
Self-Assembling of Thermally Responsive Block and Graft Copolymers in Aqueous Solutions

Janne Virtanen

University of Helsinki
Department of Chemistry
Laboratory of Polymer Chemistry
P.B. 55, FIN-00014 University of Helsinki
Finland

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PHILOSOPHY**

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Opponent

Professor Eric Goethals
Department of Organic Chemistry
University of Ghent
Belgium

Reviewers

Professor Carl-Eric Wilén
Laboratory of Polymer Technology
Åbo Akademi University
Finland

and

Professor Françoise Winnik
Faculty of Pharmacy
University of Montreal
Canada

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Preface

The work for this thesis started in 1998 in the Laboratory of Polymer Chemistry, Department of Chemistry, University of Helsinki, Finland, and was completed in the next millennium, in December 2001.

I would like to express my greatest gratitude to my supervisor and the Head of the Laboratory, Professor Heikki Tenhu for giving me the opportunity to be involved in this project with many challenges. Through this work I have learned many indispensable pieces of knowledge of modern science. I am truly indebted to his valuable scientific guidance and encouragement which he has endlessly provided. I would also like to thank him for his enormous support and friendship not only in the laboratory but also in everyday life.

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Janne Virtanen

Abstract

Various thermally responsive block and graft copolymers based on poly(*N*-isopropylacrylamide) (PNIPA) have been synthesised. Poly(ethyleneoxides) (PEO) with different chain lengths were used as hydrophilic blocks and grafts. The graft copolymers were synthesised in two steps. Firstly, two functional copolymers were prepared by free radical polymerisation of *N*-isopropylacrylamide (NIPA) with either glycidylmethacrylate (GMA) or *N*-acryloylsuccinimide (NASI). The reactivity ratios of NIPA and GMA were determined by two different methods. Secondly, an amino terminated PEO was allowed to react with the functional copolymers either in an organic solvent or in water, resulting in various amounts of side chains on the PNIPA backbone. Block copolymers were prepared by free radical polymerisation of NIPA using two PEO substituted macroazoinitiators. The copolymers exhibit reversible thermosensitivity in aqueous solutions typical to PNIPA, being completely water soluble at low temperatures but phase separate at the lower critical solution temperature (LCST) upon heating. In addition, a block copolymer of NIPA with a zwitterionic sulfobetaine, *N,N*-dimethyl-*N*-3-methacrylamidopropyl ammoniopropane sulfonate (SPP) was studied, which showed double thermosensitive properties precipitating upon cooling at the upper critical solution temperature (UCST) owing to the PSPP block, and upon heating at the LCST owing to the PNIPA block.

All the graft and block copolymers, PNIPA-*g*-PEO, PNIPA-*b*-PEO and PNIPA-*b*-PSPP, formed spherical core-shell structures in dilute aqueous solutions at their critical temperatures. The factors determining the coil-to-globule transition of the thermosensitive part include hydrophobic interactions, i.e. intra- and interchain interactions, and the solubilising effect of the hydrophilic shell on the shrinking backbone. Thermal properties of aqueous polymer solutions were studied by the combination of static and dynamic light scattering, fluorescence, EPR and UV spectroscopies, and also by viscosimetry.

The LCST of the PNIPA-PEO block and graft copolymers critically depended on the amount of PEO and on polymer concentration. Above the critical temperature, the size, size distribution and structure of the nanoscopic aggregates sterically stabilised by the PEO shell can be adjusted by various factors such as the amount of PEO, polymer concentration and the heating rate of the sample. Two copolymers grafted in water either far below or close to the LCST of the backbone turned out to be able to partly remember the original conformation of the parent backbone copolymers in which they had been grafted. The resulting copolymer solutions and the localisation of the fluorescent and spin probes inside copolymers were dependent on both the chemical nature of the probes and the method of the sample preparation.

Thermal properties of aqueous PNIPA-*b*-PSPP solutions can be influenced by the addition of sodium chloride. Salt enhances the water solubility of polysulfobetaine but on the other hand the solvent becomes poorer for PNIPA in the course of addition of salt. At room temperature, gradual addition of salt turns the mutual interactions from zwitterionic attractions between PSPP blocks to hydrophobic attractions between PNIPA blocks. At a certain concentration of salt the thermosensitivity of the PSPP block disappears. In pure water below the UCST, the block copolymers PNIPA-*b*-PSPP form loosely packed aggregates sterically stabilised by PNIPA shell whose size depends not only on the salt

concentration but also to some extent on the polymer concentration. Upon heating, the critical temperature and the size of the aggregates with a narrow size distribution were notably influenced by both the polymer and salt concentrations.

Abbreviations and symbols

Abbreviations

5-DSA	5-doxylosteoric acid
16-DSA	16-doxylosteoric acid
4HP	4-(dicyanomethylene)-2-methyl-6-(p-dimethylaminostyryl)-4H-pyran
AIBN	Azobis-(isobutyronitrile)
DLS	Dynamic laser light scattering
DSC	Differential scanning calorimetry
EPR	Electron paramagnetic resonance spectroscopy
ex. K-T	Extended Kelen-Tüdös method
F-R	Fineman-Ross method
GMA	Glycidylmethacrylate
HO-PEO	Poly(ethyleneglycolmonomethylether)
LCST	Lower critical solution temperature
MAI	Macroazoinitiator
NASI	N-acryloylsuccinimide
NH ₂ -PEO	Amino terminated poly(ethyleneoxide)
NIPA	N-isopropylacrylamide
PEO	Poly(ethyleneoxide)
PNIPA	Poly(N-isopropylacrylamide)
PNIPA- <i>b</i> -PEO, NE	Block copolymer of poly(N-isopropylacrylamide) and poly(ethyleneoxide)
PGMA	Poly(glycidylmethacrylate)
PNIPA- <i>b</i> -PSPP	Block copolymer of poly(N-isopropylacrylamide) and poly(N,N-dimethyl-N-3-methacrylamidopropyl ammoniopropanesulfonate)
PNIPA- <i>co</i> -GMA and N- <i>co</i> -G	Copolymer of N-isopropylacrylamide and glycidylmethacrylate
PNIPA- <i>co</i> -NASI	Copolymer of N-isopropylacrylamide and N-acryloylsuccinimide
PNIPA- <i>g</i> -PEO	Graft copolymer of poly(N-isopropylacrylamide) and poly(ethyleneoxide)
PSPP	Poly(N,N-dimethyl-N-3-methacrylamidopropyl ammoniopropanesulfonate)
RAFT	Reversible addition fragmentation chain transfer polymerisation
SEC	Size exclusion chromatography
SLS	Static laser light scattering
SPP	N,N-dimethyl-N-3-methacrylamidopropyl ammoniopropanesulfonate
UCST	Upper critical solution temperature

Symbols

a_N	Hyperfine coupling constant
A_2	Second virial coefficient
C_p	Polymer concentration
C_s	Salt concentration
dn/dc	Refractive index increment
D_f	Fast diffusion coefficient
D_s	Slow diffusion coefficient
E_a	Activation energy
f	Initial comonomer feed composition
F	Instantaneous copolymer composition
$I_{E,max}$	Maximum of the emission intensity
M_n	Number average molar mass
M_w	Weight average molar mass
r	Reactivity ratio
R_g	Radius of gyration
R_h	Hydrodynamic radius
R_p	Polymerisation rate
T	Temperature
w	Weight fraction of the polymer
X_2	Mole fraction of the polymer
λ_{max}	Wavelength of the maximum of the emission intensity

List of Original Publications

The thesis is based on the following six papers, hereafter referred to in the text by their Roman numerals (I-VI), but also contains some unpublished material.

- I. **Grafting of Poly(N-isopropylacrylamide) with Poly(ethyleneoxide) under Various Reaction Conditions**, Virtanen, J.; Baron, C.; Tenhu, H. *Macromolecules* **2000**, *33*, 336-341.
- II. **Thermal Properties of Poly(N-isopropylacrylamide)-g-poly(ethyleneoxide) in Aqueous Solutions: Influence of the Number and Distribution of the Grafts**, Virtanen, J.; Tenhu, H. *Macromolecules* **2000**, *33*, 5970-5975.
- III. **Fluorescence and EPR Studies on the Collapse of Poly(N-isopropylacrylamide)-g-poly(ethyleneoxide) in water**, Virtanen, J.; Lemmetyinen, H.; Tenhu, H. *Polymer* **2001**, *42*, 9487-9493.
- IV. **Studies on Copolymerization of N-isopropylacrylamide and Glycidyl Methacrylate**, Virtanen, J.; Tenhu, H. *J. Polym. Sci.: Part A: Polym. Chem.* **2001**, *39*, 3716-3725.
- V. **Dissolution and Aggregation of a Poly(NIPA-*block*-sulfobetaine) Copolymer in Pure and Saline Aqueous Solutions**, Virtanen, J.; Arotçaréna, M.; Heise, B.; Ishaya, S.; Laschewsky, A.; Tenhu, H. *Langmuir*, submitted.
- VI. **Aggregation in Aqueous Poly(N-isopropylacrylamide)-*block*-poly(ethyleneoxide) Solutions Studied by Fluorescence Spectroscopy and Light Scattering**, Virtanen, J.; Holappa, S.; Lemmetyinen, H.; Tenhu, H. *Macromolecules*, submitted.

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1. Introduction

1.1. Background

In nature, as well as in chemical laboratories, the self-organisation of amphiphilic copolymers in water can be controlled through careful polymer synthesis. The synthesis of amphiphilic block and graft copolymers is of special interest due to the unique capability of these copolymers to form stable aggregates with a core-shell structure in solution.¹⁻⁵ Amphiphilic copolymers with a hydrophobic backbone grafted with hydrophilic branches undergo self-assembling through precipitation or solvent exchange, and form stable colloidal particles in water.⁶ In the case of well-defined block copolymers, the size of the core and the dimension of the shell (or corona) in the micelles can be controlled by varying the block lengths of the copolymer.

Materials which respond in a discontinuous manner to continuous environmental changes have become of major interest.⁷⁻¹² Among this type of intelligent materials, poly(N-isopropylacrylamide) (PNIPA) is one of the most studied responsive polymers which exhibits a lower critical solution temperature (LCST) in water around 32 °C. PNIPA shows a reversible demixing with water within a range of a few degrees (1-2 °C) around the critical temperature. The copolymers of PNIPA containing blocks or grafts well soluble in water even above the LCST of PNIPA, form core-shell nanosized particles in aqueous solutions at elevated temperature.

Polymeric micelles and nanoparticles are potential materials for pharmaceutical applications.¹³⁻¹⁵ The core-shell structure of such self-assemblies is ideal to targeted and self-regulating drug delivery. A commonly used component in such polymeric carriers is poly(ethyleneoxide) (PEO), owing to its biocompatibility. In biochemical applications, surface properties of particles are often modified utilising the physicochemical properties of PEO, like low protein and cell adhesion in aqueous systems.¹⁶

Recent theoretical investigations suggest that copolymers consisting of structural units with varying solubilities may remember the conformation in which they have been synthesised.¹⁷⁻¹⁹ In this sense, amphiphilic copolymers mimic biopolymers and, in certain cases, globular proteins. Recently, Khokhlov *et al.*¹⁷ have introduced the concept of “protein-like copolymers”. This model describes the “memory” of an amphiphilic copolymer: the polymer tends to retake the conformation in which it was synthesised. An experimental verification of the concept is a challenging task for a polymer scientist.

1.2. Review

1.2.1. Thermoresponsive polymeric materials

Intelligent, stimuli-responsive polymers are polymers which undergo relatively large and sharp physical changes with small continuous changes in the environment. Recent interest in intelligent polymer systems has focused on aqueous polymer solutions, interfaces and hydrogels. In fact, the physical properties of water determine the conformation and the subsequent reactions of biopolymers responsible for life on Earth.²⁰

Intelligent polymers of special concern in this work are those which in aqueous solutions phase separate upon increasing temperature. Their transition from hydrophilic to hydrophobic substances occurs abruptly at a lower critical solution temperature (LCST). Thermodynamic requirements for the LCST were developed by Prigogine and DeFay.²¹ Briefly, at the LCST

$$(\delta^2\Delta H/\delta X_2^2)_{T,P} > 0, \quad T(\delta^2\Delta S/\delta X_2^2)_{T,P} > 0, \quad \Delta G > 0$$

where ΔG , ΔH and ΔS are excessive over ideal free energy, enthalpy and entropy of mixing the polymer with water, respectively. X_2 is the mole fraction of the polymer. It is assumed that the excess functions do not change sign. The above requirements imply that upon mixing

$$\Delta G > 0, \quad \Delta H < 0 \quad \text{and} \quad \Delta S \ll 0$$

Mixtures that positively deviate from Raoult's law, exothermically and with notable losses in entropy on formation, may exhibit an LCST.²²

Most of the synthetic thermoresponsive polymers which show the LCST behaviour belong to three types of polymers, namely poly(N-substituted (meth)acrylamides), poly(N-vinyl-N,N-disubstituted amides) and ethylene oxide –containing polymers. A general rule for LCST phenomenon of aqueous polymer solutions was developed by Taylor and Cerankowski in 1975:²²

As a polymer which is soluble in water at all temperatures is made increasingly hydrophobic, before complete water insolubility is reached, a range of compositions will be found which will have temperature inverse solubility, and the more hydrophobic the increment, the lower the LCST.

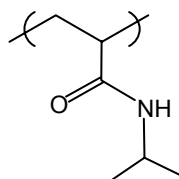
Thus, by substituting a water soluble polymer with hydrophobic groups a polymer with a LCST may be obtained, owing to entropic reasons. For instance, the entropy change upon transferring a methylene group from an organic solvent to water is around -20 J/Kmol which contributes significantly to the free energy via the term $T\Delta S$.

The LCST behaviour in aqueous polymers solutions is based on the ordering of solutes resulting from specific orientations required to hydrogen bond with the partially arranged water molecules.²³ The hydrogen bonds formed between the polar groups in the polymer and water molecules are the initial driving force for dissolution. Water molecules which are incapable to hydrogen bond with nonpolar domains of solutes have to reorientate around those regions. This phenomenon is known as the hydrophobic effect and results in

a decreased entropy upon mixing.^{24,25} At higher temperatures, the entropy term dominates over the exothermic enthalpy of the hydrogen bond formation. Therefore, the free energy change of the mixing becomes positive and phase separation takes place. The replacement of polymer-water contacts with polymer-polymer and water-water contacts is manifested by precipitation.

1.2.2. Poly(N-isopropylacrylamide), PNIPA

Poly(N-isopropylacrylamide) is a thermally responsive polymer with LCST behaviour.²⁶⁻³⁰



Poly(N-isopropylacrylamide)

The earliest report of the temperature-dependent solubility of PNIPA was published in 1963.³¹ The authors merely noted the gradual decrease in solubility with increasing temperature. The abruptness of the phase separation of PNIPA was first reported in 1967 by Scarpa *et al.*³² A report which is often cited in literature was published by Heskins and Guillet in 1968.³³ The authors observed visually the macroscopic phase separation upon heating. This method of determining the LCST is commonly known as the cloud point method.³⁴ A recent study on the demixing of water and linear PNIPAs with varying chain lengths was published by Afroze *et al.*³⁵ The authors studied the dependence of the critical temperature on polymer concentration by optical and calorimetric techniques. Within the range of the weight fraction of PNIPA from 0.4 to 0.5, a minimum in the LCST with a broad miscibility gap independent of chain length was observed. The dilution of the solution shifts the cloud point to higher temperatures, the effect being more pronounced with a decreasing chain length.

The first appearance of PNIPA in literature is found in 1956 dealing with the synthesis and polymerisation of the corresponding monomer N-isopropylacrylamide, NIPA.³⁶⁻³⁸ Ever since, the study of PNIPA in different forms such as single chains,³⁹⁻⁴⁴ microgels,⁴⁵⁻⁴⁸ macroscopic gels,⁴⁸⁻⁵³ latexes,⁵⁴⁻⁵⁷ membranes,⁵⁸ coatings⁵⁹ and fibres^{60,61} has brought together a wide range of disciplines encompassing chemistry, physics, rheology, biology, biotechnology, pharmacy and photography. Thermal properties of PNIPA can be modified by an introduction of hydrophilic groups, e.g. acrylamide,⁶² (meth)acrylic acid,⁶³ and/or hydrophobic groups, e.g. (meth)acrylates,⁶⁴ to the PNIPA chain. Also, different cosolutes, e.g. hydrophilic or hydrophobic low and high molar mass compounds, amphiphiles and salts, have an influence on the LCST of PNIPA.⁶⁵⁻⁷² These modified PNIPA polymers have been studied for several applications in various fields of material science such as in drug release,^{58,73,74} enzyme and cell immobilisation,^{75,76} treatment of water,⁷⁷ oil recovery,⁷⁸ photoresponse,^{40,79-81} film technology,⁵⁸ flocculation²⁸ and artificial muscle and polymer transducers.³⁰ Homo- and copolymers of PNIPA have been widely studied by spectroscopic,^{27,28,82-89} calorimetric^{82,90-92} and various other techniques.^{91,93-98}

1.2.3. Coil-to-globule transition of a neutral polymer

The first prediction of the coil-to-globule transition of polymers was done by Stockmayer in 1960.⁹⁹ Since then, the theory for this transition of neutral polymers in solutions has been developed by Ptitsyn and Eisner,¹⁰⁰ Lifshitz, Grosberg and Khokhlov.¹⁰¹⁻¹⁰⁵ Experimentally, the coil-to-globule transition of polystyrene ($M_w = 2.6 \times 10^7$ g/mol) in cyclohexane was first observed by Tanaka *et al.* in 1980.^{106,107}

The quality of the solvent employed and the chemical structure of the polymer, i.e. rigid or flexible, determine the conformation of the polymer in solution. With the aid of static and dynamic light scattering, by measuring the ratio of the mean-square radius of gyration, R_g , to the hydrodynamic radius, R_h , of the polymer chain it is possible to determine the conformation of the polymer chain.¹⁰⁸ The ratio R_g/R_h has been shown to have a value around 0.78 for a hard and uniform sphere and ~ 1.5 for a monodisperse random coil. Values higher than 1.5 have been encountered in polydisperse samples. Furthermore, the increasing rigidity of the chain results in higher ratios, even ~ 4 .

According to Flory, in a good solvent a polymer swells due to excluded volume interactions and elastic forces.¹⁰⁹ The former interactions cause the expansion and the latter the contraction of the chain. In this stage, excluded volume interactions prevail and the measure of the thermodynamic quality of the solvent for a polymer, the second virial coefficient, A_2 , is positive. By lowering the solvent quality by an external stimulus like temperature or solvent composition, the system reaches a state, theta point (θ), at which the polymer coil behaves like an ideal coil. The coil at θ point is in its unperturbed state and $A_2 = 0$. A further lowering of the solvent quality induces an attraction of the units of the polymer chain, finally resulting in the precipitation of the polymer from the solvent. At this state, $A_2 < 0$. The above described coil-to-globule transition of a single coil is very difficult to observe experimentally and studies on this phenomenon are restricted to very dilute solutions of monodisperse polymers with a high molar mass. In a more concentrated solution the interchain distances shorten thus increasing the attraction forces between the chains, this leading to the sticking of several collapsed chains together.

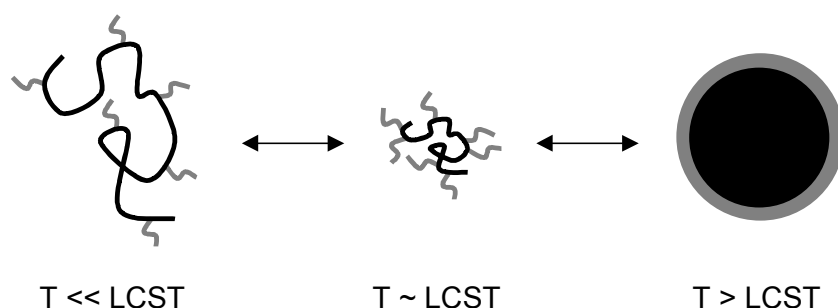
Upon heating the aqueous solution, PNIPA undergoes a coil-to-globule transition at its LCST, i.e. the θ point. In a very dilute solution, the coil-to-globule transition of a single chain of PNIPA has been observed.^{42-44,110} More often, however, a cluster or aggregate formation has been detected.

1.2.4. Polymeric self-assemblies with a micellar core-shell structure

The self-assembling of amphiphilic polymers in water leads to spherical, lamellar, cylindrical and other organised structures depending on polymer concentration and/or solvent composition owing to competing interactions between the polymer segments. In dilute aqueous solutions, the copolymers having hydrophobic and hydrophilic parts form spontaneously polymeric micelles. The association of the hydrophobic parts of the block copolymers occurs at a given polymer concentration, often regarded as a critical micelle or aggregation concentration, which greatly depends on the hydrophilic-lipophilic balance of the polymer. Double hydrophilic block and graft copolymers are materials consisting of water soluble parts with different chemical nature. In an aqueous solution, the interaction

of one or several parts of the copolymer can be triggered by the changes in the surroundings resulting in a spherical core-shell structures as an example.¹¹¹⁻¹¹⁶

The colloidal stability of micelles, liposomes, as well as other particles may be achieved sterically and/or electrostatically. The efficiency of steric stabilisation is based on the surface coverage of the hydrophilic chains on the particle. The surface coverage and the conformation of PEO grafts on spherical hydrophobic polymer particles has been studied.¹¹⁷ PEO chains lie flat on the surface of the particle when the number of PEO chains is low. With increasing number of PEO chains the packing density on the surface increases and the PEO chains extend far into the continuous medium. This change has been termed a pancake-to-brush transition.^{118,119} This model is relevant also in the present work, when considering the aggregate formation of the block and graft copolymers of PNIPA and PEO. Above the LCST the copolymers form spherical particles sterically stabilised by an outer shell of PEO. In these particles PEO chains are stretched out to the aqueous phase, perpendicular to the surface of the PNIPA core. This is schematically demonstrated below in Scheme 1.



Scheme 1. Formation of a core-shell sphere upon heating an aqueous PNIPA-*graft*-PEO copolymer solution. PNIPA (black) and PEO (grey).

1.2.5. Memory effect: The concept of the “protein-like copolymer”

The properties of biomacromolecules like proteins and peptides in living systems are based on their structure. For instance, the activity of enzymes or DNA relies on the tertiary structure, the overall conformation of the polymers. An external stimulus like a temperature raise induces the denaturation of these polymers, and the activity is lost.¹⁰⁵

The essential difference between the monomeric units of globular proteins is that some amino acid residues are hydrophilic or charged while others are hydrophobic. It is generally accepted that in globular proteins the hydrophilic units are mainly covering the surface of the globule thus preventing intermolecular association, while the core of the globule is mainly formed by hydrophobic units. A recently introduced concept of “protein-like copolymers” is an important methodological tool in understanding and mimicking the behaviour of globular proteins.¹⁷ In this model, a copolymer consisting of only two comonomers (A and B) was considered. A condensed homopolymer in its dense globular state (“parent” conformation) was “coloured” into A and B units to have an instant image of such a chain. In the model copolymer, the monomer units residing in the core of a globule were designated as B units and called hydrophobic, while the monomer units on the surface of the “instant image” of a globule were designated as A units and called

hydrophilic. It was of interest to see by computational means whether such a construction of the globule is able to remember its original conformation in which it was built up when the conditions of the system are changed. The coil-to-globule transition of the protein-like AB copolymers turned out to occur at a higher temperature and to be more abrupt than random and random-block copolymers with the same AB composition and degree of blockiness. The authors concluded that the reason for this is that the protein-like copolymer remembers some of the properties of the parent globule, thus making it easier for a dense globule to self-assemble.

Alvarez-Lorenzo *et al.*¹²⁰ have recently reported a way to synthesise a polymer which remembers its original conformation. These authors prepared weakly crosslinked PNIPA hydrogels containing a small amount of methacrylic acid which can form complexes with calcium ions. The gels were synthesised in two ways, by random copolymerisation or by using an imprinting technique. In the latter case, complexes between carboxyl groups and lead ions were formed before the polymerisation. Then these complexes were imprinted into the polymer network during the polymerisation. The capture of calcium ions by the pairs of carboxylic acids in the shrunken imprinted hydrogel was observed to be more effective than in a hydrogel without the preformed complexes.

Lozinsky *et al.*¹²¹ polymerised N-vinyl caprolactam and 1-vinyl imidazole in a DMSO/water mixture at 65 °C, after which the water-soluble part of the product was fractionated. The fraction which could not be precipitated from hot water was concluded to be stabilised by hydrophilic repeating units and to behave like a protein-like copolymer.

1.3. Aim of the study

The purpose of this work was to synthesise PNIPA based thermally responsive block and graft copolymers which upon heating in aqueous solutions form stable nanosized particles. The water solubility and steric stabilisation of the polymers and the nanoparticles was maintained by either a hydrophilic PEO block or graft, or a polysulfobetaine, PSPP, block.

Firstly, functional backbone copolymers were synthesised via free radical copolymerisation of NIPA with either glycidylmethacrylate, GMA, or N-acryloylsuccinimide, NASI. The kinetics of the copolymerisation of the former comonomer pair were studied in detail.

Secondly, the focus was on the influence of the number of PEO grafts and that of the length of a PEO block on the thermal properties of PNIPA in dilute aqueous solutions. The shrinking of the copolymers, and the formation and structures of aggregates or polymeric micelles above the LCST, were studied.

Thirdly, a block copolymer having a PSPP block attached to PNIPA was studied. The aqueous polymer exhibits both an upper critical solution temperature (UCST) and a LCST. The mechanism of the formation of micellar structures below the UCST and above the LCST, was studied.

The functional PNIPA-*co*-GMA copolymer was grafted in water at different temperatures, $T \ll \text{LCST}$ and $T \sim \text{LCST}$. Here, the main interest was to explore the memory effects in the graft copolymer, arising from the different conformations of the parent copolymer during the grafting.

Starting with radical polymerisation reactions, the aim was to prepare complex derivatives of PNIPA with well defined structures. The effects of the details of the molecular structure of the polymers on their capability of self-assembling was studied using mainly various spectroscopic methods and light scattering experiments.

2. Experimental

2.1. Polymer syntheses

2.1.1. Poly(N-isopropylacrylamide)-graft-poly(ethyleneoxide) copolymers: PNIPA-g-PEO

2.1.1.1. *Functional copolymer of N-isopropylacrylamide and glycidylmethacrylate: PNIPA-co-GMA*

Kinetic measurements: The monomers N-isopropylacrylamide (NIPA) and glycidylmethacrylate (GMA) (20 mol% of solvent) and the initiator azobis(isobutyronitrile) AIBN (0.05 mol% of monomers) were dissolved in deuterated 1,4-dioxane, and the mixtures were injected into NMR tubes sealed with septums. The samples were deoxygenated by a freeze-thaw *in vacuo* repeated six times. Molar ratios of the monomers were checked by $^1\text{H-NMR}$ at ambient temperature. Then, the samples were placed into the NMR sample cell at 70°C , and polymerised while recording the $^1\text{H-NMR}$ spectra of the mixtures for 2 hours. The polymerisations were stopped with 0.006 wt% of hydroquinone and cooling to -20°C . The product copolymers were precipitated with diethyl ether, and the precipitates were isolated by centrifugation. Then, in order to remove the residual monomers the copolymers were twice reprecipitated from acetone solutions with diethyl ether, collecting the precipitate by centrifugation. The copolymers were dried overnight in vacuum at room temperature before the analysis.

Functional copolymer for the grafting reactions: NIPA was dissolved in 1,4-dioxane and flushed with nitrogen for 30 minutes. AIBN was added when the mixture reached 70°C . GMA was added dropwise in a period of the first 2 h of polymerisation. The copolymer was precipitated twice into diethyl ether and dried *in vacuo* for 24 hours.

2.1.1.2. *Functional copolymer of N-isopropylacrylamide and N-acryloylsuccinimide: PNIPA-co-NASI*

NIPA and N-acryloylsuccinimide (NASI) were dissolved in 1,4-dioxane and flushed with nitrogen for 30 minutes. The reaction mixture was heated to 70°C and AIBN was added. After 18 h the polymer was precipitated in diethyl ether. The reprecipitation was carried out from acetone into diethyl ether. The copolymer was dried *in vacuo* for 24 h at room temperature.

2.1.1.3. PNIPA-g-PEO copolymers

PNIPA-g-PEO-6/15 and PNIPA-g-PEO-7/29: Two aqueous PNIPA-co-GMA solutions were prepared. The temperature of the reaction mixtures was set to 15 °C and 29 °C, respectively. The stability of temperatures was ensured for 48 h before amino terminated poly(ethyleneoxide), $M_w = 6000$ g/mol, (NH₂-PEO) was added to the reaction mixture. The mixtures were stirred at mentioned temperatures for a week. The number codes indicate the number of grafts/temperature in the grafting reaction.

PNIPA-g-PEO-10: PNIPA-co-GMA and NH₂-PEO were dissolved in 1,4-dioxane and the reaction mixture was stirred at refluxing temperature for 3 days (72 h). The reaction mixture was cooled to room temperature, and diethylamine was added to quench the excess epoxide groups. The mixture was stirred for 2 h. The grafted polymer was isolated by precipitation into diethyl ether.

PNIPA-g-PEO-79, 57, 51 and 43: PNIPA-co-NASI and NH₂-PEO were dissolved in 1,4-dioxane and the reactions were carried out for 5-66 h at a temperature range from 35 °C to the refluxing temperature. After this the reaction mixture was cooled at room temperature. Isopropylamine was added to consume unreacted NASI in a period of 2 h.

All the graft copolymers were purified in dialysis and dried in vacuum.

Table 1. Characteristics of PNIPA-g-PEOs.

sample	comonomer or NH ₂ -PEO		PEO ^{b)}	PEO	$M_w \times 10^{-5}$	M_w/M_n
	in feed	in polymer ^{a)}	<N>	wt%	g mol ⁻¹	
	mol%					
PNIPA-co-GMA	5.0	1.5			1.80 ^{c)}	3.4 ^{e)}
PNIPA-g-PEO-6/15	2.3	0.38	6	17	2.16 ^{d)}	
PNIPA-g-PEO-7/29	2.3	0.44	7	19	2.22 ^{d)}	
PNIPA-g-PEO-10	3.0	0.6	10	25	2.40 ^{d)}	
PNIPA-co-NASI	2.5	4.0			1.90 ^{c)}	
PNIPA-g-PEO-43	5.3	2.5	43	57	4.48 ^{d)}	
PNIPA-g-PEO-51	5.3	3.0	51	61	4.96 ^{d)}	
PNIPA-g-PEO-57	5.3	3.3	57	65	5.32 ^{d)}	
PNIPA-g-PEO-79	5.3	4.0	79	72	6.64 ^{d)}	

a) determined by ¹H-NMR or ¹³C-NMR

b) determined by ¹³C-NMR

c) measured by static laser light scattering

d) calculated by $M_w(\text{backbone polymer}) + \text{number of PEO grafts} \times M_w(\text{PEO})$

e) measured by SEC

2.1.2. Poly(N-isopropylacrylamide)-*block*-poly(ethyleneoxide) copolymers: PNIPA-*b*-PEO

Macroazoinitiators MAI₅₅₀ and MAI₁₉₀₀: The preparation of macroazoinitiators was conducted in two steps. Firstly, azobis(4-cyanopentanoic acid) was converted to the corresponding acyl chloride, 4,4'-azobis(4-cyanopentanoylchloride), by the method of Smith.¹²² ACPA was treated with phosphorous pentachloride in a molar ratio of 1:2 in benzene at room temperature. Secondly, MAI was prepared by condensation reaction of ACPC and poly(ethyleneglycolmonomethylether), $M_w = 550$ or 1900 g/mol, (HO-PEO) in dichloromethane in the presence of an excess of triethylamine for 24 h. MAI₅₅₀ was purified from unreacted HO-PEO and azoinitiator having only one PEO chain by dialysis (cutoff 1000) for 10 days at 5 °C. MAI₁₉₀₀ was purified by ultrafiltration (cutoff 10000).

PNIPA-*b*-PEOs: The copolymers were synthesised using the macroazoinitiators, MAI₅₅₀ and MAI₁₉₀₀, in dioxane at 65 °C for 20 h. The block copolymers were precipitated into diethyl ether. The purification of the copolymers was conducted by centrifugation repeated 5 times in water at 50 °C. The copolymers were dried in vacuum. In the text, the copolymers containing PEO blocks with M_w 550 g/mol or 1900 g/mol are simply called those with a short or a long PEO block, respectively. Block copolymers having an equal PEO block are called a copolymer set.

Table 2. Characteristics of PNIPA-*b*-PEOs.

sample	n(NIPA)	MAI ₁₉₀₀ ^{a)}	MAI ₅₅₀ ^{a)}	solvent	$M_n(\text{PNIPA})^b)$	NIPA/EO ^{c)}
	mmol	mol%	mol%	ml	g mol ⁻¹	
NE-A	17.7	0.01		20	734000	151
NE-B	17.7	0.1		20	334000	69
NE-C	8.8	1		10	61000	13
NE-1	17.7		0.01	20	345000	244
NE-2	17.7		0.1	20	151000	107
NE-3	8.8		1	10	39000	27

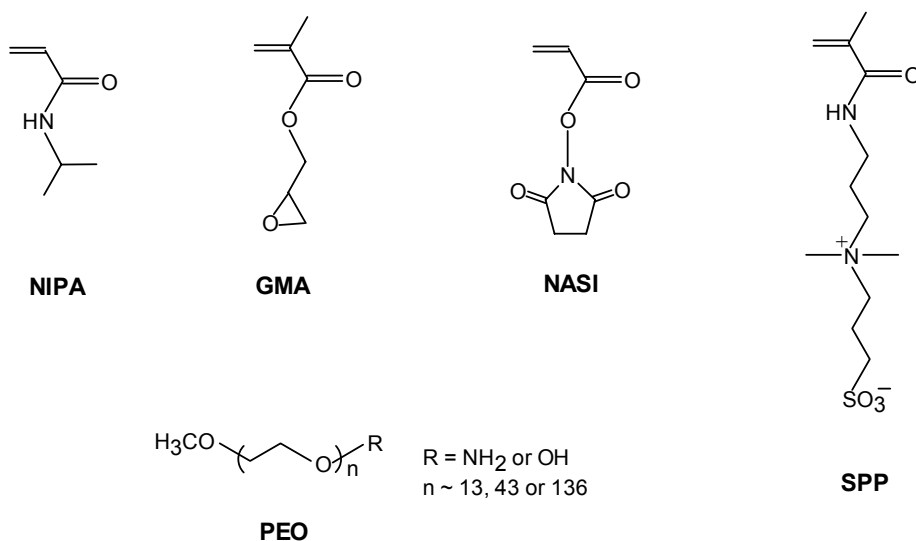
a) concentrations of MAIs are shown as molar percentages of NIPA

b) determined by ¹H-NMR

c) molar ratio of NIPA and EO repeating units in the blocks

2.1.3. Poly(N-isopropylacrylamide)-*block*-poly(sulfobetaine) copolymer: PNIPA-*b*-PSPP

The block copolymer was received from the group of Professor André Laschewsky in Belgium. The copolymer was prepared by a RAFT polymerisation technique first synthesising the PNIPA block. After the purification of the PNIPA block, N,N-dimethyl-N-3-methacrylamidopropyl ammoniopropanesulfonate (SPP) was polymerised to the chain end. The block copolymer was purified by dialysis. Molar masses of the blocks were determined viscometrically and by ¹H-NMR, $M_n(\text{PNIPA}) = 10800$ g/mol and $M_n(\text{PSPP}) = 9700$ g/mol, respectively.



2.2. Preparation of the aqueous polymer solutions

2.2.1. Polymer solutions in water

Aqueous stock solutions of the graft and block copolymers were prepared by dissolving the dry polymer in a known amount of water. No aggregate formation at room temperature was observed after dissolution. All the samples were diluted from the stock solutions and freed from dust by filtration or centrifugation before the measurements.

2.2.2. Solutions containing salt

A known amount of sodium chloride solution was added to an aqueous solution of PNIPA-*b*-PSPP in order to obtain a desired salt concentration. While adjusting the salt concentration, the polymer concentration was kept constant by adding a proper amount of a concentrated polymer solution. Before mixing, the solutions were freed from dust by filtration.

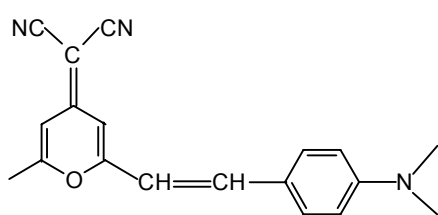
2.2.3. Solutions containing a spin or a fluorescent probe

Samples (1): Aqueous polymer samples were prepared by first dissolving the copolymer and the probe separately in chloroform. Known amounts of polymer and probe solutions were carefully mixed, and the solvent was evaporated with a stream of air. Then, known volumes of water were added on the thin films of polymer containing the probe. The solutions with 4-(dicyanomethylene)-2-methyl-6-(p-dimethylaminostyryl)-4H-pyran (4HP) were kept in the refrigerator for 24 h, and the ones with 5-doxylstearic acid (5-DSA)

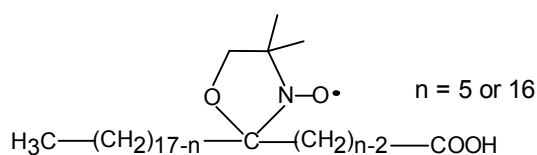
and 16-doxylstearic acid (16-DSA) at room temperature for a week for complete dissolution. The resulting samples were studied by dynamic light scattering (DLS). At room temperature, the polymers formed aggregates independent of whether the sample contained the probe or not. Therefore, the aggregation does not solely depend on the nature, i.e. hydrophobicity, of the probe but the method of the sample preparation plays an important role in the intermolecular binding of the polymers. In a film formation during the evaporation of chloroform, hydrogen bonds may form between the polar groups of the polymers, this leading to aggregates.

Samples (2): A known amount of the probe dissolved in chloroform or ethanol was placed into a vessel or in 1 mm o.d. glass capillary and the solvent was evaporated. The aqueous copolymer solution with a fixed polymer concentration was added. The solutions with 4HP were kept in a refrigerator for 3 days in order to dissolve an equilibrium amount of the probe in the polymer solution. The samples with 5-DSA were diluted with the polymer solutions until the broad singlet line due to the insolubilised probe could not longer be detected in the EPR spectrum. At room temperature these samples showed no aggregate formation by DLS.

The water used for all the measurements was purified and deionised in an Elgastat UHQ-PS purification system.



4HP



5-DSA or 16-DSA

2.3. Heating rates of the samples

In the slow heating and cooling, the samples were equilibrated at each temperature before measurement. The heating rates 0.1 and 0.2 °C/min were mainly used.

In the fast heating and cooling, the samples were placed from ambient temperature into a preheated or precooled sample holder and equilibrated at least for 20-25 minutes before measurement.

2.4. Instrumentation

¹H- and ¹³C-NMR spectra of the synthesised polymers were measured with a 200 MHz Varian Gemini 2000 spectrometer using deuterated chloroform^{I-III, VI} or deuterated acetone^{IV} as a solvent. Kinetic measurements were conducted at 70 °C in deuterated dioxane with a 300 MHz Varian ^{UNITY}INOVA spectrometer.^{IV}

Static light scattering (SLS)^{I,II,IV,VI} and dynamic light scattering (DLS)^{I-III,V,VI} measurements were conducted with a Brookhaven Instruments BI-200SM goniometer and a BI-9000AT digital correlator. The light sources were a Spectra Physics Model 127 Helium/Neon laser (632.8 nm, 35 mW)^{I,II} and a Lexel 85 Argon laser (514.5 nm, power range 15-150 mW)^{III-VI}. The refractive index increments were calculated using the equation¹²³

$$dn/dc = w_A(dn/dc)_A + w_B(dn/dc)_B$$

in which w_A and w_B are the mass fractions of pure polymers PNIPA and PEO, and $(dn/dc)_A$ and $(dn/dc)_B$ their refractive index increments, respectively. Time correlation functions were analysed with a Laplace inversion program CONTIN.

Fluorescence spectra were recorded using a Spex Fluorolog 3 spectrofluorometer at right angles.^{III,VI} Samples were excited at 469 nm. The slit widths were set at 2.0 nm for both the excitation and emission.

Electron paramagnetic resonance (EPR) spectra were recorded with a Varian E4 spectrometer.^{II,III} Microwave power 1 mW and modulation amplitude 1 G were used.

Phase transition temperatures and the corresponding enthalpy changes of the copolymers were measured with a NANO differential scanning calorimeter (DSC) from Microcal.^{II}

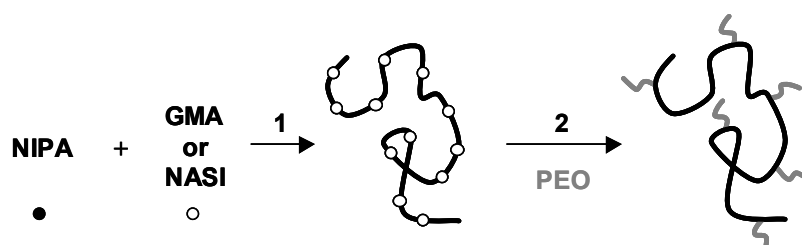
Reduced viscosities of the polymer solutions were measured with a modified Ubbelohde viscosimeter.^{II}

Turbidimetric measurements were conducted with a Shimadzu UV-1601PC spectrophotometer (UV) at a wavelength of 550 nm.^{VI}

Size exclusion chromatograph (SEC) measurements were conducted with a Waters equipment using a Waters 2410 refractive index detector.^{I,IV,VI} Poly(ethyleneoxide)^{VI}, poly(methyl methacrylate)^{IV} and polystyrene^I standards were used for the calibration.

3. Copolymers of poly(N-isopropylacrylamide) grafted with poly(ethyleneoxide)^{I-IV}

Graft copolymers were prepared in two steps, see Scheme 2. Firstly, functional backbone copolymers were synthesised by free radical copolymerisation of NIPA and either GMA or NASI. Secondly, PEO with a terminal amino group was attached to the main chain by a condensation reaction with the functional groups. The monomers NASI and GMA were chosen as functional groups because their reactivity toward primary amine groups is relatively high.¹²⁴⁻¹²⁶ The reactivity ratios of the comonomers determine the distribution of the functional comonomer along the PNIPA chain and thus, the kinetics of the copolymerisation of GMA with NIPA were studied in detail.



Scheme 2. Synthesis of the graft copolymers in two steps: (1) Copolymerisation of NIPA and GMA or NASI, and (2) grafting of PEO to the polymer backbone.

The solution properties of amphiphilic copolymers can be changed by modification with PEO. Several studies on double hydrophilic copolymers containing both PNIPA and PEO have recently been carried out.^{1,142-147} For instance, Qiu *et al.*¹ prepared a PNIPA-*g*-PEO graft copolymer with high molar mass, and also studied the coil-to-globule transition of a single chain. Lin *et al.*¹⁴⁷ prepared various block and star copolymers of PNIPA and PEO. In the investigation of these copolymers, the former group used an extremely low polymer concentration, whereas the latter group used fairly high polymer concentration, ~20 wt%, for the study of the thermal gelation of these copolymers.

In the present work, the graft copolymers with the molar masses of several hundred thousands had broad molar mass distributions (see Table 1). This chapter summarises the thermal properties of the aqueous copolymers, especially focusing on the effect of the number of PEO grafts on the shrinking and the collapse of the PNIPA main chain during heating. The studies were conducted by static (SLS) and dynamic (DLS) laser light scattering, fluorescence and electron paramagnetic resonance (EPR) spectroscopies. Among these techniques, fluorescence spectroscopy owing to its high sensitivity is an ideal method to study dilute solutions of amphiphilic polymers. Especially, the formation of hydrophobic microdomains in water-soluble polymers may well be detected by incorporating fluorescent probes into the samples.^{86,148-152} Similarly, the interactions between paramagnetic probes and the polymers, as well as the mobility of the polymers can be studied by EPR.

3.1. Grafting of poly(N-isopropylacrylamide) with poly(ethyleneoxide)

3.1.1. Kinetic studies on the copolymerisation of N-isopropylacrylamide and glycidylmethacrylate

NIPA and GMA were batch copolymerised in deuterated dioxane, varying the molar ratios of the monomers in the feed. The kinetics of copolymerisations were studied by $^1\text{H-NMR}$ spectroscopy by following the disappearance of the resonance lines of the vinyl protons of the monomers. After the polymerisation reactions, the polymers were precipitated and analysed.

Reactivity ratios were determined using both Fineman-Ross (F-R)¹²⁷ and extended Kelen-Tüdös (ex. K-T)¹²⁸ methods. Values obtained by these two ways were, $r_1=0.39$, $r_2=2.69$ and $r_1=0.30$, $r_2=2.66$, respectively. In both cases, $r_1r_2\sim 1$ which is indicative of an ideal copolymerisation resulting in a first-order Markov distribution of the monomers in the final copolymer. The dependencies of the instantaneous copolymer compositions, F , on the initial comonomer feed compositions, f , are shown in Figure 1. Table 3 shows the calculated copolymerisation rates.

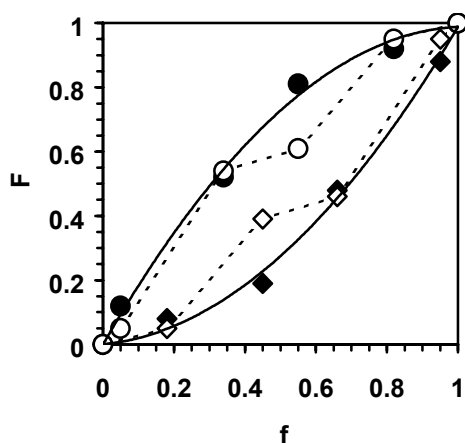


Figure 1. Dependence of the instantaneous copolymer composition, F , on the initial comonomer feed composition, f . Full symbols (circles for GMA and diamonds for NIPA) represent the values obtained from the precipitated polymers, and open symbols represent the values from the $^1\text{H-NMR}$ measurements during the polymerisation.

Table 3. Rates of copolymerisations of NIPA and GMA. Dependence of the individual ($R_{p,NIPA}$ and $R_{p,GMA}$) and overall ($R_{p,tot}$) rates of polymerisation on the molar ratios of monomers in the feed.

sample	feed		$R_{p,NIPA} \times 10^6$ mol s ⁻¹ l ⁻¹	$R_{p,GMA} \times 10^6$ mol s ⁻¹ l ⁻¹	$R_{p,tot} \times 10^{6 a)}$ mol s ⁻¹ l ⁻¹	$R_{p,tot} \times 10^{6 b)}$ mol s ⁻¹ l ⁻¹
	NIPA	GMA				
	mol%					
PNIPA	100	-	1.37	-	1.37	1.37
N-co-G-2	95	5	1.28	1.17	1.98	2.17
N-co-G-3	66	34	1.10	2.73	2.89	3.29
N-co-G-4	45	55	0.75	5.24	4.71	5.29
N-co-G-5	18	82	0.37	7.96	7.32	7.81
PGMA	-	100	-	10.8	10.8	10.8

a) r_1 and r_2 from Fineman-Ross

b) r_1 and r_2 from extended Kelen-Tüdös

Owing to the different reactivities of the two monomers it was concluded that a copolymer with a homogeneous distribution of monomers may be obtained not by a batch reaction, but by slowly and continuously adding GMA into the polymerising reaction mixture. The functional backbone copolymer PNIPA-co-GMA used for the grafting reactions was prepared by the continuous addition of GMA (total amount 5 mol% to monomers) into the reaction mixture resulting in the copolymer with 1.5 mol% of functionality. It was expected that a copolymer where the functional groups were evenly distributed along the PNIPA main chain was obtained.

3.1.2. Grafting reactions

Amphiphilic graft copolymers containing poly(ethyleneoxide) as a hydrophilic graft have been synthesised and studied.^{1,117,129-132} The simplest way to attach PEO grafts to a polymer backbone is by radical copolymerisation of a given monomer with a methacrylate end capped PEO macromonomer. However, this synthetic method often results in branching and crosslinking, and the desired linear graft copolymer is difficult to obtain.^{133,134} This was also the case in this work when NIPA was copolymerised with PEO-methacrylate under different reaction conditions. For instance, the polymerisation in water resulted in gel formation within a few minutes, whereas in THF no polymer or just oligomers were obtained.¹³⁵ Poly(ethyleneoxide) is an effective chain transfer agent in several radical polymerisation reactions.¹³⁶⁻¹³⁸ The prevention of crosslinking and/or branching of any kind in the graft polymer was one of the aims in this work.

The preparation of a well-defined functional polymer and its subsequent substitution with a reactive graft is a way to obtain a graft copolymer without side reactions. The copolymerisation with NIPA and NASI was carried out as a batch reaction, whereas GMA was slowly added in drops into the polymerisation mixture. The latter method was expected to produce a copolymer with a random distribution of GMA units in the backbone.

In the grafting reactions, long reactions times and an excess of NH₂-PEO (see Table 1) were required due to the shielding effect of a long PEO chain around the amino group.¹³⁹ The graft copolymers with varying amounts of PEO chains were synthesised in an organic solvent, except PNIPA-*g*-PEO-6/15 and PNIPA-*g*-PEO-7/29 which were prepared in water. Both PNIPA and NH₂-PEO are well soluble in dioxane whereas in water the solubilities of the polymers depend strongly on temperature. The reaction of NH₂-PEO with NASI proceeded quickly making the adjustment of the number of PEO grafts more difficult than expected. The characteristics of the copolymers are shown in Table 1.

For the following discussion, it is pointed out that the differences in the behaviour of the graft copolymers derived from PNIPA-*co*-GMA and PNIPA-*co*-NASI are due to a considerable difference in the number of PEO grafts but not to the choice of the reactive comonomer.

3.2. Thermal properties of poly(N-isopropylacrylamide)-graft-poly(ethyleneoxide) copolymers in water

3.2.1. Light scattering measurements of the collapse of the copolymers

The functionalised copolymers PNIPA-*co*-GMA and PNIPA-*co*-NASI with a polymer concentration $C_p = 1.0$ g/l phase separated from water at their critical temperatures 31.0 ± 0.1 and 31.8 ± 0.1 °C, respectively. The low LCST of the former copolymer resulted from the hydrophobic nature of the comonomer. The number of the PEO grafts in the PNIPA chain had an influence on the LCST; the critical temperatures of the aqueous solutions with $C_p = 1.0$ g/l for PNIPA-*g*-PEO-6/15, -7/29, -10, -43, and -51, were 32.5, 33.5, 33.8, 33.7 and 34.7 °C, respectively. The collapse of the copolymers became more gradual close to LCST in the course of increasing the amount of PEO. This has been observed also in an investigation on PNIPA-PEO block copolymers and microgels.^{143,144} The increase in LCST with increasing PEO content reflects the competition between the hydrophobic interactions and the solubilising effect of PEO. Although the polymer particles are covered with a PEO shell, PNIPA and PEO can not be totally phase separated owing to the architecture of the polymer.

The LCST of the solutions was also dependent on polymer concentration. The critical temperature of aggregation slightly increased with decreasing C_p , the increase of LCST, however, being more notable as the number of PEO grafts increased. For example, the concentration change from 0.1 to 5.0 g/l caused merely ~ 1.4 °C decrease in the critical temperature of a PNIPA-*g*-PEO-10 solution, whereas in the case of PNIPA-*g*-PEO-43, a smaller variation in concentration, from 0.1 to 1.0 g/l of solutions, induced a difference in the LCST higher than 2 °C. Also, the collapse seemed to be less abrupt with decreasing C_p . The dilution of the solution increases interparticle distances between the polymers, which favours the intramolecular interactions over the intermolecular attractions. On the other hand, the dilution also enhances the surface stabilisation of the polymer particles by PEO.

It has been observed that the heating rate of the sample affects the resulting aggregate size above the LCST.^{1,135} During fast heating, individual polymer chains have less chance to aggregate with each other before the collapse, this resulting in aggregates with smaller size than those induced by the slow heating. However, the differences between the LCST of the copolymers did not depend on the heating rate. The polymer solutions with $C_p = 1.0$ g/l were heated fast to 45 °C and stabilised at that temperature for 2 hours before measurements (see Chapter 2.3.). Interestingly, the average hydrodynamic sizes of the aggregates of the copolymers were similar but their size distribution broadened with increasing amount of PEO. The intensity of the light scattered by the aggregates decreased with increasing PEO content of the polymers, thus indicating that the average density of the particles decreased. This may be due to the capability of PEO to partially prevent the collapse of the PNIPA chains. It is also possible that through the whole sample series the aggregation number, $\langle N \rangle_{\text{agg}}$, decreases as the PEO content increases, as was shown to be the case with two polymers with the lowest number of PEO grafts.

At 45 °C, the $\langle R_h \rangle_{\text{agg}}$, average molar masses, $\langle M_w \rangle_{\text{agg}}$, and $\langle \rho \rangle_{\text{agg}}$ of two copolymers with the lowest number of PEO grafts decreased upon dilution. The values of $\langle R_g \rangle / \langle R_h \rangle$ are close to that of a hard sphere, 0.778. With decreasing polymer concentration the ratio $\langle R_g \rangle / \langle R_h \rangle$ decreases, even below that of a hard sphere. Wu *et al.* studied single PNIPA chains in an extremely dilute solution (~ 5 µg/ml), and stated that the low value for the $\langle R_g \rangle / \langle R_h \rangle$ equal to 0.62 was due to a higher chain density at the center than near the surface.²⁹ This is an obvious conclusion also in the present case, at low polymer concentrations. The differences of these two polymers will be discussed in Chapter 3.3. However, it is concluded that dilution enhances the surface stabilisation of the shrinking chain, thus leading to smaller aggregates.

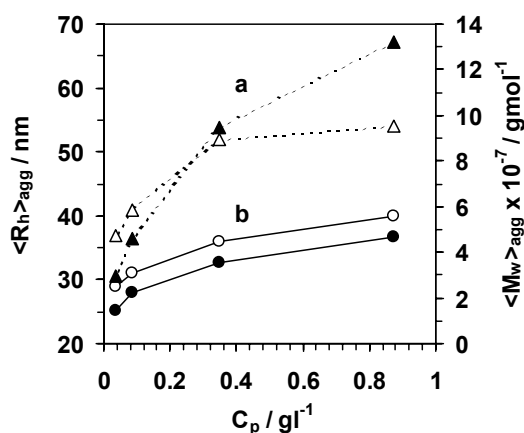


Figure 2. Concentration dependence of the average hydrodynamic radius, $\langle R_h \rangle$, (open symbols) and weight average molar mass, $\langle M_w \rangle$, (full symbols) of the aggregates of (a) PNIPA-g-PEO-6/15 and (b) PNIPA-g-PEO-7/29.

Raising the temperature from 45 °C up to 60 °C resulted in further shrinkage of the aggregates but the $\langle M_w \rangle_{\text{agg}}$ did not change.¹³⁵ Therefore, the PEO shell on the surface of the aggregate was capable of preventing further association. Figure 3 shows the formation of aggregates of PNIPA-g-PEO-51 followed by their shrinking with a gradual raise in temperature. After the aggregates are formed and stabilised with a sufficient amount of PEO, the aggregate core started to compress. Water inside the core was further expelled, and the density of the core increased.

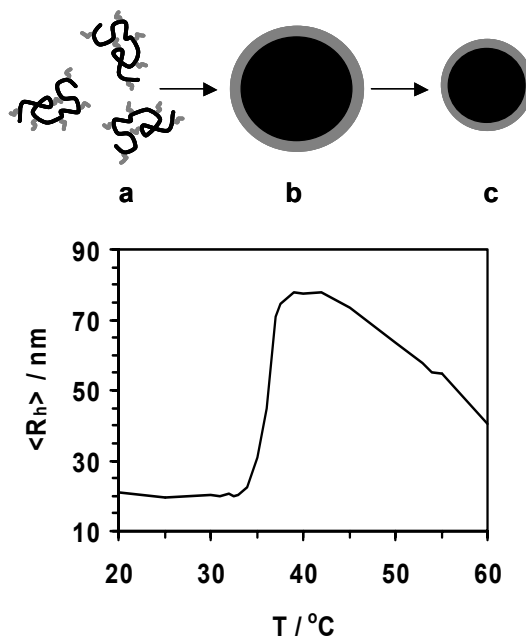


Figure 3. Formation of an aggregate and the dependence of its average hydrodynamic radius, $\langle R_h \rangle$, on temperature. The studied polymer was PNIPA-*g*-PEO-51 with $C_p = 1.0$ g/l. Above, a model describes steps of the formation of the aggregates and its shrinking upon slow heating from (a) 20 °C to (b) 45 °C and to (c) 60 °C.

Some aggregated polymer solutions were diluted with water, keeping the temperature constant at 45 °C. The diluted samples were equilibrated for 24 hours, and $\langle R_h \rangle_{\text{agg}}$ was remeasured. The purpose was to study whether the aggregates once formed decompose or not upon dilution. The sizes of the aggregates only slightly decreased with decreasing C_p which means that the aggregates once formed do not easily reorganise upon dilution. This implies that polymer chains in the aggregates core are kinetically trapped.

3.2.2. Fluorescence spectroscopy of the copolymers

The fluorescent probe 4HP used in this work exhibits an intramolecular charge transfer (ICT) property. 4HP contains both electron donor and acceptor moieties linked by an aromatic chromophore. Because both twisting and charge separation are involved in the formation of intramolecular charge transfer states, the fluorescence emission of 4HP is sensitive both to the solvent polarity and the microviscosity of the medium.¹⁵³⁻¹⁶¹ The sensitivity of 4HP to local changes in its environment makes it possible to study the formation, structure and stability of hydrophobic microdomains in a polymer system. 4HP is practically non-soluble in water but dissolves together with the polymer and localises in the hydrophobic domains of the polymer.

The samples for the fluorescence measurements were prepared by the method for the samples (1), see Chapter 2.3.3. The copolymers formed aggregates at room temperature.

Upon heating, the intensities of the emission bands increased drastically at the phase separation temperatures of the copolymers indicating an increase in the microviscosity of the surroundings of the probe. The effect of light scattering on the emission intensity at $T > LCST$ was observed to be negligible. Firstly, the samples were heated slowly, with a heating rate $0.2\text{ }^{\circ}\text{C}/\text{min}$. Figure 4A shows the maxima of the emission intensity of the probe, $I_{E,\text{max}}$, of different samples as a function of temperature. The increase in the emission intensity is highest in the case of PNIPA-*co*-GMA, and the PEO grafts slow down the increase. It is concluded that the collapse of the aggregate is increasingly prevented by the increasing number of PEO grafts inside the aggregates, this lowering the density of the core. This is well in accordance with the light scattering studies discussed in Chapter 3.2.1. During the compression of the copolymers, the environment around the probe changed to less polar than that at room temperature. This was observed as a shift of the emission bands to shorter wavelengths; above the critical temperature the wavelengths of $I_{E,\text{max}}$, λ_{max} , were around 600 nm for every sample. However, no particular dependence of the shift on the grafting degree was observed.

Secondly, the evolution of the emission bands of the samples was followed with time when the samples were placed to a preheated sample holder at $45\text{ }^{\circ}\text{C}$. In all the samples, the intensity first increased fast, the increase slowing down with time. The changes in the surroundings of the probe decreased with increasing content of PEO, see Figure 4B. Contrary to the slow heating experiments, the shift of the emission bands to shorter wavelengths upon heating was larger as the number of PEO grafts in the copolymer decreased. PEO increases the solubility of PNIPA in water especially at low temperatures, therefore the changes in polarity due to the collapse of PNIPA become more distinct as the amount of PEO increases.

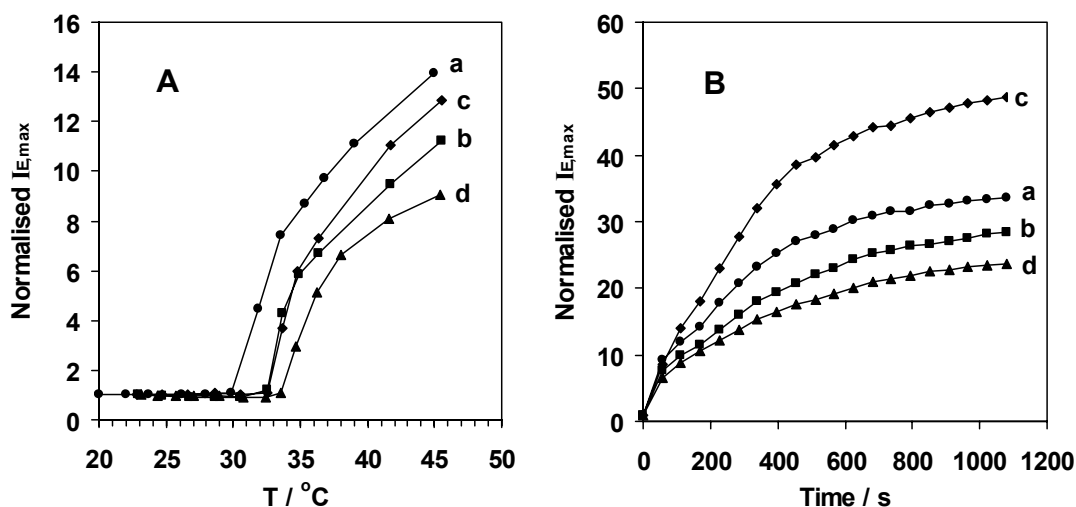


Figure 4. Normalised maximum intensities of the emission spectra of 4HP, $I_{E,\text{max}}$, during (A) slow and (B) fast heating of aqueous polymer solutions: (a) PNIPA-*co*-GMA; (b) PNIPA-*g*-PEO-6/15; (c) PNIPA-*g*-PEO-7/29; (d) PNIPA-*g*-PEO-10.

As the polymer concentration increased the changes in the microviscosity in the vicinity of the probe increased and the time for the stabilisation of the collapse of the polymer extended. This is because the diffusion of water out from the aggregate core determines the rate of collapse and an increase of the polymer concentration slows down the diffusion. Also, increasing the polymer concentration increases the size of the aggregates and thus, the collapse is slower.

3.2.3. EPR spectroscopy of the copolymers

Nitroxide spin probes derived from fatty acids have commonly been used to study organised structures such as lipid bilayer membranes or synthetic liquid crystals.¹⁶² 5-doxydstearicacid and 16-doxydstearicacid used as spin probes are amphiphiles whose solubility in water increases with temperature. The capability of the graft copolymers to solubilise the spin probes at room temperature and during the collapse of the PNIPA main chain was studied. The amphiphilic spin probes were expected to localise into more polar parts of the polymers than the fluorescent probe 4HP.

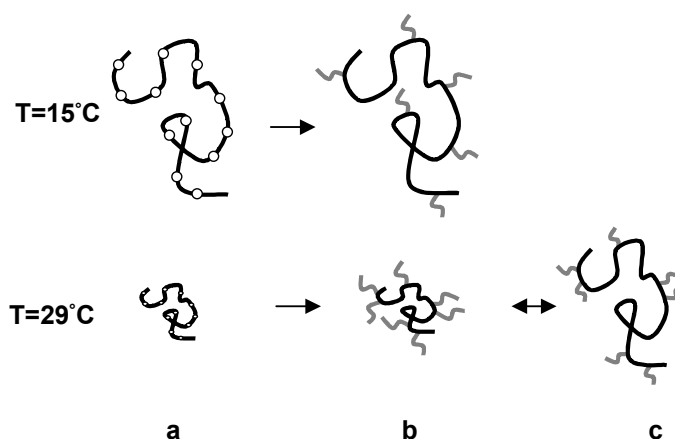
The sample preparation method determines the association behaviour of the polymer as already discussed, but also the localisation of the probe. Baglioni *et al.*¹⁶³ have studied micelles of non ionic detergents containing aliphatic and oligo(oxyethylene) segments. They showed that a doxylstearate is predominantly localised in the oligo(oxyethylene) part of the micelle. Correspondingly in this work, the nitrogen hyperfine coupling constant, a_N , of the samples (1) was 15.6 G, and that of the samples (2) was 16.4 G at room temperature. The former value approaches the coupling constant observed in a single crystal and the latter is close to that typical for nitroxides in water.^{27,88} It is concluded that the radicals are located either in the outer PEO shell of the polymers (a_N 16.4 G) or deeper inside, close to the PNIPA-PEO interface (a_N 15.6 G). The probes 5-DSA and 16-DSA did not show any difference in their coupling constants.

Upon heating, no detectable dissolution of the probe into the hydrophobic core of the collapsed polymer was observed, but all the measured EPR spectra were motionally narrowed ones consisting of three sharp lines. Also, no changes in the a_N values of the probes in either sample set during the collapse was detected.

However, an increase in the rate of rotational motion of the probe was detected. The nitroxides residing in a fairly nonpolar surroundings in the samples (1) showed only a typical thermal activation in the rate of their rotational motion, and gave similar results for every copolymer. Nitroxides close to the aqueous phase in the samples (2), however, showed a discontinuous increase in the rate of rotational diffusion upon increasing temperature. The interpretation of the collapse of the samples (2) will be discussed in Chapter 3.3.4.

3.3. Copolymers grafted in water

Recent theoretical studies suggest that copolymers consisting of structural units with different solubilities may remember their conformations taken during the synthesis.¹⁷ These polymers are called “protein-like copolymers”. Experimentally, this could be realised for instance by imprinting of chemically crosslinked gels during the polymerisation. In this work, the chain conformation of the functional backbone copolymer PNIPA-*co*-GMA during the grafting was expected to have an influence on the distribution of the PEO chains along the backbone. In aqueous solution, depending on the reaction temperature the attachment sites for PEO may be expected to be located either on the surface of the shrunken PNIPA-*co*-GMA chain ($T \sim \text{LCST}$), or randomly throughout the polymer coil ($T \ll \text{LCST}$), see Scheme 3. It is pointed out that the degree of grafting is low compared to the number of reactive groups in PNIPA-*co*-GMA, see Table 1 and thus, different distributions of the substituents in principle are possible. It was of interest to see whether the different distribution of the PEO grafts on the PNIPA main chain influences the thermal properties of the polymer and furthermore, whether the polymer grafted at elevated temperature is capable to retake the compressed conformation in which it was synthesised when aqueous solutions are heated.



Scheme 3. A model of the grafting of functional copolymers by PEO at two different temperatures in water: (a) before grafting; (b) after grafting; (c) at room temperature.

3.3.1. Light scattering measurements of the collapse of the copolymers

The phase separation of the graft copolymers during the slow heating was discussed earlier. It was concluded that the critical temperature of polymer solution increased with increasing content of PEO in the polymer, however, the biggest shift was observed at low grafting degrees. Both copolymers grafted in water, PNIPA-*g*-PEO-6/15 and PNIPA-*g*-PEO-7/29, showed a sharp increase in the scattered light intensity from aqueous solution with $C_p = 1.0 \text{ g/l}$ at 32.5 and 33.5 °C, respectively. One degree difference in the critical temperatures is significant taken into account the low number of grafts (see Chapter 3.2.1.). Therefore, it was assumed that the copolymer grafted at elevated temperature is more effectively stabilised by the PEO grafts close to its LCST than the polymer grafted at lower temperature.

The aqueous solutions of these two copolymers were heated fast to 45 °C. The size of the aggregates was not directly proportional to the number of the PEO grafts. PNIPA-*g*-PEO-7/29 formed the smallest aggregates, and the size distribution of these particles were very narrow at every concentration, see Figure 5.

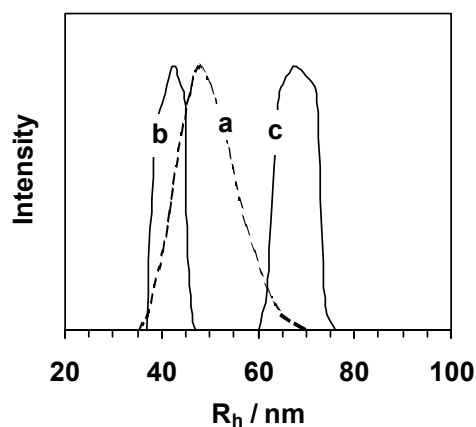


Figure 5. Size distribution of the aggregates at 45 °C. Polymer concentration 1.0 g/l. (a) PNIPA-*g*-PEO-6/15; (b) PNIPA-*g*-PEO-7/29; (c) PNIPA-*g*-PEO-10 is used as a reference though it was grafted in an organic solvent.

These observations support the hypothesis that the distribution of PEO chains along the PNIPA chain is different in every sample. The observed differences in the aggregate sizes indicate that the polymers tend to retake the conformation in which they have been synthesised.

3.3.2. Viscosimetric measurements of the collapse of the copolymers

Aqueous PNIPA-*g*-PEO-6/15 and PNIPA-*g*-PEO-7/29 solutions with $C_p = 1.0$ g/l were studied viscosimetrically as a function of temperature, see Figure 6. Since flow field tends to break the aggregates it is possible to observe the shrinking of the individual chains in the course of the temperature raise. The reduced viscosity, η_{sp}/c , of copolymer solutions approaches zero when the copolymers are in their fully collapsed, compact state and flow freely with water through the capillary. The difference in the LCSTs of the copolymers is parallel with that measured by light scattering. Above the critical temperature, η_{sp}/c increases indicative of the aggregate formation. Notably, at low temperatures the reduced viscosity of the PNIPA-*g*-PEO-6/15 is much higher than that of the PNIPA-*g*-PEO-7/29. This suggests that in a flow field the latter polymer adopts a more compact conformation than the one with fewer PEO grafts. The most obvious reason for this is a different distribution of the grafts on the PNIPA backbone. With increasing temperature it is expected that the PEO chains are more localised on the coil surface in PNIPA-*g*-PEO-7/29 than in PNIPA-*g*-PEO-6/15.

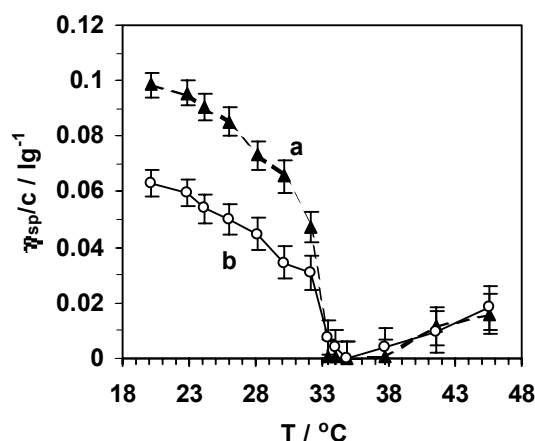


Figure 6. Reduced viscosities versus temperature of aqueous polymer solutions of (a) PNIPA-*g*-PEO-6/15 and (b) PNIPA-*g*-PEO-7/29. Polymer concentration 1.0 g/l.

3.3.3. Fluorescence spectroscopy of the copolymers

Aqueous PNIPA-*g*-PEO-6/15 and PNIPA-*g*-PEO-7/29 solutions containing a fluorescent probe were heated fast and slowly to 45 °C, see Figures 4A and 4B. As discussed earlier, the changes in the microviscosity sensed by the probe decreased with increasing number of PEO grafts. However, the exception in this order was PNIPA-*g*-PEO-7/29 which showed the highest increase in the emission intensity when the samples were heated fast. In addition, the polarity in the surroundings of the probe, observed as a change in $\Delta\lambda_{\max}$, changed most ($\Delta\lambda_{\max} \sim 17$ nm) upon the compression of PNIPA-*g*-PEO-7/29 during the slow heating. PNIPA-*g*-PEO-6/15 exhibited only a 4 nm blue shift in the emission band upon the collapse.

The shrinking copolymer and its aggregates have time to take stable conformations when the samples are heated slowly. Thus, the structural differences between the copolymers may be observed. PNIPA-*g*-PEO-7/29 may contain long strands of PNIPA segments not mixed with PEO. This possible structure is illustrated in Scheme 3c. These strands should be capable of compressing more tightly than the polymers in which PNIPA and PEO are more randomly mixed. It may be concluded that the polymer grafted in an aqueous solution at a temperature close to the LCST has the highest ability to collapse.

3.3.4. EPR spectroscopy of the copolymers

The amphiphilic spin probe 5-doxylstearic acid, 5-DSA, was dissolved in polymer solution using the preparation method for the samples (2). 5-DSA dissolved spontaneously from the wall of the sample cell, and localised in an outer part of the PEO shell of the aggregate (a_N 16.4 G). The aqueous samples of PNIPA-*g*-PEO-6/15 and PNIPA-*g*-PEO-7/29 were heated slowly from room temperature to near 50 °C. The hyperfine coupling constant did not vary during the collapse, but the probe showed a discontinuous change in its rotational motion. The logarithmic rotational correlation times (τ_R) of the probes, estimated with the classical Kivelson formalism¹⁶⁴ are shown against inverse temperature in Figure 7. The

correlation times first increase with temperature, then suddenly decrease close to the critical temperature, and start to increase again above the LCST. The interpretation of the data is as follows. Hydrophobic interactions increase in strength when the temperature is raised; the binding of the probe to the hydrophobic parts of the polymer explains the slowing down of the rotational motion of the label with increasing temperature when temperature is well below or above the LCST. Owing to the increasing water solubility of the probe with increasing temperature, the nitroxides diffuse out of the polymer core during the collapse of the PNIPA chain, this leads to an abrupt decrease of the correlation time. The activation energy of the rotational motion of a spin probe, E_a , can be estimated from the slope of a straight line in an Arrhenius plot. In the present case it is assumed that the E_a values calculated from the data in the temperature range where the PNIPA chain collapses, i.e. between the inflection points, may be used as indicators of the activation energies of the diffusion of the probe out from the polymer core.

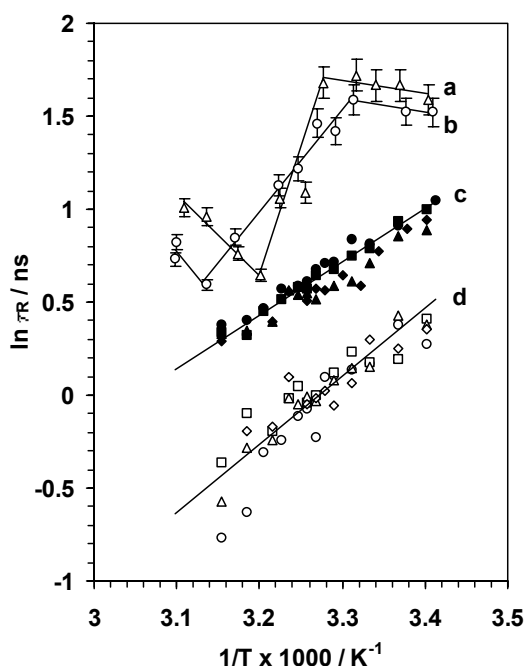


Figure 7. Logarithmic rotational correlation times, τ_R , of 5-DSA and 16-DSA versus inverse temperature of aqueous polymer solutions. (a) 5-DSA in PNIPA-*g*-PEO-6/15 and (b) 5-DSA in PNIPA-*g*-PEO-7/29 solutions at $C_p = 1.0$ g/l. (c) 5-DSA in PNIPA-*co*-GMA, PNIPA-*g*-PEO-6/15, PNIPA-*g*-PEO-7/29 and PNIPA-*g*-PEO-10 and (d) 16-DSA in PNIPA-*co*-GMA, PNIPA-*g*-PEO-6/15, PNIPA-*g*-PEO-7/29 and PNIPA-*g*-PEO-10 solutions at $C_p = 0.1$ g/l. (a) and (b) were prepared by the method for samples (2), and (c) and (d) for samples (1).

Two important conclusions may be drawn. Firstly, the collapse of PNIPA-*g*-PEO-7/29 occurs in a wider temperature range than that of PNIPA-*g*-PEO-6/15. This is in good agreement with the results of the light scattering measurements which show that increasing the number of PEO grafts makes the conformational change more gradual. The data in general shows the same tendency as the viscosity data shown above. Secondly, the activation energies E_a of the probe diffusion are 45 J/mol and 115 J/mol for PNIPA-*g*-PEO-7/29 and PNIPA-*g*-PEO-6/15, respectively. This implies that the amphiphilic nitroxide probe diffuses more easily out from the copolymer with 7 PEO grafts than from

that with 6 grafts. Evidently the hydrophobic core in which the hydrophobic tail of the probe may be embedded is bigger in PNIPA-*g*-PEO-6/15 than in PNIPA-*g*-PEO-7/29.

3.3.5. Calorimetric measurements of the collapse of the copolymers

The kinetics of the collapse of PNIPA have been studied calorimetrically by Schild *et al.*¹⁶⁵ and Grinberg *et al.*¹⁶⁶ The breakage of hydrogen bonds formed by ordered water molecules around PNIPA taking place upon heating is an endothermic process. The solubilising effect of an additional PEO chain is observed in the microcalorimetric measurements. The transition temperatures of the collapse were 33.5 and 34.3 °C for PNIPA-*g*-PEO-6/15 and PNIPA-*g*-PEO-7/29, respectively. Interestingly, it was observed that PNIPA-*g*-PEO-6/15 exhibited an enthalpy change (2.5 kJ/mole of repeating NIPA unit) twice that of PNIPA-*g*-PEO-7/29 (1.3 kJ/mol). Typical ΔH values for pure PNIPA in water vary in the range 1-7 kJ/mol.^{165,166} The difference in ΔH for PNIPA-*g*-PEOs in this work is high, taking into account the small difference in the chemical structure of the polymers. This is in good agreement with the conclusion from the EPR and viscometric measurements that the hydrophobic core is bigger in PNIPA-*g*-PEO-6/15 than in PNIPA-*g*-PEO-7/29 in which PEO grafts are concentrated on the surface of the coil.

4. Block copolymers: PNIPA-*b*-PEO^{VI} and PNIPA-*b*-PSPP^V

This chapter deals with two kinds of block copolymers in which one of the blocks is the same, i.e. PNIPA. Firstly, PNIPA-*b*-PEO block copolymers were synthesised. Their thermal behaviour and aggregate formation have been studied by static and dynamic light scattering and fluorescence spectroscopy. The purpose was to explore the effect of the length of PEO with respect to that of PNIPA on the formation of core-shell nanostructures. Secondly, a block copolymer in which the another block is a polysulfobetaine, poly(N,N-dimethyl-N-3-methacrylamidopropyl ammoniopropanesulfonate), shortly PSPP, has been studied in water and in saline aqueous solutions by dynamic light scattering. Polysulfobetaines are zwitterionic polymers which possess both positively and negatively charged moieties. They are insoluble or sparingly soluble in water owing to ionic crosslinks between two opposing charges in polyion.¹⁶⁷ Therefore, zwitterionic polymers exhibit an upper critical solution temperature, UCST.¹⁶⁸⁻¹⁷⁰ However, the addition of a salt readily enhances the water solubility of polysulfobetaine. Due to more effective site-binding ability of the cation of the salt, the polysulfonate starts to behave as a polyanion with increasing salt concentration and the chain conformation extends. On the other hand, salt promotes the phase separation of PNIPA.^{71,72} The purpose was to investigate the influence of salt on the dissolution behaviour of the copolymer and the formation of the core-shell nanostructures below the UCST and above the LCST.

4.1. Syntheses of poly(N-isopropylacrylamide)-*block*-poly(ethyleneoxide) copolymers

The usual synthetic method for the preparation of block copolymers consisting of PNIPA and PEO is a redox polymerisation using a transition metal ion such as Ce^{+IV} or Mn^{+III} and a hydroxy terminated PEO.^{147,171} In this work, PNIPA-*b*-PEO copolymers with varying block lengths were prepared by free radical polymerisation of NIPA initiated by macroinitiators which contain PEO chains symmetrically at both ends of an AIBN derivative. The polydispersities of PEOs were low which enabled the calculation of the number average molar mass for each PNIPA block from the ¹H-NMR spectra, see Table 2. In the calculations, it was assumed that the polymers were diblock copolymers, and the relative amounts of possible triblock copolymers were not studied. However, the essential point is that the polymers were linear block copolymers. Polymer concentrations, C_p, mentioned in the text are in units of moles of PNIPA segments per liter.

4.2. Thermal properties of poly(N-isopropylacrylamide)-*block*-poly(ethyleneoxide) copolymers in water

4.2.1. Cloud points of the copolymers

The cloud points of the aqueous polymer solutions with $C_p = 3 \times 10^{-7}$ M were measured by following the changes in light transmitted through the sample. Among the copolymer sets, block copolymers having a long PEO block showed an increase of the critical temperatures from 32 to 34 °C with decreasing molar ratio of NIPA/EO; see Table 2 for molar ratios. However, no difference between the copolymers having a short PEO block was observed; the critical temperature was ~ 33 °C regardless of NIPA/EO. Thus, the length of the PEO block is of importance for the solubilising of the collapsing PNIPA but has a minor effect on its cloud point.

4.2.2. Light scattering measurements of the collapse of the copolymers

Above the critical temperature at 45 °C, the block copolymers formed aggregates with narrow size distributions. As is seen in Figures 8A and 8B, the average hydrodynamic radii, $\langle R_h \rangle_{\text{agg}}$, of the aggregates increased logarithmically, whereas their weight average molar masses, $\langle M_w \rangle_{\text{agg}}$, increased linearly with increasing C_p . This result was surprising since in the studies with PNIPA-*g*-PEO graft copolymers, both $\langle M_w \rangle_{\text{agg}}$ and $\langle R_h \rangle_{\text{agg}}$ showed logarithmic tendency as C_p varied, see Figure 2. This indicates that the molecular architecture strongly affects the aggregation of the block and graft copolymers. Within a copolymer set, i.e. either with a short or a long PEO block, the $\langle R_h \rangle_{\text{agg}}$ and $\langle M_w \rangle_{\text{agg}}$ values increased when the length of the PNIPA block (and NIPA/EO) increased. It was observed, however, that the $\langle R_h \rangle_{\text{agg}}$ and $\langle M_w \rangle_{\text{agg}}$ values between two copolymer sets were more dependent on the molar ratio NIPA/EO than on the length of the PNIPA block. The only exception was the copolymer with the longest PNIPA block which formed the biggest aggregates at every concentration. As is seen in Figure 8B, $\langle M_w \rangle_{\text{agg}}$ values approached more or less the same value upon dilution. It is expected that a further dilution of polymer solutions leads to a state at which $\langle M_w \rangle_{\text{agg}}$ values are the same, however, the number of chains being different. In addition, in extreme dilution the number of chains probably approaches the value of unity. This dilute solutions could not be studied, however, due to the low scattering intensity from the sample.

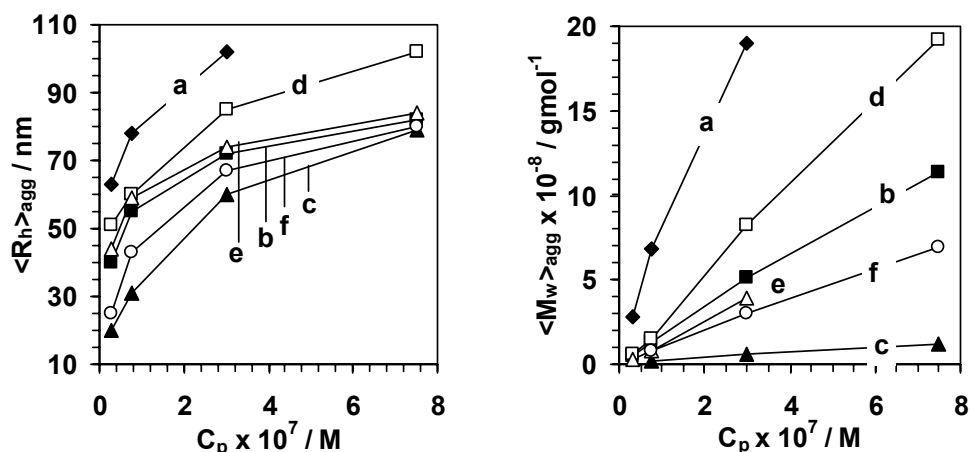


Figure 8. (A) Average hydrodynamic radii, $\langle R_h \rangle$, and (B) weight average molar mass, $\langle M_w \rangle$, of the aggregates in aqueous block copolymer solutions at different polymer concentrations, C_p given in M PNIPA blocks, at 45 °C. (a) NE-A; (b) NE-B; (c) NE-C; (d) NE-1; (e) NE-2; (f) NE-3.

The average number of polymer chains in the aggregate, $\langle N \rangle_{\text{agg}}$, at 45 °C increased with C_p . However, with decreasing length of PNIPA blocks, $\langle N \rangle_{\text{agg}}$ decreased and increased for the copolymers with a long PEO block and a short PEO block, respectively. This is understood by the steric stabilisation of the aggregates by PEO to be more effective as the length of PEO block increases.

The average densities of the aggregates, $\langle \rho \rangle_{\text{agg}}$, at 45 °C generally increased and approached the value of unity with increasing C_p , see Figure 9. Within a copolymer set, the density of an aggregate decreased as the length of PNIPA and the ratio NIPA/EO decreased. The copolymer having the lowest NIPA/EO ratio, however, behaved differently owing to the high amount of PEO buried inside the core of the aggregate. This prevents the effective shrinkage of the core. A similar behaviour was observed with PNIPA-*g*-PEO graft copolymers, as described in Chapter 3.2.1.

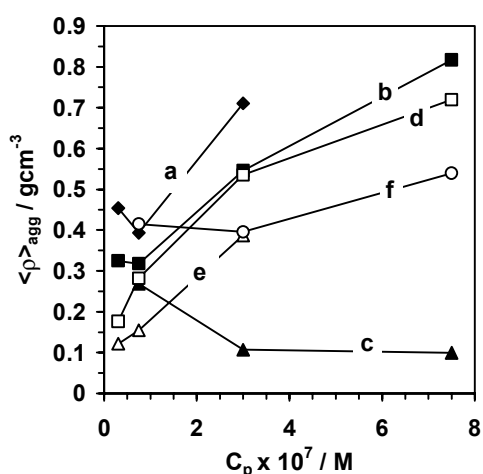


Figure 9. Average densities of the aggregates, $\langle \rho \rangle$, at 45 °C. (a) NE-A; (b) NE-B; (c) NE-C; (d) NE-1; (e) NE-2; (f) NE-3.

The $\langle R_g \rangle / \langle R_h \rangle$ values of the aggregates changed from around 1.5 (coil-like structure) to around 0.4 when lowering C_p or with decreasing the NIPA/EO ratio. The value 1.5 indicates that the shape of the aggregates may change from spherical to ellipsoidal. The assumption is justified by the narrow size distribution of the aggregates. At every polymer concentration, however, the block copolymer with the lowest NIPA/EO ratio formed globular structures whose $\langle R_g \rangle / \langle R_h \rangle$ values were even smaller than 0.78.

The aggregated polymer solutions were diluted with water, keeping the temperature constant at 45 °C. The diluted samples were equilibrated for 1 hour, and $\langle R_h \rangle_{\text{agg}}$ was remeasured. As earlier discussed with the graft copolymers, also the sizes of the aggregates formed by block copolymers only slightly decreased with decreasing C_p . Also in this case, the aggregates once formed do not break because polymer chains in the aggregate core are kinetically trapped.

4.2.3. Fluorescence spectroscopy of the copolymers

The solutions studied are denoted as samples (1) and (2) depending on the method of the sample preparation, see Chapter 2.3.3. As already noted, the samples (1) were aggregated solutions whereas the samples (2) showed no aggregation at room temperature. The hydrodynamic radii of the latter samples were similar to the polymers without the probe. For the fluorescence measurements, all the samples with $C_p = 3 \times 10^{-7}$ M were heated slowly to 40 °C. In the samples (1) the concentration of 4HP was 3.3×10^{-6} M. The original concentration of 4HP used to prepare the samples (2) was the same as above, but the resulting samples contained only an equilibrium amount of the probe.

During the heating of samples (1), the intensity of the emission of the probe increased sharply at a distinct critical temperature. The changes in the microviscosity of the surroundings of the probe within a copolymer set increased when the length of PNIPA block increased, see Figure 10A. Again, the only exception was the copolymer with the longest PNIPA segment. Comparing the copolymer sets, the changes in the microviscosity were more notable with the copolymers having the short PEO block.

During the collapse of the samples (1), the emission bands shifted indicating changes in the polarity of the surroundings of the probe. At room temperature, within a copolymer set, the emission band occurred at shorter wavelengths as the length of PNIPA increased, i.e. the environment of the probe changed to less hydrophilic one. Of the copolymer sets, the probe sensed more hydrophilic environment at room temperature in the copolymer solutions having the short PEO block than in the solutions with the long PEO block. The increased polarity around the probe is understood by the increased amount of PEO and water inside the aggregates. The difference between the copolymer sets is explained by more effective phase separation between the PNIPA and PEO as the length of PEO block increases. However, after heating up to 40 °C the probe sensed the same non-polar environment regardless of the block copolymer ($\lambda_{\text{max}} \sim 606\text{-}611$ nm).

Within a copolymer set in the samples (2), the changes in the microviscosity of the probe surroundings during the collapse increased as the PNIPA block was lengthened, see Figure 10B. It seems that these changes are solely governed by the length of PNIPA block, not by the ratio NIPA/EO. Contrary to the samples (1), these changes were more dramatic in the copolymers having the long PEO block. It was notable that the λ_{max} did not vary during

the aggregation of the samples; $\lambda_{\max} \sim 611$ nm was more or less the same as the λ_{\max} values for the samples (1) at 40 °C.

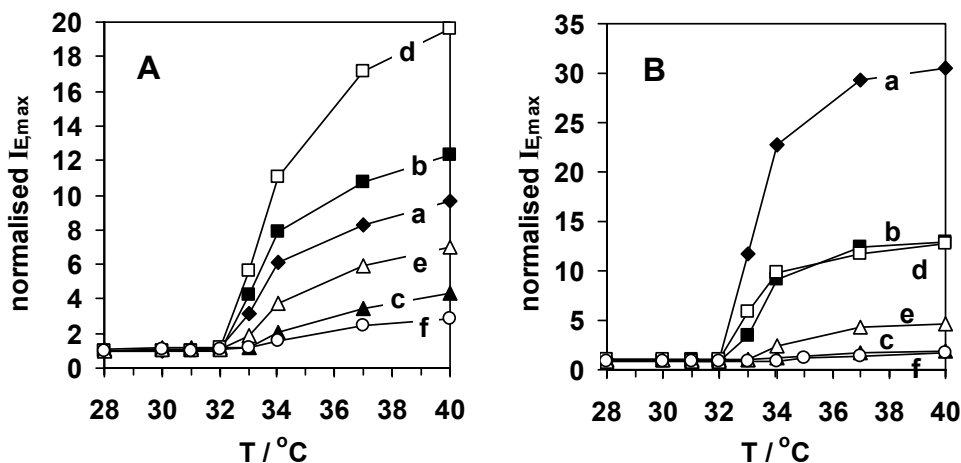


Figure 10. Normalised maxima of the emission intensities of the probe 4HP, $I_{E,\max}$, in the aqueous block copolymer solutions during the slow heating of the samples (1) and (2) in the figures A and B, respectively. (a) NE-A; (b) NE-B; (c) NE-C; (d) NE-1; (e) NE-2; (f) NE-3.

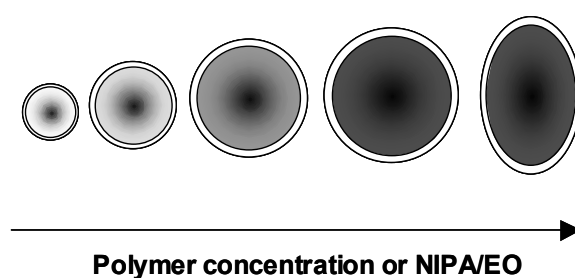
According to the changes in the polarity of the probe environment it is possible to determine the localisation of the probe in the copolymer. It is obvious that 4HP located in the hydrophobic domains of the copolymer. However, it is assumed that at room temperature the probe located in more homogeneous environment in the samples (2) than in the samples (1), the location of the probe being greatly influenced by the copolymer in the latter ones. The former conclusion is justified by the fact that λ_{\max} did not vary during the collapse of the samples (2). Also, λ_{\max} was equal for the samples (1) and (2) at elevated temperature.

4.2.4. Effect of the length of the PEO block on the aggregate formation

The block copolymers NE-B and NE-1 having closely similar PNIPA blocks but different lengths of the PEO blocks, showed different aggregation behaviour upon heating. At each C_p , the copolymer with higher NIPA/EO ratio, NE-1, formed aggregates whose $\langle R_h \rangle_{\text{agg}}$, $\langle M_w \rangle_{\text{agg}}$ and $\langle N \rangle_{\text{agg}}$ were larger than those of NE-B. The average densities of the aggregates of these two copolymers, however, showed only a slight difference. Moreover, the samples (2) of these two copolymers showed no difference in the microviscosity of the environment around the probe during heating. Therefore, the size and the molar mass of the aggregate is strongly dependent on the length of the PEO block. This is a consequence of the improved steric stabilisation of the aggregates by the shell formed by the long PEO block. However, the mass distributions in the aggregate cores differed although the average values were approximately equal. This will be discussed in the following chapter.

4.2.5. Structure of the aggregates

Following the discussion above it is suggested that the structure of aggregates is dependent on the polymer concentration and the ratio NIPA/EO, see Scheme 4. At low polymer concentration or for low values of NIPA/EO the block copolymers form aggregates where the inner part of the core is denser than the rest of the aggregate in average. This is related to the extent of the phase separation between PNIPA and PEO. Firstly, dilution favors the intramolecular interactions between the collapsing PNIPA chains. Secondly, increasing the amount of PEO in the block copolymer has not only an increased solubilising effect on the collapsing PNIPA block but it also improves the steric stabilisation of the forming aggregate. As C_p or NIPA/EO increases, the density of the aggregate increases and the mass distribution becomes more homogeneous. At high C_p values, the block copolymers form aggregates whose shape might change from spherical to ellipsoidal.



Scheme 4. The evolution of the aggregate with polymer concentration or the molar ratio NIPA/EO above the LCST.

4.3. Thermal properties of a poly(N-isopropylacrylamide)-*block*-polysulfobetaine copolymer in water and in saline aqueous solutions

4.3.1. Influence of NaCl on the mechanism of clustering at room temperature

Aqueous PNIPA-*b*-PSPP solutions were slightly opaque, however an addition of small amount of NaCl readily resulted in a completely clear solution. At the NaCl concentration around $C_s = 0.73$ M, the intensity of the scattered light increased gradually, and at $C_s \sim 0.93$ M, the intensity increased rapidly indicating the phase separation and aggregate formation of PNIPA blocks. This result is well in accordance with earlier observations on linear and crosslinked PNIPA by other groups.^{71,72} The colloidal aggregates were electrostatically stabilised by PSPP shells having polyanion character.

At room temperature the size distribution of the salt free polymer solution with $C_p = 2.0$ g/l was bimodal. The bimodality was also observed regardless of salt concentration,

however, at $C_s = 0.96$ M, the two maxima in the size distributions became narrower. From the bimodal distributions, slow and fast components of the diffusion coefficients were calculated, and are shown against salt concentration in Figure 11. The slow diffusion is a result of clustering of polymers, and the mechanism of the cluster formation changes upon the addition of salt. In a salt free solution, large temporary clusters build up due to ionic interactions, whereas in the high salt concentration regime the clustering is due to the salting out of PNIPA chains, i.e. is of hydrophobic nature. The fast diffusion is assumed to correspond to loops or mobile chain ends in the clusters. Two continuous regions for the slow diffusion coefficient, D_s , may be distinguished. At low salt concentrations ($C_s < 0.3$ M) D_s decreases linearly with increasing C_s . With $0.5 < C_s < 0.9$ M, D_s has a constant value. The salt concentration region where the sudden change in D_s takes place (between 0.3 and 0.5 M), is expected to be the region where the principal mechanism of clustering changes from ionic to hydrophobic. The fast diffusion coefficient decreased gradually with the addition of salt resulting from the restricted motional freedom of PNIPA blocks. Both the D_f and D_s values showed a sudden decrease in a high salt concentration limit, $C_s \sim 0.93$ M at which the critical temperature of PNIPA is 20 °C. In the figure, the salt concentrations have been indicated where the molar ratio of NaCl to the repeating units of SPP, $n(\text{NaCl})/n(\text{SPP})$, gets the values 31 and 155.

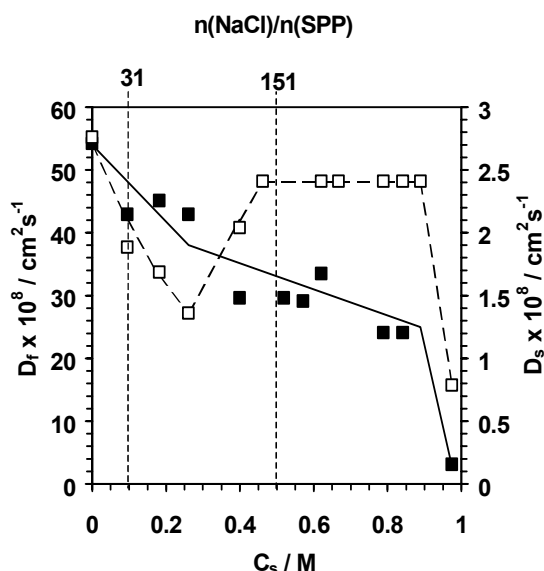
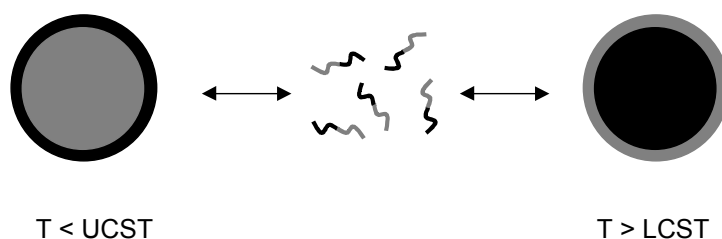


Figure 11. Slow diffusion coefficients, D_s , (open squares) and fast diffusion coefficients, D_f , (filled squares) at different NaCl concentrations at 20 °C. Polymer concentration 2.0 g/l. Vertical dotted lines show the values of $n(\text{NaCl})/n(\text{SPP})$.

4.3.2. Collapse and aggregate formation during heating and cooling

A solution in water, and two different saline polymer solutions were slowly heated and cooled. In both saline polymer solutions, the molar ratio of NaCl to the repeating units of SPP was kept constant; $n(\text{NaCl})/n(\text{SPP}) = 31$ corresponds to $C_s = 0.1$ and 0.01 M and $n(\text{NaCl})/n(\text{SPP}) = 155$ to $C_s = 0.5$ and 0.05 M when $C_p = 2.0$ and 0.2 g/l, respectively. During the cooling and heating cycles the block copolymer forms reversible core-shell aggregates as schematically illustrated in Scheme 5.



Scheme 5. A model describing the formation of the aggregates in aqueous PNIPA-*b*-PSPP solutions at different temperatures. PNIPA (black) and PSPP (grey).

The solubility of PSPP increases in the course of the addition of NaCl due to increased solvent-polymer interactions and polyanion characteristics. This results in the disappearance of the intermolecular attraction between zwitterionic groups, and no phase separation is observed. Therefore, the UCST behaviour was observed only with the pure aqueous solution upon cooling. The phase separation of PSPP occurred at around 16 °C in a solution with $C_p = 0.2$ g/l. The aggregates broke down relatively fast during a slow heating back to room temperature, but still somewhat slower than they had been assembled.

The size distribution of the dilute polymer solution with $C_p = 0.2$ g/l was monomodal at 20°C, but broadened when salt was added. At 4-6 °C the size distributions of the aggregates were narrow and monomodal with $\langle R_h \rangle_{agg} = 916$ nm ($C_p = 2.0$ g/l) and 275 nm ($C_p = 0.2$ g/l).

Aqueous solutions with polymer concentrations varying from 0.01 g/l to 0.2 g/l were cooled rapidly to 4 °C. For solutions in water, as well as for the solutions with higher salt concentrations ($n(\text{NaCl})/n(\text{SPP}) = 155$), the behaviour of the polymer did not depend on the cooling rate. However, at intermediate salt concentrations ($n(\text{NaCl})/n(\text{SPP}) = 31$), fast cooling led to the formation of aggregates, although aggregates were not formed when cooling these solutions slowly. This unusual behaviour implies that the occurrence of the phase separation is kinetically controlled in solutions with low salt concentration where zwitterions as well as anionic groups coexist. This suggests that the aggregates are metastable though this has not yet been experimentally proven.

In all cases, the size of the aggregates at 4 °C decreased with decreasing C_p , though the effect was more distinct for saline polymer solutions than for aqueous ones. The size distributions of the aggregates were rather broad, and they broadened in saline polymer solutions of intermediate salt concentration ($n(\text{NaCl})/n(\text{SPP}) = 31$). This is understood by the enhanced solubility of PSPP induced by salt, resulting in a looser packing of the aggregate core. Further, the core of the aggregate mainly consisting of PSPP blocks is polar and is expected to contain a relatively high amount of water. This prevents the compression of the core resulting in a widening of the size distribution. This hypothesis is supported by the observation that the intensity of light scattered from the aggregates is much lower from saline polymer solutions than from the solutions in water.

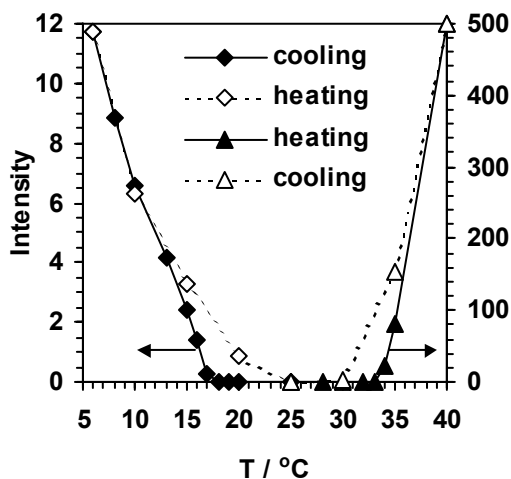


Figure 12. Intensity of scattered light from aqueous polymer solutions with $C_p = 0.2$ g/l during slow heating and cooling.

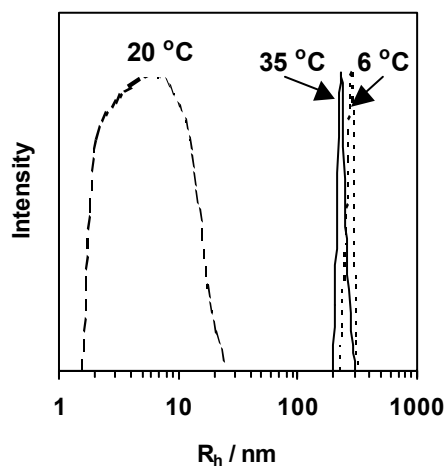


Figure 13. Size distribution of the copolymer in a saltless aqueous solution at 6 °C, 20 °C and 35 °C.

During heating, the aqueous copolymer solutions collapsed at their critical temperature. The cloud points of the samples with $C_p = 2.0$ g/l were 33 °C, 31 °C and 26 °C for a salt free solution, for $C_s = 0.1$ M and for $C_s = 0.5$ M, respectively. For more dilute solutions with $C_p = 0.2$ g/l, the cloud points were 34 °C, 34 °C and 33 °C for a salt free sample, for $C_s = 0.01$ M and for $C_s = 0.05$ M, respectively. Therefore, it can be concluded that the LCST depends critically not on the polymer concentration but on the salt concentration. The aggregates disassembled slowly when the solution were cooled to room temperature. The hysteresis effect is due to the formation of associative intrachain structures.¹¹⁰

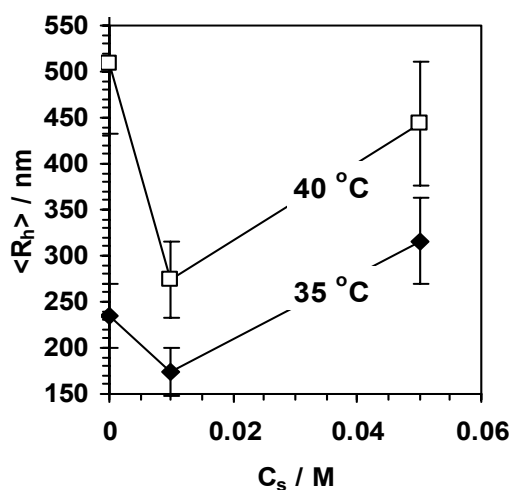


Figure 14. Average hydrodynamic radii, $\langle R_h \rangle$, of the aggregates formed during slow heating at 35 °C and 40 °C at different salt concentrations. $C_p = 0.2$ g/l.

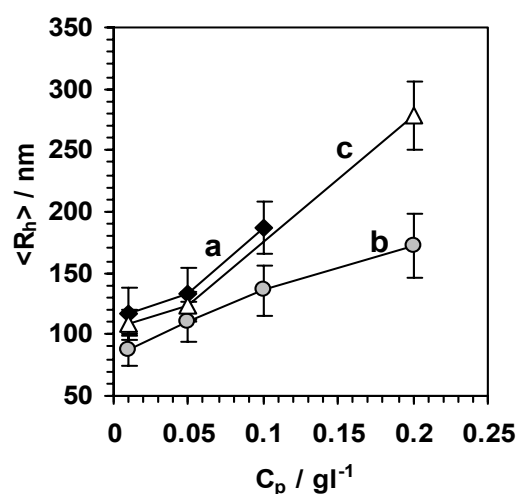


Figure 15. Average hydrodynamic radii, $\langle R_h \rangle$, of the aggregates formed during fast heating at 45 °C at different polymer concentrations. $n(\text{NaCl})/n(\text{SPP})$ equals (a) 0, (b) 31, and (c) 155.

The sizes of the aggregates with narrow size distribution in the solution with $C_p = 0.2$ g/l at 35 and 40 °C depended on the amount of added salt. A small amount of NaCl ($n(\text{NaCl})/n(\text{SPP}) = 31$) resulted in smaller aggregates than those observed in salt free aqueous solutions. With increasing concentration of NaCl ($n(\text{NaCl})/n(\text{SPP}) = 155$), however, the aggregate sizes increased again. This is understood in terms of the suggested change in the mechanism of aggregation with varying salt concentration. The difference between the sizes of aggregates in different solutions, formed during the fast heating is very similar to that observed during slow heating. Moreover, the sizes of the aggregates in each solution decreased with decreasing C_p .

5. Conclusions

A series of thermally responsive poly(N-isopropylacrylamide), PNIPA, based graft and block copolymers having poly(ethyleneoxide), PEO, or polysulfobetaine, PSPP, as the other segment have been synthesised by several radical polymerisation techniques. PNIPA-graft-PEO copolymers were obtained using linear copolymers prepared by free radical copolymerisation of N-isopropylacrylamide, NIPA, and either glycidylmethacrylate, GMA, or N-acryloylsuccinimide, NASI. The grafting reactions were conducted either in an organic solvent or in water. PNIPA-*block*-PEO copolymers were prepared by free radical polymerisation using macroazoinitiators bearing PEO chains. The synthesis of the block copolymer PNIPA-*block*-PSPP was carried out by a RAFT polymerisation technique, however, not done in this work.

In order to obtain well-defined graft copolymers it was of importance to use functional backbone copolymers to avoid any additional branching in the copolymers. Kinetic studies of the copolymerisation of NIPA and GMA showed the latter monomer to be more reactive than the former. In the preparation of the backbone copolymer PNIPA-*co*-GMA, the functional comonomer was continuously added to the reaction mixture in the course of the polymerisation to obtain a random distribution of functional groups along the PNIPA chain. The grafting reactions resulted in linear graft copolymers with a wide range of the degree of PEO substitution without any side reactions.

During the collapse at the LCST, PNIPA chains form the core of spherically shaped aggregates surrounded by a hydrophilic shell. As dilute aqueous solutions of block and graft copolymers are heated the solubilising effect of the hydrophilic segments, i.e. PEO or PSPP, competes with hydrophobic interactions of the shrinking PNIPA. The surface of the collapsing PNIPA is covered by hydrophilic segments which enhance the colloidal stability of the particle. Both effects are enhanced by increasing the amount of PEO or PSPP with respect to PNIPA and by the dilution of polymer solution. This results in the elevation of the LCST and slows down the collapse of the copolymers. The block copolymers form aggregates with a narrow size distribution, whose average size decreases with decreasing polymer concentration. This is also the case with the graft copolymers having a low number of side chains, however, the size distribution of the aggregates broadens at high grafting degrees. The size of the aggregates decreases with increasing content of PEO in the case of the block copolymers PNIPA-*b*-PEO, whereas the average size of the graft copolymers remains more or less the same.

During the collapse of PNIPA, some PEO or PSPP is buried inside the aggregate core. Therefore, increasing mixing of the phases in the core prevents the compression of the core. The block copolymers PNIPA-*b*-PEO can form very dense aggregates with narrow size distributions, whereas the core densities of graft copolymers are rather low. The number of polymers in the aggregates formed by the graft copolymers is much lower than that in the aggregates formed by the block copolymers. The difference in the aggregates may emerge from the differences in the chemical structures of the block and graft copolymers, but most probably from the different content of EO repeating units in the polymers. The densities of the cores decrease upon the dilution of the solution. Also, the

mass distribution of the core changes during dilution; the inner part of the core is more compressed than the rest of the core. In the dilution, the distance between the polymers increases resulting in the attractive forces within the PNIPA chain to dominate over the ones between the chains. This leads to an enhanced surface stabilisation by the hydrophilic parts on the shrunken PNIPA coil.

The thermal properties and the aggregate formation of the block copolymer PNIPA-*b*-PSPP in aqueous solutions depend on the salt concentration. Upon addition of sodium chloride, the solubility of PSPP increases but that of PNIPA decreases. At room temperature, the copolymers form clusters. With increasing salt concentration, the mechanism of temporary clustering of the copolymers changes from ionic into hydrophobic. The collapse of PSPP does not occur above a certain salt concentration. When phase separation occurs during cooling, the size distribution of the aggregates broadens with the addition of salt indicative of a loose packing of the core consisting mainly of polysulfobetaine. Above the LCST, the copolymer forms aggregates with a narrow size distribution whose size first decreases with salt concentration and then starts to increase with a further addition of salt.

Two graft copolymers with the lowest number of PEO grafts have been prepared by the grafting of the functional copolymer in water at two different temperatures; far below and close to the LCST of PNIPA. At the vicinity of the LCST, the capability of PEO chains to react with the functional groups is limited to the surface of the shrunken PNIPA coil. The copolymer grafted at an elevated temperature adopts a shrunken coil conformation at room temperature, and forms the smallest aggregates with a very narrow size distribution at elevated temperatures. Further, the change in the microviscosity of the polymer core is highest in this polymer. These observed differences are not directly proportional to the number of the PEO grafts but evidently reflect the different distribution of the PEO side chains along the PNIPA main chain. Therefore, the polymers evidently show a tendency of retaking the conformation in which they were synthesised. Thus, a copolymer resembling “a protein-like copolymer” has been successfully synthesised.

The thermal properties and the nanoparticle formation of the copolymers are governed by various factors. The variable density and polarity of the core of the particles offer the possibility to encapsulate different kinds of molecules with different solubilities. In addition, the biomimetic copolymers studied in this work offer a fast and efficient response to thermal treatment. The contribution of this work is the further understanding of the factors which influence the construction of nanosized polymeric micelles. The factors which still need to be studied more closely include the effect of salts on the neutral polymers, and further dilution of the aqueous and saline solution.

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