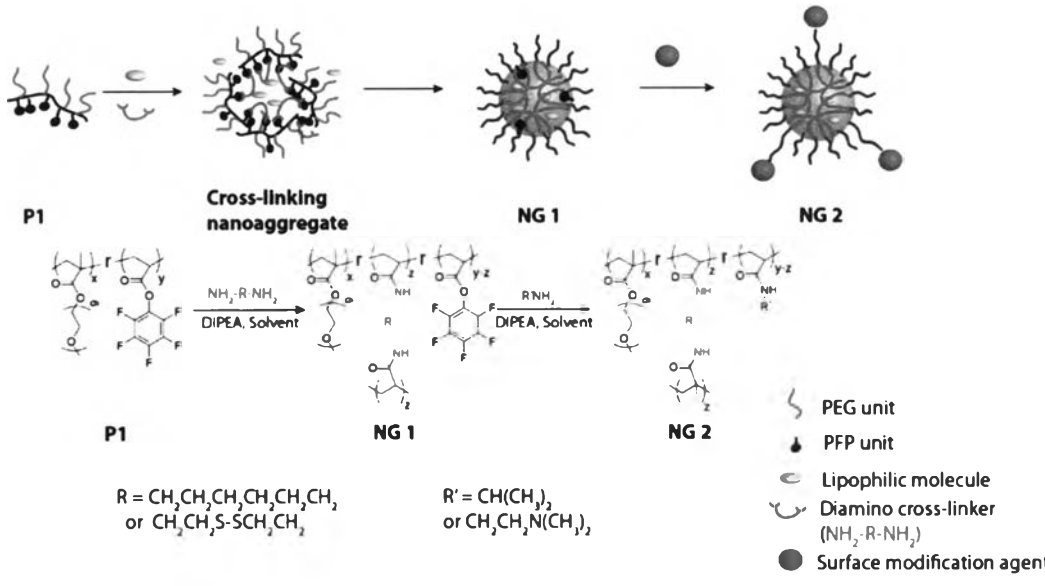

Figure 2.4. Schematic representation of design and synthesis of the cross-linked polymer nanogels [10].

2.4 Electrospinning

The electrospinning technique is recognized as a simple and versatile method for preparing micro/nanoscale fibers with a very large surface area to volume ratio, flexibility in surface functionalities, and superior mechanical performance [11]. A basic concept of electrospinning is to employ a high-voltage electric field to produce fibers from a polymeric fluid stream (solution or melt) deposited on the collector surface. Upon applying an electric field, the polymeric solution with a moderate viscosity value forms the Taylor cone (Figure 2.5) at the tip of an injector [12]. When the electric voltage applied between the injector and the collector exceeds the surface tension force of the Taylor cone, the charged jets are eventually sprinkled to the ground. During the travel of the fibrous jets, the solvent evaporates, generating a solidified nanofibrous structure on the ground. The basic experimental setup is presented in Figure 2.6.

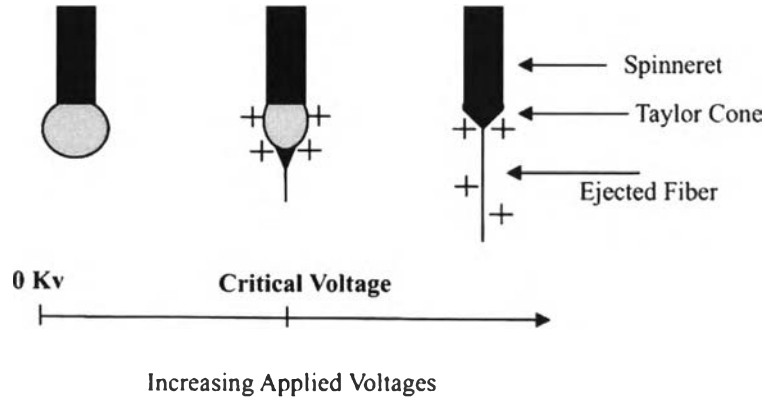

Figure 2.5. Schematic illustration of a Taylor cone formation with increase in applied voltages [12].

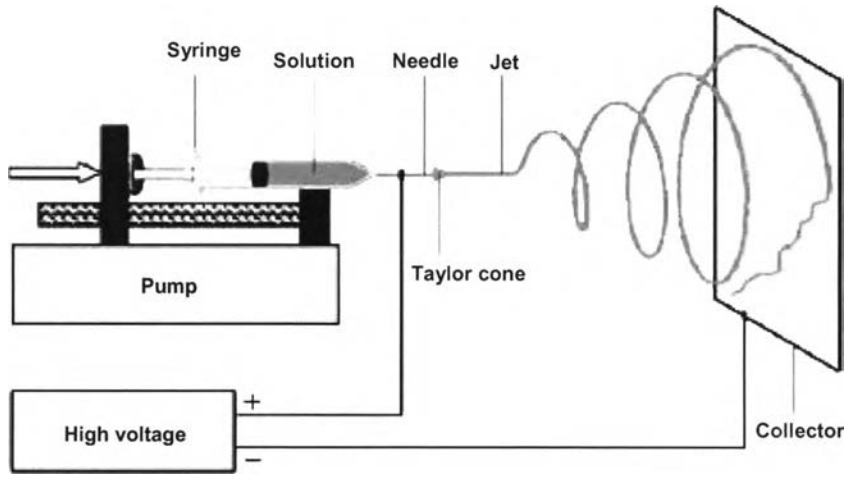

Figure 2.6. Schematic diagram of the electrospinning process [13].

To the best of our knowledge, there are a few research publications reported the use of electrospun PNIPAAm fibers for biomedical applications.

In 2012, Sharma *et al.*[14] prepared dual stimuli-sensitive polymeric nanofibers of polyaniline-carbon nanotube/poly(*N*-isopropyl acrylamide-*co*-methacrylic acid) (PANI-CNT/PNIPAAm-*co*-MAA) composite nanofibers as a conducting smart tissue engineering scaffold using electrospinning. Cellular response of the nanofibers was studied with mice L929 fibroblasts. PANI-CNT/PNIPAAm-*co*-MAA composite nanofibers supported highest cell growth and cell viability as compared to PNIPAAm-*co*-MAA nanofibers. Cell viability of 98% on the composite nanofibers

indicating that the composite nanofibers provides a better environment as a 3D scaffold for the cell proliferation and attachment suitable for tissue engineering applications.

In 2014, Hee Oh *et al.*[15] fabricated thermoresponsive polystyrene (PS) nanofibrous mats by electrospinning method and subsequent surface graft polymerization of poly(*N*-isopropylacrylamide) PNIPAAm by electron beam irradiation. Cells were well attached and proliferated on nanofibrous PS mat more than flat PS dish surface. Moreover, cells were detached more rapidly on PNIPAAm-grafted PS nanofiber surfaces than PNIPAAm-grafted PS dish surfaces probably due to the effective water supply via existing pores on nanofibers. From this view point, PNIPAAm-grafted nanofibrous mats could be a promising tool to recover intact cultured cells.

Bioengineering applications of PNIPAAm are significantly limited because of the simple structure of the PNIPAAm which does not contain any reactive functional groups that are capable of forming chemically incorporated bioconjugates with biomolecules. In order to immobilize bioactive molecules onto the surface of PNIPAAm, their surfaces must be modified to introduce active functional group for functionalization. For this reason, copolymers of PNIPAAm having multiple reactive units along their backbone should be useful precursors for multifunctional polymer preparation.

In 2003, Smith *et al.*[16] synthesized *N*-isopropylacrylamide (NIPPAm)-based thermoreversible polymers containing amine-reactive *N*-acryloxysuccinimide (NAS) groups. The NAS-containing polymers were cast as films on tissue culture polystyrene. The triamino acid sequence, arginine-glycine-aspartic acid (RGD) was then immobilized on the film. The grafted peptides support C2C12 cell attachment, as evidenced by increased cell attachment and spreading on NIPPAm surfaces conjugated with the peptide. NAS-containing thermoreversible polymers are amenable for grafting biomimetic peptides to impart cell adhesiveness to the polymers.

Designing and producing three-dimensional (3D) of functional polymer nanofibrous structure via electrospinning was investigated by Gentsch and coworkers

in 2010 [17]. They successfully prepared reactive fiber meshes of poly(pentafluoro-phenyl methacrylate) (PPFPMA) and PPFPMA/poly(ε-caprolactone) (PCL) blends, resulting in reactive nanofibers with fiber surfaces that can be functionalized with suitable bioactive entities (Figure 2.7). This fiber mesh is a versatile platform for simple immobilization of sugar molecules via polymer analogous reaction. Meshes functionalized with mannose specifically enhance the cytokine production of macrophages.

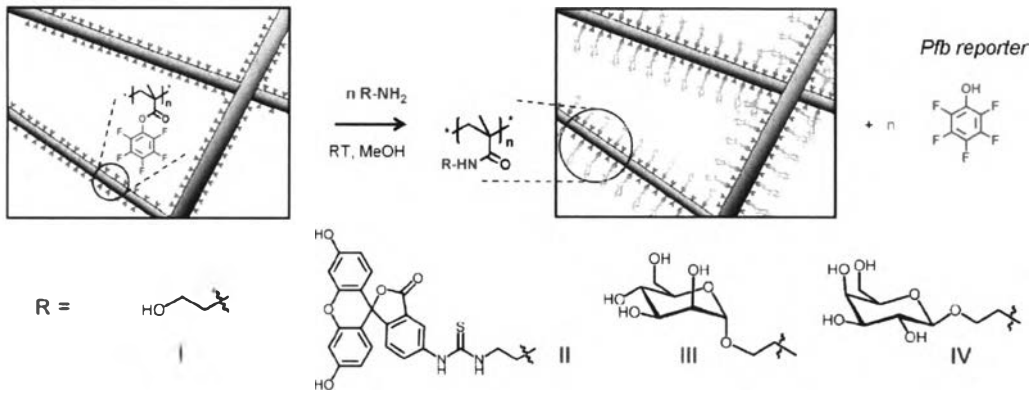

Figure 2.7. Functionalization of fiber meshes bearing activated esters at the surface allows for the introduction of different amino functional molecules [17].

2.5 Reversible addition-fragmentation chain transfer (RAFT) polymerization

In recent years, reversible addition-fragmentation chain transfer or RAFT polymerization, one kind of controlled radical polymerization, is attractive for development of living radical polymerization. RAFT polymerization has been used successfully for synthesizing a wide range of polymers with controlled molecular weight and low polydispersity index (PDI) [18-20]. Some monomers capable of polymerizing by RAFT include styrenes, acrylates, acrylamides as well as a range of other vinyl monomers. In addition, the RAFT process allows the synthesis of variety macromolecular architectures such as block, gradient, statistical, comb/brush, star, hyperbranched, and network copolymers as well as ATRP system.

RAFT is a type of living polymerization involving a conventional radical polymerization in the presence of a reversible chain transfer reagent (CTA). The CTA

or RAFT agents such as dithioesters, thiocarbamates, and dithiocarbonates (xanthates), are used to be intermediate in the polymerization via a reversible chain-transfer process. The general structure of CTA is shown in Figure 2.8. The Z group serves to activate or deactivate the reactivity of the C=S bond towards addition. The R group, a homolytic leaving group, must form a stable free radical. Like other living polymerizations, there is no termination step in the RAFT process. It is a very versatile method to form low polydispersity polymer from monomers capable of radical polymerization. The reaction is usually done with a dithioester. There are four steps in RAFT polymerization: initiation, addition-fragmentation, re-initiation and equilibration (Figure 2.9).

1) Initiation: The reaction is started by radical initators such as AIBN. In this step, the initiator (I) reacts with a monomer unit to create radical species which start active polymerizing chain.
2) Addition-Fragmentation: The active chain (P_n) reacts with the dithioester, which kicks out the homolytic leaving group (R). This is a reversible step, with an intermediate species capable of losing either the leaving group (R) or the active species (P_n).
3) Re-initiation: The leaving group radical then reacts with another monomer species, starting another active polymer chain. This active chain (P_m) is then able to go through the addition-fragmentation or equilibration steps.
4) Equilibration: This is the fundamental step in the RAFT process which traps the majority of the active propagating species into the dormant thiocarbonyl compound. This limits the possibility of chain termination. Active polymer chains (P_m and P_n) are in an equilibrium between the active and dormant stages. While one polymer chain is in the dormant stage (bound to the thiocarbonyl compound), the other is active in polymerization.

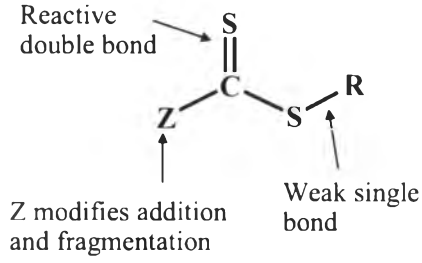

Figure 2.8. General form of RAFT chain transfer agents [20].

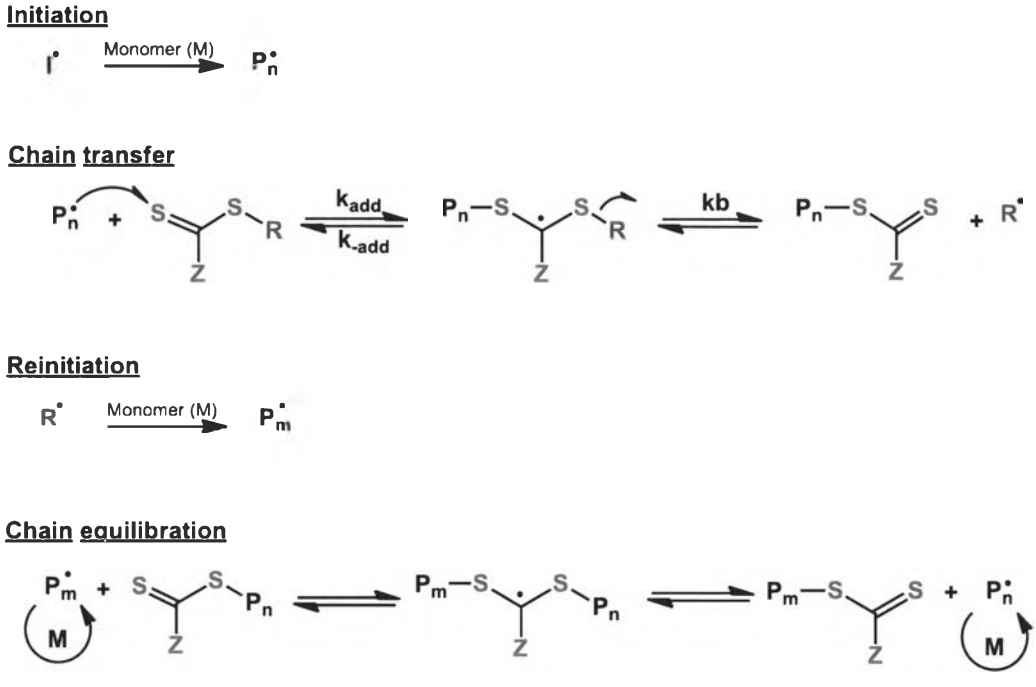

Figure 2.9. Mechanism of RAFT [20].

By controlling the concentration of initiator and CTA, it is possible to produce controlled molecular weight with low polydispersities. In RAFT polymerization, the concentration on the active species is kept low relative to the dormant species by controlling the amount of initiator and capping agent. This in turn will limit termination steps such as radical combination and disproportionation, increasing the polymer length.

First investigations of the RAFT polymerization of the copolymerization of NAS with *N,N'*-diethyl acrylamide was successful under RAFT conditions [21]. A good

control of molecular weight (5 kDa < M_n <130 kDa), narrow molecular weight distributions (PDI < 1.1), and reasonable yields of up to 70% were achieved. In similar experiments, Kane and coworkers [22] reported on the RAFT copolymerization of NMAS with *N*-(2-hydroxypropyl) methacrylamide in a wide range of molecular weights (M_n = 3-50 kDa) with narrow molecular weight distributions (PDI = 1.1-1.3), which may be used in polymeric therapeutics. Accordingly, block copolymers were synthesized by RAFT polymerization of *N,N'*-dimethyl acrylamide (DMA) to yield a first block with a molecular weight of M_n = 44.3 kDa, which had been used as a polymeric chain transfer agent for the RAFT polymerization of NAS (M_n = 7.4 kDa) [23]. Savariar and Thayumanavan [24] reported a successful copolymerization of NHSA with *N*-isopropyl acrylamide under RAFT polymerization.

A controlled RAFT homopolymerization of NAS or NMAS still causes problems, probably due to the limited solubility. An alternative approach resembles the polymerization of pentafluorophenyl acrylates (PFPA) and -methacrylates (PFPMA) [5]. For example, pentafluorophenyl methacrylate could be polymerized under RAFT conditions using cumyl dithiobenzoate or 4-cyano-4-((thiobenzoyl)sulfanyl)-pentanoic acid with a very good control over molecular weight (M_n up to 17 kDa) and narrow molecular weight distributions (M_w/M_n = 1.2) [25]. The excellent solubility of the resulting poly(pentafluorophenyl methacrylate) (PPFPMA) in common organic solvents led to the successful synthesis of diblock copolymers using RAFT polymerization.