SYNTHESIS AND CHARACTERIZATION OF THERMORESPONSIVE POLYMERS, HYDROGELS AND MICROGELS, BASED ON POLY (*N*-SUBSTITUTED ACRYLAMIDES)

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To my family and Giannis,

"The important thing in science is not so much to obtain new facts as to discover new ways of thinking about them"

Sir William Bragg
(1862-1942)

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Abstract

Stimuli-responsive polymers have the unique property of undergoing a reversible phase transition. This property has attracted much interest for their application in the field of medicine and biotechnology, such as drug delivery systems.

Linear polymers of the poly(*N*-substituted acrylamides) family and their three-dimensional macroscopic (hydrogels) or microscopic (microgels) networks demonstrate such a phase transition behavior. In particular, polymers of this family have the ability to respond to external stimuli, such as temperature and are characterized by a critical solution temperature (CST). The aim of the present thesis was the synthesis and investigation of the thermoprecipitation of the above mentioned linear polymers and their three-dimensional forms.

Polymers that are having a similar or even higher phase transition temperature than that of the well-established poly(*N*-isopropylacrylamide) is becoming increasingly attractive since they increase the range of applications even further. Optimization of their thermoresponsive behavior could be obtained by changing the surrounding environmental conditions, such as addition of salts, solvents or copolymerization.

Polymers with different thermosensitivity depending on the chemical structure of the backbone, such as poly (*N*-isopropylacrylamide), poly (*N,N'*-diethylacrylamide), poly (*N*-ethyl,*N*-methylacrylamide) and poly (*N*-pyrrolidinoacrylamide), were synthesized by chain transfer and anionic polymerization and characterized by a variety of experimental techniques. Using *N,N*-methylenebisacrylamide as crosslinking agent, macro- and microscopic network structures of poly (*N*-isopropylacrylamide) and poly (*N,N'*-diethylacrylamide) were obtained by free radical polymerization and surfactant-free emulsion polymerization, respectively.

The synthesis of thermosensitive polymers was mainly manipulated taking the behavior of oligomeric poly (*N*-isopropylacrylamide) as reference. An influence of the polymerization method and in some cases of the molecular weight and tacticity on the CST was observed. Copolymerization of poly (*N*-isopropylacrylamide) with a more hydrophilic comonomer, shifted the CST to higher values.

Co-solutes and co-solvents influence the thermoprecipitation of linear thermosensitive polymers from aqueous solution. A “salting-out” effect was noticed when inorganic

salts, except for potassium iodide, were presented in the polymer aqueous solution. Contrary to the effects observed upon the addition of simple salts as additives, the chemistry of the investigated polymers was of direct consequence for the effect of a given solvent. Also, the strength of the observed effect was related not only to the size but also the structure of the hydrophobic domain of the solvent molecule.

Despite the presence of chemical crosslinks, similarity was found between the phase transition of hydrogels with the corresponding linear polymers. Synthesis conditions seem to influence the macroscopic gel structure. Gel structure and crosslinking density showed partly an influence to the characteristic properties of the hydrogels, namely swelling ratio, reswelling and deswelling kinetics. In the presence of salts, certain parallels can be drawn to the effect observed on the phase transition of thermosensitive hydrogels in comparison with the one on polymers.
Solute permeation and pore size characterization of the two gels were investigated using dextran molecules and their molecular weight cut off was estimated to be close to 70 kDa. For the purpose of drug loading experiments, insulin and BSA were chosen as model drugs with the insulin showing higher percentage of ingress. Release experiments from the gel networks at 37°C, a temperature higher than the CST of the gels, were also realized using insulin as model drug. Between the gels, different release profiles were obtained attributed to their different hydrophobicity.

The invention of small sized thermosensitive microgels is of great interest due to their fast kinetic response. Between the polymerization processes the stirring rates were varied and an effect on the particle size was noticed; increasing stirring rate, the average particle diameter decreases. Small particles presented in the microgel solution, indicate the continuation of nucleation process and therefore the necessity for longer polymerization time.

Keywords: poly(*N*-substituted acrylamides), phase transition, thermoprecipitation, critical solution temperature, hydrogels, drug release, microgels

Résumé

Les polymères stimuli-sensibles subissent un changement de phase réversible losqu'ils sont soumis à un stimulus externe, propriété remarquable qui suscite un vif intérêt dans les domaines de la médecine et des bio-technologies en raison de l'étendue des applications possibles telles que le relargage de médicaments.
Les chaînes linéaires de polymères appartenant à la classe des polyacrylamides N-substitués de même que leurs réseaux moléculaires tridimensionnels macroscopiques (appelés hydrogels) ou microscopiques (microgels) présentent une telle transition de phase en réponse à un stimulus externe comme la température et sont caractérisés par une température critique de solution (CST). Le but de cette thèse fut la synthèse et l'étude de thermoprécipitation des polymères non réticulés et réticulés sus-mentionnés.
Les polymères caractérisés par une température de transition de phase semblable ou même supérieure à celle des poly (N-isopropylacrylamide) présentent un intérêt particulier puisqu'ils permettent d'étendre le domaine d'applications. L'optimisation de leur comportement thermosensible peut être obtenu en modifiant les conditions d'environnement par l'ajout de sels, de solvants, ou par copolymérisation.

Le comportement thermosensible, dépendant de la structure chimique de la chaîne polymérique, différents polymères ont été synthétisés tels que les poly (*N*-isopropylacrylamide), poly (*N,N'*-diéthylacrylamide), poly (*N*-éthyl,*N*-méthylacrylamide) et poly (*N*-pyrrolidinoacrylamide) par transfert de chaîne ou polymérisation anionique et ont été caractérisés par diverses techniques expérimentales. En outre, l'utilisation du *N,N*-méthylènebisacrylamide comme agent de réticulation a permis l'obtention de réseaux macroscopiques et microscopiques des poly (*N*-isopropylacrylamide) et poly (*N,N'*-diéthylacrylamide) par polymérisation radicalaire et polymérisation en émulsion en l'absence de tensioactifs, respectivement.

La synthèse de polymères thermosensibles a principalement été manipulée en prenant comme référence le comportement de l'oligomère poly (*N*-isopropylacrylamide). Une influence de la méthode de polymérisation, et dans certains cas de la masse moléculaire et de la tacticité du polymère ont été observées sur la CST. Ainsi la copolymérisation du poly (*N*-isopropylacrylamide) avec un co-monomère plus

hydrophile engendre une élévation de la CST. Par ailleurs la présence de co-solutés et co-solvants influence la thermoprécipitation des chaînes linéaires de polymères thermosensibles en solution aqueuse. Un phénomène de 'salting-out' est observé en présence de sels inorganiques dans la solution aqueuse de polymère excepté pour l'iodure de potassium. Contrairement aux observations faites lors de l'ajout de sels, l'effet du solvant dépend de la chimie des polymères, de même que l'intensité de l'effet observé est non seulement liée à la taille mais aussi à la structure du domaine hydrophobe de la molécule de solvant.

Dans le cas des hydrogels, malgré la réticulation, des similitudes furent mises en évidence entre la transition de phase observée et celle des polymères linéaires correspondants. Les conditions de synthèse semblent influencer la structure macroscopique du gel, alors que cette-dernière et le taux de réticulation affectent en partie les propriétés caractéristiques de l'hydrogel, comme l'effet de gonflement et les cinétiques de regonflement et dégonflement. En présence de sels, la même tendance est observée sur la transition de phase des hydrogels thermosensibles que pour les polymères.
La caractérisation de la taille des pores et les études de perméation des gels par un soluté ont été réalisées avec les molécules de dextran qui ont permis une estimation de la limite d'exclusion de masse moléculaire de l'ordre de 70 kDa. Dans les expériences d'absorption de médicaments le choix s'est porté sur l'insuline et la BSA comme médicaments-modèles avec un pourcentage d'absorption supérieur pour l'insuline. L'insuline a également été utilisée comme médicament-modèle dans les expériences de relargage hors des gels à une température de 37°C, supérieure à la CST des gels. Ainsi différents profils de relargage ont été obtenus en fonction du gel, que l'on attribue à leur différence de caractère hydrophobe.

L'enjeu des petites tailles conduisant à la synthèse de microgels thermosensibles présente un grand intérêt actuel en raison des cinétiques de réponse rapides. La variation de la vitesse de mélange pour différentes réactions de polymérisation influence la taille des particules obtenues. En effet, une augmentation de la vitesse de mélange engendre une diminution du diamètre moyen des particules alors que la présence de petites particules dans la solution de microgel indique que le processus de nucléation se poursuit d'où la nécessité de prolonger les temps de polymérisation.

Keywords : poly (acrylamides N-substitués), transition de phase, thermoprécipitation, température critique de solution, hydrogels, microgels, relargage de médicaments.

Thesis Outline

"Stimuli-responsive" or "Smart" Polymers and Hydrogels

Considerable research attention has been focused recently on materials that change their structure and properties in response to external chemical and / or physical stimuli (Park et al. 1992; Brazel et al. 1994; Lim et al. 1997; Zhang et al. 2000; Soppimath et al. 2002; Lee et al. 2004; Mahkam et al. 2004; Zhang et al. 2004). These materials are called "intelligent" or "smart" materials. They are also named as "stimuli-responsive" or "enviromental" polymers.

Stimulus-responsive or "smart" polymers and hydrogels can be classified according to the stimuli they respond to as: temperature-, pH-, ionic strength-, light-, electric- and magnetic field-sensitive. They undergo fast, reversible changes in microstructure from a hydrophilic to a hydrophobic state (Galaev et al. 1996). These changes are apparent at the macroscopic level as precipitate formation from a solution or order-of-magnitute changes in the size and water content of hydrogels (Galaev et al. 1999). An appropriate proportion of hydrophobicity and hydrophilicity in the molecular structure of the polymer is believed to be required for the phase transition to occur.

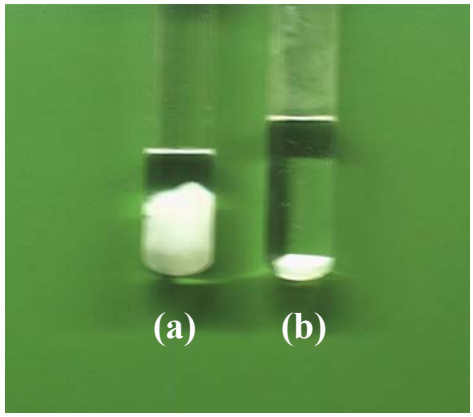

Figure 1. *Stimuli-responsive polyNIPAAM hydrogel exhibiting a thermosensitivity at* (a) *room temperature* (*27* °C) *and* (b) *50* °C.

Smart polymers could be classified in response to their physical form in to three main classes (Figure 2) (Murray et al. 1995; Jeong et al. 2002), i.e. [i] *linear free chains in solutions*, where the polymer undergoes a reversible collapse after an external stimulus is applied, [ii] *chains adsorbed or grafted on a surface*, where the polymer reversibly adsorbs or collapses on a surface, respectively, converting the interface

from hydrophilic to hydrophobic, once a specific external parameter is modified and [iii] covalently *cross-linked gels and reversible or physical gels*, which can be either micro- or macro-scopic networks and on which swelling behavior is environmentally triggered.

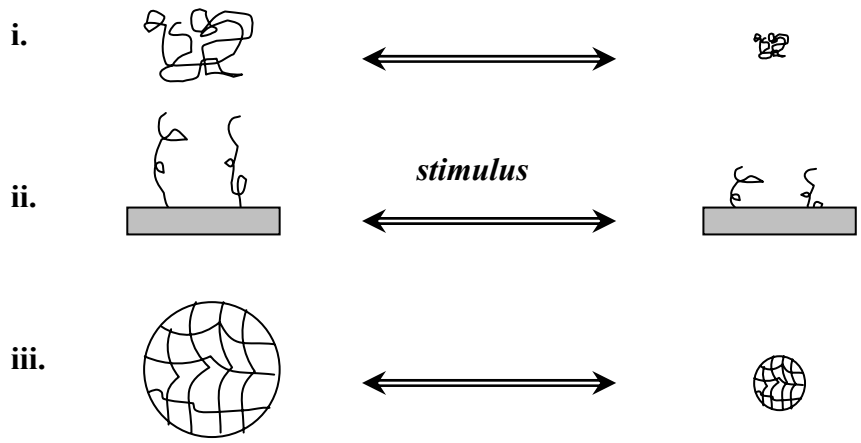

Figure 2. *Classification of the polymers by their physical form adapted from Jeong* (Jeong et al. 2002)

As the stimulus-responsive behavior occurs in aqueous solutions, these polymers and hydrogels are becoming increasingly attractive for biotechnology and medicine, for their use, among others, as basic components of *biosensors* (Chen 1998), *"switch on-off" drug release* (Ranga Rao et al. 1988; Okano et al. 1990; Yoshida et al. 1991), *drug delivery systems* (Nujoma et al. 1996; Lim et al. 1997; Okano 1998; Hoffman 2002; Kopecek 2003; Mahkam et al. 2004), *affinity precipitation* (Freitag et al. 1994; Chen et al. 1995; Freitag et al. 2001; Costioli et al. 2003), *immobilization of enzymes* (Hoffman 1988; Chen 1997; Ding 1998) and *cells* (Galaev et al. 1993), *tissue engineering* (Ohya et al. 2001) and *artificial muscles* (Matsuda 1999).

The presented thesis is dedicated to the synthesis and characterization of such "smart" linear polymers and hydrogels, which respond to temperature changes. Three parts comprise the thesis.

In the first part (**Part I**), which is divided in three Chapters, our research was focused on the linear form of thermo-responsive polymers. In the first Chapter, the synthesis by chain transfer and anionic polymerization of different poly (*N*-alkylacrylamides), namely poly (*N*-isopropylacrylamide), poly (*N,N'*-diethylacrylamide), poly (*N*-

ethyl,*N*-methylacrylamide) and poly (*N*-pyrrolidinoacrylamide), and their characterization by different analytical techniques are described. A special attention is given on their phase transition behavior and the factors, e.g. molecular weight and tacticity, which are affecting the thermoprecipitation. Modification of the surrounding aqueous environment by the addition of salts and solvents and its influence on the phase transition of the polymers is the main subject of the following two chapters, Chapter 2 and 3, respectively.

In the second part (**Part II**), which comprises the main topic of the presented investigation, we switch our research to the synthesis and characterization of the 3-dimensional form (macroscopic networks or hydrogels) of two of the previous investigated linear polymers, namely poly (*N*-isopropylacrylamide) and poly (*N,N'*-diethylacrylamide). This part is divided in four Chapters. In the first Chapter, the description of the hydrogel synthesis by free radical polymerization and the influence of the synthesis conditions on their macroscopic structure are examined. Characterizations of the hydrogels, such as measurement of the swelling ratio, reswelling and deswelling kinetics are also given. In the second Chapter, the hydrogels phase transition and swelling ratio in the presence of salts is investigated. A comparison of the additives effect on the hydrogels phase transition behavior with their linear form is also given. Solutes permeation in hydrogels using molecules (dextrans) of different sizes and the characterization of the hydrogels pores size by gel permeation chromatography is investigated in the third Chapter. The last Chapter investigates the drug (proteins) loading and release from the hydrogels in order to examine their use as drug delivery systems. Preliminary cytotoxicity test was applied to evaluate the toxicity of the hydrogels.

The last part of the thesis (**Part III**) shifts the size of the previous investigated macroscopic networks to the micro scale. The synthesis of the microgels by Surfactant-Free Emulsion Polymerization is described and a preliminary characterization, concerning mainly the size and morphology of the two investigated microgels, poly (*N*-isopropylacrylamide) and poly (*N,N'*-diethylacrylamide), is given. The characterization was done by Transmission and Scanning Electron Microscopy.

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PART I: THERMORESPONSIVE POLYMERS

General introduction

1. Thermoresponsive polymers and the phase transition phenomenon

Temperature-sensitive polymers exhibit a critical solution temperature (CST) behaviour where phase separation is induced by surpassing a certain temperature. Polymers of this type undergo a thermally induced, reversible phase transition; they are soluble in a solvent (water) at low temperatures but become insoluble as the temperature rises above the critical solution temperature (CST) (Taylor and Gerankowski 1975).

The liquid-liquid phase diagram at constant pressure of binary polymer solutions is usually determined by plotting the temperature of incipient phase separation as a function of the overall polymer concentration. Although the solution is homogeneous at low temperature, a macroscopic phase separation appears when the temperature exceeds a critical value called the *critical solution temperature* (CST) or the *cloud point* (CP) of the mixture (Durand and Hourdet 2000). The minimum in the phase diagram (known also as cloud point curve) is called the precipitation threshold, or LCST (lower critical solution temperature), since it denotes the extreme temperature at which phase separation can occur at all (Boutris et al. 1997).

Figure 1 shows a typical curve of cloud point versus composition that one might find for a thermo-responsive system. The right-hand branch of the experimental phase diagram defines the composition of the polymer that precipitates at various temperatures. It can also be viewed as the equilibrium swelling ratio of the polymer-solvent system as a function of the temperature. In a CST system, the right-hand branch of the curve is characterized by a positive slope, indicating that the polymer (or gel) will precipitate (collapses) as the temperature increases.

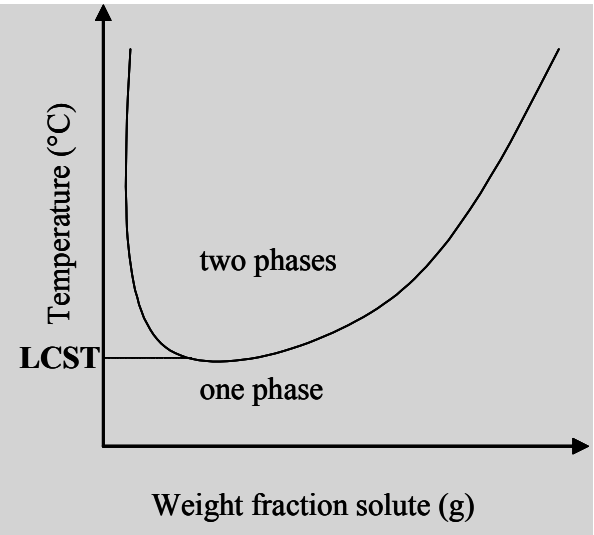

Figure 1. *Phase diagram of a system exhibiting lower critical solution temperature (LCST), adapted from Taylor and Gerankowski* (Taylor and Gerankowski 1975)

For a linear flexible polymer chain in solution, there has been much debate concerning the major determinant of the coil-to-globule transition, whether "hydrophobic effects" and / or "hydrogen bonding effects" are dominant in general in aqueous solutions (Schild 1989). Some authors (Walker 1987; Prange 1989; Otake et al. 1990; Schild 1990) favor the breakdown of polymer-water "hydrogen bonding" interactions in controlling the macromolecular contraction whereas others (Fujishige 1989; Inomata 1990; Kubota et al. 1990; Otake et al. 1990; Feil 1993; Shibayama et al. 1996; Favier et al. 2004) attribute the chain collapse to changes in the "hydrophobic effect", which induces local structure in the solvent molecules surrounding the hydrophobic substituents of the polymer. A third group of authors (Inomata 1990; Winnik 1990; Feil 1993; Volpert et al. 1998; Lin et al. 1999) argues that the CST of the thermoresponsive polymers is associated with changes in both "hydrogen bonding" and "hydrophobic interactions" within the interacting polymer-solvent system. According to the last group, at the molecular level the phase transition of temperature-sensitive polymers is a change from hydrated random coil to hydrophobic globule. As the temperature rises and approaches the phase transition point, the first step of the phase separation is the breaking up of the relatively strong hydrogen bonds, formed around the polymer coil between water molecules and the N-H or C=O groups of the temperature-sensitive polymers, followed by the collapse of the polymer molecule into a hydrophobic globule. Polymer-polymer interactions are

responsible for the aggregation and the subsequent precipitation of the polymer out of solution is taking place since hydrogen bonding becomes weaker and breaks as the temperature is raised (Fujishige 1989; Boutris 1997).

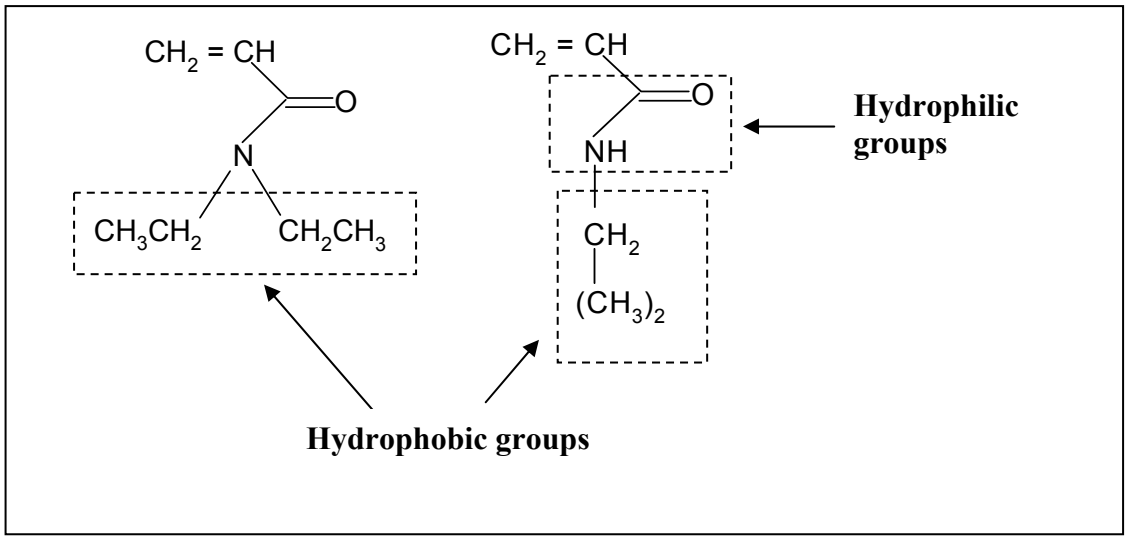

Figure 2. *Molecular structures of two monomers and identification of their hydrophilic and hydrophobic regions.*

2. Thermodynamics of polymer solutions

Specific interactions between solute and solvent are required for the occurrence of the phase transition, which result in negative values for the changes in both the enthalpy, ΔH_m, and the entropy, ΔS_m, of mixing (hydrophobic effect) (Tanford 1973; Ben-Naim 1980). In more details, the water molecules trapped by hydrogen bonds between the side chain amide groups and water form a thin shell of ordered structure around the hydrophilic part of the polymer (Horne et al. 1971). Formation of this hydration shell gives a negative enthalpy for the solution process $\Delta H_m(T_0)$ at a lower temperature T_0, at which the hydrogen bonds stay substantially unbroken (Nemethy 1962). At the same time, water molecules also form a hydrated shell of ordered icelike structure upon the hydrogen bonds between water molecules themselves around the hydrophobic part of the polymer (Horne et al. 1971). Owing to this formation a larger negative entropy change $\Delta S_m(T_0)$ is involved (Nemethy 1962). Increasing temperature tends to break the hydrogen bonds and as a result, the hydrated shells of the polymers are disordered and water molecules are excluded into the bulk and this increases $\Delta H_m(T_0)$ by δH_w and $\Delta S_m(T_0)$ by δS_w. Then, the hydrophobic groups of the polymer are approaching and they form hydrophobic bonding (Nemethy 1962). Hydrophobic

bonding can take place between the same and different polymer chains and this process is manifested as single chain collapse and intermolecular aggregation, suppressing conformation changes of the polymer and hence decreases $\Delta S_m(T_0)$ by δS_h and $\Delta H_m(T_0)$ by δH_h. Hence, $\Delta H_m(T_0)$ increases by $\delta H = \delta H_w - \delta H_h$ as a result of hydrogen bond breaking and hydrophobic bonding. The hydrophobic bonding leads to an increase in $\Delta S_m(T_0)$ given by $\delta S = -\delta S_h + \delta S_w$. Then, the above considerations allow the Gibbs free energy of mixing $\Delta G_m(T) = \Delta H_m(T) - T\ \Delta S_m(T)$ for the solution process at temperature T to be expressed by

$$\Delta G_m(T) = \Delta H_m(T_0) + \delta H(T) - T\ [\ \Delta S_m(T_0) + \delta S(T)\ ] \qquad \text{I. 1}$$

where,

$$\delta H(T) = \delta H_w(T) - \delta H_h(T) \qquad \text{I. 2}$$

$$\delta S(T) = -\delta S_h(T) + \delta S_w(T) \qquad \text{I. 3}$$

The $\delta H(T)$ term is taken as positive and increases with T to make $\Delta H_m(T) = \Delta H_m(T_0) + \delta H(T)$ positive at the phase separation (Zeng et al. 1997). The entropy change $\delta S(T)$ for hydrophobic bonding between isolated pairs of hydrophobic side chains is positive, but decreases with temperature (Nemethy 1962). The term $-T\ [\ \Delta S_m(T_0) + \delta S(T)\ ]$ is therefore also taken as positive (Zeng et al. 1997). As a consequence, the sum of the second and third terms on the right-hand-side of equation I. 1 increases monotonically with increasing T and the negative $\Delta H_m(T_0)$ is increasingly compensated by the sum of these terms, finally resulting in a positive $\Delta G_m(T)$ (Barker 2003). This predicts that, as the temperature is raised, the system becomes increasingly unstable and phase separation takes place above a critical temperature (Schild 1992; Zeng et al. 1997; Idziak et al. 1999).

Although the thermodynamic theory of phase transition and gel swelling is a classical subject, there have been a number of recent theoretical articles aimed mostly at the polyNIPAAM system (Tanaka 1979; Inomata 1994; Lele 1997; Hino 1998). Most of them are semi-empirical extensions to Flory – Huggins theory.

The **Flory – Huggins theory** (Flory 1953) is a lattice mean-field approximation to macromolecular solutions. It considers that there is an "entropy-of-mixing" and an "interaction-energy" contribution to the Gibbs free energy of mixing. The "entropy-of-mixing" contribution arises from the number of possible configurations of the solutes in solution. The larger the solute, the smaller its contribution to the "entropy-of-mixing". The "interaction-energy" contribution arises from the interactions between the monomer units on the different macromolecules and between the monomer units and the solvent molecules (Flory 1953). Traditionally the Flory – Huggins theory is applied to nonionic systems.

For a nonionic system, the *entropic contribution* is given by,

$$\Delta S_m = - k \, (n_1 \ln \varphi_1 + n_2 \ln \varphi_2) \qquad \text{I. 4}$$

where k is the Boltzmann's constant, n_i are the number of moles and φ_1 are the volume or molar fractions of 1 = solvent and 2 = polymer and are given by

$$\varphi_1 = \frac{x_1 n_1}{x_1 n_1 + x_2 n_2} \qquad \text{I. 5}$$

$$\varphi_2 = \frac{x_2 n_2}{x_1 n_1 + x_2 n_2} \qquad \text{I. 6}$$

where x_i are the number of segments in species (1 = solvent and 2 = polymer). For monomeric solvent, $x_1 = 1$.

Considering three interactions, solvent–solvent, polymer–polymer and solvent–polymer, the *interaction contribution* is given by,

$$\Delta H_m = k T \chi \varphi_2 \, n_1 x_1 \qquad \text{I. 7}$$

where $\chi \propto \frac{1}{T}$ is the polymer-solvent interaction parameter.

The polymer-solvent interaction parameter accounts for free-energy changes caused by the mixing process. Values of χ are usually between 0 and 1, with an increased of

χ indicating poorer solvents for the polymer and thus reduced degrees of polymer dissolution. It is important to recognize that χ is not a constant for a given system but is a function of temperature and concentration.

If one combines the configuration and the interaction contributions then for $x_1 = 1$, the Gibbs free energy of mixing can be expressed by

$$\Delta G_m = kT\ (n_1 \ln \varphi_1 + n_2 \ln \varphi_2 + \chi \varphi_2 n_1) \qquad \text{I. 8}$$

Chemical potential and Osmotic pressure. The chemical potential μ_1 of the solvent in the solution relative to its chemical potential μ_1^0 in the pure liquid is obtained by differentiating the free energy of mixing, equation I. 8, with respect to the number n_1 of solvent molecules (Flory 1953). The result is multiplied by Avogadro's number N in order to obtain the chemical potential per mole and the equation is given by

$$\mu_1 - \mu_1^0 = RT\ [\ln (1-\varphi_2) + (1-1/x_2)\ \varphi_2 + \chi \varphi_2^2] \qquad \text{I. 9}$$

where R is the gas constant.

For large values of x_2 and at high concentrations, equation I. 9 becomes,

$$\mu_1 - \mu_1^0 = RT\ [\ln (1-\varphi_2) + \varphi_2 + \chi \varphi_2^2] \qquad \text{I. 10}$$

For the osmotic pressure, $\Pi\ V_1 = -\ (\mu_1 - \mu_1^0)$, where V_1 is the molar volume of the solvent. Hence, according to equation I. 10 the osmotic pressure is given by,

$$\Pi = -\ (RT/\ V_1)\ [\ln (1-\varphi_2) + \varphi_2 + \chi \varphi_2^2] \qquad \text{I. 11}$$

Phenomena occurring at the CST of aqueous polymer solutions have been investigated in the literature by a wide variety of experimental techniques, including *IR spectroscopy* (Scarpa 1967; Snyder 1967), *^{1}H N.M.R. spectroscopy* (Ohta 1991; Tokuhiro 1991; Zeng et al. 1997), *viscometry* (Heskins 1968; Fujishige 1987; Kubota et al. 1990), *light scattering* (Fujishige 1989; Kubota et al. 1990; Favier et al. 2004), *fluorescence techniques* (Winnik 1990; Winnik 1990; Schild et al. 1991; Winnik 1991), *calorimetry* (Heskins 1968; Fujishige 1989; Schild 1990; Schild et al. 1991),

U.V. turbidimetry (Taylor 1975; Cole 1987; Schild 1990; Schild et al. 1991; Cho et al. 1994; Boutris 1997; Liu et al. 1999) and *visual observation* of macroscopic phase separation (Boutris 1997).

Modification of the CST can be obtain by *copolymerization* (Taylor 1975; Ringsdorf et al. 1991; Schild et al. 1991; Schild et al. 1991; Winnik 1991; Schild 1992; Feil 1993; Shibayama et al. 1996; Chee et al. 2001; Kujawa et al. 2001; Barker 2003) and by the addition of *salts* (Freitag 1994; Idziak et al. 1999; Percot et al. 2000; Panayiotou et al. 2004), *solvents* (Otake et al. 1990; Schild et al. 1991; Winnik et al. 1992) and *surfactants* in the aqueous solutions (Idziak et al. 1999; Garret-Flaudy et al. 2001).

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Chapter 1. Synthesis and Characterization of *N*-substituted acrylamide polymers

1.1 Introduction

N-substituted polyacrylamides have attracted much interest due to their unique thermal response in aqueous medium (Fujishige 1989). The phase transition temperature of this series of *N*-substituted polyacrylamide derivatives differs strictly depending on the chemical structure of the side chains (Itoh 1989). For example, the simplest analogue, polyacrylamide, is a water-soluble polymer. Poly (*N*-methylacrylamide) and poly (*N,N*-dimethylacrylamide) are also water-soluble but poly (*N*-*n*-butylacrylamide) and poly (*N*-*tert*-butylacrylamide) are not. Other analogues are soluble in cold water but separate from solution upon heating. For instance, aqueous solutions of poly (*N*-isopropylacrylamide) and poly (*N,N'*-diethylacrylamide) possess this interesting property. Their ability to exhibit thermoresponsive "smart" behavior, i.e. a critical solution temperature in aqueous solution has attracted much attention. The quickness with which the thermoreversible response of such species is established in aqueous media has been attributed to the operation of a two-stage mechanism: individual chains collapse in a coil-to-globule transition prior to aggregation of the resultant globules (Fujishige 1989; Yamamoto 1989; Wu 1995).

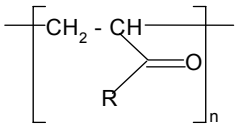
The modification of the critical solution temperature of the thermosensitive polymers is of primary interest. The CST of the mono- and di-*N*-substituted acrylamide polymers can be modified easily by copolymerization with other monomers (Biggs et al. 1993; Feil 1993; Liu et al. 1999; Chee et al. 2001; Gan et al. 2001; Nichifor et al. 2003). By copolymerization with a more hydrophilic monomer, even hydrophobic polymers can be made to posses a CST. Similarly, water-soluble polymers can be made to possess a CST by copolymerization with a hydrophobic monomer. Studies (Feil 1993; Barker 2003) have shown that a physical change is effected in the structures adopted by the copolymers both below and above their respective CSTs upon incorporation of the more polar (hydrophilic) comonomer. Below the CST of each particular system, the change in segmental mobility of the copolymer, induced by incorporation of the hydrophilic comonomer, is slight. Above the CST of the

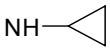
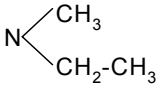
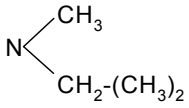
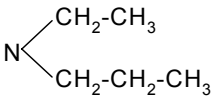
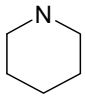
system and as its hydrophilic content is increased, the polymer coil becomes increasingly permeated by water, the segmental mobility of the macromolecule is significantly enhanced and the globules are becoming increasingly expanded and flexible.

The approach has been already used for the copolymerization of acrylamide with diacetone acrylamide (Taylor 1975) or *N,N*-diethylacrylamide (Liu 2001) and for the copolymerization of *N,N*-dimethylacrylamide with *N*-alkylacrylamides (Asada 1996), *N*-phenyl acrylamide (Miyazaki 1996), alkylacrylates (Mueller 1992) or 3-(acrylamido) phenyl boronic acid (Kataoka 1994). Besides acrylamides, other monomers such as vinyl lactams with different hydrophilicity were copolymerized in order to obtain thermosensitive polymers (Suwa 1997).

Thermoresponsive macromolecules constitute interesting materials. Especially for applications in the life sciences, the material should be as homogeneous and consistent as possible. For this reason, it has been suggested (Garret-Flaudy 2000) that oligomeric substances (mass-average molar mass < 5000 g/mol) have some advantages because they can be produced with low polydispersities and their solutions show less increase in viscosity even at fairly high concentrations. The critical temperature of the oligomers tends to be very close to that of the corresponding polymer (Eggert 1994). For this reason, mainly chain transfer polymerization (telomerization) and anionic polymerization have been applied for the synthesis of the polymers of our interest.

Table 1. *LCST of aqueous solutions of poly* (*N-substituted acrylamides*) (Galaev et al. 1993)

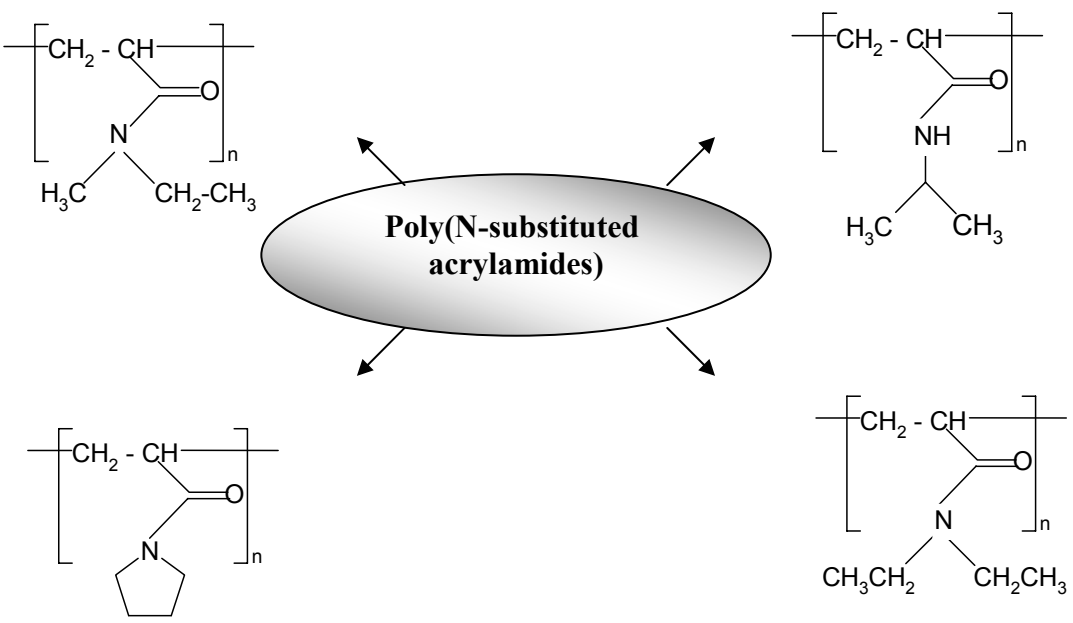


R	Poly (N-alkylacrylamide)	LCST (°C)
NH_2	Poly(acrylamide)	no LCST,soluble in water
$NH\text{-}CH_3$	Poly(N-methylacrylamide)	no LCST,soluble in water
$NH\text{-}CH_2\text{-}CH_3$	Poly(N-ethylacrylamide)	82
$NH\text{-}CH_2\text{-}CH_2\text{-}CH_3$	Poly(N-n-propylacrylamide)	22
$NH\text{-}CH\text{-}(CH_3)_2$	Poly(N-isopropylacrylamide)	32-34
	Poly(N-cyclopropylacrylamide)	47
$NH\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_3$	Poly(N-n-butylacrylamide)	no LCST,insoluble in water
$N\text{-}C(CH_3)_3$	Poly(N-t-butylacrylamide)	no LCST,insoluble in water
$N\text{-}(CH_3)_2$	Poly(N,N-dimethylacrylamide)	no LCST,soluble in water
	Poly(N-ethyl,N-methylacrylamide)	56
$N\text{-}(CH_2\text{-}CH_3)_2$	Poly(N,N-diethylacrylamide)	32-42*
	Poly(N-isopropyl,N-methylacrylamide)	25
	Poly(N-ethyl,N-propylacrylamide)	no LCST,insoluble in water
$N\text{-}(CH_2\text{-}CH_2\text{-}CH_3)_2$	Poly(N,N-dipropylacrylamide)	no LCST,insoluble in water
	Poly(N-acryloylpyrrolidine)	55
	Poly(N-acryloylpiperidine)	4

*Depending on the tacticity of the polymer

The most extensively studied polymer in the family of poly (*N*-substituted acrylamides) is poly (*N*-isopropylacrylamide). Only a few studies were conducted on poly (*N,N*-diethylacrylamide) even though this polymer behaves similarly as poly (*N*-isopropylacrylamide) without the ability to form hydrogen bridges with the oxygen of water because it lacks the proton of the amide group. This Chapter focuses on the synthesis of these two poly (*N*-substituted acrylamides) but also on the synthesis and characterization of two novel polymers of the same family, namely poly (*N*-ethyl,*N*-methylacrylamide) and poly (*N*-pyrrolidinoacrylamide) (known also as poly (*N*-acryloylpyrrolidine)), Scheme 1. These polymers have been synthesized by chain transfer polymerization (telomerization) and anionic polymerization using *n*-BuLi as initiator and mostly their critical solution temperature (CST) and molecular weight have been characterized by a variety of experimental techniques. In order to investigate the co-monomer influence on the CST of poly (*N*-isopropylacrylamide), poly (isopropylacrylamide-*co*-dimethylacrylamide) has been synthesized and comprised a small part of our research.

Poly (*N*-ethyl,*N*-methylacrylamide) **Poly (*N*-isopropylacrylamide)**



Poly (*N*-pyrrolidinoacrylamide) **Poly (*N,N*-diethylacrylamide)**

Scheme 1. *Chemical structures of the poly* (*N-alkylacrylamides*) *prepared in this study.*

1.2 Experimental Procedures

Materials

Almost all chemicals including the solvents used for the synthesis were from Sigma Aldrich Chemical (Buchs, Switzerland). *N,N'*-Diethylacrylamide (DEAAM) was obtained by Polysciences Inc. Europe (Eppelheim, Germany). The highest available purity was used throughout. Water was purified with an ELIX 3 water purification system (Millipore). Acryloylchloride, triethylamine, ethylmethyl amine and pyrrolidine were distilled for further purity before use. AIBN (2,2-azoisobutyronitrile) was recrystallised twice from diethyl ether prior to use. The chain transfer agent MPA (3-mercaptopropionic acid) was purified by distillation under reduced pressure. Solvents, such as diethyl ether and tetrahydrofuran (THF) were dried by boiling over Na wire, kept under argon atmosphere afterwards, and freshly distilled whenever needed. Unless indicated otherwise, all other substances were used as obtained from the supplier.

Preparation of the monomers and the oligomeric acrylamides

1.2.1 Synthesis of the monomers

For the synthesis of the monomers, *N*-ethyl,*N*-methylacrylamide and *N*-pyrrolidinoacrylamide (or *N*-acryloylpyrrolidine), the ethylmethyl amine and pyrrolidine, respectively, was dissolved in a mixture of ether and triethylamine and placed under nitrogen in a dry three-necked flask. The solution was cooled to 0 °C and the acryloylchloride was added drop wise over the next 1 h. The mixture was stirred for 4 h. A temperature below 3 °C was carefully maintained during the synthesis procedure. The final product was filtered under vacuum and the ether removed by a rotary evaporator. CaH_2 was added into the solution as a drying agent. The residue was distilled under vacuum. The general reaction for monomer synthesis between acryloylchloride and amine (ethylmethyl amine or pyrrolidine) is given below:

$$CH_2{=}CHCOCl + R_1R_2NH \rightarrow CH_2{=}CHCONR_1R_2 + HCl$$

Table 2. *Feed composition for the synthesis of the monomers*

Monomers	Molar ratio			
	acryloylchloride:	alkylamine:	triethylamine:	ether
N-ethyl,*N*-methylacrylamide Or *N*-pyrrolidinoacrylamide	1	1	1	19

Table 3 summarizes the experimental conditions during monomer distillation under vacuum and the yield of the monomers obtained at the end.

Table 3. *Yield of the monomer synthesis*

Monomer	b.p. (°C)	P (mbar*)	Yield
N-ethyl,*N*-methylacrylamide	25-30	0.4	60 ± 5 %
N-pyrrolidinoacrylamide	45-49	0.2	50 ± 5 %

* 1mmHg = 1.333 mbar

1.2.2 Chain Transfer Polymerization (or Telomerization) of the monomers

The advantages of chain transfer polymerization are the easy control of the molecular weight and it is also a convenient way to introduce a functional group at one polymer chain end. We employ this methodology to prepare COOH-terminated poly (alkylacrylamides) such as poly (*N*-isopropylacrylamide), poly (*N,N*-diethylacrylamide), poly (*N*-ethyl,*N*-methylacrylamide), and poly (*N*-pyrrolidinoacrylamide), and the copolymer poly (isopropylacrylamide-*co*-dimethyacrylamide), with different molecular weights.

The mechanism of the chain transfer polymerization is presented in Scheme 2.

Initiation

$$I_2 \xrightarrow{k_d} 2\,R\bullet \quad (1)$$

$$R\bullet + XY \xrightarrow{k_{tr,I}} RY + X\bullet \quad (2)$$

$$X\bullet + M \xrightarrow{k_{i,X}} X\text{–}M_1\bullet \quad (3)$$

$$[\, R\bullet + M \xrightarrow{k_i} R\text{–}M'_1\bullet \,]$$

Propagation

$$X\text{–}M_n\bullet + M \xrightarrow{k_p} X\text{–}M_{n+1}\bullet \quad (4)$$

$$[\, R\text{–}M'_1\bullet + M \xrightarrow{k_p} R\text{–}M'_{n+1}\bullet \,]$$

Chain transfer

$$X\text{–}M_n\bullet + XY \xrightarrow{k_{tr,X}} X\text{–}M_n\text{–}Y + X\bullet \quad (5)$$

$$[\, R\text{–}M'_n\bullet + XY \xrightarrow{k_{tr}} R\text{–}M'_n\text{–}Y + X\bullet \,]$$

Termination

$$X\bullet + X\bullet \xrightarrow{k_{t,X}} X_2 \quad (6)$$

$$X\text{–}M_n\bullet + X\text{–}M_p\bullet \xrightarrow{k_t} \text{Polymer} \quad (7)$$

$$[\, R\text{–}M'_n\bullet + X\text{–}M_n\bullet \xrightarrow{k_t} R\text{–}M_{2n}\text{–}X \,]$$

$$[\, R\text{–}M_n\bullet + R\text{–}M_p\bullet \xrightarrow{k_t} \text{Polymer} \,]$$

Scheme 2. *Mechanism of the chain transfer polymerization*: I = *initiator*, R = *radical*, XY = *telogen*, M = *monomer*, k_d *is the rate constant for the initiator dissociation*, k_i *is the rate constant for the initiation step*, k_p *is the rate constant for propagation*, k_t *is the rate constant for termination*. *The reactions in the brackets indicate possible competitive reaction steps due to polymerization initiated by the initiator radicals. The main reactions are*: (1) *Decomposition of the initiator,* (2) *addition of the initiator*

radical to the telogen, (3) *addition of the telogen radical to the first monomer,* (4) *addition of the propagating radical to more monomer molecules,* (5) *chain transfer of the propagating radical with the telogen,* (6) *termination of two telogen radicals,* (7) *termination of two propagating radicals.*

Chain transfer polymerization of the acrylamide monomers was performed in the presence of the chain transfer agent, MPA, according to the procedure described by Chen and Hoffman (Chen 1992). The monomer was dissolved in methanol and placed in a three-necked flask at 70 °C. The radical starter, AIBN and the chain transfer agent were added and the mixture was refluxed for 3 h under argon or nitrogen. The mixture was cooled to room temperature and the solvent was removed by distillation (rotary evaporator). In order to obtain oligomers with a narrow molecular mass distribution, fractionation was done using acetone as solvent and *n*-hexane as non-solvent, as suggested by Fujishige (Fujishige 1987). The fractionated polymer was dried in vacuum until constant weight.

The synthesis reaction for the chain transfer polymerization of the poly (*N*-alkylacrylamides) is schematically shown below:

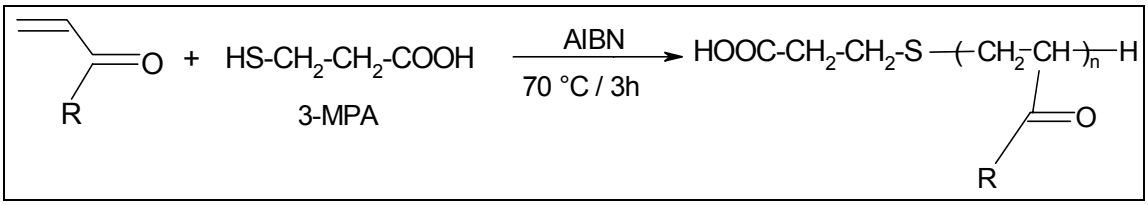


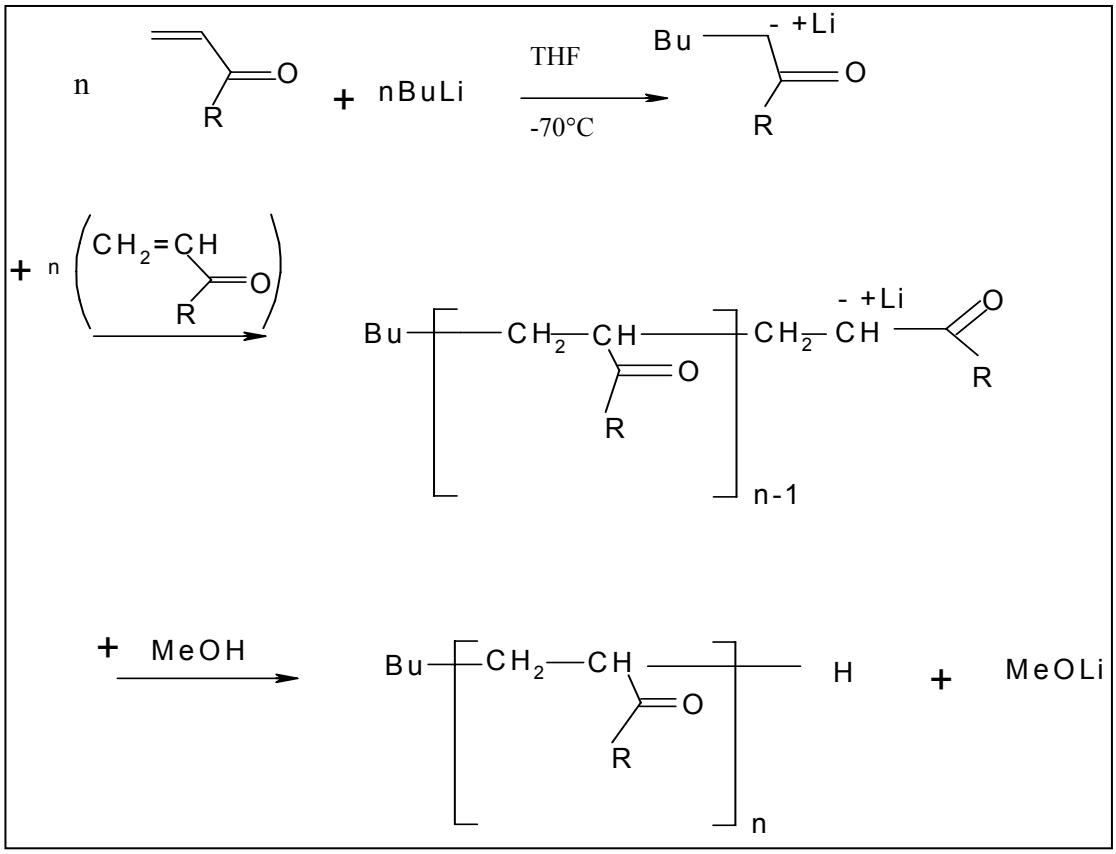
Table 4. *Feed composition of the monomer, MPA and AIBN used during the chain transfer polymerization*

Polymer	Abbreviation	Molar ratio		
		Monomer : MPA : AIBN		
Poly(*N*-isopropylacrylamide)	PolyNIPAAM	100	3	0.3
		100	1.5	0.5
		100	1.5	0.15
Poly(*N*-ethyl,*N*-methyl acrylamide)	PolyEMAAM	100	3	0.3
		100	3	0.6
		100	6	0.6
Poly(*N*-pyrrolidinoacrylamide)	PolyPLAAM	100	3	0.3
		100	6	0.6
Poly(*N,N'*-diethylacrylamide)	PolyDEAAM	100	6	0.6
		(NIPAAM+DMAAM) : MPA : AIBN		
Poly(NIPAAM-co-DMAAM)		80 + 20	6	0.6
		60 + 40	6	0.6

1.2.3 Anionic Polymerization of the monomers

Poly (*N*-ethyl,*N*-methylacrylamide), Poly (*N*-pyrrolidinoacrylamide), Poly (*N,N*-diethylacrylamide) were also prepared by anionic polymerization.

The mechanism of anionic polymerization is schematically shown below.



The monomer was dissolved in dry THF and placed under argon in a dry, septum sealed flask. The mixture was cooled at –78 °C and the polymerization initiated by rapid injection of 1mL *n*-Butyllithium (1.6 M in hexane). In all cases the molar ratio between monomer and initiator was 10:1. After 1 hour of good stirring, the polymerization was quenched by the addition of methanol. After being warmed to ambient temperature, the larger part of the solvent was removed by distillation and the polymer was precipitated twice into hexane and dried in vacuum until constant weight.

Monomer and polymer characterization

A systematic investigation of the phase transition behavior of aqueous polymer solutions was carried out in order to investigate the effect of synthesis conditions on the reported phase transition temperatures. A variety of experimental techniques has been applied, namely NMR spectrometry, MALDI-TOF, titration and cloud point measurements.

1.2.4 Characterization of the monomers

Nuclear Magnetic Resonance (NMR)

Monomers were identified using ^{1}H-NMR spectrometry. The spectra were recorded with a WM 400 (400 MHz) and a WM 200 FT spectrometer (200 MHz, both Bruker, Billerica, MA, USA). $CDCl_3$ was used as the solvent.

1.2.5 Characterization of the polymers

Nuclear Magnetic Resonance (NMR)

For polymer characterization, the same apparatus as the one indicated above for monomer characterization was used. NMR spectrometry is one of the most common experimental techniques used for the investigation of the polymers (Ohta 1991).

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS)

The molecular mass distribution was determined by high molecular weight mass spectrometry (MALDI-TOF, Atheris Laboratories, Geneva, Switzerland). Details concerning the experimental conditions and the sample preparation are given in the Appendix.

The values for the apparent mass-average molar mass, M_w, and the apparent number-average molar mass, M_n, were calculated from the mass spectra using the following formulas:

$$M_w = \frac{\sum NiMi^2}{\sum NiMi} \qquad \text{I1. 1} \qquad\qquad M_n = \frac{\sum NiMi}{\sum Ni} \qquad \text{I1. 2}$$

where Mi is the mass of a given unimolecular oligomer species in a given sample, while Ni is the number of the molecules of that weight in the preparation.

The degree of polydispersity (PD) is calculated as:

$$\text{PD} = \frac{M_w}{M_n} \qquad \text{I1. 3}$$

Polymer Titration

In the case of polymers prepared by telomerisation, the number average molecular weight M_n of the polymer was also determined by end-group titration, with a 0.1 M NaOH solution using phenolphthalein as indicator. The molecular weight of the polymer was calculated by dividing the polymer mass in the samples by the molar amount of added NaOH solution.

Cloud point measurements

The critical solution temperature (CST) was determined by cloud point measurements. For this purpose the optical density of the aqueous oligomer solution was monitored as a function of the temperature at 500 nm using a Lambda 20 spectrophotometer (Perkin-Elmer) equipped with a PTP 1 thermostat and a temperature sensor directly inserted in the reference cell. Heating rates were 0.5 °C/min and pure water was used as reference. Solutions of oligomer (0.5 wt %) were prepared by allowing the oligomer to dissolve over night in purified water at 4 °C. Since no bactericide was added, the maximum storage time of solutions was less than 1 week. CSTs were taken at the inflection point in the optical density versus temperature curves (approximated at half height). Measurements were repeated for at least three different batches of each polymer. Batch to batch deviations in the CST were < 2 °C.

1.3 Results and discussion

1.3.1 Characterization of the monomers

Nuclear Magnetic Resonance (NMR)

Figure 1 and Figure 2, show the spectra of the monomers, *N*-ethyl,*N*-methylacrylamide and *N*-pyrrolidinoacrylamide, respectively, obtained immediately after the synthesis and distillation. The chemical shifts are summarized in Table 5 and they identify the presence of the monomer.

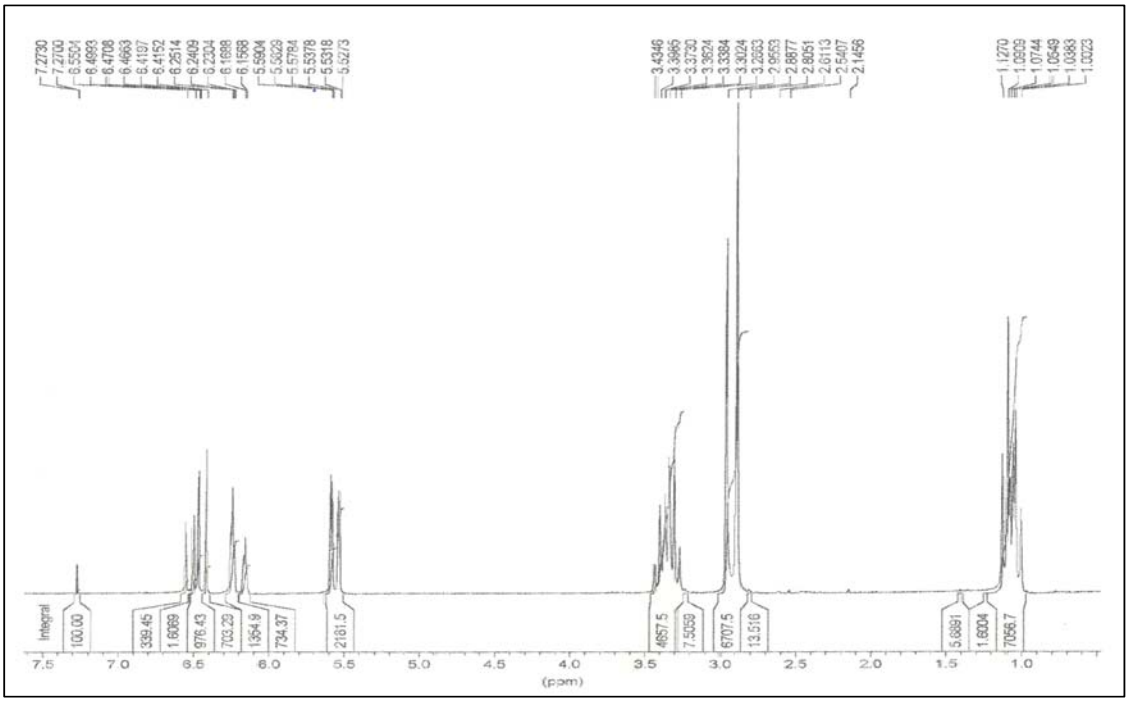


Figure 1. *^{1}H NMR spectrum of N-ethyl,N-methylacrylamide in $CDCl_3$.*

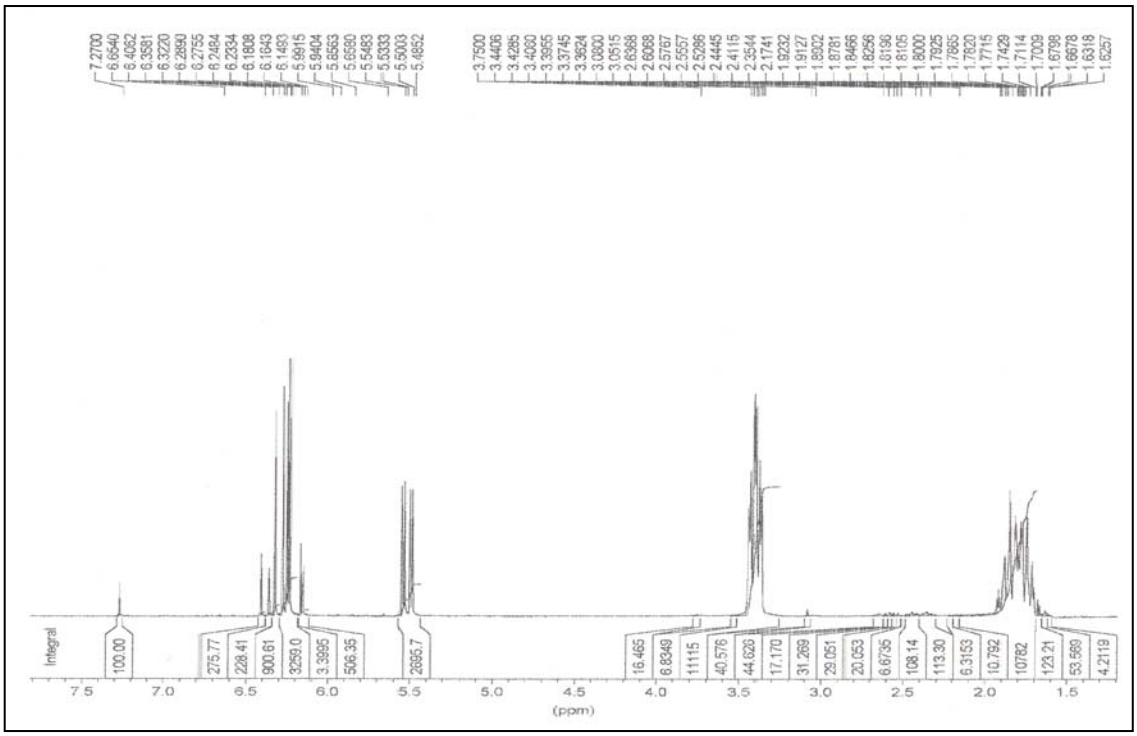
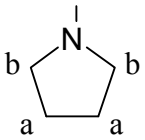


Figure 2. *^{1}H NMR spectrum of N-pyrrolidinoacrylamide in $CDCl_3$.*

Table 5. *Chemical shifts of the monomers*

Monomer	^{1}H NMR	
	δ(ppm)	proton
N –ethyl,*N*-methylacrylamide	1.1, 1.13, 1.17	- $NCH_2C\mathbf{H_3}$
	2.88, 2.95	- $NC\mathbf{H_3}$
	3.37, 3.4, 3.44	- NCH_2CH_3
	5.53, 5.6, 6.16, 6.25	$C\mathbf{H_2}$ = CHR-
	6.42, 6.47, 6.55	CH_2 = C**H**R-
N-pyrrolidinoacrylamide	a. 1.7- 1.8	
	b. 3.35- 3.45	
	5.53, 5.6, 6.2	$C\mathbf{H_2}$ = CHR-
	6.3- 6.4	CH_2 = C**H**R-

1.3.2 Preparation of the oligomeric acrylamides

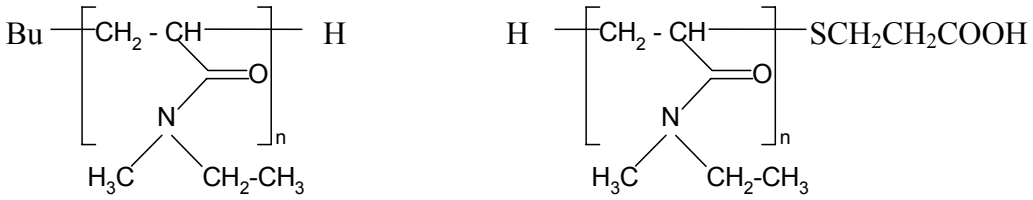
In order to investigate the thermoprecipitation of a variety of polymer chemistries, the oligomers compiled in Table 6 were synthesized. Due to the presence of the nitrogen-bound hydrogen in the side chain of the monomer unit (acidity), the polyNIPAAM could only be produced by radical polymerization (Boutris 1997). While polyNIPAAM can only be prepared by radical polymerization, including chain transfer polymerization (Garret-Flaudy et al. 2001), polyDEAAM, polyEMAAM and polyPLAAM are also accessible via other types of polymer synthesis, including anionic polymerization (Kobayashi et al. 1999; Panayiotou et al. 2004). Therefore, with the exception of polyNIPAAM all polyacrylamides were produced by chain transfer polymerization and anionic polymerization.

Table 6. *Polyacrylamides and their corresponding abbreviations prepared by chain transfer and anionic polymerization*

Polymer	Abbreviation	Polymerization
Poly(*N*-isopropylacrylamide)	PolyNIPAAM	chain transfer
Poly(*N,N'*-diethylacrylamide)	PolyDEAAM	chain transfer
		anionic
Poly(*N*-ethyl,*N*-methyl acrylamide)	PolyEMAAM	chain transfer
		anionic
Poly(*N*-pyrrolidinoacrylamide)	PolyPLAAM	chain transfer
		anionic

All polymers prepared by anionic polymerization should have a butyl-residue stemming from the initiator at the starting end and an additional proton at the other, while polymers prepared by chain transfer polymerization should carry a carboxylic acid end group at the starting end of each molecule and a proton at the other.

Figure 3 presents the chemical structure of poly (*N*-ethyl,*N*-methylacrylamide) with the end groups derived from the two polymerization methods.



I II

Figure 3. *Poly* (*N-ethyl,N-methylacrylamide*) *as it derived from* I. *anionic polymerization and* II. *chain transfer polymerization*

1.3.3 Characterization of the oligomers prepared by chain transfer and anionic polymerization

Table 7 summarizes the properties, namely CST, number-average molar mass (obtained by end group titration and by MALDI-TOF analysis), mass-average molar mass (obtained by MALDI-TOF analysis) and polydispersity, of the oligomers synthesized by chain transfer polymerization with different molar ratio between monomer, chain transfer agent and initiator.

Table 7. *Properties of the oligomer samples prepared by chain transfer polymerization*

Polymer	Molar ratio[a]	M_n [b] (g/mol)	M_n [c] (g/mol)	M_w [c] (g/mol)	PD[d]	P_n [e]	CST (°C)
PolyNIPAAM	100 :3 :0.3	4000					33
	100 :1.5 :0.5	7692					33
	100 :1.5 :0.15	6060					33
PolyDEAAM	100 :6 :0.6	2200					34
PolyEMAAM	100 :3 :0.3	3333					71
	100 :3 :0.6		2195	2895	1.32	17.1	72
	100 :6 :0.6	2380	1951	2713	1.39	17.2	72
PolyPLAAM	100 :3 :0.3	5555					74
	100 :6 :0.6	2381	2195	2896	1.32	17.1	74

[a] monomer: chain transfer: initiator, [b] obtained by end group titration, [c] obtained by MALDI-TOF MS analysis, [d] polydispersity, [e] degree of polymerization

Critical solution temperature (CST) and molecular weight of the oligomers prepared by chain transfer polymerization

PolyDEAAM CST values obtained by chain transfer polymerization tend to be almost equivalent to the polyNIPAAM ones. The same result has also been recorded in the literature even when the two polymers were synthesized via a different polymerization route, i.e. group transfer polymerization (Eggert 1994). The same observation can be made for polyEMAAM and polyPLAAM. More details are given below.

A number-average molar mass (M_n) could be determined, in the case of oligomers prepared by telomerization, by titrating the acidic end groups, which each molecule necessarily contained as a result of the starting reaction used in the synthesis, or by

MALDI-TOF analysis. A comparison of the respective critical solution temperatures should give some insight into the influence of the molecular weight on thermoprecipitation. The dependence of phase transition temperature of the thermosensitive polymers on their molecular weight is a matter of dispute. Fujishige et al. (Fujishige 1989) found that the CST of polyNIPAAM is independent of the molecular weight or the concentration (studies were carried out with polymers having an average-number molecular weight between 5 x 10^4 and 8.4 x 10^6 and concentrations lower than 1 wt %). They argued that the coil-globule transition takes place solely depending on the temperature of aqueous polymer solution at the initial stage of the phase separation, followed by the onset of aggregation of individual chain molecules due mainly to the intermolecular interaction between the hydrophobic groups distributed on the surface of the resulting globular particles of the polymer in aqueous solution. However, a certain influence of the polymer chain length on the phase transition temperature of polyNIPAAM at concentrations as low as 0.01- 0.04 wt % was found by other authors (Heskins 1968; Schild 1990; Schild 1992; Tiktopulo 1995). According to Schild et al. (Schild 1990) who studied the phase transition of polyNIPAAM samples having an average-number molecular weight ranging from 5400 to 160 x 10^3, an increase in the CST is to be expected with decreasing molecular weight and they argued that at higher concentration, where the coil-to-globule transition is followed by globule aggregation through intermolecular interactions, molecular weight should have an important influence on CST, as the overlapping concentration is dependent on the chain length. Schild et al. (Schild 1990) remarked that a sharp, low temperature transition is associated with the phase separation of a high molecular weight polyNIPAAM and a broader, higher temperature transition, with shorter chains of polyNIPAAM. The decrease in the CST with increasing molecular weight has not been established only for polyNIPAAM but also for other thermosensitive polymers such as polyDEAAM (Tong et al. 1999; Lessard et al. 2001).

When the oligomers were prepared with different molar ratio between monomer, initiator and chain transfer agent, as it was expected, different molecular weights were obtained for each molar ratio. The differences within the molecular weight of the oligomers were too small in order to show an influence on the critical temperatures of the corresponding oligomers. For example, polyNIPAAM with molecular weights of

4000, 6060 and 7692 g/mol had a critical temperature of 33 °C, regardless of the molecular weight. A similar observation can be made for the other oligomers as well. PolyEMAAM with molecular weights of 2381, 3333 and 3509 g/mol had a critical temperature of 71-72 °C (the difference of 1°C is considered negligible since it is within experimental error) and polyPLAAM with molecular weights of 2381 and 5555 g/mol had a critical temperature of 74 °C. Therefore, molecular weight differences in the range of 200 up to almost 4000 g/mol do not have any significant influence on the CST.

A possible influence of the oligomer's molecular weight on the CST can be derived in comparison with the CST values found in the literature for high molecular weight polymers. Literature values for the CST of polyNIPAAM vary between 31 and 34,3 °C (Taylor 1975; Kubota et al. 1990; Ringsdorf et al. 1991; Schild 1992; Yoshida 1995; Asada 1996; Lin et al. 1999), although slightly higher values have been observed in isolated cases (Ito 1984; Bywater 1985; Schild 1990; Galaev et al. 1993). The 33 °C measured in our investigations fall within that range, although the polyNIPAAM were synthesized by chain transfer polymerization, i.e. have small size and carry a carboxylic acid group. A CST for polyNIPAAM close to 33 °C, when it was produced under similar experimental conditions by chain transfer polymerization, can also be found in the literature (Chen G.H. 1992; Yamazaki et al. 1998; Baltes et al. 1999). It has previously been shown (Freitag et al. 1994; Favier et al. 2004) that the presence and the charge status of such a carboxylic group, which is a relatively hydrophilic group, have no significant influence (a difference of 1 °C is considered negligible) on the precipitation temperature.

PolyDEAAM samples prepared by radical polymerization in the presence of the chain transfer agent have precipitation temperatures of 34 °C, which is close to the temperature obtained by Baltes et al. (Baltes et al. 1999) and Lessard et al. (Lessard et al. 2001) for low molecular weight polyDEAAM but also corresponds well to the temperature obtained for high molecular weight polyDEAAM (Ito 1984; Idziak et al. 1999; Liu et al. 1999; Maeda et al. 2002) prepared by free radical polymerization, but differs from the value of 25 °C reported by Taylor and Cerankowski (Taylor 1975) and 30.5 °C reported by Gan et al. (Gan et al. 2001).

Surprisingly significant differences into the CST reported in the literature were obtained for polyPLAAM (74 °C) and polyEMAAM (72 °C) prepared with chain transfer polymerization. These values, which were measured with minor variation (± 2 °C) for several batches of these polymers, are well above the literature value, namely 53 °C (Asada 1996) and 55 °C (Okahata 1986) in the case of polyPLAAM and 56 °C in the case of polyEMAAM (Ito 1984). Despite the previous results for the independence of the CST of polyNIPAAM and polyDEAAM on the molecular weight, the results obtained for polyPLAAM and polyEMAAM seem to support the hypothesis that "*an increase in the CST is to be expected with decreasing molecular mass*" (Heskins 1968; Schild 1990; Schild 1992). This difference is much more significant for the longer polymer chains, since they should precipitate at lower temperatures (Patterson 1969; Wang et al. 1999).

It was demonstrated by Patterson (Patterson 1969) that the CST is proportional to the critical value of the Flory-Huggins interaction parameter (χ) (Flory 1953) and that the CST decreases with the ratio of the molar volume of the polymer to that of the solvent (r):

$$\chi = ½ (1 + r^{-1/2})^2 \qquad \text{I1. 4}$$

In equation I1. 4 if the molar volume of the polymer increases with the length of the chain, it is clear that the CST of the polymer must decrease with the molecular weight of the polymer (Lessard et al. 2001). Therefore, equation I1.4 could be used to explain the dependence of the CST from the molecular weight of the polymers.

Apart from the molecular weight influence, the differences between CSTs may also arise from the difference in the polymer samples used (i.e. polymer samples concentration, etc) and / or the conditions (i.e. heating rate, experimental technique) used in the measurements of the CST (Boutris 1997; Idziak et al. 1999) which seems to have an effect on the CST especially when it is measured using optical and spectrophotometer techniques. According to Idziak et al. (Idziak et al. 1999) and Boutris et al. (Boutris 1997) who investigated the effect of the polyDEAAM and polyNIPAAM concentrations and the heating rates on the optical measurement of their CST, CST is influenced by these factors. An increase in heating rate results in a shift to higher temperatures. At low concentrations (below 0.5 wt %) the polymer particles fail or are slow to aggregate to a size that can be detected by the

spectrophotometer. This might be attributed to the fact that the cloud point techniques are sensitive only to the macroscopic phase separation phenomena (i.e. the formation of polymer aggregates), which is a much slower process at very low polymer concentrations (Schild et al. 1991). This effect is particularly pronounced for a high heating rate of a dilute solution. Raising the temperature of the solution too quickly results in the recording of a less sharp transition and a too high cloud point. Therefore, when interpreting the UV-visible spectrophotometer results, it must be kept in mind, however, that the polymer particles should be of sufficient size in order to be detected at the selected wavelength setting. Lack of or delayed aggregation of the paricles results in an artificially high CST reading. As the spectrophotometer can only detect particles larger than the scanning wavelength, these measurements also give an indication of the influence of temperature on the size of the polymer aggregates (Idziak et al. 1999; Gan 2001). Since both of these factors were kept constant during our investigation, heating rate, 0.5 °C / min and polymer concentrations, 0.5 wt %, and they are in the range of optimum conditions for measuring the phase transition of polymers using spectrophotometer, they are not probably the main reasons for the differences between the CSTs of polyEMAAM and polyPLAAM.

Critical solution temperature of the oligomers prepared by anionic polymerization and comparison with the CST of oligomers prepared by chain transfer polymerization

Table 8 summarizes the properties, namely CST, number-average molar mass, mass-average molar mass (both obtained by MALDI-TOF analysis) and polydispersity, of the oligomers synthesized by anionic polymerization.

Turbidimetry can be regarded as the more automated counterpart of the classical visual observation of macroscopic phase separation, although it may suffer complications arising from variations in precipitated aggregated sizes and settling of precipitates (Cole 1987).

Table 8. *Properties of the polymer samples prepared by anionic polymerization*

Polymer	M : I [a]	M_n [b] (g/mol)	M_w [b] (g/mol)	PD[c]	P_n [d]	CST (°C)
PolyDEAAM	10 : 1	-	-	-	-	40
PolyEMAAM	10 : 1	i. 1336	2083	1.56	11.8	72
		ii.1563	2727	1.74	13.8	74
		iii. 773	1101	1.42	6.8	72
PolyPLAAM	10 : 1	685	922	1.34	5.34	58

[a] Molar ratio, Monomer : Initiator, [b] obtained by MALDI-TOF analysis,
[c] polydispersity, [d] degree of polymerization

The CST of polyDEAAM prepared by anionic polymerization (Table 8) shows a deviation (several degrees higher; 40 °C) from that of polyDEAAM prepared by chain transfer polymerization (see Table 7). The temperature of 40 °C is very close to the one obtained by Freitag and Baltes for similarly prepared polyDEAAM (Freitag 1994; Baltes et al. 1999). Some differences on the CST of polyDEAAM could be found in the literature. For example, Itakura et al. (Itakura et al. 2000) reported two different CSTs for polyDEAAM when they were prepared by anionic polymerization in THF using two different initiators; the polymer with a diphenyl group at the chain end had a CST around 30 °C and the second polymer, without a diphenyl group, had a CST around 38 °C. Both samples had heterotactic structure. Also, Kobayashi et al. (Kobayashi et al. 1999) reported a CST for polyDEAAM prepared by anionic polymerization in THF with an organolithium initiator, close to 31 °C. Deviations obtained between the CST for a given anionic polymer could be attributed to the different initiators used each time.

The two polyEMAAMs, prepared by anionic and chain transfer polymerization, showed hardly any difference in their respective CSTs, close to 74 and 72 °C, respectively. The difference to the CST of polyEMAAM comparing to the value for the corresponding high molecular weight polymeric polyEMAAM found in the

literature, namely 56 °C (Ito 1984), is might attributed to the preparation and characterization conditions.

Different CSTs were also observed in case of the two polyPLAAMs, although in this case the telomer had the higher CST. One possible reason for the low CST of the anionic polyPLAAM, is the very low molecular weight. On the other hand, the value of 58 °C obtained for the anionic polyPLAAM is close to the values obtained in the literature, for polymers prepared by radical polymerization, namely 55 °C (Okahata 1986).

Therefore, comparing the critical solution temperature of the oligomers, prepared either by chain transfer polymerization or by anionic polymerization, the anionic polymers, except of the anionic polyPLAAM, had a considerably higher CST. Since no direct correlation between the critical temperature and the average molecular weight within the same series of the anionic oligomers has been found, differences in the molecular weight can obviously not be responsible for the differences in the CST.
An effect of the terminal carboxylic acid group of the oligomers prepared by chain transfer polymerization, which could be assumed as another possible reason for a shift in CST, could be ruled out. First of all it is the anionic polyDEAAM (butyl end group) that shows the deviation from the typical polyDEAAM CST of 32 °C (Ito 1984). Secondly it could be shown that the coupling of the terminal carboxylic acid group of the polyDEAAM telomer with a variety of charged and uncharged ligands does not change the CST to a measurable extent. Hence, it can be deduced that the carboxylic end group, whether charged or not, does not influence the CST (Eggert 1994). According to some authors (Freitag 1994; Garret-Flaudy 2000; Garret-Flaudy et al. 2001) the configuration of the polymer is playing an important role which seems to be responsible for the observed difference in their critical solution temperature. Tacticity is influencing the tendency for association in aqueous solution and especially in the case of small oligomers, any structure that stabilizes the individual molecules may influence the CST (Freitag 1994). Therefore, tacticity seems to be a powerful key for the explanation of the differences obtained within the CSTs of oligomers prepared by telomerization and anionic polymerization and it will be discussed in more detail below.

Nuclear Magnetic Resonance (NMR)

NMR spectrometry is one of the most common experimental techniques used for the investigation of the polymers (Ohta 1991). The chemical shifts found in the ^{1}H-NMR spectra of the oligomers prepared by telomerization and anionic polymerization are compiled in Table 9.

^{1}H-NMR measurements enable the estimation of the portion of isotactic, syndiotactic and heterotactic structures within a given polymer (Klöpffer 1984). The chemical shifts of the methine proton should increase in that order.

Figure 4 and Figure 5, show the ^{1}H- NMR spectra for polyEMAAM and polyPLAAM, respectively, prepared by telomerization. Taking the ^{1}H- NMR spectra into account, polyEMAAM (Figure 4), has mostly heterotactic structure and some methine protons seem to be placed in an isotactic one, where no syndiotactic groups seem to be presented.

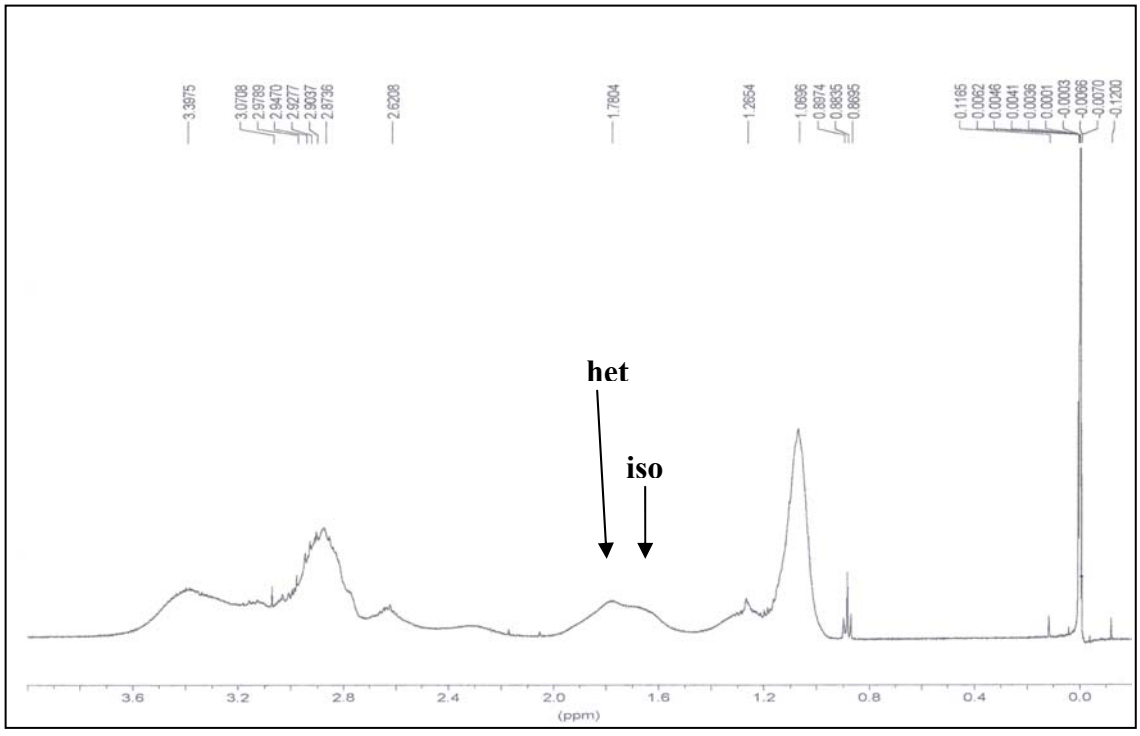


Figure 4. *^{1}H NMR of polyEMAAM prepared by chain transfer polymerization*

Similar observation can be made for polyPLAAM (Figure 5) with the exception that some methine protons can be observed in a syndiotactic structure. In the case which

polyEMAAM prepared by anionic polymerization, a highly heterotactic structure can be reported and few methine groups are placed in an isotactic one (Figure 6). The similarity in the tacticity (heterotacticity) between polyEMAAM prepared by chain transfer polymerization and anionic polymerization, could be used to explain the small difference between their CSTs, with the highly heterotactic oligomer (the anionic one) having a slightly higher CST.

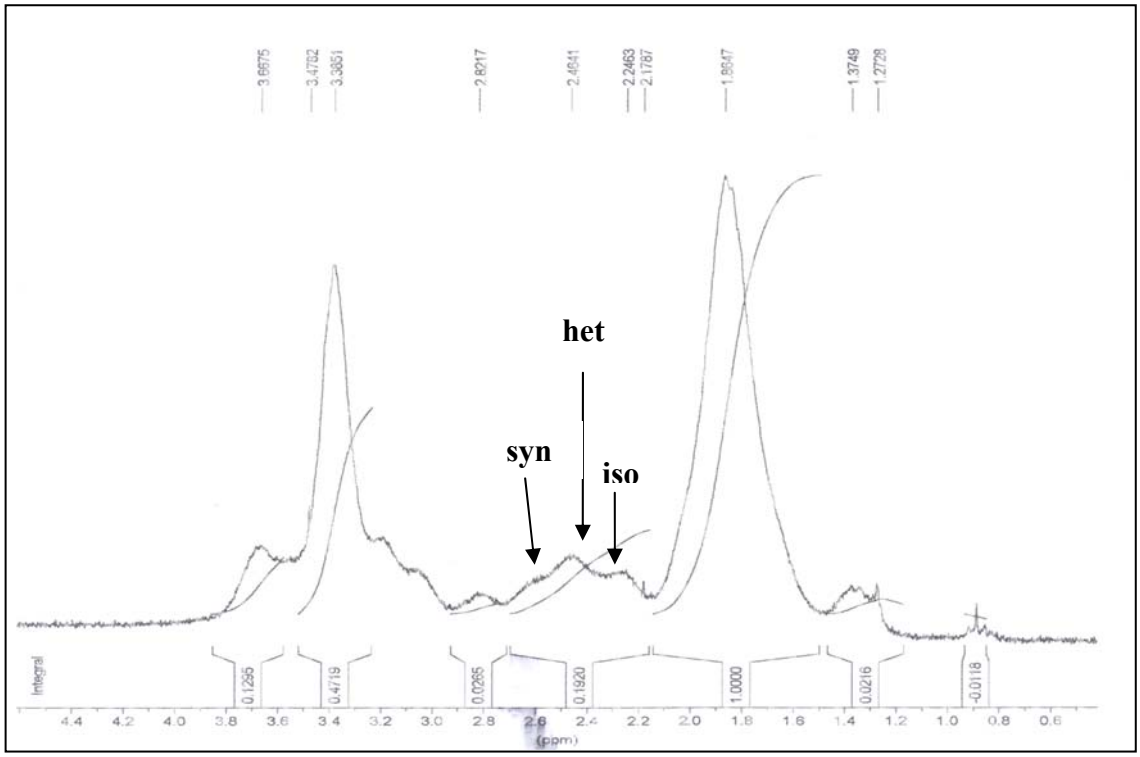


Figure 5 *^{1}H NMR of polyPLAAM prepared by chain transfer polymerization*

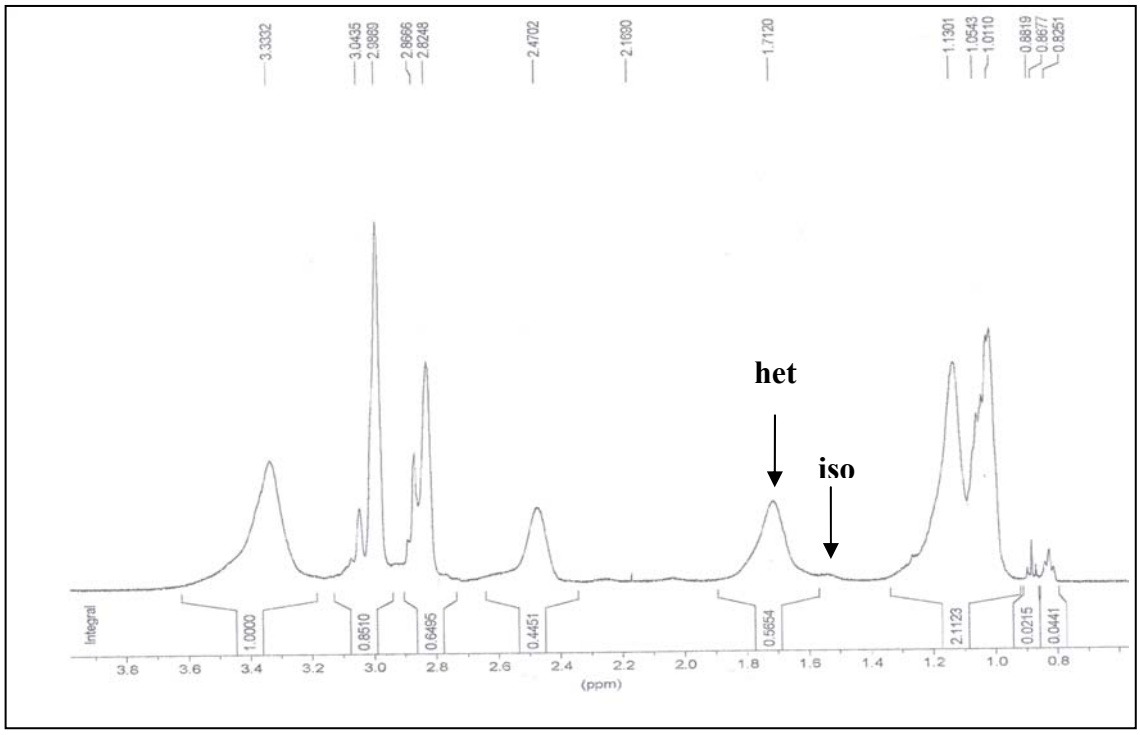


Figure 6. *^{1}H NMR of polyEMAAM prepared by anionic polymerization*

An isotactic structure prevails in polyPLAAM anionic oligomer (Figure 7), whereas some heterotactic groups seem to be present. The result concerning the configuration of anionic oligomers, is supported by many authors (Huynh et al. 1980; Huang 1983; Ilavsky et al. 1985; Freitag 1994), where a pronounced tendency to form isotactic structures is reported mostly for anionic polymers, i.e. poly (*N,N*-diethylacrylamide) and poly (*N,N*-dimethylacrylamide), although hetero- and syndiotacticity has been also observed in some cases for anionic poly (*N,N*-diethylacrylamide), poly (*N,N*-dimethylacrylamide) and poly (*N,N*-dipropylacrylamide) (Kobayashi et al. 1999), especially when they are measured under certain conditions (i.e. in the presence of additives) (Kitayama 1989; Kitayama 1994; Kobayashi et al. 1999).

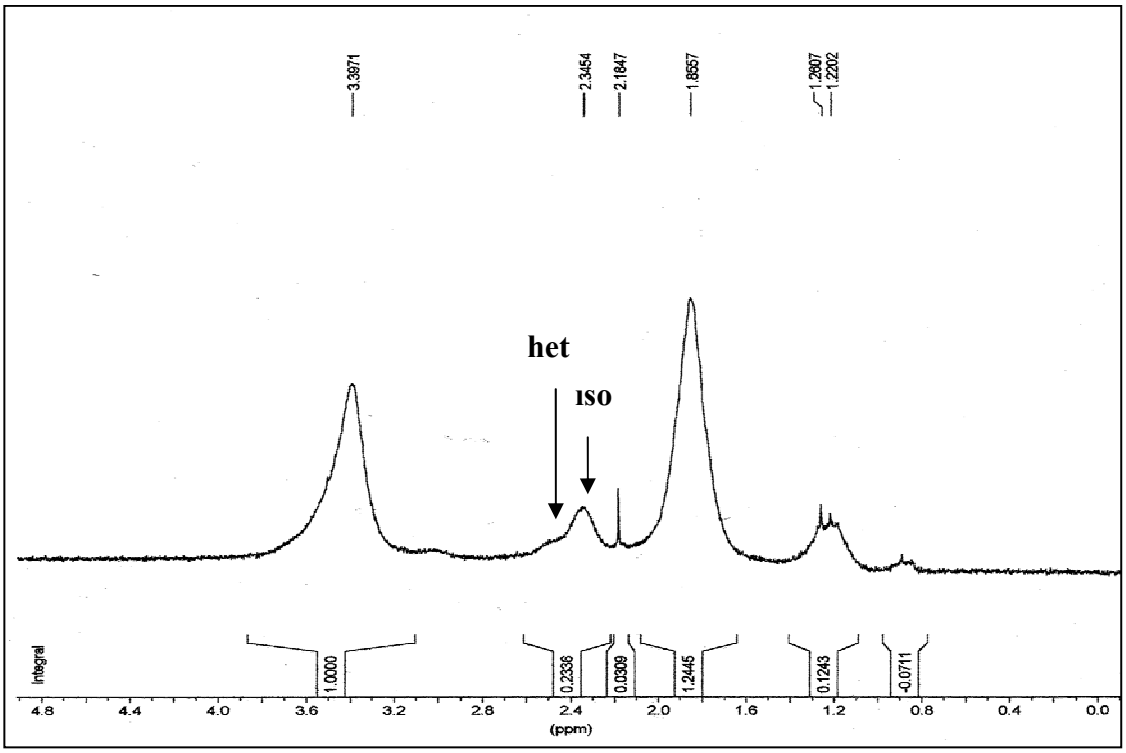
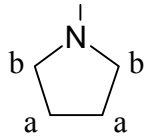
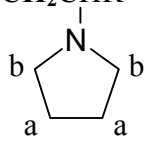


Figure 7. *^{1}H NMR of polyPLAAM prepared by anionic polymerization*

Table 9. *Chemical shifts found in the ^{1}H-NMR spectra of polyEMAAM and polyPLAAM prepared by telomerization and anionic polymerization*

Polymer	Polymerization	^{1}H-NMR	
		δ(ppm)	proton
polyEMAAM	chain transfer	3.397	- NC**H$_2$**CH$_3$
		2.927	- NC**H$_3$**
		2.62	- CH$_2$C**H**R-
		1.78	- **CH$_2$**CHR-
		1.069, 1.265	- NCH$_2$**CH$_3$**
	anionic	3.361	- NC**H$_2$**CH$_3$
		2.839, 2.88, 3.00, 3.057	- NC**H$_3$**
		2.486	- CH$_2$C**H**R-
		1.712	- C**H$_2$**CHR-

		1.011, 1.13	- $NCH_2C\mathbf{H_3}$
polyPLAAM	chain transfer	2.246, 2.464, 2.821	- $CH_2C\mathbf{H}R$-
		1.864	- $C\mathbf{H_2}CHR$-
		a. 1.272, 1.375	
		b. 3.385, 3.667	
	anionic	2.345	- $CH_2C\mathbf{H}R$-
		1.855, 2.184	- $C\mathbf{H_2}CHR$-
		a. 1.22, 1.26	
		b. 3.397	

The literary standpoint that polymers having isotactic structure show higher CST compared to the polymers having heterotactic (Garret-Flaudy 2000) or even syndiotactic (Freitag 1994) is not applicable in the case of polyPLAAM, where oligomers with an heterotactic structure showed considerably higher CST values (74 °C) compared to the CST of the oligomers having an isotactic structure (58 °C).

Matrix-assisted laser desorption / ionization time-of-flight mass spectrometry (MALDI-TOF MS)

High molecular weight mass spectrometry (MALDI-MS) was used to characterize the individual polymers. MALDI-TOF MS analysis reveals for each individual polymer chain present in the sample, its absolute molecular weight and end group structure (Favier et al. 2004). The MALDI technique allows the transfer of intact macromolecules into the gas phase and the assessment of the molecular weight by TOF (time of flight) detection. During that process the macromolecules usually acquire a hydrogen, sodium or potassium ion originating from matrix impurities, which increases the molar mass detected by 1, 23 or 40 mass / charge units, respectively.

In the spectra (Figure 8 and Figure 9) each molar mass causes a double peak whose masses correspond to the sum of the mass of the respective oligomer with hydrogen, sodium or potassium ion attached. MALDI spectra of the other oligomers are not shown.

The height of the peak corresponds to the relative frequency of the respective species in the preparation and the distance between individual peaks corresponds to the mass of the respective repetitive units; 127.2 g/mol for polyDEAAM, 113.2 g/mol for polyNIPAAM and polyEMAAM, and 128.2 g/mol for polyPLAAM (Baltes et al. 1999). MALDI-TOF MS is most reliable for molecules below 100.000 g/mol, so the relevant mass of the synthesized oligomers could be recorded.

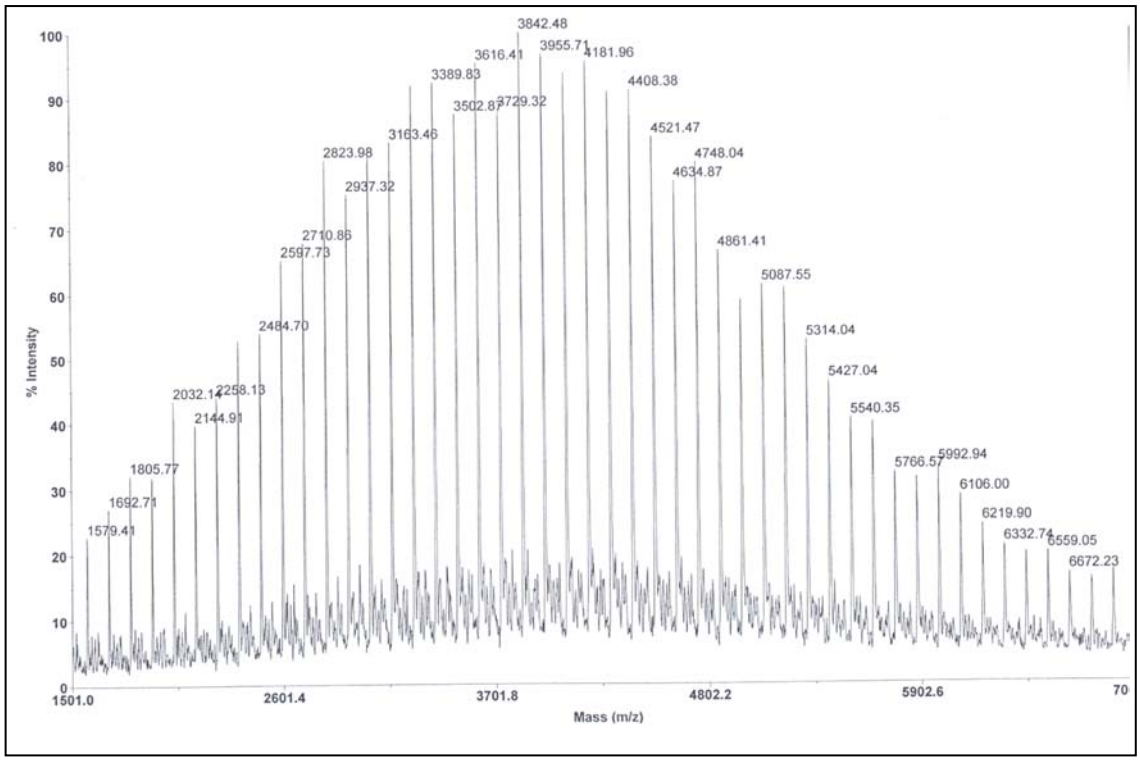


Figure 8. *MALDI-TOF of polyNIPAAM*

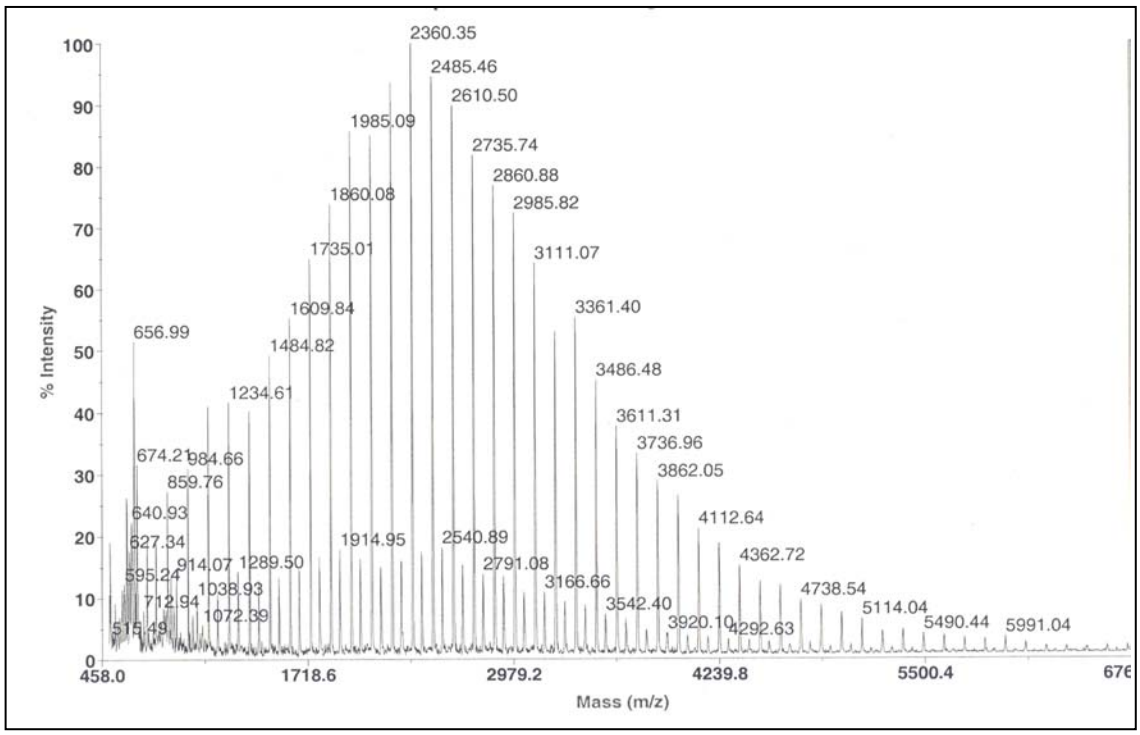


Figure 9. *MALDI-TOF of polyPLAAM prepared by chain transfer polymerization*

Table 7 and Table 8 summarize the mass data for the oligomers prepared by chain transfer and anionic polymerization, respectively. Low polydispersities (< 1.4) were calculated for oligomers prepared by chain transfer polymerization as well for polyPLAAM and polyEMAAM (sample iii) prepared by anionic polymerization, indicating that the polymerizations occurred with few side reactions. Higher polydispersities (1.56 and 1.74) were calculated in the case of anionic polyEMAAM (samples i and ii) indicating the occurrence of deactivation of the growing polymer chain (Xie et al. 1996). Broad polydispersities were obtained in the case of different poly (*N,N*-dialkylacrylamides) by Kobayashi et al. (Kobayashi et al. 1999) when the polymers produced by anionic polymerization. The cause of the polydispersity broadening may have been due to the fast propagating rates during the polymerization (Kobayashi et al. 1999) but also to residual ethylmethylamine that was difficult to remove completely from the monomer during the monomer preparation. The resulting protonation of the polymeric anions can cause several termination reactions and broadens the polydispersity (Xie et al. 1996; Kobayashi et al. 1999).

An average degree of polymerization of 5.34 and 6.8 is calculated for the anionic polyPLAAM and polyEMAAM (sample iii), respectively. These values do not come

close to the degree of polymerization of ten, which was predetermined by the molar ratios chosen for the monomer and the initiator. One possible reason for this finding is due to mass discrimination taking place during the MALDI-TOF MS measurements. This effect would cause the higher molecular weight oligomers to be under-represented (Eggert 1994). However, such a strong influence seems to be unlikely in view of the relatively good agreement between MALDI measurements and titration that has been obtained in this investigation (see Table 7), but also in view of the good agreement (at least for the low molecular weight samples) found in the published literature between MALDI-TOF MS based measurements of the molecular weight of polymers and the standard techniques in general (Kobayashi et al. 1999; Ganachaud et al. 2000). In view of the fact that quantitative polymerization did not take place, the occurrence of terminating reactions seems to be more likely, resulting in lower than the predetermined value.

1.3.4 Copolymerization of *N*-isopropylacrylamide with *N,N*-dimethylacrylamide

Polymerization of NIPAAM with hydrophilic or hydrophobic monomers results in a shift of CST to higher or lower values, respectively (Beltran 1991; Feil 1993; Yoshida et al. 1994; Bignotti 2000; Bokias 2000; Benrebouh 2001; Liu 2001). The copolymerizations of *N*-isopropylacrylamide with the hydrophilic *N,N*-dimethylacrylamide were carried out in methanol, which is a good solvent for both the monomers and the corresponding copolymers. Table 10 summarizes the results of the CST obtained, using UV spectrophotometer, for the poly (NIPAAM-*co*-DMAAM) prepared using two different molar ratios between the two comonomers. The CST of the copolymers always lies in between the CSTs of the two homopolymers. The homopolymer of *N,N*-dimethylacrylamide is water soluble with a critical temperature higher than 100 °C (Asada 1996; Nichifor et al. 2003).

Table 10. *CST of the poly(NIPAAM-co-DMAAM)*

Copolymer		% molar ratio NIPAAM : DMAAM	CST (°C)
Poly(NIPAAM-co-DMAAM)	i.	80 : 20	43
	ii.	60 : 40	53

Increasing the DMAAM content of the poly(NIPAAM-*co*-DMAAM) leads to an increase of the CST (relative to that of polyNIPAAM) to an extent which depends on the amount of the more hydrophilic comonomer (DMAAM) present in the system. The results are as might be expected from the predictions of Taylor and Cerankowski (Taylor 1975) and on the basis of other studies (Feil 1993; Shibayama et al. 1996; Barker 2003) in which the hydrophilic / hydrophobic balance of NIPAAM-based systems was altered. It is reasoned that incorporation of a small amount of hydrophilic monomer (DMAAM), reduces the amount of hydrophobic groups and increases the hydrophilicity of the copolymer, due to to the strong interactions (hydrogen bonding) between water and hydrophilic groups on the polymer. This causes the CST value to increase, since the hydrophobic interactions, which increase with temperature, are compensated for up to a higher temperature by the increased polymer-water interactions (Feil 1993; Barker 2003).

The experimental CST values, at a given DMAAM content, are 3 °C higher than those quoted by Barker et al. (Barker 2003) and a bit lower than the CSTs given for 56 % content of NIPAAM by Asada et al. (Asada 1996), namely 56 °C. The differences obtained compared with the results of Barker et al., could be attributed to the different sample concentration (0.01 wt %) and to the different technique used to measure the phase transition (optical) by them. In our case, a spectrophotometer was used to measure the phase transition of the sample.

For a given chemical composition, the CST of the copolymer can be estimated by the use of the following equation (Liu et al. 1999):

$$T = \frac{\mu_1 T_1 + K'\mu_2 T_2}{\mu_1 + K'\mu_2} \qquad \text{I1. 5}$$

where μ is the molar fraction of a given monomer (note that $\mu_1+\mu_2= 1$), *T* is the CST of the corresponding homopolymers and the subscripts 1 and 2 denote comonomers 1 and 2, respectively, and *K' is* a weighting parameter which can be deduced from curve fitting of the experimental results. In the ideal case, *K'* has a value of 1 and the relationship between comonomers 1 and 2 becomes linear.

Fitting the experimental results on equation I1. 5 and a hypothetical CST for the homopolymer polyDMAAM, have been calculated equal to *K'* = 0.998 and CST_{hyp}= 82.95 °C. This hypothetical CST of polyDMAAM does not exceed the literature reported CST of 100 °C (Asada 1996; Nichifor et al. 2003) and it may be attributed to the lack of experimental points. Still, the hypothetical CST value of polyDMAAM confirms its hydrophilicity and hence the increase of the poly (NIPAAM-*co*-DMAAM) CST.

Temperature measurements in microfluidic systems: A novel application of thermoresponsive copolymers

Note: *The presented application is a result of collaboration with the Microsystems Laboratory (STI-LMIS4) at EPFL*

The manipulation of living biological cells in microfluidic channels by a combination of negative dielectrophoretic barriers and controlled pressure driven flows is widely employed in lab-on-a-chip systems. However, electric fields in conducting media induce Joule heating. Many of today's microfluidic devices do not allow the use of external sensors to measure temperatures at the point of interest, and the integration of dedicated sensors complicates the fabrication and characterisation processes in several ways. For that reason, several methods for detecting temperatures *in situ* have been applied, particularly to monitor temperature fields within working electrophoresis equipment.

Water-soluble polymers, which exhibit thermo-precipitating properties, may be used to detect temperature thresholds near nDEP (negative dielectrophoresis) - barriers in microfluidic channels. It is possible to sense temperature variations of a few degrees with this method.

The measurements with sample i (Table 10) thermosensitive poly (NIPAAM-*co*-DMAAM) in PBS (the recipe of PBS is given in the Appendix), CST 39.8 °C in PBS,

is made under working nDEP conditions. The heat generated in the volume between the electrodes pair is witnessed by the formation of optically detectable precipitate if the medium locally reaches or exceeds a temperature of 39.8 °C. As the effect of precipitation is reversible, the interface between the zones of transparent solution and precipitation corresponds to a temperature-zone at CST (Figure 10 a). By changing the voltage applied to the electrodes during the experiments, this zone can be shifted back and forth, the precipitation zone always indicating temperatures of CST and above (Figure 10 b).

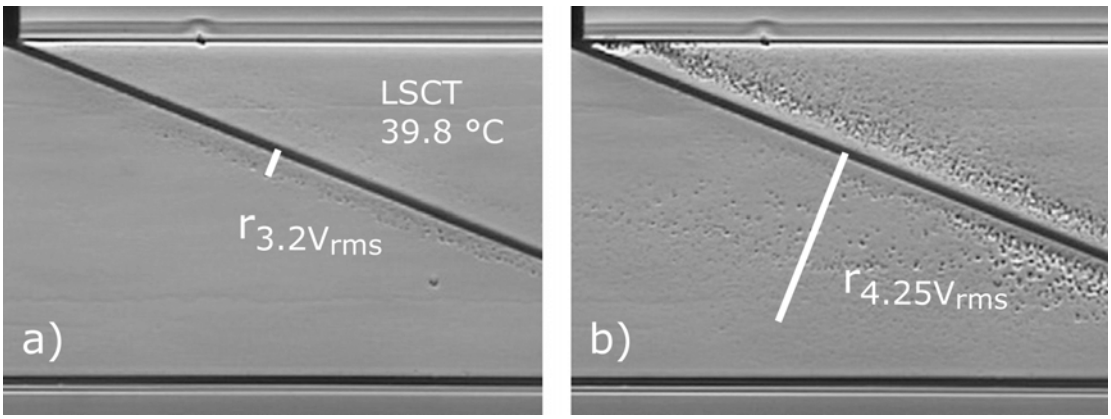


Figure 10. *Photographic top-views of a 300 µm large microchannel with an nDEP-barrier electrode pair reaching in from the side. Poly(NIPAAM-co-DMAAM) in PBS buffer is flowing from right to left.* a) *A sinusoïdal signal of* 3.2 V_{rms} *at* 1 MHz *is applied to the electrode pair; the generated Joule heat induces a visible temperature zone between the precipitate close to the electrodes and the clear solution. The distance electrodes – temperature-zone is indicated by the white bar perpendicular to the electrode pair.* b) *The supply voltage is increased and so is the temperature- zone distance* r. *At greater distances, accuracy decreases. Typical delays are in the order of seconds.*

With this kind of *in situ* temperature measurement it is not possible to obtain two-dimensional temperature distributions in one shot, but by sweeping through a range of supply voltages and correlating the distance r from the electrode pair to the temperature-zone, useful information can be retrieved. Such a correlation is plotted in Figure 11. A non-linear behavior of this distance vs. supply-voltage relationship is observed; the nonlinear behavior is in agreement with the fact that the injected electrical power goes with the square of the voltage. Again, critical temperatures well

above the physiological value of 37 °C are attained when too much power is applied to the nDEP-barrier.

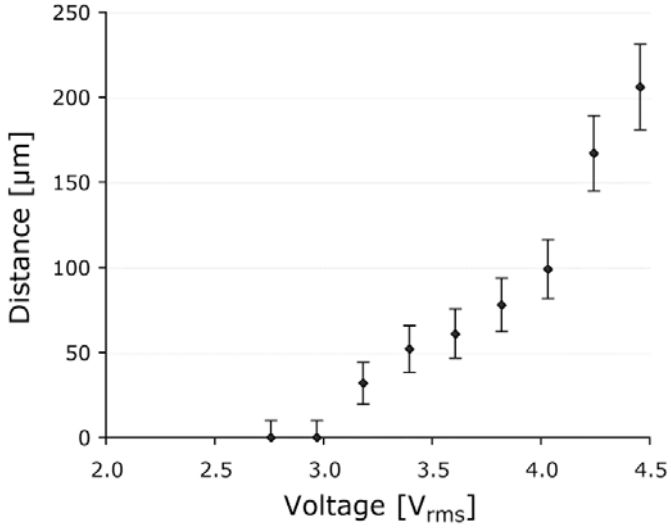


Figure 11. *Plot of the distance between an active nDEP-barrier and temperature-zones at CST* 39.8 °C *as a function of the applied voltage.*

The advantage of this method, using thermosensitive polymers, lies in its simplicity and in its intrinsic capability to locally probe absolute temperatures independently of optical intensity calibration. This is the case regardless of channel geometries and of heating mechanisms. Moreover, since CST of the thermosensitive polymers can be tailored, this allows the user to explore different ranges of temperatures in microfluidic channels or in any other liquid containing system.

1.4 Summary

Two polymerization routes, namely chain transfer and anionic polymerization were used for the synthesis of oligomers (polyNIPAAM, polyDEAAM, polyEMAAM and polyPLAAM) with low molecular weights and relatively small polydispersities. Depending on the initiator used in the two polymerization methods, the resulted oligomers carry different end groups. The properties of the oligomers were characterized using methods such as titration, MALDI-TOF, proton NMR, and cloud point measurements. The last method was used to investigate the critical solution temperature (CST) of the oligomers. The results concerning the CST of the oligomers prepared by chain transfer polymerization demonstrated a similarity between polyDEAAM and polyNIPAAM and between polyEMAAM and polyPLAAM.

Despite the low molecular weight, the polyNIPAAM and polyDEAAM oligomers prepared by chain transfer polymerization had a CST close to the CST of the corresponding polymers with high molecular weight found in the literature; hence no significant correlation between the average molecular weight and the observed CST could be established for these oligomers. On the contrary, the CST of the polyEMAAM and polyPLAAM showed a great dependence on the molecular weight and a significantly higher CST compared with the literature reported CST values of the polymers having high molecular weight were observed. The CST of the anionic oligomers showed a deviation (few degrees higher) from the oligomers prepared by chain transfer polymerization. Higher deviation was recorded between the two polyDEAAM species and a smaller one between the polyEMAAM ones, while an inverse behavior was observed for polyPLAAM (higher CST for the telomer). These CST differences were attributed mainly to the different tacticity of the oligomers as evidenced by the ^{1}H NMR spectra. The similarity in the tacticity (heterotacticity) between telomeric and anionic polyEMAAM is the reason for the small difference between their CST, while the isotactic, anionic polyDEAAM had a significantly higher CST compared to the heterotactic, telomeric one.

Copolymerization of polyNIPAAM with the more hydrophilic polyDMAAM led to an increase of the CST, relative to that of polyNIPAAM, depending on the amount of the more hydrophilic comonomer (DMAAM) present in the system. The poly(NIPAAM-*co*-DMAAM) has been applied, for the first time, for temperature measurements in microfluidic systems.

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Chapter 2. Investigation of the effect of Inorganic Salts on the phase transition of the oligomers

2.1 Introduction

The influence of salts, detergents and organic solvents on polymer thermoprecipitation from aqueous solution has been studied since the 1960's, albeit mainly for molecules such as poly(ethylene glycol) and poly(vinylpyrrolidone) (Von Hippel 1964; Saito 1969; Eliassaf 1978; Ataman 1982; Florin 1984). Since a rather delicate free energy balance involving hydrophobic, hydrophilic and H-bridge mediated interactions determines the solubility of a given polymer in water, the salt effect is hardly surprising. The interpretation of the observed effects is usually based on the assumption of an interaction of the co-solute with the dissolved polymer or an influence of the co-solute on the solvent water (structure, availability). Simple salts, e.g., are generally assumed to exert their influence by acting on the water structure (salting in / salting out) and the resulting behavior can be interpreted as a consequence of the "hydrophobic effect" (Tanford 1980).

A common explanation of the cosolute effect relies on the assumption of a competition for the water molecules available for solvation (Tanford 1980). With increasing charge density of the ions or, in case of an organic cosolvent with increasing hydrophobicity, the influence on the cloud point temperature becomes more pronounced. Therefore, any additive to the polymer/water system can be expected to influence the CST. As thermoresponsive materials become candidates for putative applications ranging from drug delivery to artificial muscles and parts for microanalytical systems, the understanding of such complex systems has increased in importance.

The addition of a simple salt generally results in a decrease of the cloud point temperature ("salting out" effect) as a function of the salt concentration (Saito 1971; Eliassaf 1978; Ataman 1982; Florin 1984; Schild 1990; Park et al. 1993), although some exceptions are known, e.g., in the case of certain thiocyanates or quaternary ammonium salts (Saito 1971; Schild 1990; Suwa et al. 1998; Baltes et al. 1999).

The cosolute effect has already been observed more than 100 years ago and the phenomenon has been named as the Hofmeister effect (Hofmeister 1888). The

Hofmeister effect refers to an ordered sequence of ions, the *Hofmeister series*, also called the *Lyotropic series* (Von Hippel 1969; Melander and Horvath 1977; Piculell 1990; Salomäki et al. 2004). The Hofmeister series originally categorized salts in regard to their "salting out" potential toward proteins from aqueous solution (Hofmeister 1888; Von Hippel 1964; Von Hippel 1969; Ataman 1987). Starting from the anion that has the greatest ability of "salting in" some hydrophobic proteins, the Hofmeister series goes as follows: ClO_4^-> SCN^-> I^-> NO_3^-> Br^-> **Cl^-**> CH_3COO^-> $HCOO^-$> F^-> OH^-> HPO_4^-> CO_3^{2-}> SO_4^{2-} (Dhara and Chatterji 2000). The anions in the series can be divided into two classes defined by the location of chloride, which can be treated as a median (Salomäki et al. 2004). The anions on the left of chloride in the above mentioned series are *chaotropic ions* (water structure breaking), which exhibit weaker interaction with water than water itself. The ions on the right of chloride are *cosmotropic* (water structure making), which exhibit strong interactions with water. A similar series holds for cations, but their effect on stabilizing macromolecules is generally smaller than that of the anions (Von Hippel 1969; Leontidis 2002).

The cosmotropic and chaotropic properties of the ions can be described by the viscosity *B* coefficient of the Jones-Dole (Jones 1929) empirical expression (Jenkins 1995), where the viscosity (n) of the salt solution depends on the ion concentration (c). The relation is given by the following expression,

$$n/n_0 = 1 + Ac^{1/2} + Bc \qquad \text{I2. 1}$$

where n_0 is viscosity of water at the same temperature. The values of the *A* coefficients are rather small compared to the *B* coefficient values. Therefore, the *A* term affects the viscosity values in the concentration range $c < 0.05$ mol/L, and above that, the *B* term is dominant (Jenkins 1995). The *B* coefficient of an ion is a constant in a specified solvent at a given temperature. It is an additive quantity meaning that the *B* coefficient of an electrolyte is a sum of individual ionic *B* coefficients of the ions present in the solution. Roughly, for a cosmotropic ion, the *B* coefficient is positive and for a chaotropic ion negative in water at 25 °C.

In this chapter, the effects of different salts and their concentrations on the thermoresponsiveness of aqueous oligomeric solutions are investigated.

2.2 Experimental Procedures

Materials

All salts were obtained from Sigma Aldrich Chemical (Buchs, Switzerland) and were used without further purification.

2.2.1. Sample preparation

For the sample preparation, stock solutions of oligomer (5 wt %) were prepared by allowing the oligomer to dissolve over night in purified water at 4 °C. Aliquots of these solutions were added to the inorganic salt stock solutions. These solutions were then diluted with purified water to the desired sample concentration and stored over night at 4 °C before the measurements. The final sample solution for the cloud point measurements was 1 wt %. Samples with potassium salt solutions having a concentration range from 0 to 3 M, and with ammonium sulphate solution from 0 to 0.5 M, were prepared. Since no bacteriocide was added, the maximum storage time of stock and sample solutions was less than 1 week.

2.2.2. Measurement of the critical solution temperature, CST

The CST was determined by cloud point measurements using a spectrophotometer at 500 nm. Heating rates were 0.5 °C/min and CSTs were taken at the inflection point in the optical density versus temperature curves. Pure water was used as a reference. Data concerning the spectrophotometer are given in Chapter 1.

2.3 Results and Discussion

For the salt effects on the solubility of aqueous oligomeric solutions, ammonium sulphate ($(NH_4)_2SO_4$) which is one of the strongest salting out agents known (Baltes et al. 1999) and a series of potassium salts (anion effect); KI, KNO_3, K_2CO_3, KOH, KCl, KF, KBr, were investigated. It is generally accepted that the anion is important in the salt effect while there is no difference between cationic species, such as Na^+, K^+, in the “salting out effect” (Suwa et al. 1998; Lee 1999; Freitag et al. 2002; Okamura et al. 2002). PolyEMAAM and polyPLAAM, prepared by telomerization and anionic polymerization were the oligomers used for the salts influence study.

It has been already reported that some salts, the chaotropic agents, increase the CST ("salting in" effect), whereas other salts, the cosmotropic ones, decrease the CST ("salting out" effect) (Park et al. 1993; Lee 1999). The salts effect (except KI), used in our investigation, on the present oligomers is clearly due to the "salting out" effect. The observed "salting out" process can be explained as a combination of several effects, i.e. changes of the water structure in the polymer hydration sheath and changes of the interactions between the polymer and the solvent, due to the presence of salts (Suwa et al. 1998; Eeckman et al. 2001; Freitag et al. 2002). It is known that the addition of electrolytes to water changes the normal hydrogen bonded water structure. Several models have been proposed for the structure of water in the presence of ionic solutes (Frank 1957; Samoilov 1972; Tanford 1980; Florin 1984; Ataman 1987). According to the model of Frank and When (Frank 1957) for aqueous salt solutions, water consists of three regions:

- A region: It is composed of water molecules which are immobilized through ionic-dipole interactions
- B region: It consists of water molecules partially ordered by the electric field of region A, being more random in organisation than "normal" water
- C region: Consists of "normal" water.

The extent of region B is a measure of the ability of the ion to destroy water structure. In general, small and polyvalent ions have large A regions and are called *structure makers* or "positively hydrated". On the other hand, large and monovalent ions have small A regions and large B regions and are called *structure breakers* or "negatively hydrated". It is known that the addition of structure breakers to water decreases the viscosity of the solutions and vice versa. This is the reason why the phase transition phenomenon of aqueous polymer solutions is associated with changes in the normal structure of water induced by electrolytes (i.e. salts).

When dissolved in water, polyEMAAM or polyPLAAM is surrounded by an extended so called hydration sheath where water has lost its normal structure. According to the type and the concentration of ions, the hydration sheath will be more or less destructured (in the case of "salting out" ions) or strengthened (in the case of "salting in" ions) (Ataman 1987).

It has been well established that the initial thermal response of thermoresponsive polymers is the individual chain collapse in a coil-to-globule transition (Barker 2003). Two factors have been thought to contribute to the coil-to-globule transition, which include the breakdown of polymer-water hydrogen bonding in controlling the macromolecular contraction and chain collapse due to changes in the "hydrophobic effect" (Otake 1989; Inomata 1990; Otake et al. 1990). Furthermore, from the point view of mechanism, two factors may affect the self-associations of the amphiphilic polymers. On one hand, the addition i.e. of KCl increases the hydrogen bonding among water molecules therefore decreases that among water and hydrophilic chains. Subsequently the hydrogen bonding among the hydrophilic chains becomes dominant, which results in a stronger tendency for the polymers to associate and decreases their CST (Bromberg 1996; Liu et al. 2003; Liu et al. 2004). On the other hand, the presence of i.e. KCl will undoubtedly increase the polarity of the aqueous medium, thus enhancing the hydrophobic-hydrophobic interactions (Idziak et al. 1999). The stronger hydrophobic-hydrophobic interaction indicates the stronger tendency for the oligomers to self-aggregate; a feature that will result in lower solubility of the polymer in water, hence a decrease in CST (Garret-Flaudy 2000). Therefore, both mechanisms account for the decrease in the CSTs of the oligomers.

The solubility of a polymer in an aqueous salt solution can be expressed by the empirical Setschenow equation (Park et al. 1993; Okamura et al. 2002):

$$\log(S_i^0 / S_i) = k_s C_s = K_s C_s \qquad \text{I2. 2}$$

The equation I2. 2 can be transformed into

$$\log(S_i) = - K_s C_s + \log(S_i^0) \qquad \text{I2. 3}$$

where S_i^0 and S_i represent the solubility of the polymer in pure water and in a salt solution, respectively, k_s or K_s is the salting out coefficient for a given salt and C_s is the salt concentration. According to this empirical equation, the solubility of any particular water-soluble polymer decreases gradually with increasing ionic strength.
From the above equation, it can be easily deduced that, in non-ionic polymers, salt will not lead to an abrupt change in the solubility at a "critical" concentration, because

it is believed (as mentioned before) that increasing the salt concentration gradually changes either ion-dipole interactions between the ion and the polar group in the polymer or the water structure associated with the nonpolar group (Park et al. 1993).

Figure 1 and Figure 2 show that the lowering of CST is nearly a linear function of the salt concentration, supporting the observations found in the literature by different authors (Suwa et al. 1998; Baltes et al. 1999; Lee 1999; Okamura et al. 2002; Xiuli 2004). The Figures demonstrate how the CST of a 1 wt % polyEMAAM and polyPLAAM oligomer solution prepared by chain transfer polymerization, changes as a function of the counterion (i.e. the anion) and the concentration of different potassium salts.

As can be observed in Figure 1 and Figure 2 save for small amounts of KI, which seems to have a "salting in" effect, the CST drops in a linear fashion with increasing salt concentration. Similar "salting in" effect by I^- has been observed by many authors (Suwa et al. 1998; Lee 1999; Freitag et al. 2002).

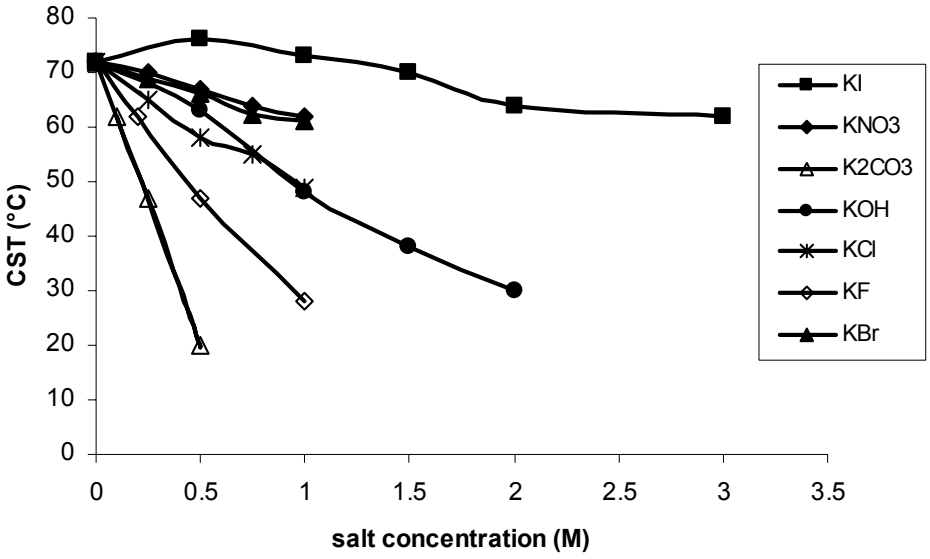

Figure 1. *Influence of various potassium salts on the CST of a 1 wt % aqueous solution of polyEMAAM prepared by chain transfer polymerization.*

In the presence of only 0.1 M of K_2CO_3 the precipitation temperature of the solutions is lowered by approximately 55 °C, and K_2CO_3 has obviously the strongest "salting

out" effect among the investigated salts. It is followed by KF; 1M of KF reduces the CST to 30 °C, causing a decrease of around 45 °C. A strong effect could be also observed for KOH. The CST of the solutions is decreased by 40 °C in the presence of 2M KOH. For iodide the effect is less pronounced and, as mentioned before, low concentrations of this anion even tend to slightly elevate rather than depress the cloud point ("salting in" effect). At higher concentrations of KI, however, a linear decrease of the cloud point temperature with increasing salt concentration is again observed, albeit to the lowest degree of all investigated salt species. Moreover, the observed absolute changes in the CST correspond in magnitute to those previously observed by Freitag and Garret-Flaudy (Freitag et al. 2002) for polyNIPAAM and polyDEAAM.

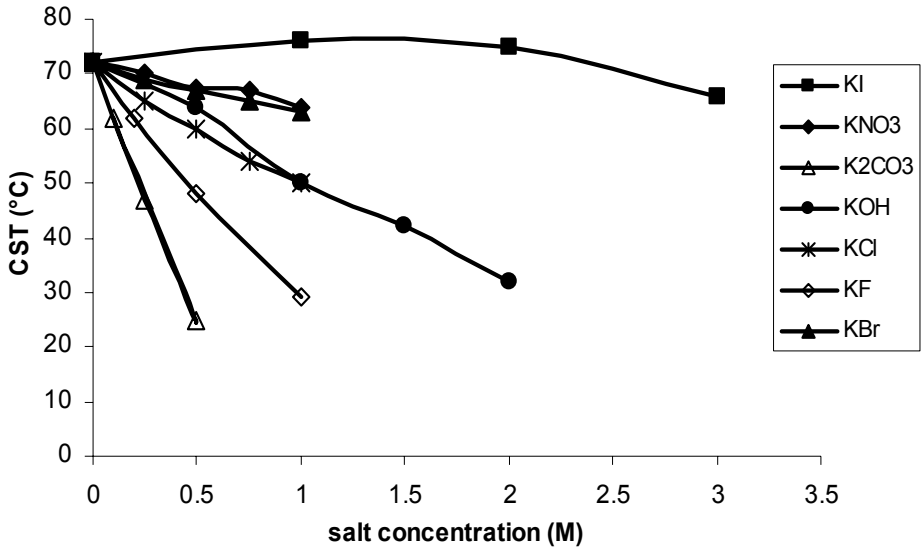

Figure 2. *Influence of various potassium salts on the CST of a 1 wt % aqueous solution of polyPLAAM prepared by chain transfer polymerization*.

Salts can be ranked in the following series with respect to the "salting out" effectiveness of aqueous oligomeric solutions, as it is derived from our investigation: KI< KNO_3< KBr< KOH< KCl< KF< K_2CO_3. This sequence is in accordance with the Hofmeister series (Ataman 1987) but also in agreement with other authors (Horne et al. 1971; Suzuki 1993). From the ranking obtained it can be seen that the valence of the anion plays an important role in the "salting out" process (Suwa et al. 1998; Eeckman et al. 2001; Okamura et al. 2002; Xiuli 2004). This means that salts

containing divalent anions like CO_3^{2-} are more effective at "salting out" the investigated oligomeric chains than those containing monovalent anions like Cl^-, Br^- etc. From the halide series, F^-> Cl^-> Br^-> I^-, it can be seen that the size of the anion also plays a role in the "salting out" process (Eeckman et al. 2001). In particular, a large ion such as I^- (1.32 Å) is classified as a water structure breaker, while small ions such as F^- (0.57 Å) and Cl^- (0.97 Å) are water structure makers and consequently show a strong "salting out" effect.

The drop in the CST values upon the addition of a certain quantity of a given salt is very similar in all cases, including the effect on the polyEMAAM and polyPLAAM prepared by anionic polymerization. The effect on the anionic polymers is demonstrated in Figure 3 and Figure 4, for polyEMAAM and polyPLAAM, respectively.

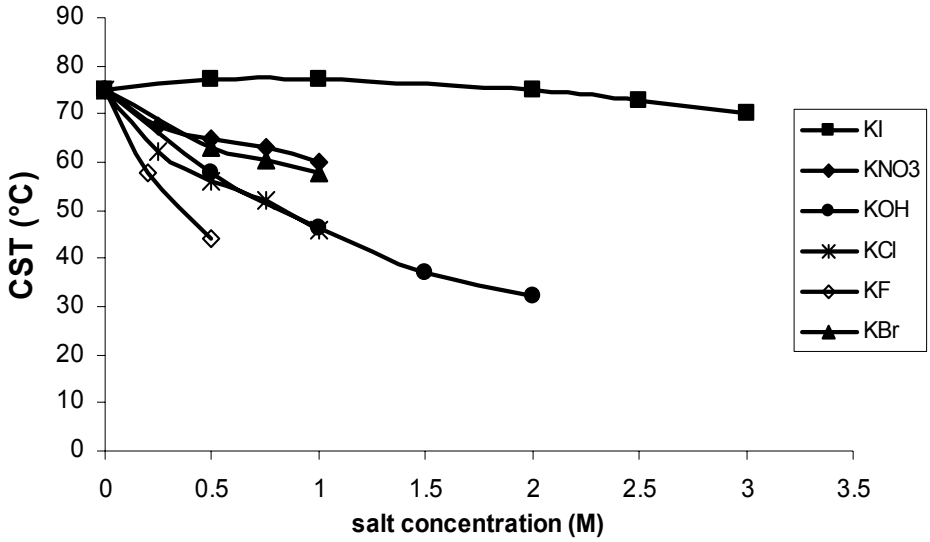

Figure 3. *Influence of various potassium salts on the CST of a 1 wt % aqueous solution of polyEMAAM prepared by anionic polymerization*

Even in the case of anionic polymers, the ranking of the salts' ability to reduce the CST remains the same, confirming the observation reported by Baltes et al. (Baltes et al. 1999) who investigated the influence of NaCl and ammonium sulphate on polymers (mostly polyDEAAM) prepared by radical, anionic and group transfer polymerization.

Among the investigated salts in the case of anionic polymers, KF seems to have the strongest "salting out" effect. Again, KI causes a slight increase on the CST ("salting in" effect) at salt concentrations less than 0.5 M, followed by a linear decrease, as can be seen in Figure 3.

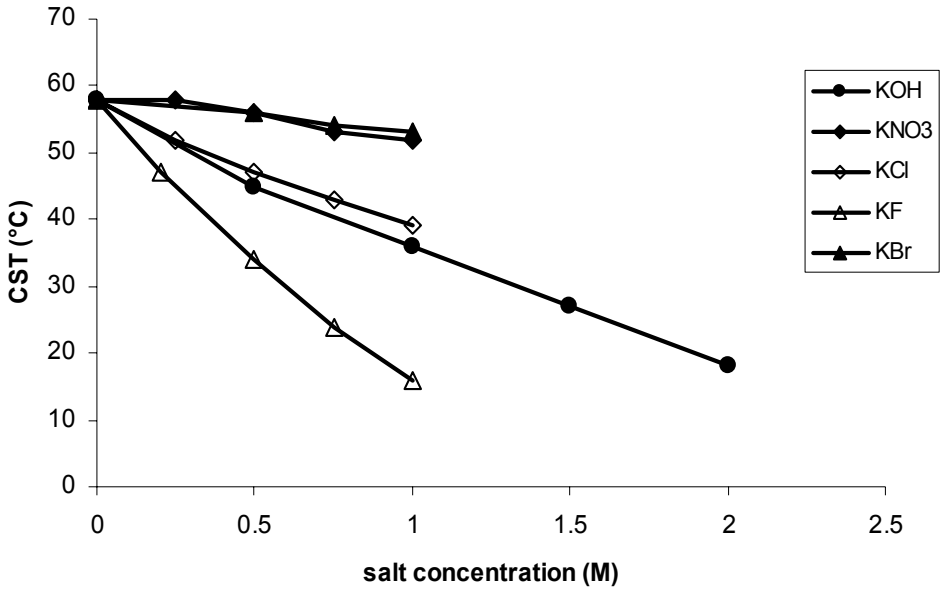

Figure 4. *Influence of various potassium salts on the CST of a 1 wt % aqueous solution of polyPLAAM prepared by anionic polymerization.*

The similarity to the behavior of telomers and anionic oligomers in the presence of a given salt is also visible in Figure 5, which shows the CST of the four polyEMAAM and polyPLAAM species as a function of the ammonium sulphate concentration. As expected, a much stronger effect is observed for ammonium sulphate than for potassium salts (Baltes et al. 1999). From the results obtained here, the salt's effect was found to be more or less independent of the chemical nature and the original cloud point temperature of the oligomer. As shown in Figure 5, the ΔT measured for a given salt concentration was nearly identical in all investigated cases. For example, in the presence of 0.1 M ammonium sulphate, all cloud points are lowered in a similar manner by approximately 9 - 10 °C. Hence, the "salting out" effect does not seem to depend on the structure of the oligomer. It seems therefore likely that the salting out/in effect exerted by a salt operates indeed via the water structure and not by acting directly on the dissolved species (Freitag et al. 2002).

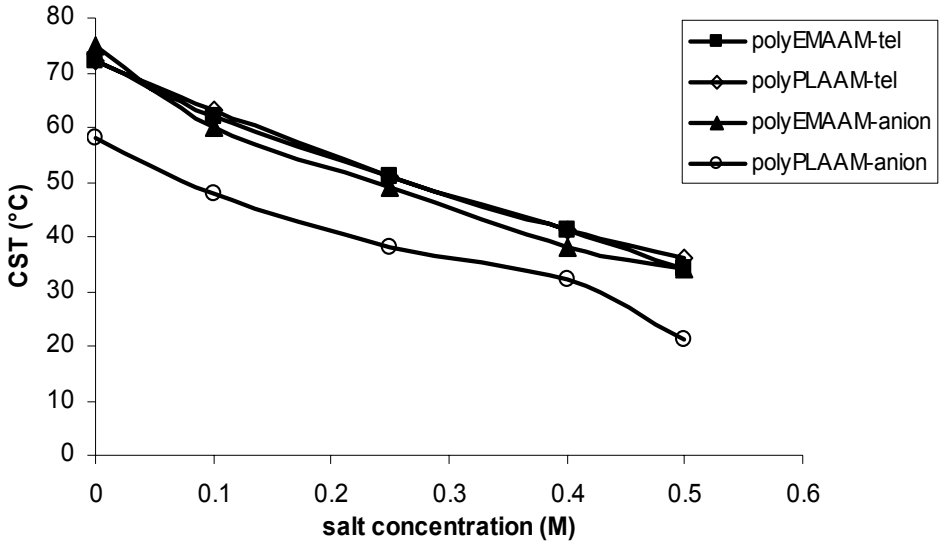

Figure 5. *Influence of increasing amounts of ammonium sulphate on the CST of 1 wt % aqueous solution of polyEMAAM and polyPLAAM, prepared by chain transfer and anionic polymerization*

Jones and Dole (Jones 1929) proposed to use the viscosity *B* coefficient as an empirical constant representing the ion-solvent interaction, to be used as a measure of the relative ordering/disordering tendency of ions on the solvent structure including the solvent water. Values of the viscosity *B* coefficient of different ions can be found in the literature (Samoilov 1972).

Salting-in anions or anions with a rather large negative viscosity *B* coefficient break the structure of the water ("structure breaker") and as a result stabilize e.g. hydrophobic hydration; the coil-like structure of the thermo-responsive oligomers is favored in these solutions. In the case of our investigated oligomers, this would mean that dissolution is aided and that precipitation occurs at higher temperature than in pure water. Salting-out anions or anions with a positive or less negative viscosity *B* coefficient, on the contrary, function as "structure makers" for water and as a result, they strengthen hydrophobic interactions among the side chains and promote the formation of the globular structures (collapse-state) of the polymers (Fuoss 1948; Suwa et al. 1998).

Table 1 summarizes the values of the viscocity *B* coefficient of the investigated anions and the correlation between the corresponding values is plotted against the relative change in transition temperature (ΔT) of the polyEMAAM and polyPLAAM oligomers solutions, prepared by chain transfer or anionic polymerization, containing 1 M of the indicated salt in Figure 6. It is observed that almost a linear correlation is found between the measured ΔT and the viscosity *B* coefficient.

Table 1. *Classification and Value of viscosity B Coefficients of ions in water at 25* °C (Samoilov 1972)

Class	Ion	Viscosity *B* Coefficient (L/mol)
structure-disordering ion	I^-	-0.068
structure-disordering ion	NO_3^-	-0.046
structure-disordering ion	Br^-	-0.042
structure-disordering ion	Cl^-	-0.007
structure-ordering ion	F^-	0.096
structure-ordering ion	OH^-	0.112
structure-ordering ion	SO_4^{2-}	0.208

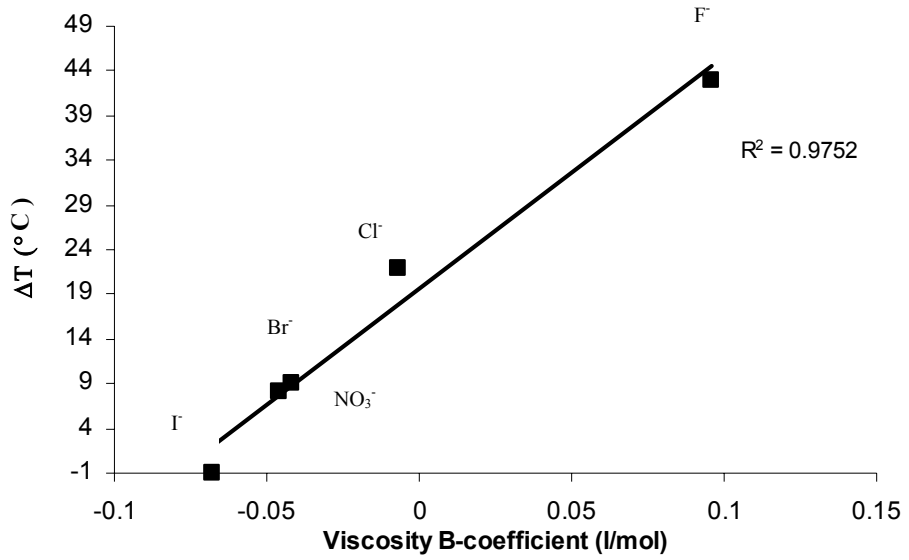

Figure 6. *Correlation between the viscosity B coefficient of several anions and the transition temperature decrease (Δ*T*) of 1 wt % aqueous polyPLAAM solutions to which 1 M of the respective potassium salts has been added.*

2.4 Summary

The effect of a series of potassium salts and ammonium sulphate on the phase transition of the investigated oligomers was the main subject of this chapter. The salts' effect on the present oligomers could be clearly characterized as "salting out" since a linear drop to the CST was observed in most of the cases. Exceptions were seen for potassium iodide (KI) which at low concentration, exhibits a slight "salting in" effect followed by a "salting out" one when the salt concentration increases. The found sequence of the "salting out" effectiveness of the salts on the aqueous oligomeric solutions was in accordance with the Hofmeister series, with the divalent anion CO_3^{2-} having a more pronounced "salting out" effect than a monovalent one, like Cl^- or F^-. Within the halide series, the size of the anion seems to play an important role, with the biggest ion, i.e. I^- to increase the CST ("salting in") of the oligomer and being classified as a "structure breaker". The chemical structure of the oligomers seemed to have no influence on the "salting out" effect and oligomers prepared either by chain transfer or anionic polymerization, exhibited similar behavior in the presence of salt. The viscosity *B* coefficient was used as a qualitative measure of the effect of

salts on the water structure and the results correlated well with the ability of a salt to behave as "structure maker" or "structure breaker".

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Chapter 3. Cononsolvency Phenomenon: Solvent influence on the phase transition of the oligomers

3.1 Introduction

Organic solvents constitute another type of additive, which is of obvious importance. The addition of solvents may promote a drastic change in the CST, as has been demonstrated by swelling experiments with polyNIPAAM gels (Hirokawa 1984; Katayama 1984; Hirotsu 1987; Inomata 1993; Mukae 1993; Hirotsu 1995) and cloud point or calorimetric measurements of linear polyNIPAAM molecules in solution (Otake et al. 1990; Winnik 1990; Schild et al. 1991; Winnik et al. 1992). PolyNIPAAM is soluble in a number of organic solvents, provided that they are capable of hydrogen bonding. Examples include acetone, dimethylformamide (DMF), dioxane, ethanol, methanol and tetrahydrofuran (THF). In these solvents, no phase transition is observed and polyNIPAAM is soluble to the solvent's boiling point. Therefore, it may be assumed that the addition of such an organic solvent to an aqueous polyNIPAAM solution should raise the CST and extent the solubility range. However, initial investigations have shown that this is not necessarily the case (Bywater 1985; Winnik 1990; Schild et al. 1991; Schild 1992; Winnik et al. 1992; Winnik et al. 1993; Asano 1995). Instead, the addition of small amounts of a good solvent such as methanol to polyNIPAAM / water solutions initially decreases transition temperature, and only a further addition of solvent promotes an increase (Schild et al. 1991). In a general manner, the polyNIPAAM solubility is reduced within a range of intermediate solvent concentrations in binary hydro-organic solutions, showing a rather rare phenomenon called "cononsolvency" (or antagonistic solvency), which describes the situation of polymers soluble in two pure solvents but insoluble in their mixtures, for some mixture compositions (Wolf 1978). Cononsolvency has been observed for polyNIPAAM for hydro-organic mixtures containing methanol, dioxane or tetrahydrofuran (Fujishige 1987; Schild 1992).

Cononsolvency phenomenon

Even if the phenomenon of cononsolvency is not completely understood, it may be explained by picturing a dehydration of polymer chains caused by the presence of solvent molecules with hydrophobic groups. It is known that the structure of liquid water, produced by a hydrogen-bonded network, is modified by changes in temperature, pressure and by the addition of ionic or non-ionic solutes (Frank 1945; Frank 1957; Franks 1968). When a small amount of a low polarity organic solvent is added to water, these solute molecules are surrounded by cages of water molecules forming a cluster shell, known as hydrophobic hydration shell (Nemethy 1962; Iwasaki 1977; Ben-Naim 1980; Matubayashi 1994; Nakahara 1995). The shape of this hydration shell depends on the molecular structure of the solute; it is stabilized by the hydrogen bonds formed among water molecules in a configuration different from that in pure water. Therefore, the formation mechanism and the structure of the hydration clusters around alcohols depend strongly on the type of alcohol involved, especially on the properties and the shape of the molecule (Suzuki et al. 1997). The low polarity molecules are also called "structure makers" due to their ability to increase relaxation time of "clusters" of water molecules. Since solute-solvent interactions are poor, the solute does not destroy the water structure, but enhance its stability. Alcohols and ketones are examples of "structure makers", while compounds like glycerol, DMSO (dimethylsulfoxide) and amides are considered to be "structure breakers" because their favourable interactions with water decrease stability of the hydrogen-bonded water structure (Franks 1968). Nevertheless, the referred "structure promotion" is limited to the capability of water to hydrate certain amounts of solute, beyond which the so-called hydrophobic interaction may be prevalent, ultimately leading to phase separation. A more hydrophobic solute demands a larger number of water molecules to hydrate it and is more likely to cause rupture of its hydration shell.

3.2 Experimental procedures

Materials

All solvents (alcohols and non-alcohols) were from Sigma Aldrich Chemical (Buchs, Switzerland). The highest available purity was used. DMF (*N,N*-dimethylformamide) was purified and dried by conventional methods in the laboratory.

3.2.1. Sample preparation and CST measurements

The same procedure as the one used to prepare the salt solutions has been followed also in the case of stock and final solvent solution preparation. The final sample solution for cloud point measurement was typically 1 wt % in polymer content and the maximum storage time of stock and sample solutions was less than one week.

The CST was determined by cloud point measurements using a spectrophotometer at 500 nm. Heating rates were 0.5 °C/min and CSTs were taken at the inflection point in the optical density versus temperature curves. Pure water was used as reference.

3.3 Results and discussion

Solvents for this study were selected according to the similarity of their molecular structures. By using methanol, ethanol, 1-propanol, 2-propanol, *tert*-butanol and *iso*-butanol, it was possible to evaluate the influence of size and shape of the hydrophobic groups on the phase separation temperatures. DMF and dioxane were the two, non-alcoholic, organic solvents included in this investigation.

3.3.1. CST changes in aqueous oligomer solutions in the presence of alcohols

Our investigation of possible cononsolvency effects started with a study of the thermoprecipitation of polyNIPAAM telomers from mixtures containing alcohols. Other acrylamides besides polyNIPAAM, namely polyDEAAM, polyEMAAM and polyPLAAM, were also investigated.

At room temperature, oligomeric polyNIPAAM is well soluble in alcohols such as

pure methanol, ethanol, propanol and butanol. However, when even small amounts of any of these alcohols are added to water, the solubility of the oligomer in that particular solvent mixture was reduced, as evidenced by a lowered CST, Figure 1. In such cases the CST appears to decrease almost linearly with the alcohol concentration for the investigated range (< 2 M alcohol) confirming the well established results found in the literature on the cononsolvency phenomenon using polyNIPAAM (Winnik 1990; Schild et al. 1991).

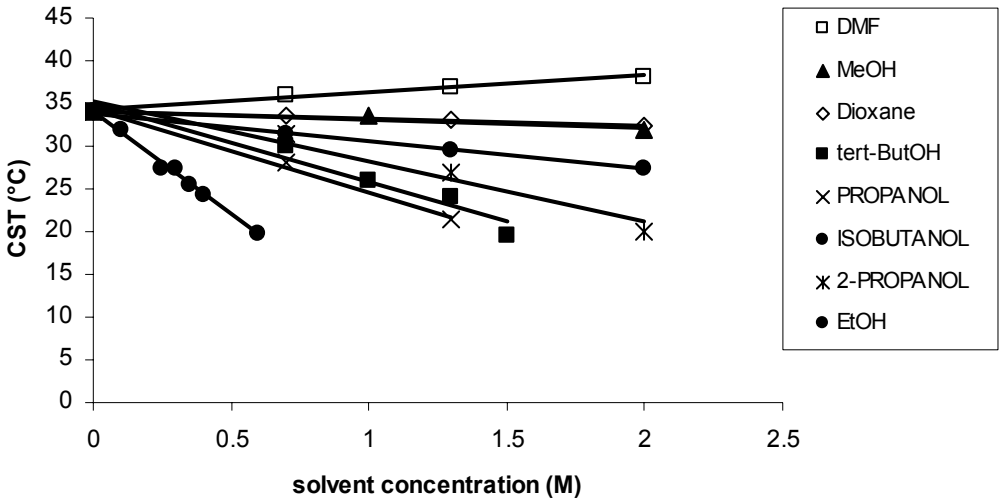

Figure 1. *Influence of various co-solvents (alcohols and non-alcohols) on the CST of a 1 wt % aqueous polyNIPAAM solution.*

The extent of the variation of the CST for a given "co-solvent" depends in a characteristic manner on the length of the alcohol's alkyl chain (Horne et al. 1971; Suzuki et al. 1997; Costa et al. 2002; Shimizu et al. 2003). In particularly, the cloud point shifts to lower temperature with an increasing number of carbons in the alcohol molecule. According to the hydrophobic hydration formation mechanism the cluster shell around the butyl alcohol molecule should be stiffer and more stable than that around the propyl and ethyl alcohol (Suzuki et al. 1997). If the effects of methanol, ethanol, propanol and butanol are compared, the effects on the CST become more pronounced as the chain length of the alcohol increases. The fact that a more hydrophobic alcohol causes a larger decrease in CST is related to the larger number of

water molecules required to form the hydration shell. Hence, one *iso*-butanol molecule disturbs the system much more significantly than one propanol molecule considering the hydrophobic volumes that need to be accommodated in the water structure. Similarly, a propanol molecule is hydrated by more water molecules than an ethanol or a methanol molecule.

In addition to the length, the structure of the alkyl chain also exerts a strong influence on the magnitude of the observed effect, as can be seen in Figure 1, Figure 2 and Figure 4. Solutions containing *tert*-butanol show a significantly different behavior than the *iso*-butanol ones. In fact even 1-propanol shows a more pronounced effect than *iso*-butanol. The CST trend is also influenced by the position of the alcoholic group on the alkyl chain (Pandya et al. 1993). As the comparison of 1- and 2-propanol demonstrates, the effect of an alcoholic group in the *n*-terminal position (1-propanol) on the CST is more efficient in decreasing the CST than that of one located closer to the center of the molecule (i.e., 2-propanol). *Iso*-butanol shows the strongest effect, causing the sharpest decrease on the CST of the oligomers, among the investigated solvents (Figure 2, Figure 4).

The difference between the effect of the investigated alcohols leads to an explanation, again involving hydration shells. Spherical hydrophobic solutes (such as *tert*-butanol) are more miscible with water than their linear analogues because they permit the formation of a cage structure with less distortion between hydrogen bonds in the normal structure of water (Hayashi 1990). Although both 1-propanol and 2-propanol are miscible with water in any proportion, each solvent demands a different hydration structure. According to Hayashi et al. (Hayashi 1990), the hydration shell involving 1-propanol is less stable and it is more likely to mobilize a larger number of water molecules when compared with 2-propanol, thus promoting a larger decrease in the CST.

The effect of a given cosolvent shows a strong dependence on the chemistry of the oligomer. In the case of polyDEAAM prepared by chain transfer and anionic polymerization, Figure 2 and Figure 3, respectively, all investigated solvents save for propanol and butanol increased the CST. 2-propanol increased the CST in the case of the predominately polyDEAAM prepared by anionic polymerization, whereas the

CST of the polyDEAAM prepared by chain transfer polymerization was lowered by this solvent under otherwise similar circumstances.

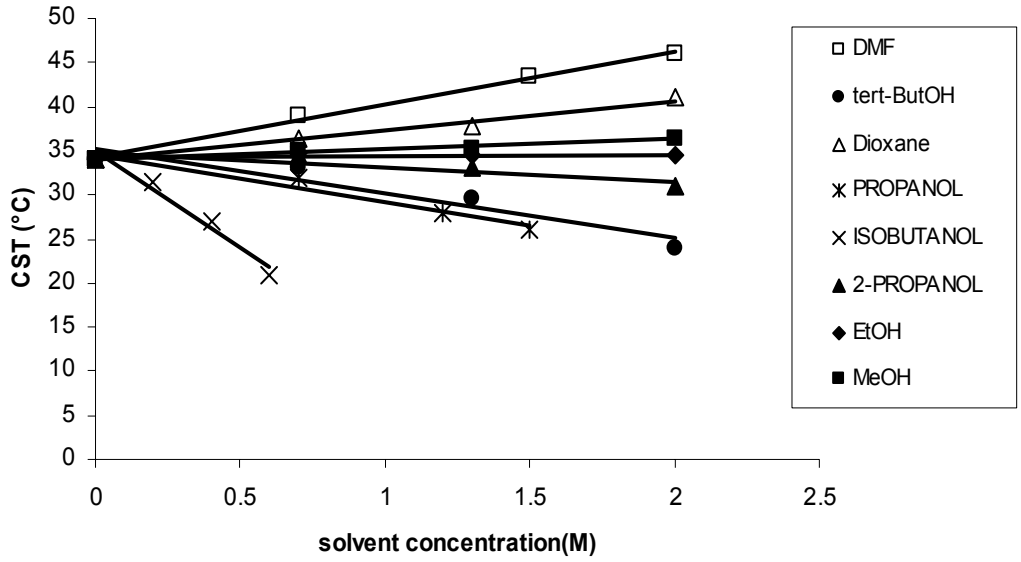

Figure 2. *Influence of various organic solvents on the CST of a 1 wt % aqueous polyDEAAM solution prepared by chain transfer polymerization.*

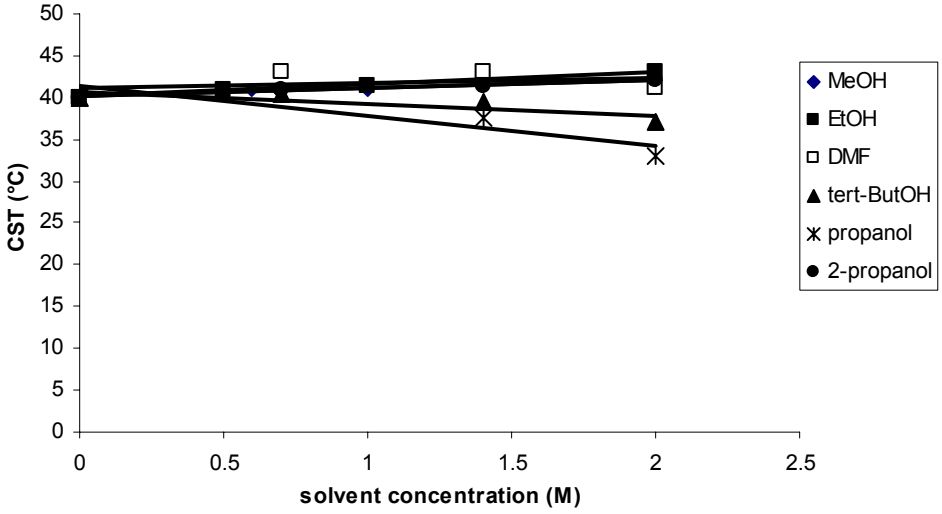

Figure 3. *Influence of the alkyl chain length of various alcohols as co-solvent on the*

CST of a 1 wt % aqueous polyDEAAM solution prepared by anionic polymerization.

Such behavior is not observed in the case of polyEMAAM oligomer prepared either by chain transfer or anionic polymerization. The tacticity of the molecule may also be of consequence. In the case of polyEMAAMs, which are both heterotactic, even methanol, which has the smallest hydrophobic domain, shifts the CST of polyEMAAM (Figure 4 and Figure 5) to lower temperatures and the effect is even more pronounced than that of the more hydrophobic solvents, such as propanol and *tert*-butanol (Figure 4).

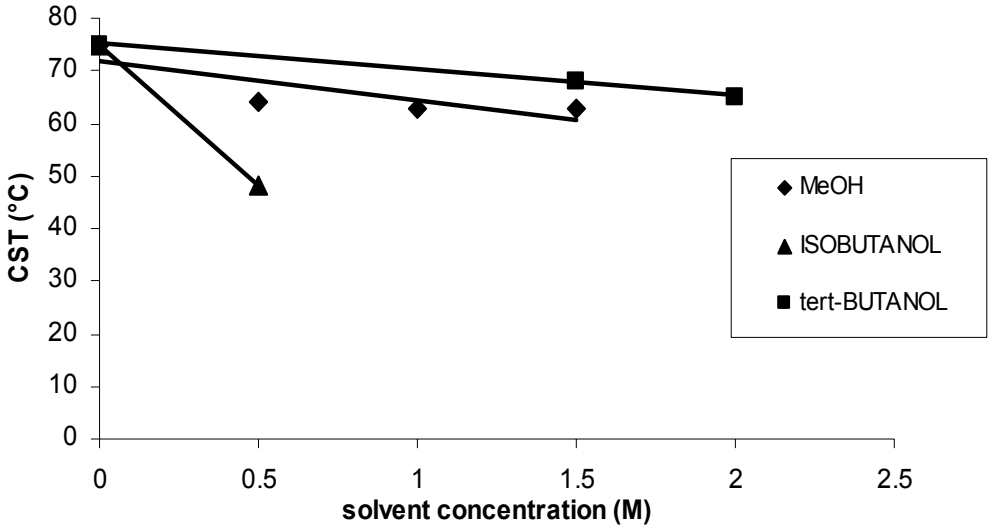

Figure 4. *Influence of various organic solvents on the CST of a 1 wt % aqueous solution of polyEMAAM prepared by chain transfer polymerization.*

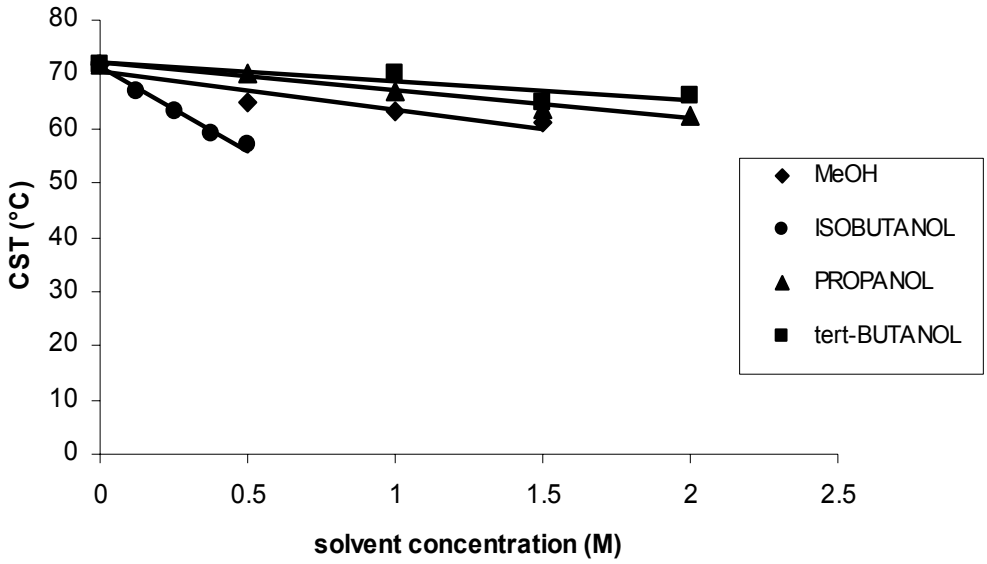

Figure 5. *Influence of various organic solvents on the CST of 1 wt % aqueous solution of polyEMAAM prepared by anionic polymerization.*

The effect of a variety of alcohols on the CST of polyPLAAM prepared by anionic polymerization is presented in Figure 6.

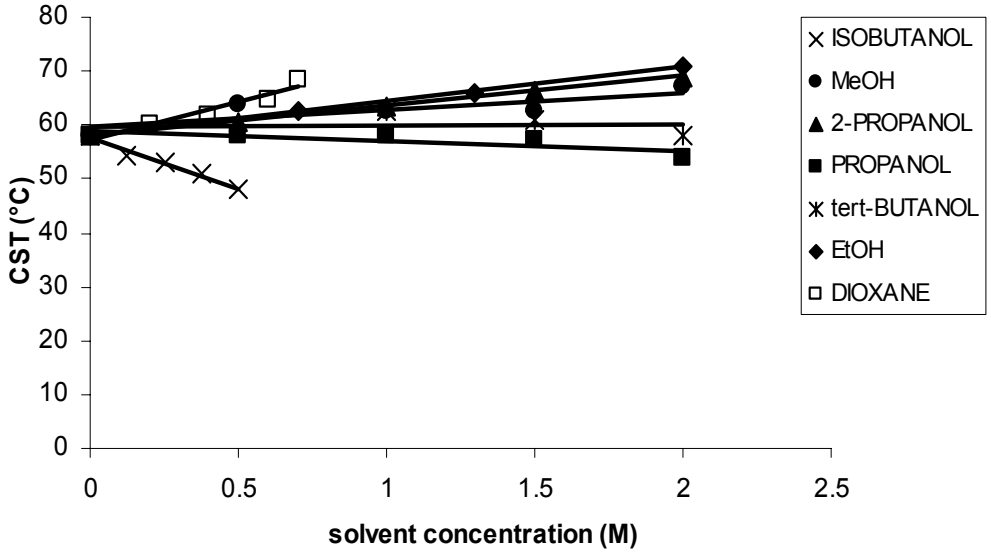

Figure 6. *Influence of various organic solvents on the CST of 1 % wt aqueous solution of polyPLAAM prepared by anionic polymerization.*

PolyPLAAM prepared by chain transfer polymerization (data not shown) shows the most extreme behavior. For these molecules no CST is recorded in most solvent mixtures save for those that contain *iso*-butanol. The fact of CST could be explained in two ways, both of them related with the spectrophotometer; firstly, either the polymer aggregates may not have sufficient size to be detected at the selected wavelength (Schild et al. 1991; Idziak et al. 1999; Gan et al. 2001) and secondly, either due to the use of a thermostat connected with the spectrophotometer, there was a maximum limit of temperature (75 °C) that could be applied and no valuable data could be recorded above this temperature. But as was mentioned before, the addition of a structure maker decrease the CST leading to a small size polymer aggregates having a globular structure. Since *iso*-butanol, which has the strongest effect among the investigated solvents shows results, the first hypothesis of no-CST record could be rule out and the second hypothesis is becoming more possible. In the case of anionic polyPLAAM (isotactic) a similar behavior as for polyDEAAM can be seen, i.e. solvents with large hydrophobic domains such as *iso*-butanol lower the CST; in solvent mixtures that contain organic solvents with small hydrophobic domains, namely methanol and ethanol, a CST is still observed but a higher value than in pure water. Given that the tendency for formation of aggregates increases with increasing size of the hydrophobic domain, the formation of mixed aggregates can not explain the steady increase of the CST observed, e.g. when methanol was added to the polyDEAAM solutions.

A similar increase in CST had previously been observed by Horne et al. (Horne et al. 1971) and later confirmed by Schild et al. (Schild et al. 1991). They demonstrated that the cloud points of PVME (poly(vinyl methyl ether)) in water / methanol and water / ethanol mixtures were shifted to higher temperatures as the volume fraction of methanol or ethanol, respectively, increased. Clearly the simple water structuring argument is not sufficient to explain all observed effects. Similar observations have also been made by Pandya et al. (Pandya et al. 1993) during the investigation of the phase behavior of an ethylene oxide-propylene oxide block copolymeric surfactant Pluronic L-64 in the presence of various additives, including alcohols. They observed that lower alcohols (C_1 –C_3) had a slight increasing effect on the cloud point of L-64, while higher alcohols decreased the cloud point and they interpreted the effect by

assuming that short-chain alcohols prefer a water environment, decreasing the polarity of the solvent, thereby increasing the solubility of L-64 and also the cloud point, while long-chain alcohols are effectively attracted to the L-64 molecules to an extent that depends on the chain length of the alcohol. Long-chain alcohols tend to associate with the L-64 molecules, making the polymer more hydrophobic and therefore also less water-soluble.

The generally observed decrease in the CST upon the addition of the organic solvents could also simply be linked to the solvent's effect on the water structure. The viscosity *B* coefficient, which has been proposed as a qualitative measure of the effect of additives on the water structure (Jones and Dole 1929) and has been previously used in the discussion of the effect of an electrolyte (salt), could also be applied in the case of solvents as additives. A positive viscosity *B* value implies positive hydration of the solute molecules and a strengthening of the water structure. Hydrophobic interactions are promoted under these conditions. In the cases considered here, this would lead to the collapse and subsequent aggregation / precipitation of the acrylamide molecules, especially when their concentration is high compared to that of the additive. Table 1 compiles the viscosity *B* coefficient values for most of the alcohols used in this study and it seems to correlate well with an alcohol's ability to suppress the CST of most of the investigated oligomers.

Table 1.*Viscosity B coefficient of the alcohols at 25* °C (Herskovits and Kelly 1973)

Alcohol	viscosity *B* coefficient (l/mol)
methanol	0.087
ethanol	0.170
1-propanol	0.250
2-propanol	0.273
iso-butanol	0.311
tert-butanol	0.373

3.3.2. CST changes in aqueous oligomer solutions in the presence of DMF and Dioxane

Not only alcohols give rise to cononsolvency effects. The results obtained with organic co-solvents other than alcohols are compiled in the Figure 1, Figure 2, Figure 3 and Figure 6, showed above (non-filled symbols). For the concentration range considered here, both DMF and dioxane elevate the CST to some extent in the case of polyDEAAM (Figure 2), while no CST was observed anymore upon the addition of both solvents to solutions of polyEMAAM, prepared by chain transfer (Figure 4) or anionic polymerization (Figure 5) and polyPLAAM (data not shown). An explanation for the absence of CST has been given before and could be used to explain the lack of recording a CST in the presence of DMF and dioxane, as well. Addition of DMF elevated the CST of polyNIPAAM (Figure 1) and a linear drop of the CST is observed with increasing concentration of dioxane. No CST was observed any more upon adding DMF to solutions of the anionic polyPLAAM (Figure 6), while dioxane caused a slight increase in its CST. The opposite effect is observed for anionic polyDEAAM (no CST when adding dioxane, increased CST when adding DMF), (Figure 4). Based on these observations one could confirm the influence of the chemistry of the oligomers, as well as the influence of the properties and shape of the solvents on the hydration shell, resulting in different phase separation behavior. Solvents that disturb the water structure, e.g. DMF, increase the CST. An explanation for the behavior of DMF is given by Costa et al. (Costa et al. 2002), who suggested that the slight increase in the CST caused by DMF is related to its amide group, which is able to form a strong hydrogen bond with water (Visser 1977). Assuming that low concentrations of DMF break water structure very efficiently, DMF molecules would be able to avoid its own hydrophobic hydration and even to favour hydration of oligomeric segments, thus increasing the oligomer's compatibility with water. Excluding the hydration effects, that would be the expected behavior when a good solvent for the polymer is added in the solution (Costa et al. 2002).

3.4 Summary

A series of solvents including alcohols such as methanol, ethanol, 1- and 2-propanol and *iso*- and *tert*-butanol, and two non-alcohols, DMF and dioxane, were used for the

investigation of the cononsolvency effect on the CST of the oligomers prepared in this investigation. Comparing the influence of the alcohols, the effect becomes more pronounced shifting the CST to lower temperature (1) with increasing the number of carbons of the alcohol molecule and (2) when the alcoholic group of the solvent is located to the *n*-terminal in comparison to the influence of the solvent having the alcoholic group located closer to the center of the molecule. Opposed to the effect observed for salts, the effect of the solvents depends on the chemistry and tacticity of the oligomer. Solvents with the smallest hydrophobicity (i.e. methanol, ethanol) caused a slight increase in the CST of most anionically prepared oligomers, while they decreased the CST in the case of the oligomers prepared by chain transfer polymerization. Influence of the chemistry of the oligomer is also observed in the case of the investigated non-alcohols, DMF and dioxane, which either increased or decreased the CST. Absence of the CST phenomenon in some cases could be attributed to the size of the aggregated particles, which were too small to be recorded by the spectrophotometer and to the upper-limit of the temperature in the measurements due to the thermostat control. Again, in the case of cononsolvency, the viscosity *B* coefficient shows good correlation with an alcohol's ability to suppress the CST of most of the investigated oligomers.

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PART II: THERMORESPONSIVE HYDROGELS

General Introduction

1. Hydrogels

Hydrogels are three-dimensional water-swollen structures composed of mainly hydrophilic homopolymers or copolymers. Hydrogels may absorb from 10-20 % (an arbitrary lower limit) up to thousands of times their dry weight in water (Hoffman 2002).

These materials are for the most part insoluble due to the presence of chemical or physical crosslinks of hydrophilic polymer chains.

Hydrogels are called “reversible” or “physical” gels when the networks are held together by molecular entanglements and/or secondary forces including ionic, H-bonding or hydrophobic forces (Campoccia 1998; Prestwich 1998). Physical hydrogels are not homogeneous, since clusters of molecular entanglements or hydrophobically- or ionically-associated domains can create inhomogeneities. The crosslinks provide the network structure and physical integrity.

Hydrogels are called “permanent” or “chemical” gels when they are covalently-crosslinked networks. Chemical hydrogels may be generated by crosslinking of water-soluble polymers, or by conversion of hydrophobic polymers to hydrophilic polymers plus crosslinking to form a network (Allcock 1993; Laschewsky et al. 1999; Snellings et al. 2003; Xu et al. 2003; Lee et al. 2004). In the crosslinked state, crosslinked hydrogels reach an equilibrium swelling level in aqueous solutions which depends mainly on the crosslink density and also on the hydrophilic character of chains. Like physical hydrogels, chemical hydrogels are not homogeneous. They usually contain regions of low water swelling and high crosslink density, called “clusters” that are dispersed within regions of high swelling and low crosslink density. This may be due to the hydrophobic aggregation of the crosslinking agents during polymerization, leading to high crosslink density clusters (Drumheller 1995). In some cases, depending on the solvent composition, temperature and solids concentration during gel formation, phase separation can occur and water filled “voids” or “macropores” can form. In chemical gels, free chain ends represent gel network “defects” which do not contribute to the elasticity of the network (Hoffman 2002).

There are many different macromolecular structures that are possible for physical and chemical hydrogels. They include, crosslinked or entangled networks of linear homopolymers, linear copolymers and block or graft copolymers; polyion-multivalent ion, polyion-polyion or H-bonded complexes; hydrophilic networks stabilized by hydrophobic domains; and IPNs or physical blends (Hoffman 2002).

Hydrogels can be also classified in a number of other ways. They can be neutral or ionic based on the nature of the side groups. They can also be classified based on the network morphology as amorphous, semicrystalline, hydrogen-bonded structures, supermolecular structures and hydrocolloidal aggregates. Additionally, in terms of their network structures, hydrogels can be classified as macroporous (large pores between 0.1 and 1 μm), microporous (100 and 1000 Å) or nonporous (10 and 100 Å) (Peppas 2000).

The permeability and the swelling behavior are two of the most important characteristics of a polymeric gel and they are strongly dependent on the chemical nature of the polymer(s) composing the gel as well as of the structure and morphology of the network.

A polymer network in an organic or aqueous solvent may have an intrinsic temperature dependence of swelling on the specific heat of mixing of the polymer chain and solvent. Most polymer networks show increased swelling in organic solvents with increased temperature, due to the enhanced compatibility of the chains and solvent, as predicted by conventional polymer solution theory (Flory 1953). However, the temperature dependence of hydrogel swelling may demonstrate a different pattern and is closely related to the chemical properties of the main chain and pendent groups. Aqueous solutions of some water-soluble polymers show phase separation on cooling; the maximum temperature for phase separation is termed the upper critical solution temperature (UCST). However, other polymers demostrate demixing when the temperature is raised until a lower critical solution temperature (LCST) occurs (Billmeyer 1970).

2. Phase Transition of *N*-alkylacrylamide gels in water

The property, which makes the smart hydrogels unique, is that the swelling or shrinking occurs by very small changes in the environmental conditions. The volume phase transition of gels is known to result from the interactions between the polymer chain and solvent molecules at shrunken and swollen states. Concerning interactions for gels, van der Waals interaction, hydrogen bonding, hydrophobic interaction and ionic interaction have been considered to explain the phase transition and swelling behavior (Otake 1989; Ilmain 1991).

With respect to temperature dependence, three types of phase transitions have been reported:

- **Thermoswelling type** or expansion with temperature; corresponding to Upper Critical Solution Temperature (UCST) for polymer solutions (Tanaka 1980).
- **Thermoshrinking type** or collapse with temperature; corresponding to Lower Critical Solution Temperature (LCST) for polymer solutions (Tanaka 1980).
- **"Convexo" type**, a mixture of the two types mentioned above, causing firstly expansion and then shrinkage with temperature; corresponding to hour-glass type for polymer solution (Katayama 1985).

Main examples of thermoshrinking hydrogels are composed of monomers like *N*-ethylacrylamide, *N,N*-diethylacrylamide and *N*-isopropylacrylamide whose hydrophobic substituents make them less hydrophilic.
Gels whose monomers are composed of both hydrophobic and hydrophilic groups have the possibility of undergoing a thermoshrinking type phase transition in water. For instance, the molecular structure of *N*-isopropylacrylamide, which does not have only hydrophilic groups (NH, C=O) but also a hydrophobic group (isopropyl) shows that hydrophobic interaction as well as hydrophilic interaction may play an important role in the thermoshrinking type transition.

3. Hydrophobic Hydration / Hydrophobic Interactions

When hydrophobic solutes are introduced into water, two phenomena are simultaneously observed (Ben-Naim 1980; Nakanishi 1984; Okano et al. 1990):

- **Hydrophobic hydration**, in which the water molecules form cage-like structures around the hydrophobic solutes
- **Hydrophobic interactions**, which is the association of hydrophobic solutes.

Generally, an increase in temperature results in a reduction of the total number of water molecules structured around the hydrophobic solutes, which promotes hydrophobic interaction. As schematically shown in Figure 1, hydrophobic hydration is an exothermic and entropically unstable process while hydrophobic interaction is endothermic and entropically stable process. Consequently, a rise in temperature strengthens the hydrophobic interaction.

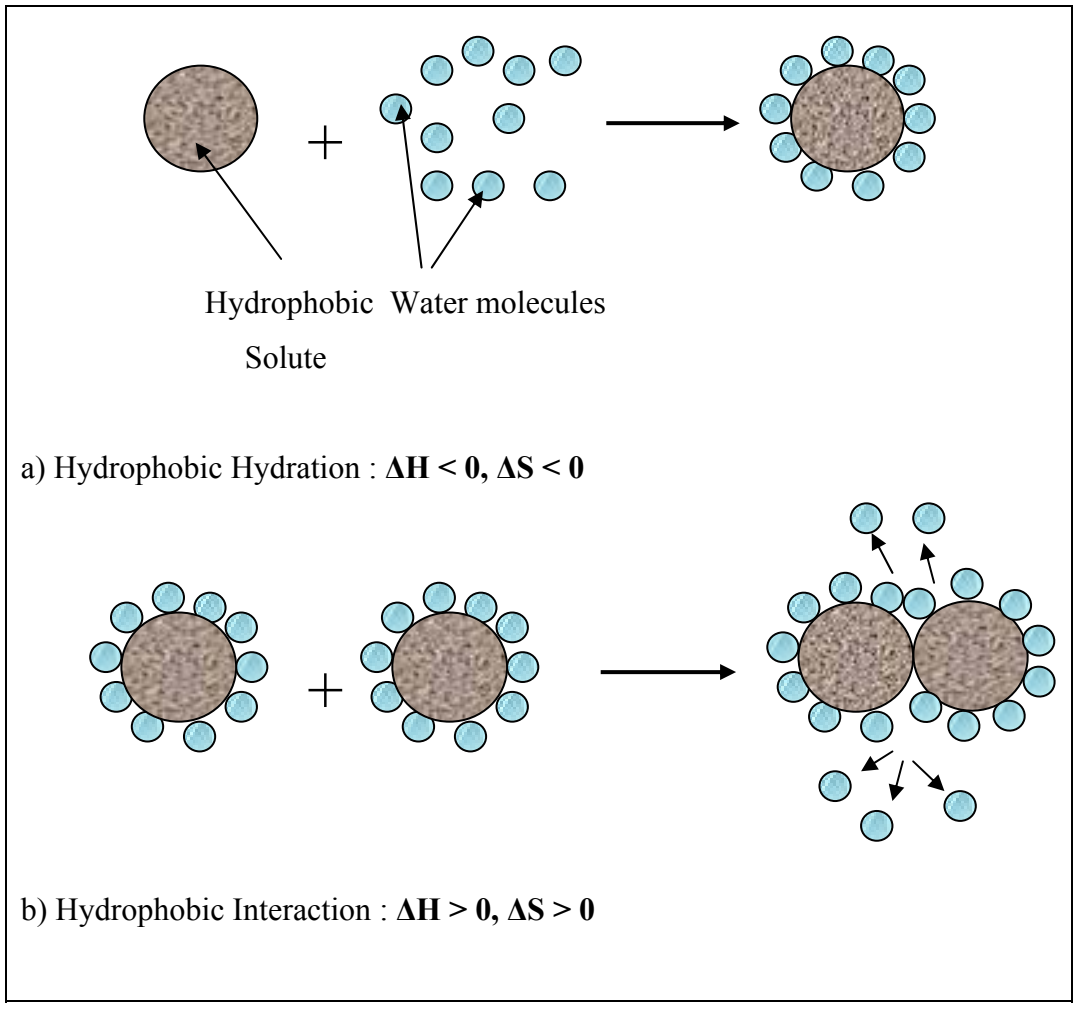

Figure 1. *Schematic representation of* a) *Hydrophobic hydration and* b) *Hydrophobic interaction.*

4. Equilibrium swelling theory

Hydrophobic interaction has been shown to play an important role in the phase transition of *N*-alkylacrylamide gels in water (Otake et al. 1990). Therefore, it is expected that the change in hydrophobicity of the gel network or property of the solvent being used, bring about the observed change in the swelling behavior of the gels. Inomata and co-workers showed that the swelling behavior of different *N*-alkylacrylamide gels was strongly dependent on the hydrophobicity of the specific *N*-alkyl group but it was remarkably similar in both the completely collapsed and expanded regions (Inomata 1990). This suggests that the hydrophobicity of the *N*-alkyl group in the side chain significantly affects the transition temperature of the thermally induced phase transition, whereas the swelling ratio in the swollen state is mainly influenced by the elastic contribution or the nature of the network backbone.

It is well known that the phase transitions of gels are induced by a continuous change in various conditions of the surroundings, such as the ionic concentration of the network, temperature, pH or solvent composition. Thermodynamically, the swelling equilibrium of gels can be deduced from the free energy of these materials which results from the elasticity, the free energy of mixing and the osmotic pressure of the counterions (Tanaka 1980).

Since *N*-alkylacrylamides are non-ionic, their swelling equilibrium is governed by the elasticity of the gel and by the free energy of mixing that results from the interaction between the polymer network and the water molecules; Flory-Rehner theory (Flory 1943; Flory 1953).

At equilibrium, these two forces are equal. Equation II.1 describes the physical situation in terms of the Gibbs free energy.

$$\Delta G_{total} = \Delta G_{elastic} + \Delta G_{mixing} \qquad \text{II. 1}$$

where $\Delta G_{elastic}$ is the contribution due to the elastic retractive forces developed inside the gel and ΔG_{mixing} (entropic component) is the result of the spontaneous mixing of the fluid molecules with the polymer chains. The term ΔG_{mixing} (enthalpic component) is a measure of the compatibility of the polymer with the molecules of the surrounding fluid. This compability is usually expressed by the polymer-solvent interaction parameter χ.

A suitable expression ΔG_{mixing} is obtained from equation I. 8 (PART I - General Introduction) bearing in mind that the number n_2 of polymer molecules is equal to one owing to the absence of individual polymer molecules in the network structure. Thus, ΔG_{mixing} is given by

$$\Delta G_m = kT\,(n_1 \ln \varphi_1 + \chi \varphi_2\, n_1) \qquad \text{II. 2}$$

and $\Delta G_{elastic}$ is given by the rubber elasticity theory (Flory 1953)

$$\Delta G_{elastic} = (kT\nu_e / 2)\,(3\,\alpha_s^2 - 3 - \ln \alpha_s^3) \qquad \text{II. 3}$$

where ν_e is the effective number of chains in the network and $\alpha_s = \alpha_x = \alpha_y = \alpha_z$ are the factors related with an alteration of the network dimensions and

$$\alpha_s^3 = V / V_0 = 1 / \varphi_2 = (V_0 + n_1 V_1 / N) / V_0 \qquad \text{II. 4}$$

where V and V_0 are the volume of the swollen gel and unswollen polymer (the volume of the relaxed network), respectively, V_1 is the molar volume of the solvent and N is the Avogadro's number.

Equation II. 3 is now given by

$$\Delta G_{elastic} = (kT\nu_e / 2)\,[3\,(1 / \varphi_2)^{2/3} - 3 - \ln (1 / \varphi_2)] \qquad \text{II. 5}$$

Therefore, the total Gibbs free energy could be determined by,

$$\Delta G_{total} = kT\,[(n_1 \ln \varphi_1 + \chi \varphi_2\, n_1) + (\nu_e / 2)\,[3\,(1 / \varphi_2)^{2/3} - 3 - \ln (1 / \varphi_2)]] \qquad \text{II. 6}$$

Chemical potential. The chemical potential difference is expressed as

$$\mu_1 - \mu_1^0 = \Delta\mu_{elastic} - \Delta\mu_{mixing} \qquad \text{II. 7}$$

where $\Delta\mu$ is the chemical potential of the penetrating solvent, μ_1 is the chemical potential of the solvent in the polymer gel and μ_1^0 is the chemical potential of the pure solvent and it could be calculated by differentiating equation II. 6 with respect to the number n_1 of solvent molecules, while keeping the temperature and pressure constant. The derived equation is given by

$$\mu_1 - \mu_1^0 = RT\,[\ln (1-\varphi_2) + \varphi_2 + \chi \varphi_2^2 + V_1\,(\nu_e / V_0)\,(\varphi_2^{1/3} - \varphi_2 / 2)] \qquad \text{II. 8}$$

At equilibrium, the difference between the chemical potential of the solvent outside and inside the gel must be zero. Therefore, the changes of the chemical potential due to mixing and elastic forces must balance each other.

Osmotic pressure. Compositional changes in various acrylamide copolymers seem to result in changes in the gel's osmotic pressures, owing to interactions between the polymer segments and water (mixing term in the equilibrium swelling theory). Therefore, the contribution of the mixing term of the gel's osmotic pressures may not control the swelling in the expanded and collapsed states, but does so in the transition region.

The osmotic pressure Π of a hydrogel during swelling is given as the sum of the pressures due to polymer-solvent mixing (Π_{mix}) and due to deformation of the network chains to a more elongated state (Π_{elas}). For gels with ionisable groups, the terms Π_{ion}, represents osmotic pressure arising from a concentration difference of ions between the gel and solution, while Π_{elec} accounts for the electrostatic interactions of charges on the polymer chains. In a non-ionic gel system, the terms Π_{ion} and Π_{elec} can be ignored and the relationship of the osmotic pressure of a hydrogel is given by

$$\Pi = \Pi_{mix} + \Pi_{elas} \qquad \text{II. 9}$$

According to the Flory-Huggins theory, Π_{mix} is given by,

$$\Pi_{mix} = - (RT/ V_1) [\ln (1-\varphi_2) + \varphi_2 + \chi\varphi_2^2] \qquad \text{II. 10}$$

and the elastic contribution, Π_{elas}, by,

$$\Pi_{elas} = - RT\rho (\varphi_2^{1/3} - 0.5\ \varphi_2) \qquad \text{II. 11}$$

where ρ is the effective crosslink density. Hence, if a gel swells in the thermodynamic equilibrium state, equation II. 9 can be expressed as,

$$- \frac{1}{V_1} [\ln(1-\varphi_2) + \varphi_2 + \chi\varphi_2^2 + \rho (\varphi_2^{1/3} - 0.5\ \varphi_2)] = 0 \qquad \text{II. 12}$$

5. Hydrogels Swelling Mechanism

In the swelling process, following penetration of water into a glassy matrix, the following three steps are proposed to occur in succession (Yoshida et al. 1994):

- Step 1: Diffusion of water molecules into the polymer network
- Step 2: Relaxation of polymer chains with hydration
- Step 3: Expansion of the polymer network into the surrounding bulk water upon relaxation.

The swelling behavior differs, depending on which step becomes dominant in determining the rate. Depending on the dominant factor, the mechanism of transport for solvent penetration into the polymers can be classified as either Fickian diffusion (Case-I) or anomalous (non-Fickian) and Case-II transport.

- Step 1 : The solvent uptake behavior in the course of step 1 can be described by **Fick's second law** (Crank 1975).

For a slab-shaped gel, the Fickian law is expressed by the following equation:

Case-I: Fick's second law: $$\frac{M_{st}}{M_{s\infty}} = 4\left[\frac{Dt}{\pi l^2}\right]^{1/2} \quad \text{for } 0 \leq \frac{M_{st}}{M_{s\infty}} \leq 0.6 \qquad \text{II. 13}$$

M_{st} and $M_{s\infty}$ are the total amount of water sorbed by the gel at time t and at the equilibrium state, respectively, D is the diffusion coefficient of the solvent in the polymer and l is the gel thickness.
The total amount of solvent uptake by diffusion increases in proportion to the square root of time at the early swelling stages.

- Step 2: In the extreme case that the diffusion rate is much faster than the relaxation rate, relaxation processes become rate-determining.

This results in a clear interface between the completely swollen gel region at the surface side and the internal glassy polymer core. If solvent penetrates into the polymer matrix devices of various geometries by a **Case-II transport** mechanism at constant speed, the solvent absorption rate can be expressed by (Alfrey 1966):

Case-II transport : $$\frac{M_{st}}{M_{s\infty}} = 1 - \left[1 - \frac{tk_0}{\alpha C_0}\right]^N \qquad \text{II. 14}$$

where k_0 is the Case-II relaxation constant, C_0 is the equilibrium concentration of a solvent in a polymer, a is the radius for a cylindrical and spherical polymer and the half-thickness for a slab polymer and N = 1 for slab, = 2 for cylindrical and =3 for spherical gels.
Contrary to Fickian diffusion, the amount of absorbed solvent is proportional to time in the case of slab geometry.

When no structural changes of the polymer network occur throughout the whole process or when the rate of diffusion or penetration is much less than that of the polymer hydration relaxation process, the penetration of solvent into the polymer is governed by solvent molecule diffusion through the polymer network (Step 1). In the extreme case that the solvent diffusion rate is much faster than the relaxation rate, the relaxation process becomes the rate-determining step (Step 2). When the gel expansion process dominates, the swelling kinetics are governed by collective diffusion (Tanaka 1979; Sato 1988).
Above the phase transition temperature, the gel is in a shrunken state, governed by polymer-polymer interactions and remains in a glassy state. The amount of water absorbed by the gel from a dry state increases in proportion to the square root of time. This means that water is absorbed into the gels by a diffusion mechanism. The gel, however, hydrates and swells as the temperature is reduced below the phase transition temperature. Since the gel hydrates and swells, polymer relaxation processes affect the swelling behavior.

When the rate of solvent uptake rapidly increases in the late stage of Case-II transport with alteration of temperature and solvent composition, the mechanism is called **super Case-II transport.**

- Step 3: The swelling kinetics in step 3 are governed by **collective diffusion** (Tanaka 1979; Sato 1988).

According to Tanaka (Tanaka 1979) and Sato (Sato 1988), the motion of a polymer network upon swelling has been shown to obey a diffusion equation. The swelling kinetics is described by

$$\Delta R(t) = \frac{6\Delta R_0}{\pi^2}\sum_{n=1}^{\infty}\frac{1}{\pi^2}\exp\left(-\frac{n^2\pi^2 D_c}{R^2}t\right) \cong \frac{6\Delta R_0}{\pi^2}\exp\left(-\frac{n^2\pi^2 D_c}{R^2}t\right) \quad \text{II. 15}$$

where D_c is the collective diffusion coefficient, R is the radius of equilibrium swelling for a spherical gel, $\Delta R(t)$ is the radius changes at time t and ΔR_0 is the total radius change. The gel changes its radius exponentially in the swelling process and it was shown that the network relaxation time (τ) is proportional to the square of the characteristic length of the gel ($\tau = R^2 / \pi^2 D_c$). Therefore, smaller gels swell or shrink faster.

When the temperature is increased from the swelling temperature (which is below the phase transition temperature) to the shrinking temperature (above the phase transition temperature), the outer surface of the gel exposed to warmer water immediately shrinks to form a surface layer denser than the bulk matrix. Until the onset of this skin formation, the shrinking process obeys the "collective diffusion" behavior of polymer networks (Sato 1988). The layer formed on the surface of the gel is often dense enough to retard the subsequent outflow of water from the gel interior, preventing the gel from shrinking further and interrupting the shrinking process for a certain time period. So, changes in the deswelling process show an initial rapid shrinking followed by slow deswelling due to skin formation (Yoshida et al. 1991). Such a shrinking process, forming a heterogeneous skin structure, is attributed to the high thermosensitivity of the gels.

The swelling / deswelling changes affect the physical properties and appearance of the gels. The gels are transparent or nearly transparent (depending on the polymer) in the swollen state, but start to become turbid (white) immediately with increasing temperature. Decrease in transmittance (increase in turbidity) signifies that the gel forms heterogeneous structures with a dense skin layer on the surface (Yoshida et al. 1992).

Gels shrink much faster at higher shrinking temperatures. This means that shrinking forces increase with increasing shrinking temperatures and large internal accumulating hydrostatic pressures rapidly expel the interior water. The gel's swelling/deswelling speed depends on their size according to collective diffusion theory, as mentioned above (Sato 1988).

6. Factors affecting swelling of hydrogels

There are three factors that affect the swelling of hydrogels (Peppas 2000):

Crosslinking ratio: is defined as the ratio of moles of crosslinking agent to the moles of monomeric repeating units and is one of the most important factors that affects the swelling of hydrogels. The higher the crosslinking ratio, the more crosslinking agent is incorporated in the hydrogel leading to a tighter structure. Crosslinking hinders the mobility of the polymer chain, hence lowering the swelling ratio.

Chemical structure: the chemical structure of the polymer may also affect the swelling ratio of the hydrogels. Hydrogels containing more hydrophilic groups swell to a higher degree compared to those containing hydrophobic groups. Hydrogels containing hydrophobic groups collapse in the presence of water, thus minimizing their exposure to the water. As a result, the hydrogels will swell much less compared to hydrogels containing hydrophilic groups.

Stimuli: in the case of environmentally sensitive hydrogels, swelling can be affected by specific stimuli. Swelling of temperature-sensitive hydrogels can be affected by changes in the temperature of the swelling media.

7. Solute diffusion

Three phenomenological equations were introduced to characterize different types of solute diffusion in polymers.

- The common equation (Ritger et al. 1987),

$$M_t / M_\infty = K\, t^{n'} \qquad \text{II. 16}$$

represents the fraction of released drug molecules and is a function of time, t. M_t is the swelling ratio as a function of the time t, M_∞ is the swelling ratio at equilibrium, K is a constant related to the characteristics of the gel and n' is the exponent describing the Fickian or anomalous swelling mechanism. For n'> 0.5, non-Fickian diffusion is observed, while n'= 0.5 represents a Fickian diffusion

mechanism. The case of n'= 1 stands for a Case II-transport mechanism in which drug release from hydrogel having slab geometry will be zero-order.

- The **Debora number** (Vrentas 1975),

$$De = \lambda_m / \theta_D \qquad \text{II. 17}$$

where λ_m is the mean relaxation time of the hydrogel / solvent system and θ_D is defined by L^2 / D_s, with L the sample thickness and D_s the solvent diffusion coefficient. When De >>1 or De <<1, Fickian diffusion in either glassy or rubbery states occurs. If De ≈1, non-Fickian diffusion, including Case II-transport, is to be anticipated.

- The **Swelling interface number** (Korsmeyer 1981),

$$S_w = v\, \delta_{(t)} / D_{sw} \qquad \text{II. 18}$$

where v is the velocity of the penetrating swelling front, $\delta_{(t)}$ the time-dependent thickness of the swollen phase and D_{sw} the drug diffusion coefficient in the swollen phase. For the case of S_w <<1, drug diffusion through the swollen phase is expected to be much faster than the rate at which the glassy-rubbery front advances and zero-order drug release is shown. Fickian release is observed in the case of S_w >>1 since the swelling front advances faster than drug release. For values of S_w ≈1, non-Fickian drug release is anticipated.

The characteristics for drug diffusion in hydrogels are summarized in Table 1.

Table 1. *Summary of the Characteristics for Drug Diffusion in Hydrogels*

Type	M_t / M_∞	De	S_w
Fickian diffusion	n'= 0.5	<<1 or >>1	>>1
Non-Fickian diffusion (anomalus)	n'> 0.5	~ 1	~ 1
Case II diffusion	n'= 1	~ 1	<< 1

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Chapter 1: Synthesis and Characterization of *N*-substituted acrylamide hydrogels

1.1 Introduction

Temperature-sensitive (or thermoresponsive) hydrogels have gained considerable attention in the field of medicine and biotechnology due to the ability of the hydrogels to swell or deswell as a result of changing the temperature of the surrounding fluid (Galaev et al. 1999; Jeong et al. 2002).

Thermosensitive hydrogels can be classified as positive or negative temperature-sensitive systems. A positive temperature sensitive hydrogel has an upper critical solution temperature (UCST). Such hydrogels contract upon cooling below the UCST. Negative temperature-sensitive hydrogels have a lower critical solution temperature (LCST). These hydrogels contract upon heating above the LCST (Peppas 2000). The LCST phenomenon has been characterized by negative enthalpy of mixing and negative entropy of mixing (Patterson 1969; Liddell 1970). In an aqueous system, these conditions are associated with hydrogen bonding and / or hydrophobic interactions.

One of the best studied negative thermoresponsive polymers is *N*-isopropylacrylamide (NIPAAM). Aqueous solutions of the NIPAAM polymer exhibit a lower critical solution temperature (LCST) in the vicinity of 32°C in water (Bae et al. 1990; Inomata 1990; Inomata 1995; Zhang et al. 2002). Below the LCST, the polymer chains hydrate to form expanded structures. However, they rapidly dehydrate and aggregate to form compact structures above the LCST.

Okano et al. (Okano et al. 1990) compared the swelling behavior of different alkyl-substituted acrylamide gels, such as crosslinked poly(*N*-ethylacrylamide), poly(*N,N*-dimethylacrylamide), poly(acrylamide), poly(*N*-pyrrolidinoacrylamide), poly(*N,N*-diethylacrylamide) and poly(*N*-isopropylacrylamide) gels. In the case of poly(*N*-isopropylacrylamide), the phase transition results in sharp swelling / deswelling changes. The sharp transition of the temperature responsive polyNIPAAM is characteristic of any material whose functions can be controlled simply by changing the temperature without changing the chemical structure (Okano 1998). Considering these results and monomer structures, that is, unsubstituted (AAM), monosubstituted

(EAAM, NIPAAM), disubstituted (DMAAM, DEAAM, PLAAM (five-membered ring)) monomers, it was suggested that the size and stereospecific configuration of the alkyl group on the backbone may affect the thermosensitivity more than the total hydrophobicity. In addition, the mobility of the side chain may contribute the hydrophobicity or thermosensitivity (Bae et al. 1990).

Closest to the polyNIPAAM swelling behavior should be the behavior observed in the case of poly(*N,N*-diethylacrylamide) (polyDEAAM). However, not much work can be found in the literature for this molecule. The purpose of this second part of the thesis is the synthesis and characterization of both polyNIPAAM and polyDEAAM hydrogels. Using polyNIPAAM gel as reference, we try to investigate the optimum conditions for the synthesis of polyDEAAM gel, as well as, perform a characterization of both gel types in order to prepare their use in the field of biotechnology, particularly as drug delivery devices.

Gels having the same nominal composition may display different swelling degrees and transition temperatures. There are several reasons for these discrepancies. One of these is variable monomer purity. It is known that copolymerising NIPAAM with different monomers will cause shifts in the transition temperature, swelling degree and the sharpness of the volume change at the transition even at relatively low levels of comonomers content. Hydrophilic and ionic comonomers increase swelling and the transition temperature; the opposite is the case for hydrophobic comonomers (Chiklis 1970; Taylor 1975; Hirotsu 1987). In addition, ionic comonomers may induce a discontinuous transition (Hirotsu 1987). Secondly, increasing crosslink density reduces swelling below the transition temperature, possibly affecting this temperature, while the crosslink density is in turn affected by a number of variables, including extent of reaction, oxygen concentration and initiator concentration. The extent of reaction depends upon the total time allowed for the reaction and the polymerization rate; in turn, the rate of polymerization is a function of the initiator type as well as the concentration, oxygen content and temperature. Finally, the temperature of the synthesis affects physical properties, such as the optical clarity and the heterogeneity of the microstructure (Kabra 1991).

Therefore, the synthesis variables have the potential for significantly affecting gel swelling above and below the transition temperature, the transition temperature itself

and the sharpness in the change of swelling at the transition, as well as the physical appearance and microstructure.

For the reasons mentioned above, the synthesis and characterization of the polyNIPAAM and polyDEAAM gels, was realized under the same experimental conditions.

1.2 Experimental Procedures

Materials

N-Isopropylacrylamide (NIPAAM), *N,N*-methylenebis-acrylamide (BIS), ammonium Persulfate (APS), *N,N,N',N'*-tetramethylethylenediamine (TEMED) were obtained from Sigma Aldrich Chemical (Buchs, Switzerland) and used as received, with no further purification. *N,N'*-diethylacrylamide (DEAAM) was obtained from Polysciences Inc. Europe (Eppelheim, Germany). Water was purified using an Elix-3 system (Millipore, Bedford, MA).

1.2.1 Synthesis of the hydrogels

Hydrogels were prepared by free radical polymerization in aqueous solutions of NIPAAM or DEAAM, in the presence of BIS as crosslinking agent. APS was used to initiate the reaction and TEMED was used as an accelerator. The well-known *W* x *C* nomenclature was used to characterise the composition of the gels (Gehrke 1992). In this nomenclature, *W* denounces the weight in grams of the combined monomers per 100 mL of water and *C* the mass of crosslinker expressed as a percentage of the total amount of monomer plus crosslinker. Table 1 summarises the composition of the gels prepared in this investigation. For the polymerization, monomer, cross-linker agent and water were mixed together in a glass vessel at room temperature (27 °C) for 2 hours, under N_2 atmosphere. Then radical starter and accelerator were added and they were mixed together for 5 minutes. Afterwards, the solution was poured into moulds and kept at room temperature for at least 24hours during which time the polymerization took place. The gels were then removed from the moulds and placed in distilled water at room temperature for at least 2 days in order to remove any unreacted material. The water was exchanged several times during this period.

Table 1. *Feed composition for the preparation of polyNIPAAM and polyDEAAM gels*

Component	Gel composition (*W* x *C*)		
	10 x 4	10 x 2	10 x 1
Monomer[a]	2.4 g	2.45 g	2.475 g
BIS	0.1 g	0.05 g	0.025 g
H_2O	25 ml	25 ml	25 ml
APS	7.5 mg	7.5 mg	7.5 mg
TEMED	4.87 µl	4.87 µl	4.87 µl

[a] NIPAAM or DEAAM

1.2.2 Measurements of the Swelling Ratio (SR)

The swelling ratio of the gels was measured gravimetrically in distilled water in the temperature range from 22 to 45 °C for polyNIPAAM and 22 to 50 °C for polyDEAAM. Before the measurement, the gel was incubated in distilled water for at least 24 h at every particular temperature.

The swelling ratio was calculated using the following expression (Zhang 1999; Zhang et al. 2000),

$$SR = W_s / W_d = (W_w - W_d) / W_d \qquad \text{II1. 1}$$

where W_s is the weight of water in the swollen gel after the equilibrium has been reached in distilled water at a particular temperature, W_w is the weight of the wet sample and W_d is the dry weight of the gel dried (at 50 °C) in vacuum overnight.

1.2.3 Measurements of the Deswelling kinetics (Water Retention, WR)

The deswelling kinetics (Water Retention, WR) were determined as follows. Gel samples were equilibrated in water at room temperature and were at t = 0 quickly transferred into hot distilled water (50 °C for polyNIPAAm and 42 °C and 50 °C for polyDEAAm). The deswelling kinetics were measured gravimetrically. The weight changes of each gel were recorded every 10 min for at least 1 hour. The water retention (%) was then calculated as (Zhang 1999; Zhang et al. 2000)

$$WR = 100 \times (W_t - W_d) / W_s \qquad \text{II1. 2}$$

where W_t is the weight of the gel at regular time intervals and the other symbols are the same as defined above.

1.2.4 Measurements of the Reswelling kinetics (Water Uptake, WU).

The reswelling kinetics (Water Uptake, WU) of the gels were measured gravimetrically at 20 °C for polyNIPAAM and 15 °C for polyDEAAM after incubating the dry samples in hot water for a few hours. The weight changes of gel were recorded every 10 min for 1hour.
The water uptake (%) was calculated as (Zhang 1999; Zhang et al. 2000),

$$WU = 100 \times (W_t - W_d) / W_s \qquad \text{II1. 3}$$

The symbols are the same as defined above.

The experimental conditions of the swelling ratio, the deswelling and reswelling kinetics are summarized in Table 2.

Table 2. *Experimental conditions*

	Hydrogel			
	polyNIPAAM		polyDEAAM	
	T (°C)	t (min)	T(°C)	t (min)
SR	22 - 45	24 x 60	22 - 50	24 x 60
WR	50	10 - 60	42, 50	10 - 60
WU	20	10 - 60	15	10 - 60

1.3 Results and Discussion

1.3.1 Synthesis

The hydrogels were synthesized by free radical polymerization in aqueous solution. The polymerization of NIPAAM or DEAAM is initiated by the addition of ammonium persulfate ($(NH_4)_2S_2O_8$) and the base *N,N,N',N'*-tetramethylenediamine (TEMED). TEMED catalyses the decomposition of the persulfate ion to give a free radical:

$$S_2O_8^{2-} + e^- \rightarrow SO_4^{2-} + SO_4^{-\bullet}$$

The time required for gel formation varies depending upon the kind of accelerator and the gel preparation temperature (Rathjen et al. 1995). TEMED was used as an accelerator because it is better for e.g. NIPAAM polymerization according to the literature (Rathjen et al. 1995) and the gelation occurs in matter of minutes. The polymerization temperature was controlled to be at room temperature.
After the synthesis, the gels appeared to be transparent, translucent or opaque (Table 3).

Table 3. *Physical properties of the polyNIPAAM and polyDEAAM gels*

Hydrogel	Gel composition	Physical property
polyNIPAAM	(10x4)	opaque
	(10x2)	transparent
	(10x1)	transparent
polyDEAAM	(10x4)	opaque
	(10x2)	opaque
	(10x1)	translucent

Table 3 summarises the physical properties of the gels below the CST. The results for the polyNIPAAM and polyDEAAM gels in Table 3 indicate that the appearance of the gels is opaque (heterogeneous state (Lee et al. 2000)) for the (10x4) gels, while differences in the appearance are observed for the (10x2) and (10x1) gels.

PolyNIPAAM (10x2) and (10x1) gels appear to be transparent (homogeneous state (Lee et al. 2000)) while the polyDEAAM (10x2) gels appear opaque and the (10x1) gels appear translucent. It is well known that the network of opaque gels is composed of dense and coarse parts, while that of transparent gels is homogeneous macroscopically. As the temperature is increased above their CST, all the gels turn opaque. Similar observations can be also found in the literature (Wu et al. 1992; Zhang 1999).

Even if a phase separation during the formation of the gel network is usually taken to be responsible for the formation of heterogeneous porous structures (Kabra 1991; Okay 2000), which will be the case when synthesizing the gels at temperatures above their critical solution temperature (Gehrke 1992; Gotoh 1998), some literature reports similar observations; the gel turns out to be opaque even when it was synthesized at temperatures below the LCST (Rathjen et al. 1995; Gotoh 1998). Suzuki et al. (Suzuki 1992) explains that in the pre-gel phase and at high preparation temperatures, inhomogeneities may form as the result of clusters of the monomer (i.e. NIPAAM) formed at the initial reaction stage, becoming "compact" and "rigid" due to the insolubility of the polyNIPAAM or polyDEAAM polymer and crosslinks that occur within the cluster. These insoluble clusters are then incorporated into the gel network during the gelation process. It is believed that the inhomogeneities formed within the gel network during polymerization at high polymerization temperatures (i.e. room temperature) contribute to the opaque appearance of the gels (Rathjen et al. 1995).

Therefore, one possible reason for these discrepancies, i.e. opaque gels obtained at a polymerization temperature below the CST, is the temperature of synthesis, which is known to affect the optical clarity and the heterogeneity of the microstructure (Kabra 1991; Gehrke 1992). During the polymerization, the temperature rises because of the highly exothermic polymerization reaction, whose rate increases as a result of the reduced termination rates of the growing chains due to the rapidly increasing solution viscosity near the gel point and the decreasing polymer / solvent compatibility with increased conversion and temperature (Gehrke 1992). The temperature change of the solution during polymerization depends upon different variables, such as mold geometry, initiator type and concentration (Gehrke 1992). In our case, mold geometry was the same for all the synthesized gels but the proportion between monomer and the concentration of the initiator (APS) were different. The concentration of the initiator was constant (7.5 mg) independently of the gel composition (W x C), so it can be

considered as “high” for the (10x4) gels and “low” for the (10x1) gels. Increasing the concentration of the initiator causes an increase in the temperature. Taking into account the above considerations, during polymerization the temperature of the solution expected to rise and be higher than the initially adjusted temperature (27°C) (Rathjen et al. 1995). As can be seen below, the gel transition temperature for polyDEAAM gels was around 30°C and around 32°C for polyNIPAAM, which are quite close to the initial solution temperature (27°C). Therefore, during the polymerization the rise in temperature caused by the exothermic polymerization reaction and the contribution of the initiator’s concentration, was close to the CST of the gel solutions, leading especially to a considerable temperature increase during polymerization, which most likely suppressed the CST and hence caused the formation of the clusters during gel formation and finally the opaque appearance of the corresponding (10x4) gels.

1.3.2 Hydrogels Characterization

Swelling Ratio

The swelling of the gels in water is strongly dependent on the structure of the monomer composing the gels. PolyNIPAAM is expected to have a critical solution temperature in pure water between 32 °C and 34 °C (Bae et al. 1990; Inomata 1995; Zhang et al. 2002). For polyDEAAM polymers a critical solution temperature in the same region, i.e. around 30 °C is given (Zhong 1996; Gan et al. 2001). Whereas polyNIPAAM macromolecules typically have a very sharp phase transition within a fraction of a degree centigrade, the turbidity curves published for polyDEAAM show a phase transition spanning several degrees centigrade (Baltes et al. 1999).

Figure 1 shows the swelling ratio of the polyNIPAAM and polyDEAAM hydrogels with a *W* x *C* composition of 10 x 4 as a function of the temperature.

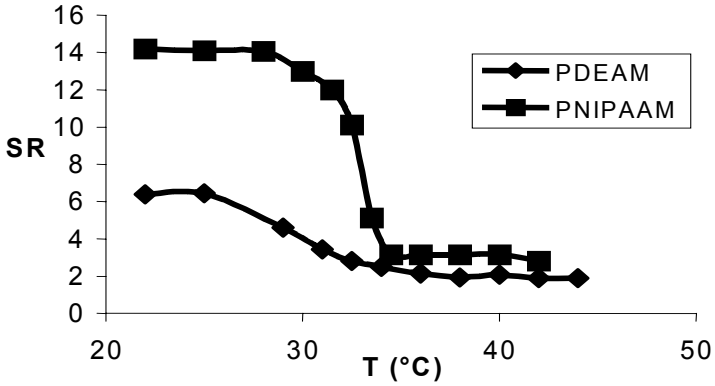

Figure 1. *Swelling ratio of the polyNIPAAM and polyDEAAM (10x4) gels*

The behavior is reminiscent of that of the linear polymers of the same type as discussed above. In the case of the polyNIPAAM gel the swelling ratio decreases sharply once the critical solution temperature (CST) is passed and drops from 14 to 3 between 30 °C and 35 °C. The point of inflection of the swelling ratio versus temperature curve is observed at 32 °C, i.e. at a value that is identical with the point of inflection of the typical turbidity curves recorded for linear polyNIPAAM molecules. The change in the swelling ratio occurs over a much broader temperature range in the case of the polyDEAAM gel. A first effect is already observed at 25 °C. In addition, the swelling curve of the polyDEAAM gel includes a point of inflection around 30 °C followed by a gradual further reduction of the swelling ratio that tapers off to a value of 2 at 38 °C. In spite of the similar *W* x *C* composition, i.e. presumably a similar degree of crosslinking, the swelling ratio at low temperature (<< CST) is twice as high in the case of the polyNIPAAM gel (14) compared to the polyDEAAM one (6.2). At elevated temperatures (>> CST) both gels show approximately the same residual swelling ratio (2-3).

These differences in the swelling behavior can presumably be related to the different nature of the isopropylamino and diethylamino groups in the side chains of the monomeric units. The temperature-induced collapse of thermo-responsive hydrogels in water and the thermo-precipitation of the corresponding linear polymers in aqueous solution are induced by an aggregation of the polymer segments due to hydrophobic interaction (Heskins 1968; Otake 1989; Otake et al. 1990; Baltes et al. 1999). Generally, the strength of the hydrophobic interaction is proportional to the number of

water molecules that form the hydrophobic hydration and increases with temperature (entropy effect). It can be therefore presumed that the gel whose hydrophobic group has a larger hydrophobic surface (contact) area undergoes phase transition at lower temperatures. Between the two alkyl-substitutes of the acrylamide used in this study the surface area of the diethylamino group of DEAAM is larger than that of the isopropylamino group of NIPAAM. This would explain the earlier onset of shrinking in the polyDEAAM-based gels, but also the lower swelling ratio (lower tendency for water uptake) for a given monomer / crosslinker ratio observed in these gels. According to Bae et al. (Bae et al. 1990), the characteristic feature of the swelling for each network is evident in the polymer-water interaction parameter χ.

The polymer-solvent interaction parameter χ can be calculated from the equation of equilibrium swelling of a polymer network formed in the presence of diluent and it is given by (Bae et al. 1990),

$$\chi_H = \left(\frac{\partial \chi}{\partial \left(\frac{1}{T} \right)} \right) \frac{1}{T} = -T \left(\frac{\partial \chi}{\partial T} \right) \qquad \text{II1. 4}$$

where χ_H is the enthalpic polymer-solvent interaction parameter, T the absolute temperature and $\chi = \chi_H + \chi_S$, where χ_S is the entropic polymer-solvent interaction parameter.

At low temperatures the χ values of polymer networks are similar, regardless of the size and configuration of the alkyl side groups. This assumes that the hydration of the polymer network at low temperatures is dependent on hydrogen bonding interactions between water molecules and amide groups, but not on hydrophobic interactions and that the size and configuration of the alkyl groups in the side chains do not affect the swelling levels and thermosensitivity. At higher temperatures, the polymer networks undergo hydrophobic interactions among side groups, intermolecularly or intramolecularly. The length and configuration of an alkyl side group on an amide nitrogen affects the thermosensitivity rather than the total hydrophobicity and the mobility of the side chain may contribute to the hydrophobicity and thermosensitivity.

The degree of hydrophobicity of the side groups is related to the characteristic temperature dependence of χ for each network in water: DEAAM > NIPAAM > APY > DMAAM > EAAM (Bae et al. 1990).
Therefore, as a source of thermosensitivity, the major interactions between water and polymers containing neutral, polar and hydrophobic / hydrophilic balanced side groups will be hydrogen bonding and hydrophobic interactions.

- ***Dynamic swelling kinetics***

Above the phase transition temperature, the gel is in a shrunken state due to aggregation of the polymer chains by segment-segment interaction. In this shrunken state, the polymer chains are physically crosslinked by intermolecular bonding due to aggregation. Below the gel phase transition temperature, the diffusion rate of water in the gel or the polymer relaxation processes also influence the swelling behavior.

To investigate the applicability of the **diffusion model** for the gels, the swelling ratio, M_t as a function of the time t is analysed according to the following equation (Ritger et al. 1987)

$$M_t / M_\infty = K\, t^{n'} \qquad \text{II1. 5}$$

Equation II1.5 should allow distinguishing between the Fickian and non-Fickian release behavior. M_∞ is the swelling ratio at equilibrium, K is a constant related to the characteristics of the gel and n' is the exponent describing the Fickian or anomalous swelling mechanism. Using the natural logarithm of equation II1.5, equation II1.5 is given by

$$\ln (M_t / M_\infty) = \ln K + n' \ln t \qquad \text{II1. 6}$$

where the values of n' and K are calculating from the slope and intercept of the plot of $\ln (M_t / M_\infty)$ against $\ln t$, respectively (Table 4).

Table 4. *Values of* n' *and K of the polyNIPAAM and polyDEAAM (10x4) gels*

Hydrogel	n'	*K*
PolyNIPAAM	0.3618	0.3030
PolyDEAAM	0.1183	0.4315

The values of n' and *K* indicate that the transport mechanism of polyNIPAAM and polyDEAAM gels belong to Fickian transport, for $M_t / M_\infty \leq 0.6$. The values of n' and *K* were close to the experimental values obtained by Lee et al. (Lee et al. 1997; Lee et al. 2002) in water. According to their experimental results, the swelling ratio and the n' values increase with an increase in the pore size of the gels. From our results, it can be deduced that the pore size of the polyNIPAAM gels are higher than that of the polyDEAAM gels.

- ***Effect of crosslinking on the SR of the gels***

The effect of a crosslinking point on the swelling behavior of the polyNIPAAM and polyDEAAM gels was investigated by preparing the gels with various crosslinking densities. From previous work, done by Hirokawa et al. (Hirokawa 1984) and Inomata et al. (Inomata 1995), it was expected that the swelling ratio of a gel decreases as the crosslinking density of the gel increases.

A similar behavior is observed when the two gel types are prepared with a lower degree of crosslinking, Figure 2 and Figure 3. The relative crosslinker concentration affects the value of the possible swelling ratio, but not the general shape of the curve, the broadness of the phase transition, or the transition temperature. A lower degree of crosslinking, as expected, leads to a higher swelling ratio. The effect is especially pronounced in the case of the polyDEAAM gels at temperatures below CST.

PolyNIPAAM gels (Figure 2) consistently show a sharper phase transition and a higher swelling ratio compared to the corresponding polyDEAAM gels (Figure 3).

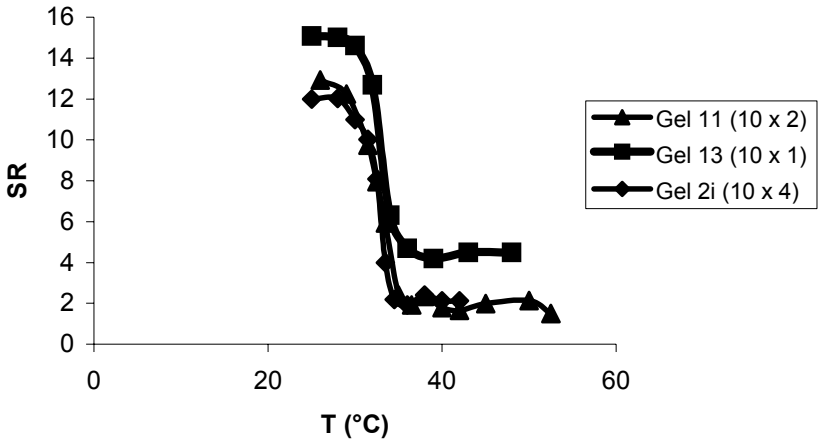

Figure 2. *Swelling ratio of polyNIPAAM gels with different crosslinking densities.*

Save for the 10 x 2 polyNIPAAM gel, all investigated gels show in the collapsed state a swelling ratio around 2. Whereas a reduction of the crosslinker concentration from 10 x 4 to 10 x 2 nearly doubles the swelling ratio below CST in the case of the polyDEAAM gels and a much smaller effect (increase ca. 20 %) is observed in the case of the polyNIPAAM gels.

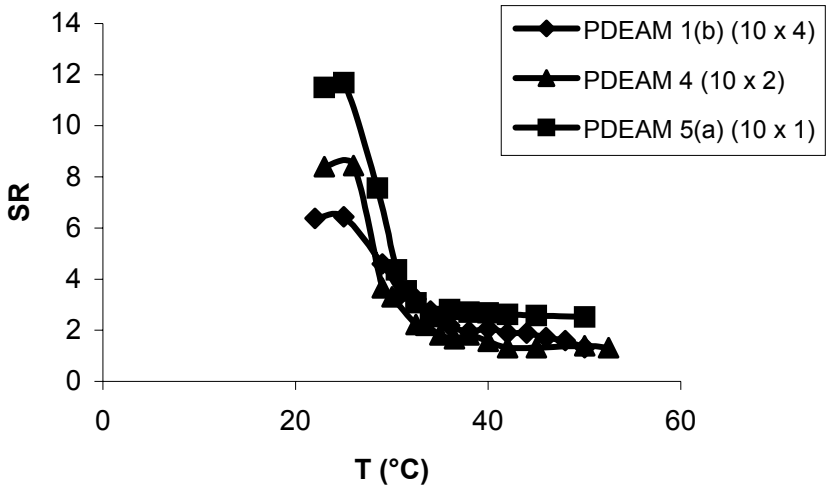

Figure 3. *Swelling ratio of polyDEAAM gels with different gel composition*

From Figure 2 and Figure 3, it can be concluded that the equilibrium swelling ratio is controlled by the crosslinker concentration. Differences in microstructure do not necessarily affect important properties of the gels, such as the equilibrium swelling degree (Gehrke 1992) but they cannot be completely ignored. According to Suzuki et al. (Suzuki 1992) these inhomogeneities are clusters of insoluble polyNIPAAM or polyDEAAM that are trapped inside the gel network. These inhomogeneities break the continuity of the polymer and may reduce the elasticity of the hydrogel, resulting in lower swelling capability (Rathjen et al. 1995). Because transparent gels should have fewer inhomogeneities, they have greater swelling capability. So, this difference to the swelling capacity may to some extent also be due to the presence or absence of inhomogeneities within the gel network.

- ***Effect of the dry sample mass on the SR of the gels***

To understand the swelling kinetics of a gel in water and the effect of the dry gel mass on the swelling ratio, polyNIPAAM gels with three different dry masses were used for swelling ratio measurements. The results (Figure 4) show that the dry mass can influence the analytical results. All the samples exhibit the same swelling behavior in a parallel way, with the swelling ratio of the smallest sample being higher than that of the biggest one.

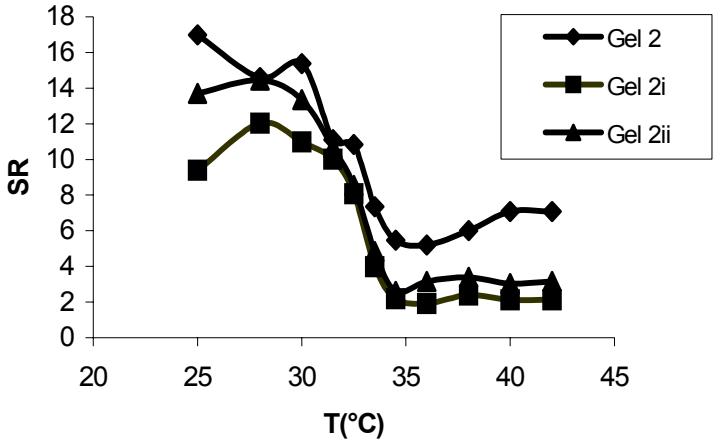

Figure 4. *Influence of the gel dry mass (sample size) on the swelling ratio* (*Gel 2:* 0.0373g*; Gel 2i:* 0.1444g*; Gel 2ii:* 0.0891g)

The equation of Fickian law for $0 \leq (M_t / M_\infty) \leq 0.6$ (Part II - General Introduction, Equation II.15), confirms the above observation, where the swelling ratio is decreased as the size of the gel is increased.

Deswelling Kinetics

For non-ionic gels, such as polyNIPAAM and polyDEAAM, the gel is swollen and the polymer chains expand in water at temperature below the CST. Due to the H-bonds between the hydrophilic groups and water, water molecules are structured around the hydrophobic groups, such as the isopropylamino and diethylamino groups. These structured water molecules act cooperatively to form a cage-like structure, which leads to the good solvation of the whole gel system. As the temperature is raised, these H-bonds are destroyed, the hydrophobic groups are naked and the hydrophobic interactions become stronger. The polymer chains begin to dehydrate. When the temperature is above the CST, the hydrophobic interactions become dominant and the polymer chains collapse and aggregate abruptly (Heskins 1968; Otake et al. 1990; Tokuhiro 1991). When the expulsion of water from the collapsed gels is followed at 50 °C (polyNIPAAM) and 42 °C (polyDEAAM) respectively,

Figure 5, it is evident that both gel types respond quickly and in a very similar manner (kinetics). 80 % of the stored water is released within 10 minutes, after which no further loss of water can be observed.

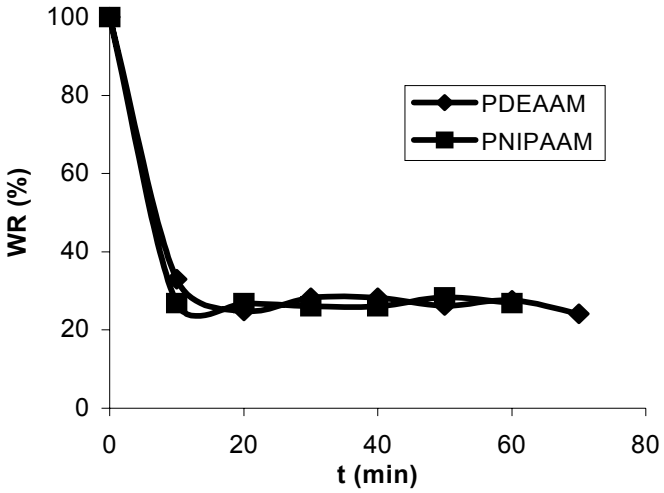

Figure 5. *Deswelling Kinetics of polyNIPAAM* (*at 50* °C) *and polyDEAAM (10x4)* (*at 42* °C) *gels*

If we presume the collapse of the gels above the critical temperature to be due to a destruction of the solubilising H-bridges at that temperature followed by an enforced interaction of the 'naked' hydrophobic groups in order to increase the entropy of the

system, i.e. an effect mainly driven by the surrounding water molecules and less so by the solubilised polymer chains, the similarity in the deswelling kinetics of the two gels types is to be expected as long as both gels are placed into an aqueous environment well above the critical temperature.

On the other hand, due to the increased thermal energy (at the temperature above the CST), the thermal motion of the polymer chains increases. The attractive forces induced by the increased hydrophobic interactions also drive the chains to collapse and entangle with each other (Tokuhiro 1991) and the structured water is expelled into the bulk water. During this course, the entropy of the polymer chains is decreased and the entropy of the pristinely structured water surrounding the polymer chains of gel increases. But as a whole, the total entropy of the system, including the polymer chains and the surrounding water is increased. The collapse of the sensitive gels is an *entropy driven process* (Okazaki 1983; Otake et al. 1990; Luan 1991; Tokuhiro 1991); the phase separation phenomenon of polyNIPAAM and polyDEAAM gels can be elucidated by the second law of thermodynamics.

From the above standpoint of the entropy, we can presume the following: at the swollen state, due to the expanded polymer chains of the polyNIPAAM and polyDEAAM gels, the number of structured molecules around them is large and the whole entropy of gel system is decreased. Thus, the expanded gel network is easy to shrink and to force to undergo phase separation according to the second law of thermodynamics. When the temperature is raised above the CST, the expanded polymer chains will quickly dehydrate and the gel system will exhibit a rapid deswelling rate.

The deswelling kinetics of the different polyNIPAAM hydrogels are given in Figure 6.

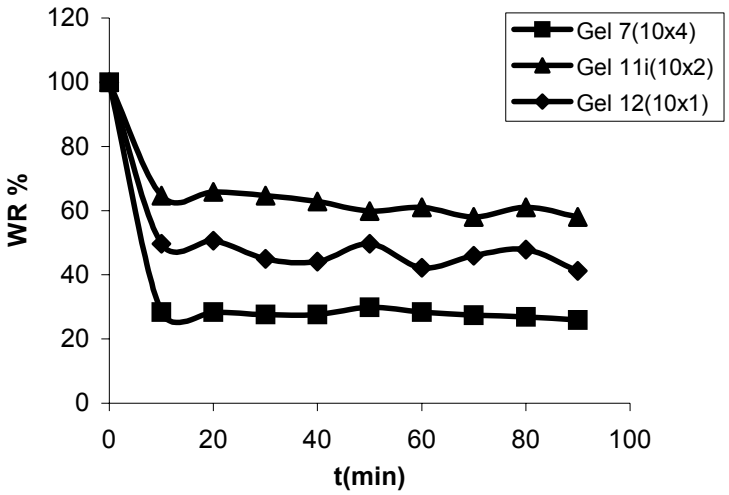

Figure 6. *Deswelling Kinetics of polyNIPAAM gels with different gel composition*

Comparing the deswelling behavior of the gels, having different gel composition, we observed that the deswelling kinetics are controlled by a contribution of the crosslinking density and the opaque or transparent structure of the gels. PolyNIPAAM (10x2) deswells slower than polyNIPAAM (10x1). It is noticeable that apart from the gel composition and structure, all investigated gels reach the equilibrium after 10 minutes. PolyNIPAAM (10x2) gels lose almost 40% of water within 10 minutes, while the (10x1) and (10x4) gels loose 50% and almost 80%, respectively.

The same conclusion is applicable in the case of the polyDEAAM gels (Figure 7), where gels that appeared to be opaque, which are the gels with the higher crosslinking density, the (10x2) and (10x4) gels, allow rapid collapse, losing almost 75 % and 60%, respectively within the first 10 minutes. PolyDEAAM (10x2) gel reaches the equilibrium some minutes later while the polyDEAAM (10x4) gel kept loosing water after the first 10 minutes, reaching 20% water retention within the next 60 minutes. The translucent (10x1) polyDEAAM gel showed slower collapse and lost 60% of the water within the first 10 minutes and then reached equilibrium. Wu et al. (Wu et al. 1992) have observed similar results, comparing a conventional hydrogel with a macroporous one.

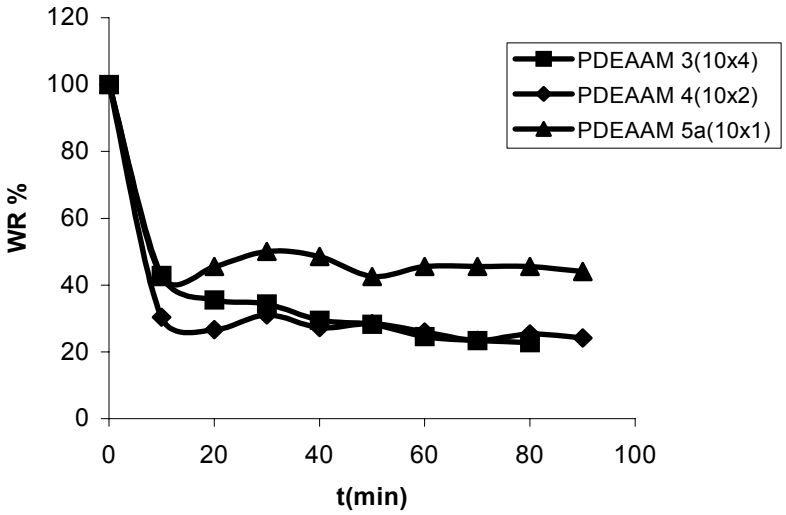

Figure 7. *Deswelling Kinetics at* 42°C *of polyDEAAM gels with different gel composition*

Increasing the shrinking temperature for polyDEAAM gels, no differences to the water retention were observed, since both of the experimental temperatures (42°C and 50°C) are above the CST of the polyDEAAM gels. (Figure 8). At temperatures above their CST (around 30°C), the gels are already in a glassy state.

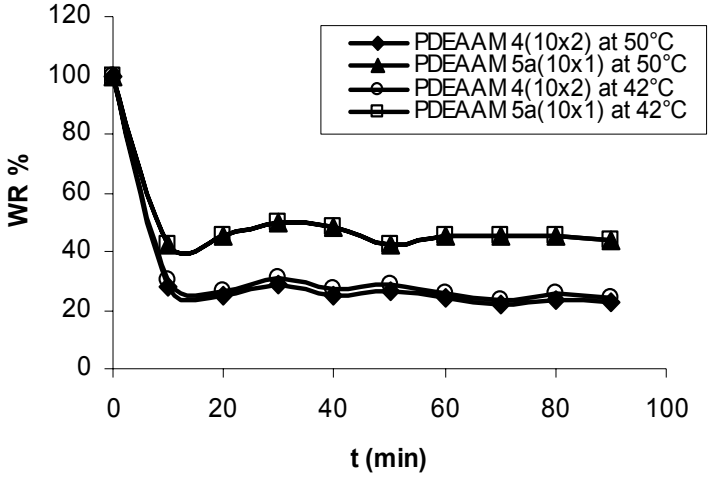

Figure 8. *Deswelling Kinetics at* 42 °*C and at* 50 °C *of polyDEAAM gels with different gel composition*

However, we must consider the other factor, which also influences the shrinking rate; the dense skin layer possibly formed during the shrinking process (Bae 1987; Sato

1988). When the gel starts to shrink, it quickly dehydrates in the surface layer and the collapsed chains in the surface region of gel may form a dense skin layer, which is less permeable to water. During the shrinking process, the excluded water in the gel can diffuse out timely and quickly through the network of the gels and the rapid deswelling rate is achieved. However, the networks of both of the gels prevented the forming of a very thick dense skin layer and the water in gel is not prevented from diffusing out quickly. The shrinking process above the CST is greatly influenced by combinations of surface skin formation and internal pressure as well as polymer network diffusion. From the above results, we assume that the skin layer formed on the transparent / translucent gels is more thick and dense, comparing to the one formed on the opaque gels, leading to slower deswelling kinetics.

Reswelling Kinetics

PolyDEAAM gels show slower reswelling rates than the corresponding polyNIPAAM gels. When the reswelling kinetics were recorded at 20 °C (polyNIPAAM) and 15 °C (polyDEAAM) respectively, some differences can be observed, Figure 9.

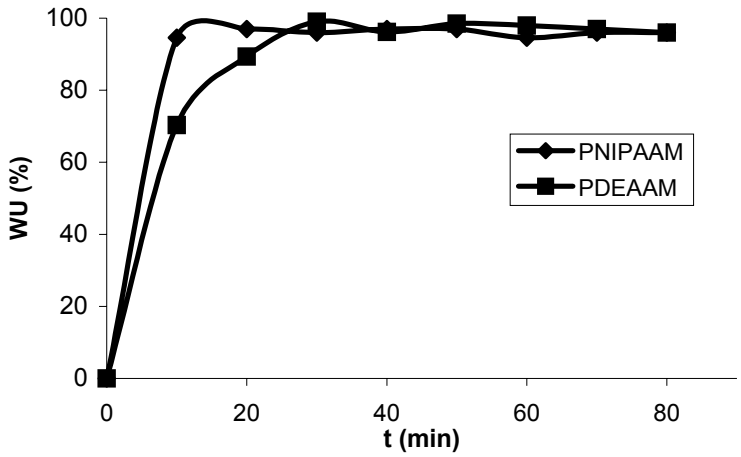

Figure 9. *Reswelling Kinetics of polyNIPAAM and polyDEAAM (10x4) gels*

Under the same conditions, polyNIPAAM gels reswell faster than polyDEAAM gels. PolyNIPAAM absorbs 95% of the water within the first 8 min and reaches equilibrium (> 99%) within the first 10 minutes. PolyDEAAM requires 10 minutes to absorb 70% of the equilibrium water and at least 30 minutes to reach full equilibrium. This may be attributed to differences in the hydrophobicity of the DEAAM and

NIPAAM side chains. If the hydrophobic interactions between the DEAAM chains are stronger during the dehydrating process of polyDEAAM gels, slower reswelling kinetics and, incidentally, a lower swelling ratio for a given crosslinking degree (see also Figure 1) are to be expected. During the reswelling process, these strongly aggregated chains of the dried gel are difficult to rehydrate. From this point of view, polyDEAAM gel should indeed reswell more slowly.

According to Zhang et al. (Zhang et al. 2002), the reswelling of a gel depends on the pre-treatment before reabsorbing the water. In our case, the reswelling rate was measured of the xerogel, which shrunk in hot water. If we compare the deswelling and reswelling kinetics (Figure 5 and Figure 9, respectively), it can be seen that in the case of polyDEAAM the time scales are much smaller for the collapse (~ 10 min) than the reswelling process (~ 20 min). This is probably due in the latter case to the slow process for the aggregated chains to break their hydrophobic interactions and rehydrate as compared to the deswelling case where the free and hydrated chains are in a much more mobile state during the collapse process (Wu et al. 1992). In other words, the gels swell from the shrunken state in the reswelling process and the shrunken gel has a larger resistance against the diffusion of water into the gel. On the other hand, in the shrinking process, the gel shrinks from the swollen state through which the diffusion resistance of water is considered to be small (Gotoh 1998). Since the hydrophobicity of polyDEAAM is higher than that of polyNIPAAM, the time that the aggregated chains of polyDEAAM needs to rehydrate is longer than the time needed for the aggregated chains of polyNIPAAM.

During the reswelling process, three steps have been proposed to occur in succession (Yoshida et al. 1994): (1) water molecules diffuse into the hydrogel network, (2) the hydrated polymer chains become relaxed and (3) the polymer network expands into the solvent. If the first step plays the key role, the amount of water absorption is directly proportional to the square root of time (Grank 1975). If the second step is the dominant factor, water uptake increases in direct proportion to the time (Enscore 1977).

Experimentally we find that the water uptake of the polyNIPAAM (Figure 10) and polyDEAAM (Figure 11) hydrogels is proportional to the time.

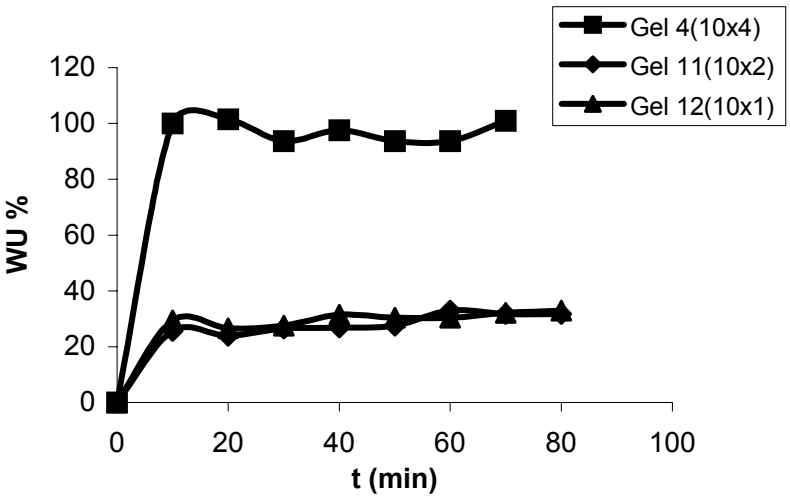

Figure 10. *Reswelling Kinetics of polyNIPAAM gels with different gel composition*

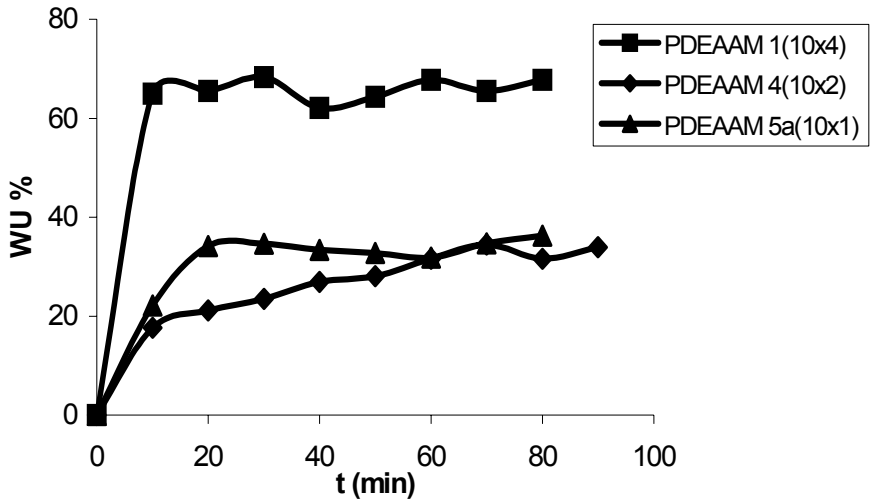

Figure 11. *Reswelling Kinetics of polyDEAAM gels with different gel composition*

This indicates that the reswelling process of the hydrogels is mainly determined by the relaxation of the hydrated polymer chains, hence the relaxation processs becomes rate-determining for reswelling. Such linear increase in water uptake behavior can be explained in terms of a Case II transport mechanism. When polymer chains become hydrated, polymer-polymer interactions are disrupted by water. As the macromolecular relaxation rate with hydration is much slower than the diffusion rate of water in the gel, the relaxation process may become rate limiting for swelling. Then the polymer matrix is divided into a glassy core and the swollen region separated by an interface (Okuyama et al. 1993). The interface (swelling front) proceeds towards

the inside of the gel at a constant rate. As a result, the amount of absorbed water increases in proportion to time (Yoshida et al. 1994).

The observations referring to the crosslinking density in contribution with the gel structure (opaque or transparent) can be also done in the case of reswelling kinetics, with the (10x4) gels allowing the water to penetrate faster into the interior of the gel. Especially in the case of the polyNIPAAM gels, the contribution of their gel structure on the reswelling kinetics is more pronounced than their crosslinking density.

1.4 Summary

Two alkyl-substituted acrylamides, *N*-isopropylacrylamide (NIPAAM) and *N,N*-diethylacrylamide (DEAAM), with three different crosslinking densities, were used for the synthesis of thermoresponsive hydrogels by free radical polymerization. Some characterization of them, including the swelling ratio, deswelling and reswelling kinetics, was performed and the influence mainly of the crosslinking densities and temperature changes on the properties of the gels was investigated.

The gels appeared to be opaque or transparent and their different physical properties were attributed mainly to the temperature of polymerization (nominally room temperature in all cases), which rises due to the exothermic polymerization reaction and can be assumed to reach values above the CST in certain cases. The temperature of polymerization seems to increase partly by the initiator concentration leading to the creation of a heterogeneous gel structure. The gel appearance is influenced by the inhomogeneities formed during the polymerization due to the insolubility of the polyNIPAAM and polyDEAAM polymers and crosslinks.

PolyNIPAAM generally has a greater swelling ratio than polyDEAAM, a sharp thermosensitivity and a transition temperature around 32°C while polyDEAAM has an enhance thermosensitivity and a transition temperature close to 30°C. The gel structure (opaque or transparent) and the different crosslinking density influence the swelling ratio, reswelling and deswelling kinetics. Water diffusion during swelling follows a Fickian behavior meaning that it is controlled by the diffusion mechanism and not the relaxation one. The results can be influenced by the dry sample mass; smaller samples have higher swelling ratio.

Increasing the crosslinking density the swelling ratio decreases but the transition temperature is not affected. Differences to the microstructure may influence partly the

swelling of the gels but mostly the reswelling and deswelling kinetics. Both of the gels lose the water and collapse almost as fast as they absorb water and reswell. The deswelling kinetics is controlled by the formation of a dense skin layer formed on the gel surface, internal pressure as well as polymer network diffusion. Under the same conditions polyNIPAAM gels reswell faster than polyDEAAM gels and this is due to the different hydrophobicity of the isopropylamino and diethylamino groups. The reswelling process of the hydrogels is mainly determined by the relaxation of the hydrated polymer chains and the water uptake behavior can be explained in terms of a Case II transport mechanism.

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Chapter 2: Influence of Inorganic Salts on the swelling kinetics of the hydrogels

2.1 Introduction

There have been many reports of attemps to control the thermally induced volume phase transition of *N*-alkylacrylamide gels, for example through the use of mixed solvents (Hirotsu 1987; Otake et al. 1990), the addition of salt (Inomata 1992; Dhara et al. 2000), the copolymerization of an electrolyte monomer (Hirotsu 1987) and the addition of surfactants (Hirotsu 1987; Inomata 1992).

Park and Hoffman (Park 1994) were the first to demonstrate that aqueous NaCl can induce volume phase transition in non-ionic polyNIPAAM hydrogels. They monitored the effect of a series of sodium salts and concluded that the chloride ion is responsible for causing this transition.

Liquid water has a distinctive structure feature called an “iceberg”; a change in this structure is evoked by the presence of ionic solutes. For the structure of water in the presence of ionic solutes, Frank and Wen (Frank 1957) proposed a model, which was derived from numerical analyses of the entropy of the hydration and it was explained in Chapter 2 of Part I. Depending on whether the ion is a structure breaker or not, the normal hydrogen bonded structure of water is destroyed or retained.

The viscosity *B* coefficient (Jones 1929) is generally considered to represent a measure of hydrate structure. An ion with positive viscosity *B* coefficient is a structure maker and tends to enhance the hydrophobic interaction, while one with a negative value, a structure breaker, tends to stabilize hydrophobic hydration. Table 1 shows the viscosity *B* coefficient values of the ions used in this study.

Table 1. *Viscosity B Coefficient of Ions in water* (*25* °C) (Samoilov 1972; Inomata 1992)

Ion	Viscosity B Coefficient (L/mol)
K^+	-0.007
I^-	-0.069
Cl^-	-0.007
HO^-	0.112
SO_4^{2-}	0.208

The effects of low molecular weight additives on the transition temperature can be explained by the hydrophobic hydration and interaction. The hydrophobic and hydrophilic groups of the gels strongly promote the hydration structure around them cooperatively (Nakanishi 1984; Tanaka 1984). When the additives are added to an aqueous solution, they interact with the bulk water and decrease the entropy through hydration. This is one reason for the depression of the transition temperature following the addition of low molecular weight additives.

Our experiments showed that the phase transition of the two investigated gels, polyNIPAAM and polyDEAAM gels, occur at a temperature close to the critical solution temperature of their aqueous solutions. To examine whether this similarity holds even in the presence of additives, we compare the effects of the additives (inorganic salts) on the phase transition and the swelling ratio of the gels with the effect on linear polyNIPAAM and polyDEAAM polymers.

2.2 Experimental Procedures

Materials

Potassium iodide (KI), potassium chloride (KCl), potassium hydroxide (KOH) and potassium sulfate (K_2SO_4) were obtained from Sigma Aldrich Chemical (Buchs, Switzerland) and were used without any further purification.

2.2.1. Swelling Ratio measurements

For the purpose of the additives influence on the swelling ratio of the hydrogels, aqueous potassium salt solutions having concentrations in the range from 0 to 2 M, were prepared.

Dry samples of the two investigated polyNIPAAM and polyDEAAM (10x4) gels, having an average mass of 0.1g (dry mass), were immersed in 10 ml salt solution and their swelling ratio was measured gravimetrically in the temperature range from 2 to 45 °C. Before the measurement, the gel was incubated in distilled water for at least 24 hours at every particular temperature. The SR was calculated using the same expression as in Chapter 1.

2.3 Results and Discussion

When the polymer is crosslinked and formed into a gel, the change in solubility (or viscosity) for a nonionic uncrosslinked polymer in the salt solution can be reflected as a change in the equilibrium swelling degree of the gel (Park et al. 1993). The decreased swelling degree directly implies a decrease in the solubility (or viscosity).

Park and Hoffman (Park 1994) observed that the phase transition is dependent not on the cation but on the anion and roughly on its position in the Hoffmeister series (Kaminsky et al. 1969; Durand et al. 2000). Similar observations, using aqueous hydroxides and chlorides of Na and K, have been done by Dhara et al. (Dhara et al. 2000). For instance, in the series of alkali halides, the efficiency of lowering CST follows the ranking I^- <Br^- <Cl^- <F^-, while remaining independent of the cation. The essential difference between an anion and a cation could be in the manner in which they interact with water. But if we consider that central to water structure formation is the interaction of the amide group of the polymer with an anion then the issue is settled. Because anions are more inclined to interact with the amide group than the cations (Dhara et al. 2000).

Taking in consideration the above observation, we investigate the influence of the anionic species (I^-, HO^-, Cl^-, SO_4^{2-}) of different potassium salts on the phase transition and the swelling ratio of the gels.

Figure 1 shows the influence of inorganic salts; KI, KOH and KCl, on the phase transition of polyNIPAAM (10x4) gels and Figure 2 show the influence of the KI, KCl, KOH and K_2SO_4 on the phase transition of the polyDEAAM (10x4) gels. The

inorganic salts influence the critical temperature of the hydrogels in the same way as the critical temperature of the corresponding linear polymers (PART I - Chapter 2). The critical solution temperature (CST) is decreased by the addition of the salt, except for low concentrations of KI on the CST of polyNIPAAM and KI and KC on the CST of polyDEAAM, which tend to slightly elevate rather than depress the CST (salting in effect) at low concentrations and cause the CT to decrease linearly with increasing salt concentration. From the three investigated salts in Figure 1, KOH has the strongest influence on the CST ("salting out" effect) of the polyNIPAM hydrogel.

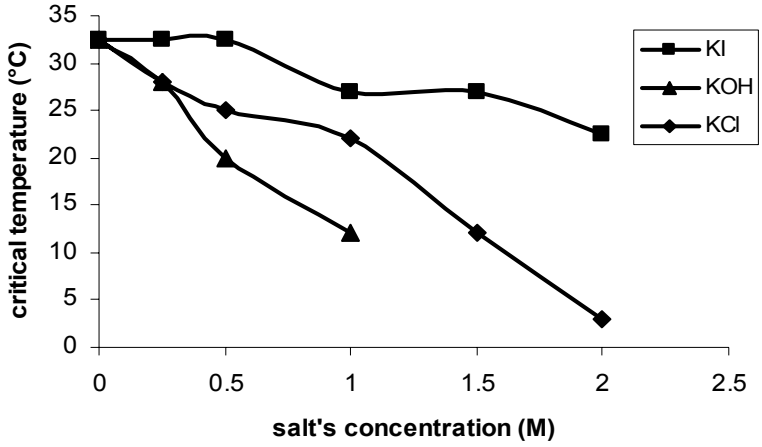

Figure 1. *Influence of inorganic salts on the critical temperature of polyNIPAAM (10x4) gels*

In Figure 2 we observe that K_2SO_4 has the strongest salting out potential of all investigated anions on the CST of the polyDEAAM hydrogel.

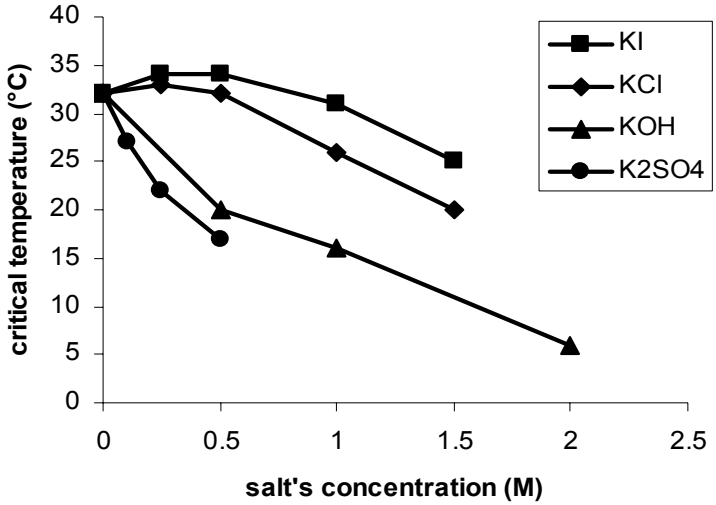

Figure 2. *Influence of inorganic salts on the critical temperature of polyDEAAM (10x4) gels*

The phase transition exhibits the usual dependence on temperature (Figure 3 and Figure 4). At each temperature the water content decreases sharply for a change in the salt concentration.

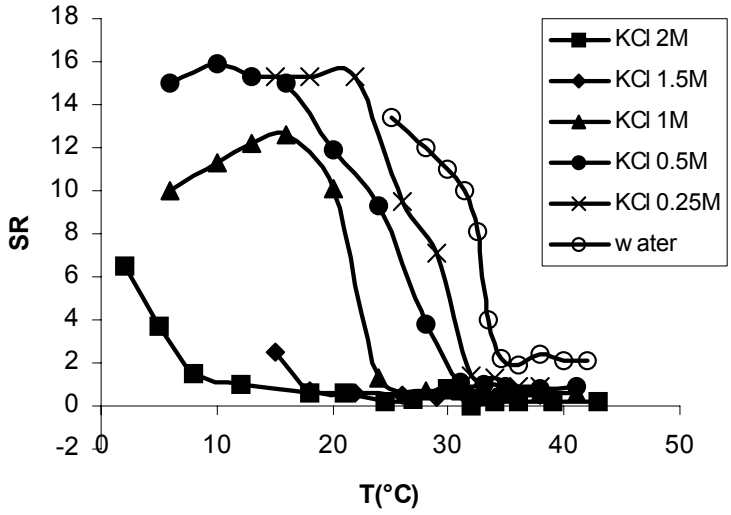

Figure 3. *Influence of different concentrations of KCl on the swelling ratio of polyNIPAAM (10x4) gels*

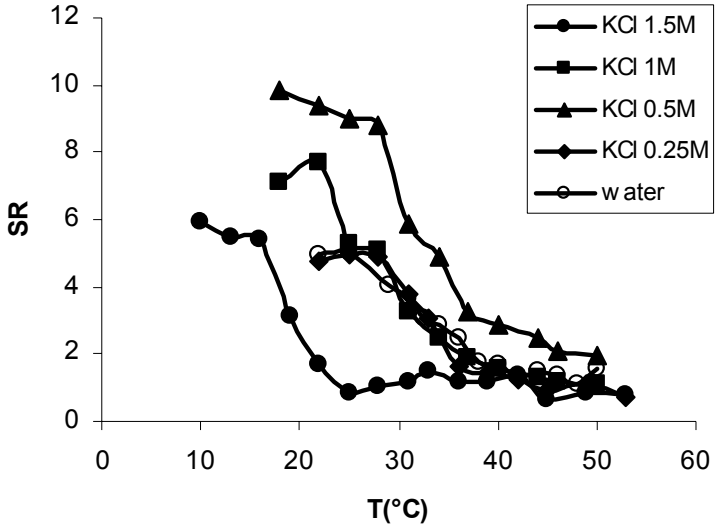

Figure 4. *Influence of different concentrations of KCl on the swelling ratio of polyDEAAM (10x4) gels*

Therefore, this series of experiments reveals that the change in the polyNIPAAM and polyDEAAM gels phase transition strongly depends on the salt concentration and the effect of the salt increases in the following order: KI< KCl< KOH< K_2SO_4, which is in accordance with the Hoffmeister series (Ataman 1987), but also with the results obtained by other authors (Annaka et al. 2000; Yang et al. 2001).

It is known that the viscosity *B* coefficient represents the ability of hydration structure formation. The order of the viscosity *B* coefficient is in good agreement with the degree of transition temperature depression of the additives. Thus, as a large viscosity *B* coefficient implies the formation of a large hydration structure, this also supports the existence of a hydrophobic interaction.

Figure 5 shows the relationship between the viscosity *B* Coefficient values of the anions and the change in transition temperature (ΔT) of polyDEAAM (10x4) gels. As seen in Figure 5, the ΔT is almost proportional to the viscosity *B* Coefficient value for the monovalent and divalent anions ("structure breakers") used in this work.

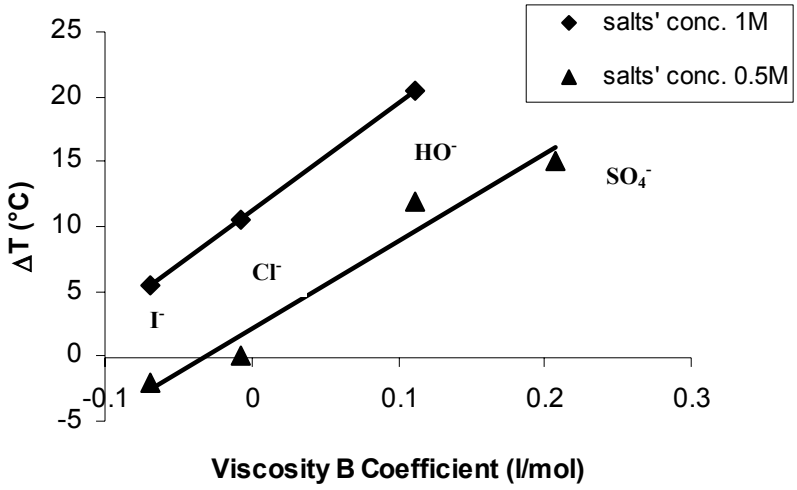

Figure 5. *Correlation between the viscosity B coefficient of several anions and the transition temperature decrease (*ΔT *) of polyDEAAM (10x4) gels to which 0.5 M and1 M of the respective potassium salts has been added.*

The viscosity *B* Coefficient value is closely related to the degree of change in the transition temperature of the gel resulting from the addition of inorganic salts. This may be attributable to the fact that inorganic ions form hydrates through simple ion-dipole interactions. When electrolytes have other interactions as well as the ion-dipole interaction are added, the additives would be expected to have different effects on the transition temperatures of the polymers and gels (Inomata 1992).

2.4 Summary

The effect of low molecular weight additives on the critical temperature and on the swelling ratio was investigated using a series of potassium salts. The critical temperature and the swelling ratio are decreased sharply by the addition of most of the investigated salts; exception is low concentrations of KI on the critical temperature and swelling ratio of polyNIPAAM and polyDEAAM gels and of KCl on the critical temperature and swelling ratio of polyDEAAM. The phase transition and the swelling ratio of the gels depends on the anionic species (I^-, HO^-, Cl^-, SO_4^{2-}) following the Hofmeister series.

The inorganic salts influence the critical temperature of the hydrogels in the same way as the critical temperature of the corresponding linear polymers and their influence

can be explained by the hydrophobic hydration and interaction of the polymer chains. Viscosity *B* coefficient represents the ability of hydration structure formation and its relationship with the change in the transition temperature (ΔT) is linear.

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Chapter 3: Pores size characterization and solute permeation in hydrogels

3.1 Introduction

Thermosensitive hydrogels, especially those based on polyNIPAAM, have been used to sorption-desorption of hydrophilic solutes (Hoffman 1986), for control of solute release from polyNIPAAM grafted nylon capsules with assymetrical pore structure (Okahata 1986), for pulsatile control of release of hydrophobic solute from simple monolithic devices (Bae 1987), for the temperature controlled delivery of bioactive compounds (Hoffman 1988), for the regulation of the activity of embedded enzymes (Hoffman 1986; Hoffman 1987; Hoffman 1988) and as extraction solvents for proteins (Gehrke 1986; Freitas 1987). Most of these applications are clearly based on the changes of permeability of solutes through the polymer matrix by temperature changes.

The character of water in a hydrogel can determine the overall permeation of molecules into and out of the gel. When a dry hydrogel begins to absorb water, the first water molecules entering the matrix will hydrate the most polar, hydrophilic groups, leading to *"primary bound water"*. As the polar groups are hydrated, the network swells and exposes hydrophobic groups, which are also interacting with water molecules, leading to hydrophobically bound water or *"secondary bound water"*. Primary and secondary bound water are often combined and simply called the *"total bound water"* (Hoffman 2002).

After the polar and hydrophobic sites have interacted with the water molecules, the network will imbibe additional water, due to the osmotic force driving the network chains towards infinite dilution. This additional swelling is opposed by the covalent or physical crosslinks, leading to an elastic network retraction force. Thus, the hydrogel will reach an equilibrium swelling level. The additional swelling water that is imbibed after the ionic, polar and hydrophobic groups become saturated with bound water is called "free water" or "bulk water" and is assumed to fill the space between the network chains and/or the center of larger pores, macropores or voids (Hoffman 2002).

As mentioned before, the amount of water in a hydrogel, i.e. the volume fraction of water and its free vs. bound water 'character' will determine the absorption (or partitioning) and diffusion of solutes through the hydrogel. Pores may be formed in hydrogels by phase separation during synthesis, or they may exist as smaller pores within the network (Hoffman 2002). The average pore size, the pore size distribution and the pore interconnections are important factors of a hydrogel matrix that are often difficult to quantify. These factors are mostly influenced by the composition and crosslink density of the hydrogel polymer network (Park 1994). This is also confirmed by the results obtained by Bae et al. (Bae et al. 1989) using crosslinked polyPLAAM and copolymers. In that case, the permeability of proteins (insulin) through thermosensitive hydrogels, was simply related to the degree of hydration, regardless of the chemical structure of the hydrophobic comonomers, composition and applied temperature. Their results indicated that the major factor affecting solute permeation through thermosensitive polymers is the hydration of the polymers, which the role of temperature is an external stimulus that induces a swelling change for a given polymer. This phenomenon was similar to the results of indomethacin release from a thermosensitive monolithic device, obtained again by Bae et al (Bae 1987). The solute size and shape, its relative hydrophilic and hydrophobic character and the availability of water molecules for hydrating the solute molecules are also important factors governing solute permeation through any particular hydrogel (Rosiak et al. 1999).

From the free-volume theory of diffusion in swollen hydrogels, it can be expected that the permeation of solutes through the hydrogel membrane is dependent on the swelling level of the hydrogel and on the solute molecular size. The solubility of the solute through the hydrogel increases with hydrogel hydration and decreases with the solute size (Sato et al. 1984).

According to this theory (Yasuda 1969),

$$\frac{D_m}{D_w} = \psi(q)\exp\left(-B\left(\frac{q}{V_w}\right)\left(\frac{1}{H}-1\right)\right) \qquad \text{II3. 1}$$

or

$$\ln\left(\frac{D_m}{D_w}\right) = \psi(q)\left(-B\left(\frac{q}{V_w}\right)\left(\frac{1}{H}-1\right)\right) \qquad \text{II3. 2}$$

where q is the cross-sectional area of the solute, $\psi(q)$ is the probability of pores having larger cross-sectional area than q, B is the proportional constant and V_w is the free volume of water. The ratio of the diffusion coefficient within the gel (D_m) to that in water (D_w) is proportional to the water content (H) within the gel; thus the solute permeability increases with increase in gel water content (swelling ratio). This equation implies that the separation of the mixture of molecules having a distinct difference in molecular size may be achieved by varying the degree of swelling of the hydrogel.

Separation of solutes can be perfomed based on predictions of the free-volume theory concerning the influence of the swelling level and the solute size on the permeability.

As the crosslinker density increases, the pore size becomes smaller (Park 1994). The swelling ratio of the gel increases; hence a higher hydration, as the crosslinking density of the gel decreases, leading to an increased permeation. So, hydrogels, having a large swelling ratio, are permeable to a higher amount of solute. This observation has also been made recently by Lee et al. (Lee et al. 2003) using copolymers of polyNIPAAM and investigating their permeation to different solutes.

Labeled molecular probes of a range of molecular weights or molecular sizes are used to probe pore sizes in hydrogels. Fluorescein-labeled dextrans, proteins and oligo- and polysaccharides are among the most commonly used probe solutes (Freitas 1987; Dong 1994; Park 1994).

When labelled molecules are used, the test solution is equilibrated with the hydrogel and the concentration of the probe molecule in the gel at equilibrium is measured. Assuming that only the free water in the gel can dissolve the probe solute, one can calculate the free water content from the amount of the imbibed probe molecule and the known (measured using e.g. UV spectrophotometer (Freitas 1987) or gel permeation chromatography (Park 1994)) probe molecule concentration in the external solution. Then the bound water is obtained by difference of the measured total water content of the hydrogel and the calculated free water content.

Some assumptions and limitations for use of this technique, given by Hoffman (Hoffman 2002) are: (a) the solute does not affect the free and bound water distribution in the gel, (b) all of the free water in the gel is accessible to the solute, (c) the solute concentration in the hydrogel's free water is equal to the solute

concentration in the external solution and (d) the solute does not interact with the gel matrix chains.

In general, it is difficult to determine the porous structure of a swollen material because of its fragility. Hydrogels may consist of 90 or even 99 wt % water and only 10 or 1 wt % polymer network. The porous structure of a hydrogel exists only in contact with an aqueous solution; when dehydrated, the network collapses into a compact mass. Therefore, techniques for measuring the porous structure of a hydrogel must consider the polymer - aqueous solution interaction that is required to preserve the structure. So, for measuring gel microstructure, it is not possible to use common methods such as mercury porosimetry or nitrogen adsorption because gels are swollen in a liquid medium; therefore pores are not readily accessible to mercury or nitrogen (Kremer 1994). Optical methods such as scanning electron microscopy have been applied to determine the microstructure of hydrogels, yielding a three-dimensional image of the structure. However, results from these methods strongly depend on the preparation technique of the hydrogel which modifies the structure (Kremer 1994; Park 1994).

3.2 Experimental procedures

Materials

Dextrans of different molecular weights (5-70 kDa) were all obtained from Sigma Aldrich Chemical (Buchs, Switzerland) and used as received.

3.2.1. Molecular Ingress

Dextran standards with molar masses ranging between 5 and 70 kDa were used as model permeants for the purpose of the estimation of molecular ingress into the gels as a function of the chemistry and the size of the molecules. For such measurements the fully swollen (0.9 % NaCl solution), hydrogels were placed at room temperature (i.e. T < CST) in solutions (0.9 % NaCl + 0.01% sodium azide as bactericide) containing 1mg/mL of the probe molecules. Known volumetric amounts of the gels were incubated in a known volume of the probe molecule at room temperature and the concentration of the probe molecule in the external solution was evaluated initially and after 3 and 24 hours by gel permeation chromatography. For this purpose,

samples were injected into a liquid chromatograph system (pump: HITACHI L-7110, Detector: KNAUER, RI K-2300) equipped with a Shodex protein KW-804 column and a KW-G guard column. All the samples were filtered before the injection into the column.

3.3 Results and discussion

The accessibility / permeability of the gels to molecules of differing sizes was estimated using four dextran standards (5, 15, 40, and 70 kDa). The advantage of using dextran is that its molecules are neutral random coils and the diffusion through the porous matrix primarily depends on the correlation of molecular size / pore size. Thus, the apparent pore size of the gel can be estimated by measuring the exclusion of dextran solutes (Angelova 2001).
As long as the predominant transport mechanism of solutes in hydrogels is governed by the water channels or pore mechanism, the water content will be a strong factor affecting solute permeation. From our previous investigation of the swelling ratio (see Chapter 1), a larger pore size is expected for polyNIPAAM gels; hence higher hydration, leading to a better solute permeation.

For the permeability experiments, fully swollen gels equilibrated with buffer were placed into solutions containing the probe molecules at a temperature below CST (room temperature). Then the ingress of the molecules into the gels was followed chromatographically (gel filtration) over a period of 24 hours.

The diffusion process was evaluated as a function of time. For short contact times between the dextran solution and the gels, it is quite likely that the low percentage of the standard diffused is due to the low number of pores with a given size. This delayed the time to reach the equilibrium dextran distribution. To evaluate the influence of the time on diffusion, the molecule concentration was measured initially and after 3 and 24 h.

The gel's selective absorption depends on solute size. It is essential to know the relationship between solute size and molecular weight. The relevant relationship for dextrans is given by the following equation (Brissova 1996):

$$R_n = 0.026\, M_w^{0.495} \qquad \text{II3. 3}$$

Table 1 gives the corresponding values (R_n, M_w) of the probe molecules used to our experiments.

Table 1. *Characteristics of dextrans*

Sample	M_w	R_n (nm)
Dextran-5	5,200	1.8
Dextran-15	15,000	3.03
Dextran-40	40,000	4.93
Dextran-70	70,000	6.5

The viscosity radius (R_n) can be used to explain the different ingress between the probe molecules (dextran).

Small differences to the ingress % are found between the (10x4), (10x2) and (10x1) polyNIPAAM and polyDEAAM gels as shown in Figures 1-6. According to the free volume theory, the swelling of the gels plays an important role to the solutes permeation, therefore no significant differences were expected among the gels having different crosslinking density, since their swelling does not differ in a very high range (see Chapter 1).

As mentioned before, decreasing the gel's crosslinking increases solute absorption. This means that use of a highly crosslinked gel may allow separations of smaller solutes. However, a gel does not suddenly become effective, meaning that it does not show an abrupt molecular weight cut-off but the tendency for partitioning gradually decreases with solute molecular weight (Freitas 1987). The Figures indicate that there is no significant reduction in molecular weight (or molecular size) cut off values as the crosslinking density increases and this is probably attributed to the existence of noncovalent hydrophobic interactions between polymer network chains. This observation corresponds to previous reports on the solute penetration experiments for gel membranes by Freitas and Cussler (Freitas 1987) and for hydrogel beads by Park and Hoffman (Park 1994). In addition to the covalent bridges between polymer chains in the network, there may be inter- and intramolecular hydrophobic interactions between the isopropyl and diethyl groups in the side chains of NIPAAM and DEAAM, respectively. At low concentrations of crosslinker, the hydrophobic

interactions would be weak, since the distance between hydrophobic groups located in the polymer backbone would not be within the range of intramolecular interactions.
As the crosslinker density increases, the pore size becomes smaller (Park 1994). However, at high concentrations of the crosslinker, hydrophobic interactions between the polymer chains would become more important and these noncovalent interactions might also contribute to the estimation of the molecular weight cut off. Therefore, there may be little influence of the crossliker density on the pore size beyond a certain concentration.

Still, some differences can be noted. As one expected, the ingress of Dextran 5 kDa is higher, with small or bigger deviations, than that of the other molecules. In the case of the (10x4) gels (Figure 1 and Figure 2), the probe molecules follow similar kinetics with small ingress difference between each other. Presumably the pore sizes are too small to allow a noticeable ingress in this case within the measurement time. After 24 hours, Dextran 5 and 15 kDa, reach a percentage of ingress around 25% and have an increasing tendency, while Dextran 40 and 70 kDa, reach 15% and 12%, respectively (Figure 1). The percentage of Dextran 70 kDa ingress remains stable at 12 % within 3 and 24 hours.

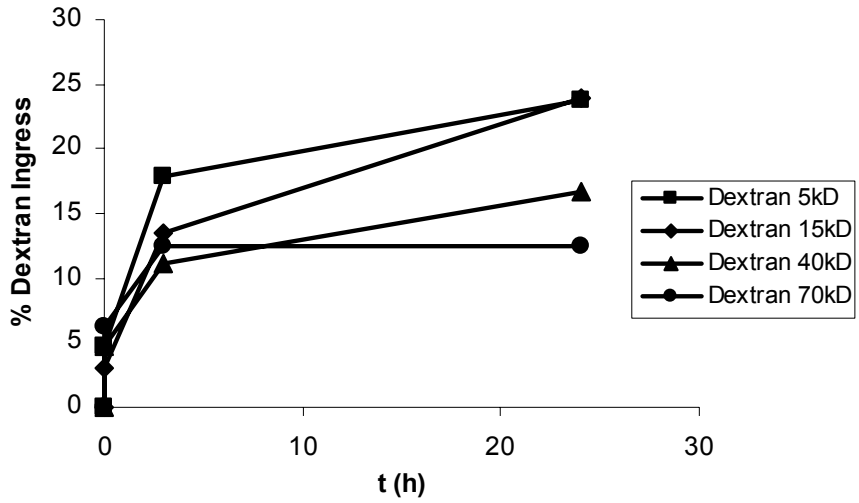

Figure 1. *Dextran Ingress in polyNIPAAM (10x4) gels*

Higher percentages of ingress have been obtained in the case of the (10x4) polyDEAAM gels. After 24 hours, almost all the investigated molecules (Dextrans) reach around 32% of ingress and show the tendency to enter more in the pores of the gel.

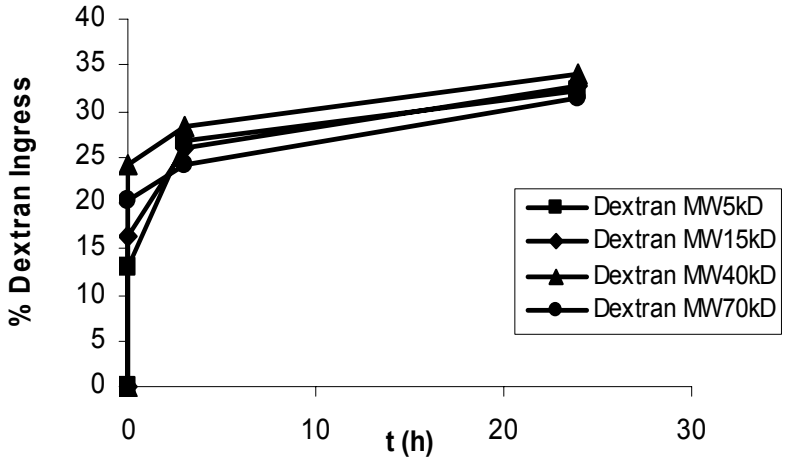

Figure 2. *Dextran Ingress in polyDEAAM (10x4) gels*

When a gel with a lower crosslinking degree (composition 10 x 1) is chosen, Figure 3 and Figure 4, ingress reaches slightly higher proportions, especially for the smaller molecules. For some of the larger ones lower ingress values are observed in some cases. The most noticeable difference is that in the 10 x 1 gels maximum ingress is generally already observed after 3 hours, whereas in the case of the 10 x 4 gels significant ingress still takes place between 3 and 24 hours.

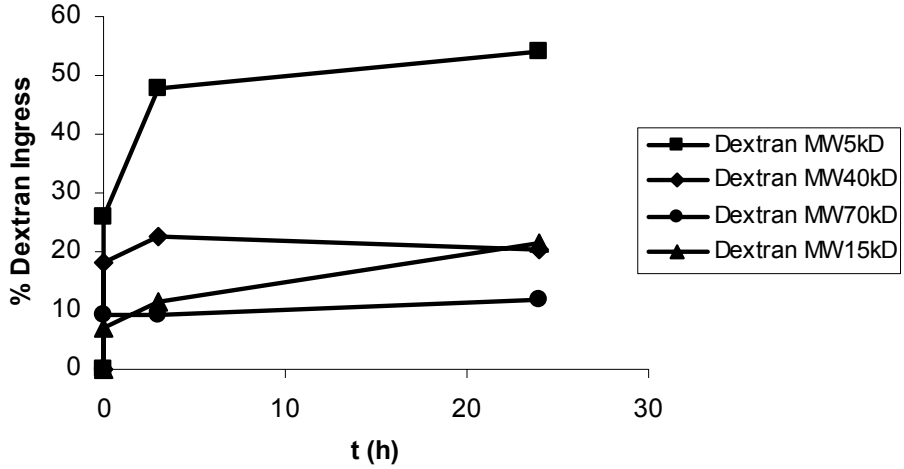

Figure 3. *Dextran Ingress in polyNIPAAM (10x1) gels*

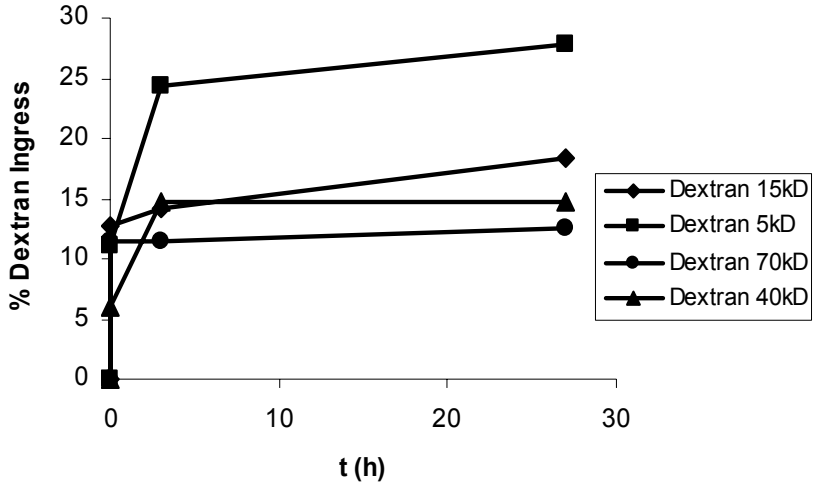

Figure 4. *Dextran Ingress in polyDEAAM (10x1) gels*

Comparing the ingress between the two gels, the dextran standards show higher and faster ingress into the polyDEAAM gels than into the polyNIPAAM ones. An intermediate behaviour is exhibited in the case of (10x2) gels without noticeable differences to the profile of Dextran ingress in the case of polyNIPAAM gels. Their profiles are closer to the polyNIPAAM (10x4) rather than to polyNIPAAM (10x1) gel. In the case of polyDEAAM, it is remarkable that apart from 5 kDa Dextran, the rest reach the maximum ingress (close to 15 %) after 3 hours. Dextran 5 kDa reaches almost 27% after 24 hours and has the tendency to increase its ingress.

The Figures of the Dextran Ingress in (10x2) gels are given below (Figure 5 and Figure 6).

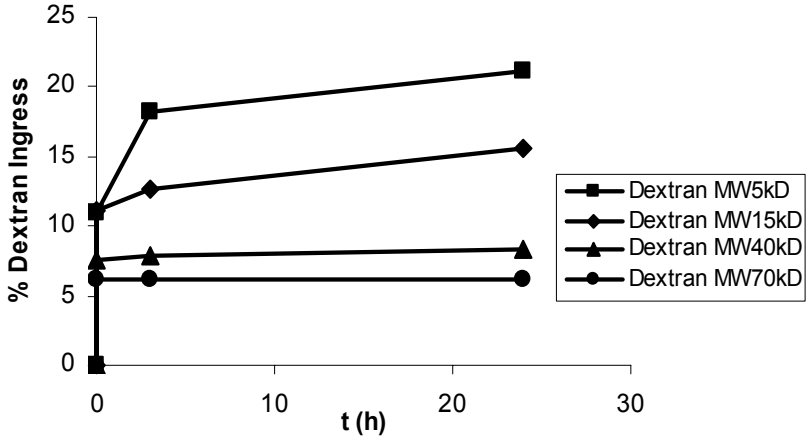

Figure 5. *Dextran Ingress in polyNIPAAM (10x2) gels*

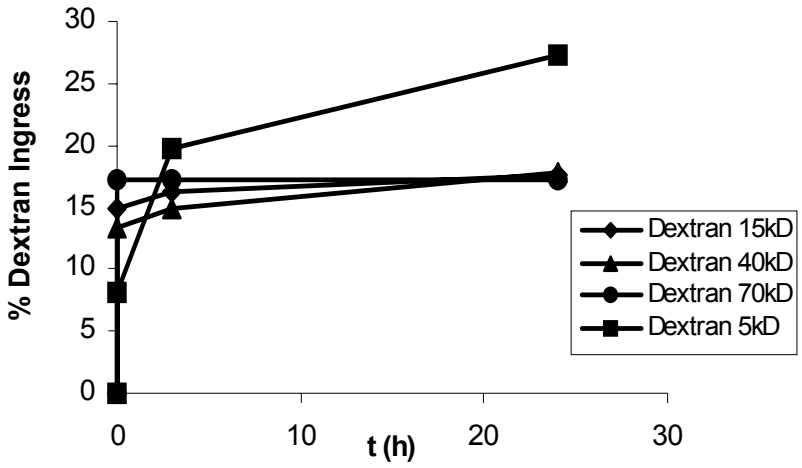

Figure 6. *Dextran Ingress in polyDEAAM (10x2) gels*

The Dextran Ingress into the polyNIPAAM and polyDEAAM gels, is proportional to the different swelling behaviour of the gels caused by crosslinking density in contribution with the gel structure.

3.4 Summary

Dextran molecules having different molecular weight were used in order to investigate the solute permeation into the network of the hydrogels and to estimate the molecular weight cut off and by inference the pore size. The viscosity radius (R_n) was used to explain the differences in the ingress observed among the gels. In most of the cases, the dextran standards showed higher and faster ingress into the polyDEAAM gels than into the polyNIPAAM ones. Dextran 5 kDa reaches the highest percentage of ingress into the gels, followed by the Dextran 15 kDa, 40 kDa and 70 kDa. Not much difference between the ingress as a function of the crosslinking density was observed. This is analogous to the small differences observed in their swelling ratio (free volume theory), but it is probably also linked to an influence of the gel structure. In general, the molecular weight cut off of the gels was estimated to be a bit higher than 70 kDa or 6.5 nm for both of the gels, polyNIPAAM and polyDEAAM.

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Chapter 4. Drug Loading and Release

4.1 Introduction

The abrupt shrinking of temperature responsive gels above the critical temperature resulting in such a drastic change in their swelling degree has called forth a rather extensive research effort directed toward the applications of such gels to the controlled release of drugs and of proteins in particular. Covalently crosslinked temperature-sensitive gels currently are perharps the most extensively studied class of environmentally sensitive polymer systems in drug delivery (Hoffman 1987; Bae et al. 1989; Bae et al. 1990; Okano et al. 1990).

Changing the temperature can control solute permeability through thermoresponsive hydrogels, such as polyNIPAAM. A sudden temperature increase above the transition temperature of these gels results in the formation of a dense, shrunken layer on the gel surface ("skin layer") which immediately hinders the permeation (or leakage) of the drug from the inside of the gel to the enviroment (Sato 1988). By lowering the temperature, below the transition temperature, the deswollen surface recovers its equilibrium swelling and allows permeation of the drug. So, the dense skin layer formed on the surface immediately after increasing temperature prevents drug permeation through the gel or drug release from the gel.

Increased permeation rate is expected below the CST, which is caused by increased hydration, as well as an increased effective surface area. The surface area increase is due to an overall dimensional increase of the membrane at lower temperature.

Loading or release of a macromolecular drug to or from a hydrogel can be controlled by the pore volume fraction, the pore sizes and their interconnectivity, the size of the drug molecule and the type and strength of interactions of the drug with the polymer chains that make up the hydrogel network. In turn, the key factors that control the pore volume fraction, the pore sizes and their interconnections are the composition of the network polymer chains and the crosslink density. The interactions of the drug molecules with the network chains can be determined by their respective compositions (Hoffman 2002). These characteristics can all be manipulated by changing the preparation conditions. Thus, in designing a hydrogel network for controlled release of a drug, it will be necessary to "match" the polymer composition

and crosslink density with the particular size and composition of the drug molecule to be delivered.

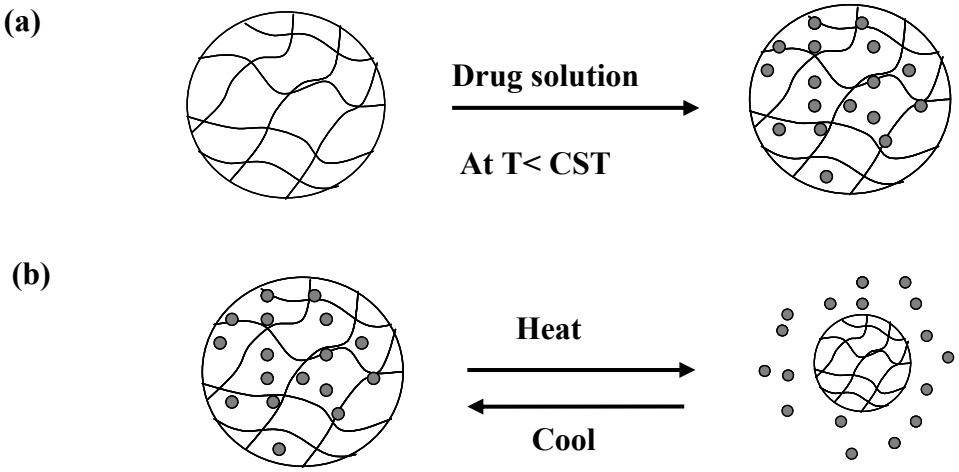

Figure 1. *Schematic representation of* (a) *permeation and* (b) *release of substances using thermoresponsive hydrogels adapted from Hoffman* (Hoffman et al. 1986).

By varying the preparation conditions, the gel can be obtained with a different network density, pore size and pore size distribution thus resulting in different permeabilities. This is also a function of the gel size. As with capsules (Angelova 2001), small spheres having equal total volume with large spheres, expose larger surfaces and thus higher number of pores. As a result, the diffusion of the probe / drug molecules is faster and reaches equilibrium concentration after a shorter time.
Most of the hydrogels are glassy in their dehydrated state and drug release generally involves simultaneous absorption of water and desorption of drug via a swelling-controlled mechanism (Ranga Rao et al. 1988)**.** The rate-controlling factor mediating drug delivery is the resistance of the polymer to an increase in volume and change in shape. A glassy hydrogel, upon coming into contact with water or any other thermodynamically compatible medium, allows solvent penetration into free spaces on the surface between the macromolecular chains. The presence of the solvent in a glassy polymer causes the development of stresses that are accommodated by an increase in the radius of gyration and end-to-end distance of the polymer chains, which is seen macroscopically as swelling (Ranga Rao et al. 1988).

There are relatively few techniques of gel loading acceptable for drug delivery. The first method involves mixing the drug with the appropriate monomer, crosslinker and

initiator solution, which then is allowed to polymerize, entrapping the drug within the formed matrix (Song 1981; Kim 1992; Angelova 2001). A second method is to mix a drug with an already formed polymer and then crosslink it (Gehrke 1992). Both techniques suffer from the possibility of side reactions that can denature the protein / drug. A third method, most widely used, is to allow a preformed purified gel to swell to equilibrium in a drug-containing solution. The exclusion of large molecules, like proteins, from the hydrogel networks is a serious limitation of this approach, since loading level achieved by solution sorption are often less than 0.1 wt% (Antonsen 1993). This limitation, which stems from steric repulsion forces between hydrated polymer coils and proteins, may be overcome if a dry gel network is allowed to swell in a non-aqueous protein solution, then dried and reswollen in water (Bromberg 1996). A fourth strategy was proposed by Gehrke et al. (Gehrke 1991; Gehrke 1995) who achieved very high loading of proteins in gels by partitioning of proteins from polymer solutions of low affinity (i.e. poly(ethylene oxide) solutions) into the gel phase of higher affinity (such as dextran). The additional advantage of the latter technique is that the hydrogel plus any solute loaded from low affinity solution enhance the bioactivity of the loaded proteins (Gehrke 1991; Gehrke 1995).

The ability of molecules of different sizes to diffuse into (drug loading) and out of (drug release) hydrogels allows the possible use of dry or swollen polymeric networks as drug delivery systems (Gupta 2002). Practical applications of crosslinked hydrogels in e.g. drug delivery require high purity and as a consequence non-toxicity should be one of their essential features. Especially, with gels that are made by chemical reaction, most of the problems with toxicity associated with hydrogel carriers are the unreacted monomers, oligomers and initiators that leach out during application (Peppas 2000). Several measures have been taken to solve this problem, including modifying the kinetics of polymerization in order to achieve a higher conversion and extensive washing of the resulting hydrogel. Also, the formation of hydrogels without any initiators (e.g. by gamma irradiation) has been explored to eliminate the problem of the residual initiator (Nedkov 1994; Akkas 1999; Peppas 1999). Cell culture methods, also known as cytotoxicity test, can be used to evaluate the toxicity of hydrogels.

4.2 Experimental procedures

Materials

Insulin and bovine serum albumin (BSA) were obtained from Sigma Aldrich Chemical (Buchs, Switzerland) and used as received.

4.2.1. Drug (Insulin and BSA) loading

For the loading of the proteins, we follow exactly the same method as the one described for the dextran (Chapter 3) and the concentration of the probe molecule (proteins) in the external solution was evaluated initially and after 3 and 24 hours by gel permeation chromatography.

4.2.2. Insulin Release experiments

Insulin was used as a model drug in the release experiments. Dry gels were immersed in 10 ml of a 0.1 % solution of insulin in distilled water and in PBS (phosphate buffered saline, pH 7.4). The gels were left to soak in the solutions for 3 days, under mild agitation at room temperature. This permitted the hydrogels to swell. Then, the gels were transferred in a water bath at 37°C for the release experiments. The amount of insulin released into the medium was determined using a Lambda 20 UV-spectrophotometer (PERKIN ELMER) at 214 nm (the insulin calibration curve, Figure 4, is given in the Appendix). The insulin release was calculated according to (Traitel 2000),

$$\text{Insulin Release} = M_t / M_{in} \qquad \text{II4. 1}$$

where M_t is the amount of insulin released at time t and M_{in} is the amount of insulin initially present in the gel at time 0.

4.2.3. Cytotoxicity Test of the polyNIPAAM and polyDEAAM gels

The cytotoxicity test, which has been applied on the polyNIPAAM and polyDEAAM (10x4) gels, was realized using Jurkat cell line (ATCC TIB 152), a human T cell leukaemia line. Cells were cultured at 37°C, 5% CO_2 in culture medium (RPMI-1640

medium supplemented with 10% heat inactivated fetal calf serum, 1% non-essential amino acids, 2 mM L-glutamine, 1 mM sodium pyruvate, 1% HEPES, 50 U/ml penicillin and 50 µg/ml streptomycin). 2 ml of the cell suspension was added to each well of a 12-well cell culture plate (density around 10^5 c/ml) and 0.5 cm^3 of polyNIPAAM or polyDEAAM hydrogel (swollen in PBS) was added. Triplicate samples were collected and observed continuously for 6h. Trypan blue viability assay was used to assess the viability of the cell populations utilizing trypan blue, which is taken up by nonviable cells. A hemocytometer was used to count the average number of cells present in each well. By counting the alive and the dead cells (cells binding trypan blue dye), this assay provides information about cytotoxicity of the drug solution. Cell density and viability were compared with the viability obtained in a control well (without hydrogels).

Images taken during the cytotoxicity test are presented below.

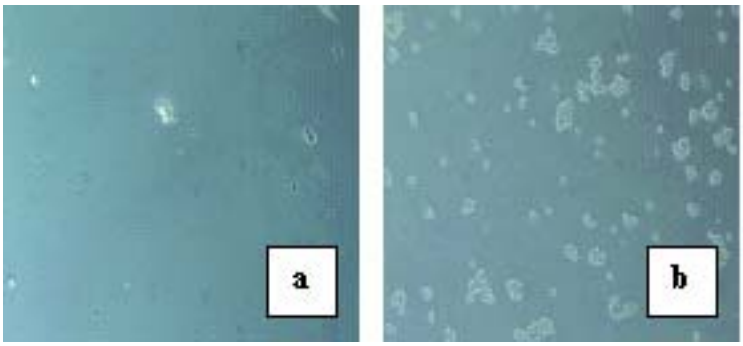

Figure 2. (a) *PolyNIPAAM gel and* (b) *Jurkat Cell line on the polyNIPAAM gel after 6 hours*

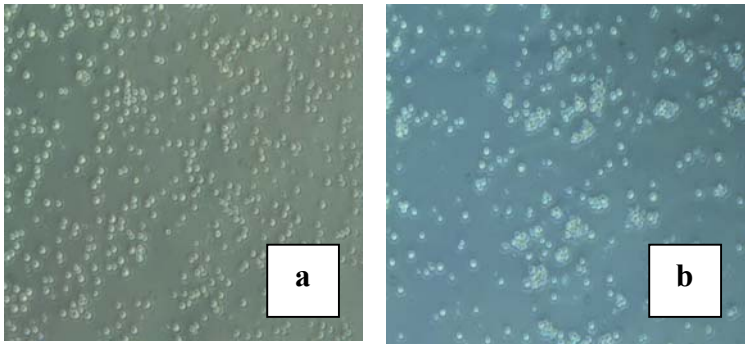

Figure 3. *Jurkat Cell line* (a) *without any gel (blanc),* (b) *on the polyDEAAM gel after 6 hours*

4.3 Results and discussion

4.3.1. Insulin and BSA loading

Insulin and bovine serum albumin were used as model drugs. Insulin is a relatively small polypeptide (5.7 kDa) and its permeability was expected to be high since the upper limit of molecular weight exclusion in the polyNIPAAM and polyDEAAM gels was less than 70 kDa, as described before (Chapter 3). BSA (66 kDa) was chosen as the second model drug, since its high molecular weight will limit its diffusion through the thermosensitive hydrogels. So, in order to investigate the permeability of a small (insulin) and a big (BSA) drug, BSA was the choice for the latter case.

As mentioned before (Chapter 3), the gel's selective absorption depends on solute size and it is essential to know the relationship between solute size and molecular weight.

The relevant relationship for proteins is given by the following equation (Brissova 1996):

$$R_n = 0.051\ M_w^{0.378} \qquad \text{II4. 2}$$

Table 1, gives the corresponding values (R_n, M_w) of the proteins used to our experiments.

Table 1. *Characteristics of proteins* (Brissova 1996)

Sample	M_w	R_n (nm)
Insulin	5,700	1,34
BSA	66,000	3.62

Based on the literature data available for globular proteins (Table 2-Appendix) and dextrans (Table 3-Appendix), we plotted values of R_n (viscosity radius) as a function of molecular weight (Figure 3-Appendix).

The dependence of $R_n = f(M_w)$ for dextrans deviates remarkably from that of globular proteins (Figure 3-Appendix), especially in the high molecular weight region, which points out the compactness of the three-dimensional protein structure as opposed to the statistical coil presumably formed by the dextrans. Moreover, in the case of dextrans, the macromolecular dimension increases almost monotonously with the

molecular weight of the solute, while in the case of proteins, the data exhibit scatter around the best fit curve (up to 17% of R_n), suggesting that even proteins of substantially different molecular weight may assemble into structures of the same size (Brissova 1996). So, if one uses proteins for the permeability measurements it becomes very important to know their macromolecular dimension, otherwise results expressed in terms of molecular weight will be misleading.

This consideration can be used to explain the higher percentage of ingress of insulin (R_n = 1.34 nm) (Figure 4 and Figure 5) comparing to the closest MW that of Dextran 5 kDa (R_n = 1.8 nm) but also the fact of BSA ingress in almost all the investigated gels, independent of the gel compositions (Figure 6 and Figure 7). The ingress of Dextran 5 kDa (see Chapter 3) varies from 22 to 25 and 50 % for PolyNIPAAM (10x2), (10x4) and (10x1) gels, respectively while for polyDEAAM (10x2), (10x4) and (10x1) gels the corresponding values are 27, 28 and 32 %, respectively. As can be seen from Figure 4, after 24 hours, the minimum insulin ingress is close to 35 % for polyNIPAAM (10x2) gels and reaches higher values up to 50 and 55 % for polyNIPAAM (10x1) and (10x4) gels, respectively.

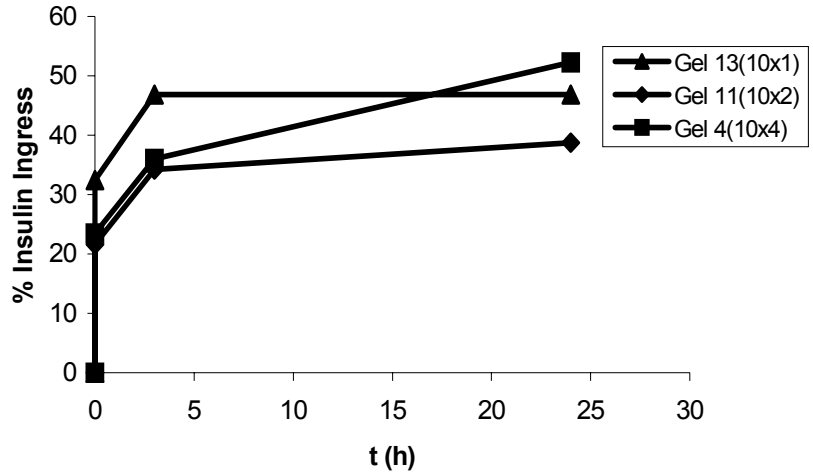

Figure 4. *Insulin loading into polyNIPAAM gels*

Figure 5 shows the insulin ingress in polyDEAAM gels. The lower ingress is close to 30 % for polyDEAAM (10x4) gels and increases to 45 and 55 % for polyDEAAM (10x1) and (10x2) gels, respectively. In the case of (10x1) and (10x4) gels, insulin

ingress remains stable within 3 and 24 hours, while it shows a slight tendency to enter more the pores in the case of polyDEAAM (10x2) gels.

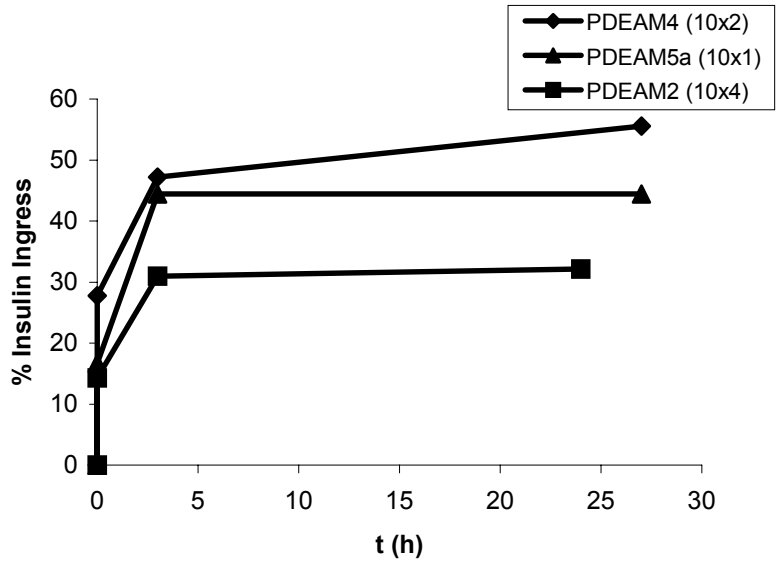

Figure 5. *Insulin loading into polyDEAAM gels*

A difference is also observed in the case of the 10x2 gels between dextran 40 kDa (R_n = 4.93) and BSA (R_n = 3.62). Even if the latter has nominally the higher molecular weight, its viscosity radius, is smaller than that of dextran 40 kDa, which can explain the higher % of ingress.

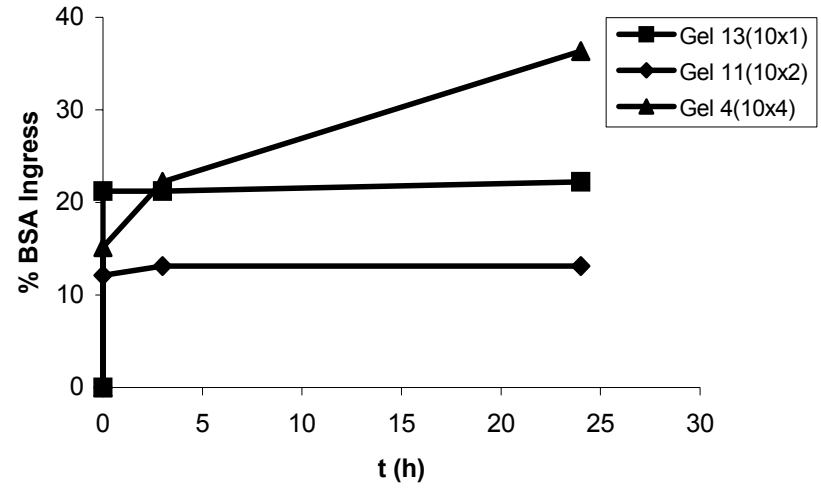

Figure 6. *BSA loading into polyNIPAAM gels*

It is remarkable that especially in the case of polyNIPAAM (10x4) gels, dextran 40 kDa ingress is close to 15 % after 24 hours, while BSA reached an ingress value of 37 % and shows the tendency for higher ingress. Similar percentage ingress of dextran 40 kDa and BSA, close to 20 %, can be observed for polyNIPAAM (10x1) gels, while a slightly higher ingress of BSA (12 %) compared to the ingress of dextran 40 kDa (7 %) is given in the case of polyNIPAAM (10x2). BSA ingress in polyNIPAAM (10x2) and (10x1) gels remains stable between 3 and 24 hours.

Different behavior, with the dextran 40 kDa having higher percentage of ingress (32 %) compared to the ingress of BSA (25 %) can be noted in the case of polyDEAAM (10x4) gels after 24 hours. A tendency for higher ingress of BSA in the gel is observed.

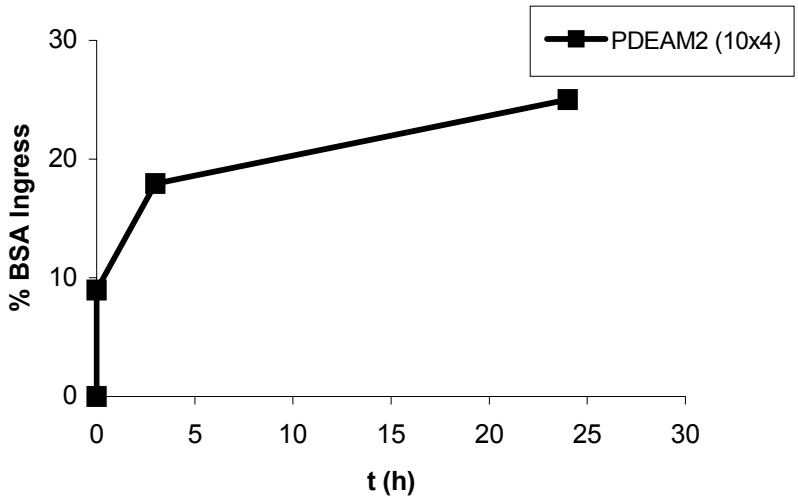

Figure 7. *BSA loading into polyDEAAM gels*

Comparing the (10x4) gels, proteins seem more prone to enter the polyNIPAAM (10x4) gels than the corresponding polyDEAAM (10x4) ones, as insulin after 24 hours reached an ingress value of 50 % in the case of the polyNIPAAM gel compared to only 30 % in the case of the polyDEAAM one (BSA: 35 % versus 23 %).
Because the BSA molecule is larger than the insulin molecule, we can observe that the diffusion amount of BSA is lower than that of insulin in all gels. BSA is excluded completely from the polyDEAAM (10x2) and (10x1) gels (Figure 7).

4.3.2. Insulin Release

The ability of the gels to release a drug upon stimulation was investigated using insulin as model drug. The gels (polyNIPAAm and polyDEAAm, both (10 x 4) were equilibrated in a solution containing 0.1 mg/mL of insulin until equilibrium had been reached. Then the gels were transferred to water or buffered (PBS) solution (T = 37 °C > CST, collapse of the structure) and the insulin release was followed photometrically.

Diffusion of insulin molecules occurs only when the pore size of the matrix becomes larger than the size of single insulin molecule. The change in the pore size that controls the insulin release rate from the hydrogel matrix can be modulated by the external stimuli. Such environmental variations elicit a change in the pore size of the gel matrix, allowing the insulin molecule to be released in a stimuli-responsive fashion (Park 1999). Since the insulin molecule (M_w 5700) is a relatively small polypeptide, the permeability of the insulin through a hydrogel matrix would be a sensitive function of the changing hydration degree of the gel. The water content of the gel is a key variable in controlling the pore size (Park 1994). A free volume theory has been successfully applied to the transport of many solutes through hydrogel matrices. At 37°C, the polymer chains aggregate due to the hydrophobic interaction driving force among the hydrophobic groups and the pore size of the gels decreases (Zhang et al. 2004).

Comparing Figure 8, Figure 9, Figure 10 and Figure 11, we observe that the release profiles of polyNIPAAM and polyDEAAM are different; two stages of release are observed for polyNIPAAM and one for polyDEAAM. No significant change can be observed between the release profiles, either of polyNIPAAM or of polyDEAAM, depending of the release medium (distilled water and PBS).

Insulin is rather rapidly released at the initial stage (burst release), and then slowly diffuses out thereafter. Before the release experiment, the gel was equilibrated in the protein solution and then was transferred to a 37°C environment to carry on the release study. When the temperature was increased above the CST, the hydrogel shrunk quickly due to the occurrence of phase transition. The fast and linear release at the initial stage (up to 10 hours in the case of polyDEAAM) might be related to the rapid squeeze out of insulin molecules located near the gel surface through the pores

together with the water molecules (Afrassiabi 1987; Okano et al. 1990; Cheng 2003; Zhang et al. 2004). Thus, solute transport in this initial period may occur primarily through movement of bulk solution out of the hydrogel pores as a skin forms and the polymer network collapses. After the gel had completed this major initial deswelling, a slower release rate was sustained for periods of 30 hours. However, for the insulin molecules loaded in the inner part of the gel network could not move as quickly as the front ones. An important contributing factor to the slower release rate may be due to the closure of large interconnected pores (Afrassiabi 1987).
The drug release mechanism can be explained using the "skin layer" theory: when the gels shrink, the formation of the not completely permeable surface skin layers stops the release of drug molecules, which are inside the gel. Accumulation of the internal pressure affects this drug release pattern and can be altered with different internal pressure (Yoshida et al. 1991; Yoshida et al. 1992).

The drugs are squeezed out of the gels with the pressure caused by the drastic volume decrease at 37°C. As the gels deswelled at 37°C (above their transition temperature), the gels collapsed and formed a hydrophobic layer in the gels (Park 1994). This layer could hinder the release of the drug from the gel. So, during the initial gel shrinking process, the surface skin formation restricts water permeation and drug release from the gel interior. During this skin formation, both water and drug are released by diffusion mechanisms. Eventually, skin formation practically prevents water and drug release from gel. After a certain period, drug is released again rapidly through the skin bubbles with the convective outflow of water caused by accumulated internal pressure. Consequently, the internal pressure resulting from gel surface skin formation in the shrinking gels affects the drug release stopping pattern (Kaneko et al. 1995). A second stage of release through the skin bubbles is observed in the case of polyNIPAAM (release in PBS) (Figure 8) after 24h of slow release; during the first stage, a burst release is observed the first hour reaching 25% and then insulin slowly diffuses out reaching 40 % of release in 24 hours. Insulin is rapidly released again for the next 6 hours and then slowly diffuses out. Faster release is observed during the second stage, reaching almost 90 % within the 6 hours.

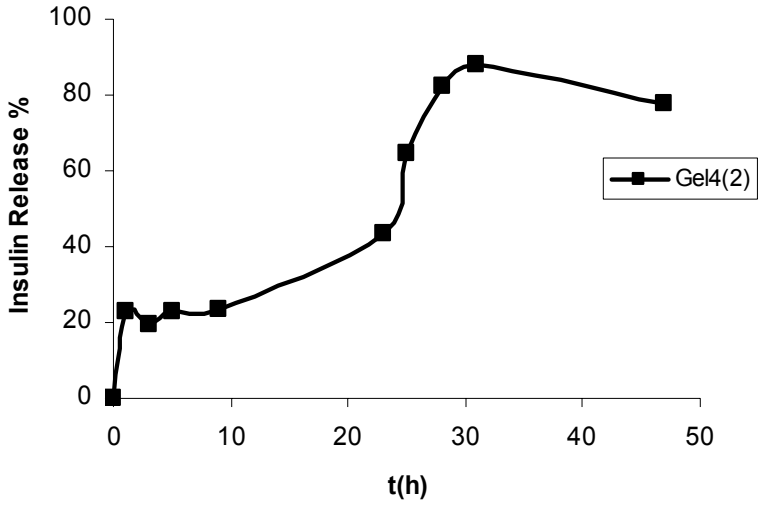

Figure 8. *Insulin Release in PBS from polyNIPAAM (10x4) gels*

When water was used as the release medium (Figure 9), an initial release was observed the first 5 hours, reaching 40% of release and then insulin slowly diffuses out within the next 20 hours, following by a second fast release reaching almost 90% within the next 4 hours.

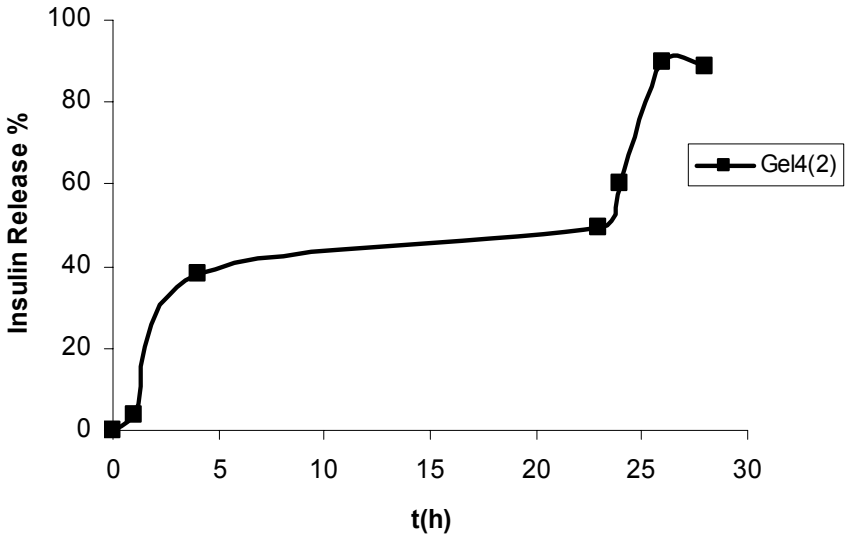

Figure 9. *Insulin Release in water from polyNIPAAM (10x4) gels*

Such a second stage is not as clearly observed in the case of polyDEAAM (10x4) hydrogels in which insulin release is fast and linear and reaches 80 % within the first 10 hours (initial stage) in PBS (Figure 10).

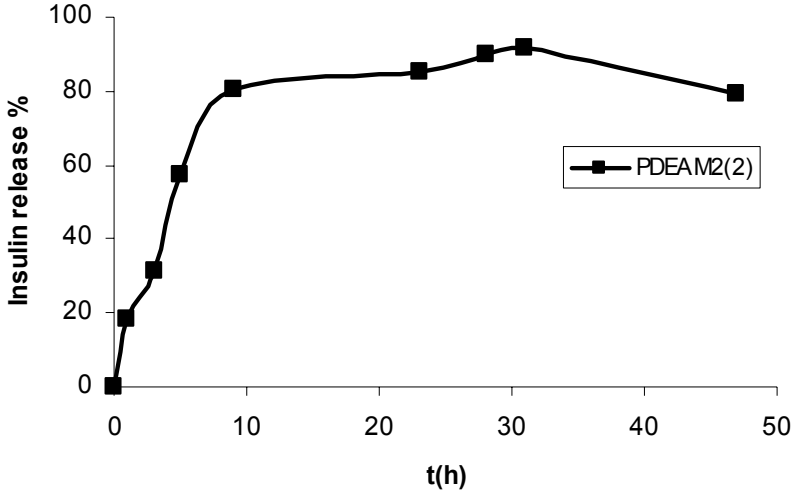

Figure 10. *Insulin Release in PBS from polyDEAAM (10x4) gels*

In the case of using water as the release medium (Figure 11), a fast release up to 40%, is observed within the first 5 hours followed by a gradual release reaching 80% of release the next 40 hours.

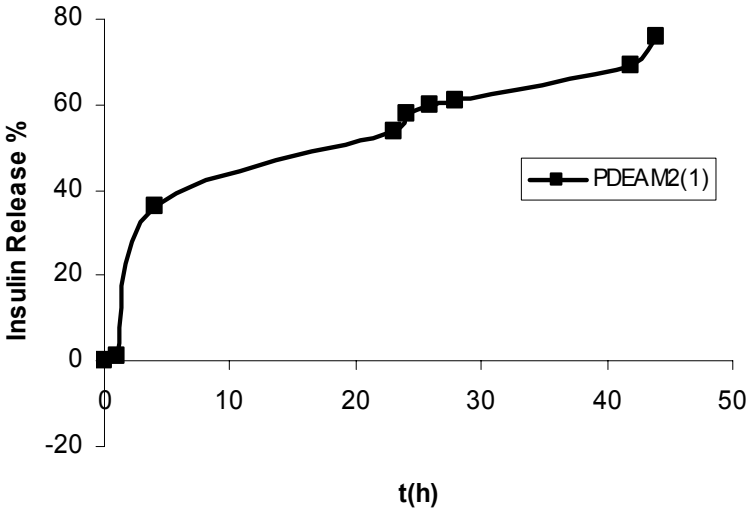

Figure 11. *Insulin Release in water from polyDEAAM (10x4) gels*.

Comparing the release rates (using the release vs. time scale) and the release profiles of the polyNIPAAM and polyDEAAM (10x4) hydrogels in the two different media (water and PBS) some differences can be noted (see above) and they may be attributed to the different release media. The influence of the medium is more pronounced in the polyDEAAM release profile. According to the literature, the

equilibrium swelling ratio of the polyNIPAAM in PBS is lower than its swelling ratio in water (Yoshida et al. 1994). Similar effect is expected on the swelling ratio of polyDEAAM in PBS, because of the similar behavior of the two hydrogels (see Chapter 2). These result in an enhanced aggregation force between polymer chains due to hydrophobic interactions of alkyl side chains and to the formation of a denser and more stable skin layer on the surface of the hydrogels at high temperatures (higher than CST) (Yoshida et al. 1994; Zhang et al. 2004) comparing to the layer formed on the hydrogels surrounded by water. This thick, dense layer on their surface restricts water molecules from being squeezed out (Bae 1987; Sato 1988) and results in a slow response rate. The burst release at the initial stage is caused by the release of the drug on the surface of the gels.

For both gels, close to 90 % of the insulin initially present within the gel matrix are released after the gel had been forced to undergo temperature induced phase transition. After release, 10 % of the protein still remains in the gel matrix, which means that the protein cannot be completely released from the hydrogel matrix. Probably the gel network somehow fixes the protein molecules inside the matrix. So, some drugs entrapped within the gel cannot be released. This phenomenon was also observed in some previous studies (Dong 1986; Hoffman et al. 1986; Bae 1987; Sato 1988; Park 1999; Lee et al. 2003).

We should not completely ignore the existence of electrostatic repulsive force between the insulin molecules, which have a negative net charge at pH value above its isoelectric point (pI~5.3) and the polyNIPAAM or polyDEAAM gels. Even if the two gels were prepared by free radical polymerization and are expected to be nonionic, an anionic initiator residue (- SO^{4-} group) probably remains at the chain ends. Although the electrostatic forces between the polymer chains and drug molecules are not very strong in our system, the electrostatic force remains one of the most dominant factors determining the adsorption in our systems. The hydrophilicity of the gels would be the second important factor having a remarkable influence on the adsorption in a system where an electrostatic attractive force exists between the protein molecules and the thermosensitive gels (Kawaguchi 1992). Stronger effects have been observed between the drug bupivacaine (which is a cation in medium with a pH smaller than the pK_a of the drug bupivacaine (pK_a =8.16)) and the carboxylic group of MMI (monomethyl

itaconate), copolymer of the gel poly(acrylamide-co-monomethyl itaconate) (Blanco 2003). Therefore, the drug establishes electrostatically attractive interactions with the ionised side groups of the hydrogels, which prevents bupivacaine release. Makino et al. have also observed similar results (Makino 2001).

4.3.3. Cytotoxicity test

Figure 12 shows the record of cell viability versus time while Jurkat cells were cultured in the presence of polyNIPAAM and polyDEAAM. We can remark that there is no significant decrease in the percentage of the viability. For cells in either condition, the viability was close to that observed in the absence of the hydrogels. In fact, after 6 hours, the percentage, of the samples with gels, does not decrease more than 2.5 % and the reference sample (without gels) indicates a decrease of 1.5 %. We observe that the two curves (with and without gel) have an average difference of 1.5 %. It is thus apparent that neither preparation caused any acute cytotoxic effect resulting that the gels are not toxic for the Jurkat cell lines within a period of 6 hours.

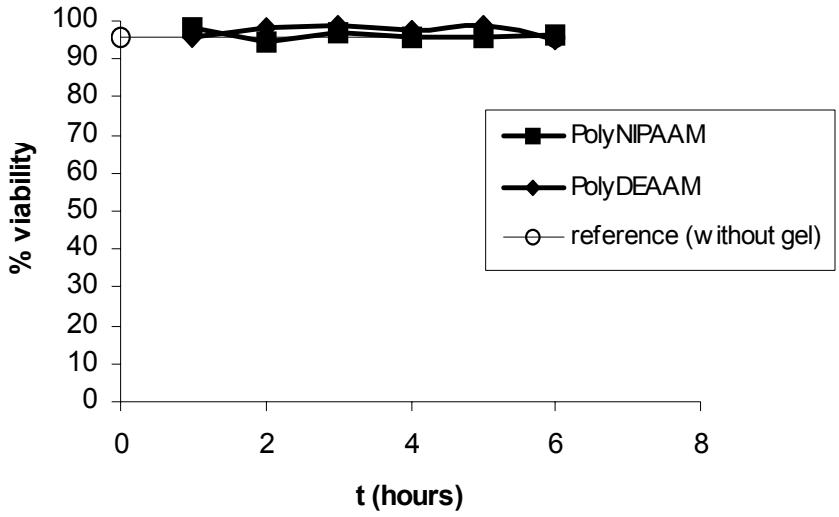

Figure 12. *Viability of the Jurkat cells with and without the polyNIPAAM and polyDEAAM (10x4) gels.*

4.4 Summary

Insulin and BSA were used as model drugs for the purpose of drug loading / release investigation of polyNIPAAM and polyDEAAM gels. Insulin as expected due to its smaller, more compact structure compared to that of BSA had a greater potential to enter the matrix of the gels and hence gave higher percentage of ingress. The viscosity radius (R_n) of the proteins in comparison with the radius of the dextrans (Chapter 3) was used to elucidate the differences between the ingress of the two proteins and the dextrans. Proteins, even those of higher nominal mass than the dextran molecular weight, have a smaller viscosity radius, as a result of their compact, three dimensional structure.

Between the two proteins, insulin was used as a model drug for the investigation of the drug release from the gel matrix of polyNIPAAM (10x4) and polyDEAAM (10x4) gels. In polyNIPAAM (10x4) gels, two stages of release were observed while in polyDEAAM (10x4) gels a second stage was not clearly observable. The release experiments were realized at 37°C, a temperature higher than the CST of the two gels. The release is controlled by the dense skin layer formed on the surface of the gels and prevents the drug to be completely released. The differences observed to the release profile between the gels surrounded by water or PBS solution were attributed to the formation of such a thick, dense layer. Increasing the hydrophobicity, the formation of the layer is more pronounced. A burst release, which was observed initially, was due to the release of drug molecules close to the gel surface.

Almost 90% of the insulin could be released from the gel and the remaining 10% is believed to be entrapped in the gel matrix and thus to be prevented from leaving the gel further.

The release can be also influenced by the electrostatic interactions that may a charged protein (in our case insulin at a pH higher than its isoelectric point) can have with the polymer backbone.

Preliminary results of acute cytotoxicity test on the gels give a first estimation of their toxicity; more advanced tests should be applied in order to give us a total “image” about the possible toxicity of the gels.

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PART III: MICROGELS

General Introduction

1. Microgel particles

A general definition for a microgel is given by Saunders et al. (Saunders et al. 1999) who defined a "microgel particle" as a crosslinked latex particle which is swollen by a good solvent. It may also be defined as a disperse phase of discrete polymeric gel particles, which are typically ranging in size between 1 nm and 1 μm, which are uniformly dispersed in a continuous solvent medium, and which are swollen by a good solvent (Murray et al. 1995). Microgels are intermediate between branched and macroscopically crosslinked systems (Murray et al. 1995). The overall dimensions of microgels are comparable with high molecular weight linear polymers (approximately 10^6 Da). However, their internal structure resembles a typical network (Murray et al. 1995).

A number of other terms, in addition to the term "microgel", are used in the literature to describe small crosslinked particulate systems, such as "polymer microparticles" (Andrew 1979; Andrew 1980), "hydrogel microsphere" (Kawaguchi et al. 1992; Kawaguchi et al. 1993), "latex particle" (Pelton et al. 1986; McPhee et al. 1993; Snowden 1993), "submicron gel bead" (Hirose 1987), "ultrafine microsphere" (Sawai et al. 1991) or "nano-particle" (Park et al. 1992).

Microgels have similar properties to polymers and water-swollen gels (hydrogels or macrogels) but are discrete particles with characteristics that are dependent on the method of synthesis, crosslinking density, monomer concentration, monomer composition and solvency conditions (Funke 1989; Snowden 1995; Antonietti 1998). Depending on their monomeric composition, microgels may be swellable in aqueous or non-aqueous solvents. Because of their porous "sponge-like" structure, microgels swell in suitable solvents (Lowe et al. 1998); this swelling has a finite limit depending on the degree of crosslinking. The polymer may exhibit sensitivity in terms of their swelling behavior, to various environmental conditions such as temperature (Pelton et al. 1986; Hirose 1987), pH (Sawai et al. 1991), electric field (Tanaka 1982) or ionic strength (McPhee et al. 1993; Park et al. 1993). Therefore, microgels are insoluble and do not form solutions,

unlike linear or branched polymers, but may be considered to form colloidal dispersions (Murray et al. 1995).

2. Preparation of microgel particles

Four methods have been reported for the preparation of microgel particles:

- Emulsion polymerization (Clarke 1981; Pelton et al. 1986; McPhee et al. 1993; Wu et al. 1994)
- Anionic copolymerization (Antonietti et al. 1995)
- Crosslinking of neighbouring polymer chains (Baker 1949; Frank 1991)
- Inverse micro-emulsion polymerization (Hirose 1987; Neyret 1997)

Emulsion polymerization is a versatile technique, which yields narrow particle size distributions (Gilbert 1995). It can be performed in the presence of added surfactant, *Conventional Emulsion Polymerization* (McPhee et al. 1993; D'Emanuele et al. 1995; Oh et al. 1998; Hatto et al. 2000; Chai et al. 2003; Xiao et al. 2004) or in the absence of added surfactant, *Surfactant-Free Emulsion Polymerization* (Kawaguchi et al. 1992; Snowden 1992; Ole Kiminta et al. 1995; Weissman 1996; Wu et al. 1996) Conventional emulsion polymerization enables preparation of very small microgel particles (i.e. particle diameters less than 150 nm) and suffers from the difficulty of completely removing residual surfactant. Surfactant-free emulsion polymerization yields microgel particles with diameters between 100 and 1000 nm and does not suffer from residual surfactant contamination (Saunders et al. 1999).

2.1 Surfactant-Free Emulsion Polymerization (SFEP)

Some authors call this procedure “Precipitation Polymerization” (Shiroya 1995; Huang et al. 2004) or “Surfactant-Free Precipitation Polymerization (SFPP)” (Saunders 2004). The continuous phase must have a high dielectric constant (e.g. water) and ionic initiators are employed (e.g. $K_2S_2O_8$). The polymerization is conducted at 60-70 °C in order to generate free radicals by the decomposition of the persulfate initiator. Also, in cases

where thermoresponsive polymer chains, such as polyNIPAAM chains, are presented in the polymerization process, elevated temperature is required, in order to assure that growing chains phase separate to form colloidal particles. The key feature of SFEP is that the particle nucleation period is very short (of the order of minutes), which ensures a narrow particle size distribution (Gilbert 1995; Saunders et al. 1999).

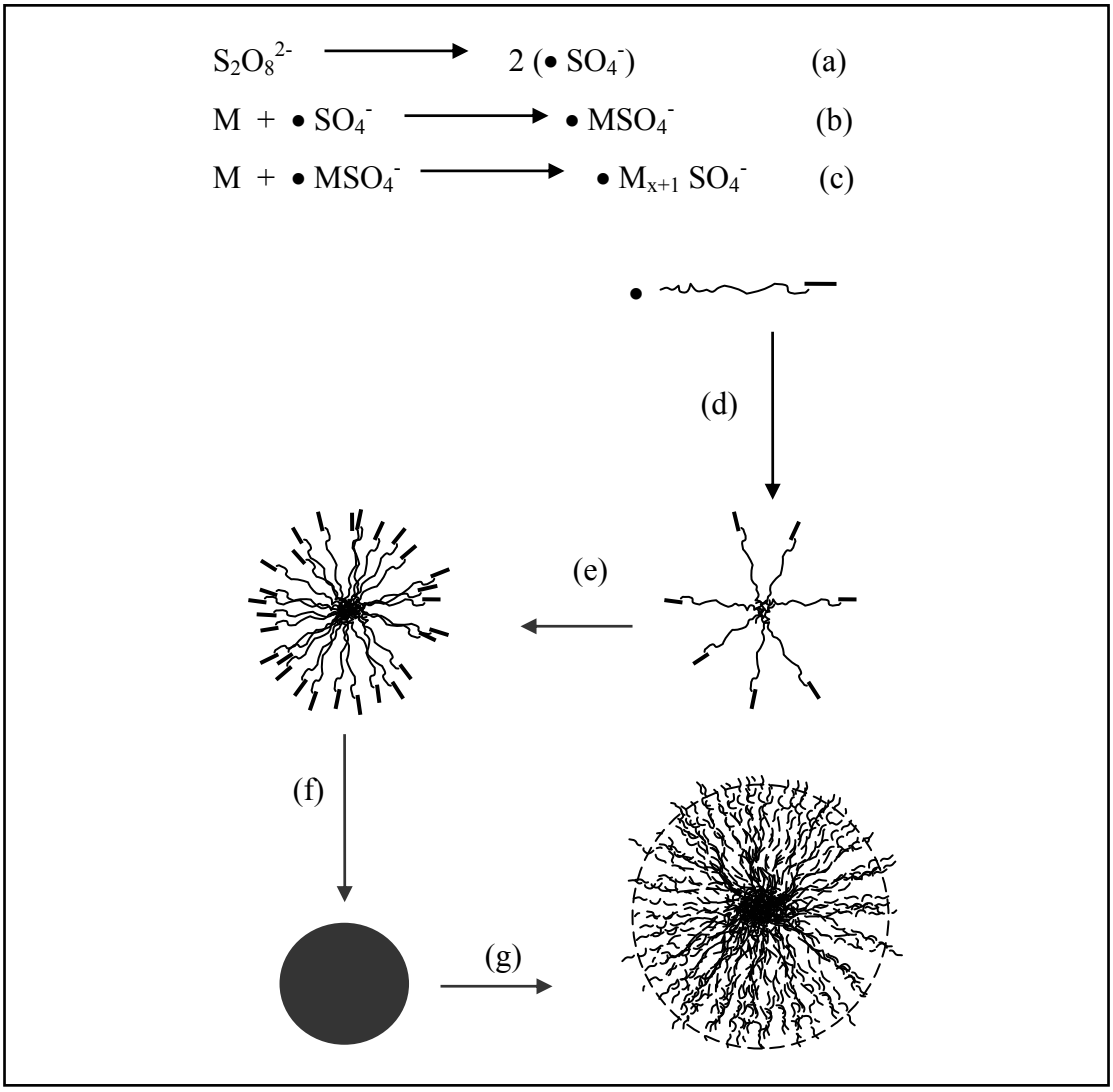

Figure 1. *Mechanism for the preparation of microgel particles by SFEP adapted from Saunders* (Saunders et al. 1999). *The steps shown are* (a) *initiator decomposition,* (b) *initiation,* (c) *propagation,* (d) *particle nucleation,* (e) *particle aggregation,* (f) *particle growth (in a poor solvent),* (g) *particle swelling (in a good solvent).* M *represents a vinyl monomer.*

According to this mechanism (Figure 1) the thermal decomposition of the ionic initiator ($S_2O_8^{2-}$) initiates free-radical polymerization (Figure 1 a). A water-soluble sulphate radical initiates a chain by reacting with a water-soluble monomer (Figure 1 b) which then grows in solution, chain propagation, (Figure 1 c) until it reaches a critical chain length (that exceeds the solubility limit of the solvent) after which the growing chain collapses to become a colloidal unstable "precursor particle". The precursor particles follow one of two competing processes. Either they deposit onto an existing colloidal stable polymer particle or they aggregate with other precursor particles until they form a particle sufficiently large to be colloidal stable. Polymerization continues within the particles until another radical species enters the growing particle and termination occurs (Saunders et al. 1999; Pelton 2000). Termination can also occur when a free radical contacts oxygen (Xiao et al. 2004).

3. Colloid stability

Aqueous colloids are considered stable if they do not aggregate in the time scale of interest. Colloidal stability depends upon the balance of van der Waals attraction, which causes aggregation and steric or electrostatic forces that oppose aggregation. Below the CST, thermoresponsive particles are swollen and thus consist mainly of water. Under these conditions, the van der Waals attractive forces are relatively small. Furthermore, polymer tails extend from the gel structure to act as steric stabilizers further enhancing colloid stability. If charged groups (from initiator and surfactant) are incorporated (at the surface and / or in the interior) into the particles during polymerization then electrostatic interactions contribute to colloidal stability below the CST (Saunders et al. 1999). For instance, surface groups on polyNIPAAM particles have been shown to be responsible for the fact that these dispersions remain stable during synthesis, even though the synthesis temperature was much higher than the CST of polyNIPAAM in water, such that the particles are collapsed (Wu et al. 1994; Ole Kiminta et al. 1995). At elevated temperatures the water content of the gels is reduced giving a higher density, thereby increasing the van der Waals forces, which in turn tend to aggregate the gels. On the other hand, at low ionic strength the electrophoretic mobility of polyNIPAAM microgels is high indicating that electrostatic stabilization is operative (Pelton et al. 1989).

4. Methods applied for the characterization of microgels

A variety of methods have been suggested in the literature for the characterization of the morphology and the thermodynamic properties of microgels. Light scattering and electron spectroscopy are the most common used experimental techniques for the investigation of, among the other features, the size, shape and the molecular weight of the microgels. Using these techniques, the water content and the polymer concentration in the gel could be calculated (Pelton 2000). These methods with some of the corresponding references are summarized in Table 1. Combination of different methods can provide a total "image" of the characteristic features of the microgels.

5. Applications of microgels

The major applications of thermoresponsive microgel particles have been in the surface coatings industry (Murata 1980; Nakayama 1982; Aihara 1986) and particularly in the automotive industry (Bradna 1995). In addition such structures have been used for improving the rheological properties of paints (Wolfe 1989) and the performance of films (Bromley 1989). Microgels have also found wide application in other areas such as proteins (Kawaguchi et al. 1992; Fujimoto et al. 1993), cells (Achiha 1995) and enzyme immobilization (Seitz 1979; Williams 1989; Shiroya 1995; Yasui 1997), but also show promise in the printing industry (Sasa 1994) and for oil recovery from petroleum reservoirs (Snowden 1993). In addition, microgel dispersions have potential use as environmentally sensitive optoelectronic devices (Sawai et al. 1991; Weissman 1996). The controlled uptake and release of large molecules has been also demonstrated (Snowden 1992; Snowden 1993; Kato 1994). Thermosensitive microgels having either cationic or anionic surface charge groups have been shown to have potential applications for the removal of ionic contaminants from waste water (Snowden 1993). The applications related with the use of microgel systems are schematically summarized in Figure 2.

Microgels may be "customised" to meet the requirements of specific applications by varying monomer (Kawaguchi et al. 1993) and initiator (Xiao et al. 2004) composition, crosslink density (Kawaguchi et al. 1992; McPhee et al. 1993; Kratz 2001; Hazot et al.

2002), particle size (Kawaguchi et al. 1993; Chai et al. 2003), or the nature of the surface charge (Goodwin 1978) or solvent type (Paine 1990; Lowe et al. 1998).

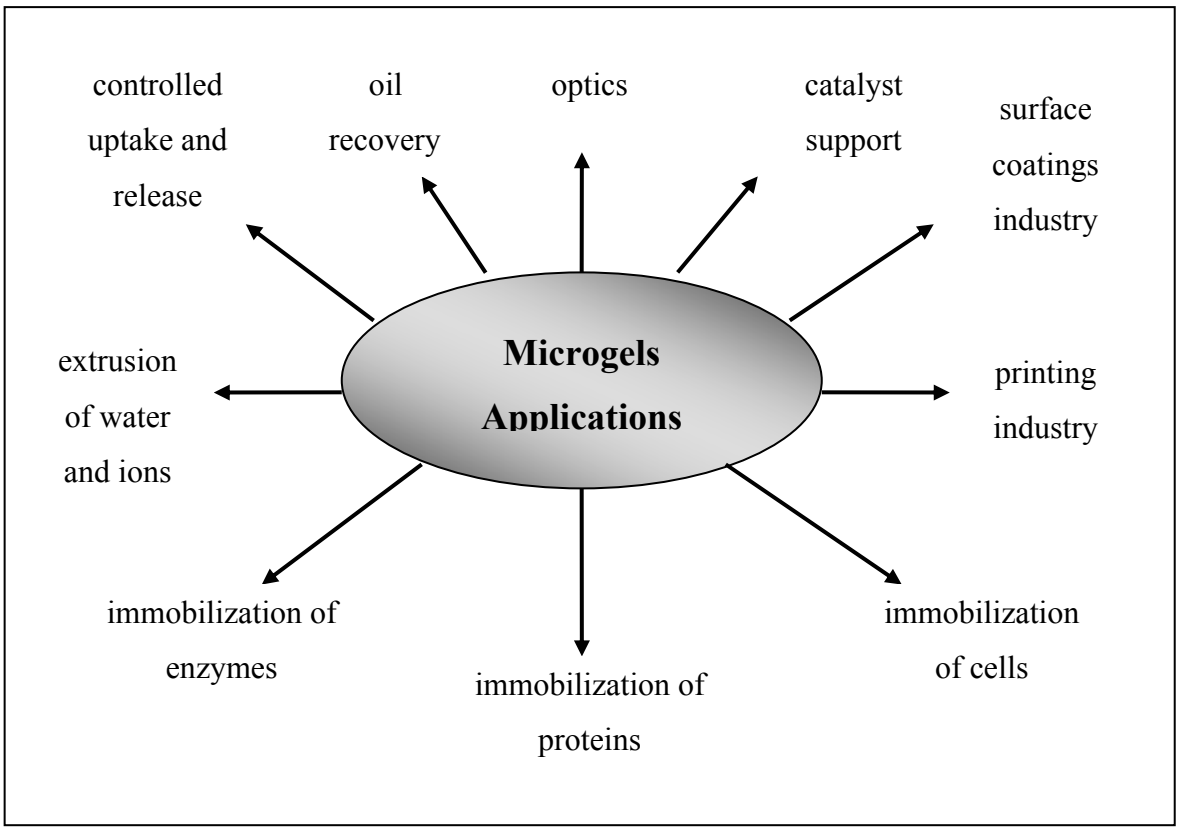

Figure 2. *Applications of microgels*

Table 1. *Experimental Techniques for microgels characterization*

Experimental technique	Measurement	References
Transmission Electron Microscopy (TEM)	Particle size and shape	(Kawaguchi et al. 1992) (Kawaguchi et al. 1993) (Hatto et al. 2000)
Scanning Electron Microscopy (SEM)	Particle size and shape	(Kawaguchi et al. 1993) (Chai et al. 2003) (Xiao et al. 2004)
Static Light Scattering	Particle molecular weight	(Antonietti et al. 1988) (Kunz et al. 1986)
Photon Correlation Spectroscopy or ***Dynamic Light Scattering***	Hydrodynamic size	(Hirose 1987) (Lopez et al. 2004) (Wu et al. 1994) (Kratz 2001)
Gel Permeation Chromatography	Weight and NumberAverage Molecular Weight	(Kunz et al. 1986)
Ultracentrifugation	Weight Average Molecular Weight	(Nieuwenhuis 1981)

Table 2. *Experimental Techniques for microgels characterization (continued)*

Experimental technique	Measurement	References
Conductometric and Potentiometric titration	Surface charge	(McPhee et al. 1993) (Stacey 1980)
Small Angle X-ray Scattering (SAXS)	Internal Structure	(Seelenmeyer et al. 2001)
Small Angle Neutron Scattering (SANS)	Internal Structure	(Seelenmeyer et al. 2001) (Kratz 2001)
Atomic Force Microscopy (AFM)	Surface morphology	(Kratz 2001)
Turbidimetric methods	Stability of microgel dispersions	(Snowden 1992)
High Sensitivity Differential Scanning Calorimetry	Thermodynamic Properties	(Murray 1994)
Differential Scanning Calorimetry	Thermodynamic Properties	(Ole Kiminta et al. 1995) (Lopez et al. 2004)
Bohlin Rheometer	Rheological measurements	(Ole Kiminta et al. 1995)

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Chapter 1. Synthesis and characterization of microgels

1.1 Introduction

Macroscopic gels have the disadvantage that the swelling / deswelling process occurs on a rather long time scale. Fast response to external stimuli, however, is a prerequisite for most potential applications. Because the diameter (< 1 μm) of a microgel is much smaller than the diameter (usually > 1 mm) of a macrogel, the microgel responds to environmental stimuli much faster than the macrogel (Gao et al. 2002). Therefore, decreasing the size of the hydrogels to micro- or nano-dimensions can circumvent the problem of delayed stimuli-response. Because of their fast kinetic response (Tanaka 1985), microgels are good candidates to gain more insight into the physical nature of the volume phase transition (Hirokawa 1984; Oh et al. 1998; Pelton 2000) and research into synthesis and characterization of colloidal microgels has been intensified recently (Kratz 1998; Hatto et al. 2000; Xiao et al. 2004; Xiao et al. 2004). In addition, a small dimension is necessary for the stimuli-responsive hydrogels to be applied in certain areas e.g. as drug delivery systems (Little 1962). Consequently, the fabrication of small sized environmental stimuli-responsive microgels is of both scientific and technological interest.

It was found that neutral colloidal polyNIPAAM particles with a diameter of some hundred nanometers have similar characteristics as their macroscopic counterparts, concerning the transition temperature and swelling capacity at the volume phase transition (Kratz 2001). PolyNIPAAM can form monodisperse colloidal microgel particles having a narrow size distribution (Baker 1949; Pelton et al. 1986; Schild 1992) when synthesized using surfactant-free emulsion polymerization. Similar to the behavior of polyNIPAAM macrogels, polyNIPAAM microgels are swollen at room temperature, but collapse as the temperature is increased above 32 °C (the CST of polyNIPAAM) (Hirotsu 1987; Pelton et al. 1989; Shibayama 1993). PolyNIPAAM microgel dispersions were first synthesized by Pelton and Chibante (Pelton et al. 1986).

PolyNIPAAM lattices appear to be colloidally stabilized by a combination of steric and electrostatic repulsive forces below the CST whereas only electrostatic effects

appear to be operative above the CST (Pelton et al. 1986). The surface properties of a latex particle, in the absence of added surface active agents, are determined by the functional groups (SO_4^-) of the initiator ($K_2S_2O_8$), which provide the colloid stability to the particles.

Most of the work found in the literature involves microgels based on NIPAAM. However, other monomers also yield polymers which have a CST in water, as has been already discussed in detail in the previous Chapters, and a few of these monomers, including *N*-isopropylmethacrylamide (Duracher 1999), *N*-ethylacrylamide (Lowe et al. 1998), *N*-acryloylpyrrolidine (Kawaguchi 1986), *N*-acryloylpiperidine (Hoshino 1987) and *N*-ethylmethacrylamide (Hazot et al. 2002) have been used to make temperature-sensitive microgels. In this Chapter, Surfactant-Free Emulsion Polymerization is applied for the synthesis of polyNIPAAM and polyDEAAM microgels. The work presented here is believed to be the first reported preparation of a polyDEAAM microgel system. Some results derived from electron microscopy, particularly TEM and SEM, characterizations are also reported.

1.2 Experimental procedure

Materials

N-Isopropylacrylamide (NIPAAM), *N,N*-methylenebis-acrylamide (BIS), potassium persulfate (KPS), were obtained from Sigma Aldrich Chemical (Buchs, Switzerland) and used as received, with no further purification. *N,N'*-diethylacrylamide (DEAAM) was obtained from Polysciences Inc. Europe (Eppelheim, Germany). Water was purified using an Elix-3 system (Millipore, Bedford, MA).

1.2.1 Synthesis of microgels

The particles were prepared using Surfactant-Free Emulsion Polymerization (SFEP) in water. The method adopted is similar to that used by Goodwin et al. (Goodwin 1978) for the preparation of polystyrene lattices and which was later applied for the first time by Pelton et al. (Pelton et al. 1986) for the preparation of polyNIPAAM lattices. The monomer (2.5g) and the crosslinker BIS (0.25g) were dissolved in 100mL of distilled water in a round-bottomed flask equipped with a condenser, a nitrogen inlet and a stirrer. The mixture was degassed to remove oxygen and the

temperature of the flask was raised to 70°C. After a thorough mixing of both NIPAAM (or DEAAM) and crosslinker, potassium persulfate (0.1g) was added to initiate the polymerization. The reaction was continued for 4h under mild stirring and nitrogen atmosphere. The stirring rate between individual polymerization processes was varied and the applied rates are given in Table 1. The gel particles were purified by four successive centrifugations, using a High Speed Refrigerated Centrifuge (HSRC: Kontron Instruments, Centrikon T-124), at 9000 rpm for 45 minutes each centrifugation and at 20 °C, each followed by decantation and redispersion in distilled water. Before the characterizations the microgel solution was filtered using a glass filter (POR 4: 11-16 μm).

Table 1. *Stirring rate during polymerization*

Microgels	Code name	Stirring rate
polyNIPAAM	m3	300 rpm
	m4	500 rpm
polyDEAAM	md1	400 rpm
	md3	500 rpm
	md4	700 rpm

1.2.2 Characterization of microgels

Transmission (TEM) and Scanning Electron Microscopy (SEM)

A Philips XL30-SFEG scanning electron microscope and a Philips CM20 transmission electron microscope were employed to study and record the morphology of the microgel particles. The samples were prepared using an original spin-coating method adapted for electron microscopy supports in CIME (Centre Interdisciplinaire de Microscopie electronique) at EPFL. The SEM images were recorded without metallic sputtering working with low-voltage acceleration. The particle size analysis was performed from different TEM images using Opti-lab software.

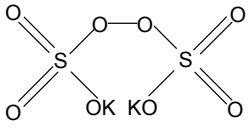
1.3 Results and discussion

1.3.1 Synthesis of polyNIPAAM and polyDEAAM microgels

PolyNIPAAM and polyDEAAM microgels were synthesized by a method analogous to the Surfactant-Free Emulsion Polymerization (SFEP) of styrene (Goodwin 1978), in aqueous solutions of NIPAAM and DEAAM, respectively. The reaction was initiated by potassium persulfate at 70 °C. The method results in polymer particles stabilized by surface charge groups originating from the potassium persulfate initiator. Some features of the potassium persulfate are given in Table 2. Thus, it is expected that many of the end groups on the polyNIPAAM and polyDEAAM chains will be sulphate groups (Pelton et al. 1989).

Pelton and Chibante (Pelton et al. 1986) found that polyNIPAAM microgels could only be formed above the temperature of 55 °C, whereas below this temperature no particles are formed. Normally a temperature is chosen at which initiator decomposition occurs at a satisfactory rate. At a polymerization temperature of 70 °C, which is well above the CST, decomposition of the persulfate initiator provides sulphate groups which are integrated into the polymer network and stabilize the growing polyNIPAAM and polyDEAAM microgel particles colloidally by electrostatic repulsion (Goodwin 1974). The surface potential of the polyNIPAAM and polyDEAAM microgels should influence the latex colloid stability (Pelton et al. 1989). Once formed the microgels at low temperatures, are also stabilized sterically by the hydrophilic polyNIPAAM and polyDEAAM chains. Above the critical temperature, polyNIPAAM and polyDEAAM have low affinity for water and steric stabilization is no longer operative (Pelton et al. 1989).

Table 2. *Potassium persulfate characteristics for the synthesis of microgels* (Goodwin 1978)

Initiator	Structure	Latex charge	Surface groups
potassium persulfate		negative	mainly $-SO_4^-$ can be also -COOH, -OH

The choice of potassium persulfate as initiator in our investigation was based on its good suitability to initiate NIPAAM and to form colloidally stable polyNIPAAM microgel particles (Pelton et al. 1986). Pelton and Chibante (Pelton et al. 1986) investigated the effect of different types of initiators on microgel dispersions of polyNIPAAM and they found that the maximum monomer content possible for stable monodisperse lattices, prepared under similar experimental conditions, varied depending on whether ABA (2,2'-azobis(2-amidinopropane, which provides the particle surface with an overall positive charge) or KPS (potassium persulfate) was used. The latter could be used for the synthesis of good lattices in the presence of higher monomer concentrations (25 g/l) compared to the corresponding value (10 g/l) when ABA was used.

During polymerization, it was necessary that nitrogen should be bubbled into the mixture to remove oxygen, which acts as a radical trap and would therefore terminate the polymerization. The reaction was then done under a blanket of nitrogen (Ole Kiminta et al. 1995).

There are several methods employed, either alone or in combination, to clean microgel dispersions, such as centrifugation, dialysis, filtration through glass wool, dispersion in mixtures of solvents, using ion exchange resins etc. (Hearn et al. 1981; D'Emanuele et al. 1995; Ole Kiminta et al. 1995; Makino 2001; Gao et al. 2002; Huang et al. 2004; Lopez et al. 2004; Saunders 2004). Centrifugation, followed by decantation and redispersion in clean water is an effective cleaning procedure and it

can successfully remove low molecular weight impurities (Pelton et al. 1986; Pelton et al. 1989; McPhee et al. 1993; Wu et al. 1994; Shiroya 1995; Hazot et al. 2002).

In order to investigate the influence of the stirring rate on the size of the microgels, stirring rate was varied between different polymerization processes (Table 1).

1.3.2 Morphological characteristics of microgels

The size and appearance of the microgel particles were observed by transmission and scanning electron microscopy. Electron microscopy, particularly transmission electron microscopy (TEM) is a method often used to analyse microgel dispersions (Yu 1982; Theodore 1985; Pelton et al. 1986; Kawaguchi et al. 1992). It has the major advantage that a visual assessment can be made of the size, shape and size distribution of the particles. The particular advantages of electron microscopy with respect to visual assessment have enabled the observation of interparticle bridge formation (Wilkinson 1974) and in some cases, anomalous particle formation (Goodall 1975) and presence of a fraction of smaller particles resulting from secondary nucleation (Hearn et al. 1970) to which most other techniques would be very insensitive. The disadvantages of the technique lie in the fact that the particles are not viewed in the aqueous phase but have to be dried and therefore can only be observed in the collapse state. It is therefore not possible to measure the swollen diameters. Also, microgel dispersion is exposed to possible swelling and shrinkage in the electron beam under conditions of reduced pressure and irradiation and surface tension effects may cause some aggregation (Hearn et al. 1981; Murray et al. 1995). Scanning electron microscopy (SEM) gives a 3-dimensional image of the particles and has proved valuable for examining particle morphology (Cox et al. 1977; Kawaguchi et al. 1993).

SFEP was successfully used to prepare polyNIPAAM microgels in the size range 880-1100 nm (Figure 1 and Figure 3) and polyDEAAM microgels in the size range 800-875 nm, by varying the stirring rates. The results are summarized in Table 3. In the case of polyNIPAAM microgels an increase from 886 to 1110 nm is observed with a decrease of the stirring rate from 500 to 300 rpm. The effect is less pronounced in the case of polyDEAAM, where no big variation to the size range could be observed. High speed stirring rate breaks the latex particles; correspondingly the diameter of the produced microgels becomes smaller (Chai et al. 2003; Xiao et al. 2004). When the

stirring rate was lower or equal to 400 rpm (Figure 1 and Figure 5), the microgels were more or less uniform and their size distributions were narrow (Figure 2). However, when the stirring rate was higher than 400 rpm (Figure 3, Figure 6 and Figure 7) nonuniform particle sizes are observed. According to Xiao (Xiao et al. 2004) this may be due to the fact that the chance of meeting between latex particles and the precursor particles increased too much at high stirring, which led to the product particle sizes becoming nonuniform. Similar effect of the stirring rate on size distribution of microspheres has been also observed by D'Emanuele et al. (D'Emanuele et al. 1995) for poly(NIPAAM-*co*-AA) microspheres and by Xiao et al. (Xiao et al. 2004) for poly(NIPAAM-*co*-St) microspheres.

Table 3. *Average diameter of microgels*

Microgels	Code name	Average diameter (nm)[a]
polyNIPAAM	m3	1110
	m4	886
polyDEAAM	md1	866
	md3	812
	md4	875

[a] obtained by TEM

Transmission and Scanning electron micrographs of the polyNIPAAM and polyDEAAM microgels are presented in the figures below. The number and size of microgels prepared by SFEP depend on the frequency of particle nucleation and the stability of the nuclei (Tseng et al. 1986; Paine 1990).

The transmission and scanning electron microscopy images show the polyNIPAAM microgels (Figure 1) to be monodisperse spheres having a regular size and shape with narrow size distribution (Figure 2).

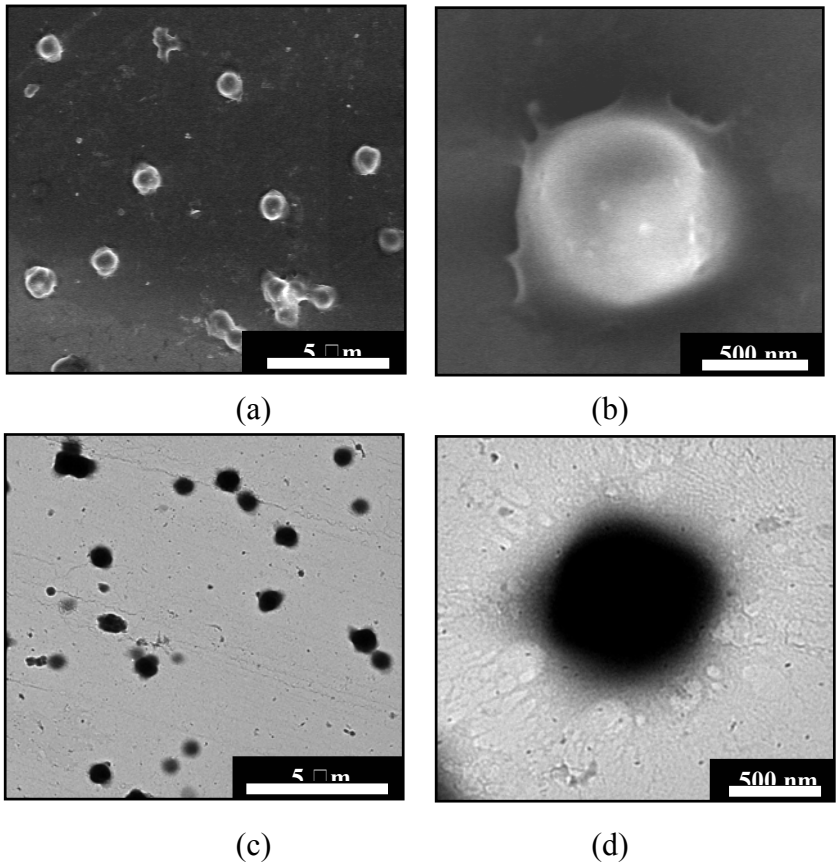


Figure 1. *Morphological characteristics of polyNIPAAM (m3) particles by* (a-b) *low-voltage SEM and* (c-d) *TEM*

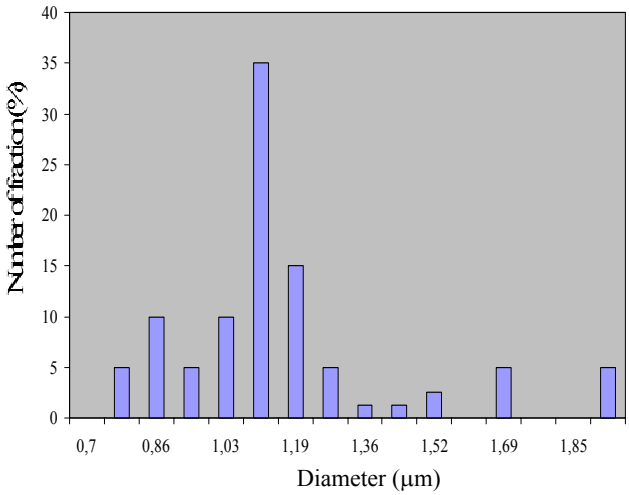
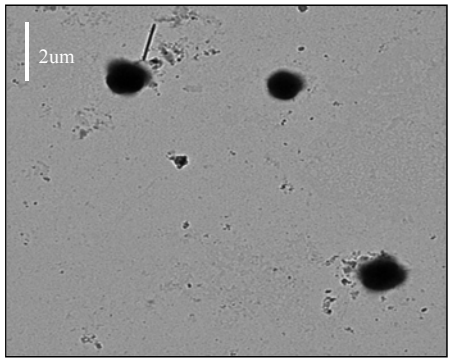
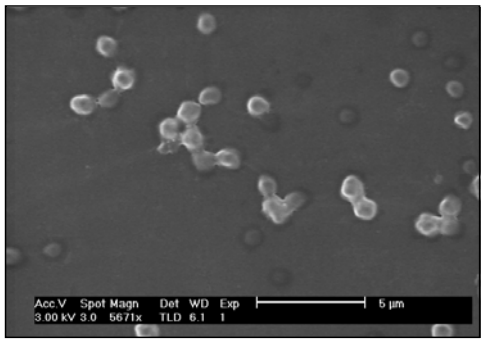


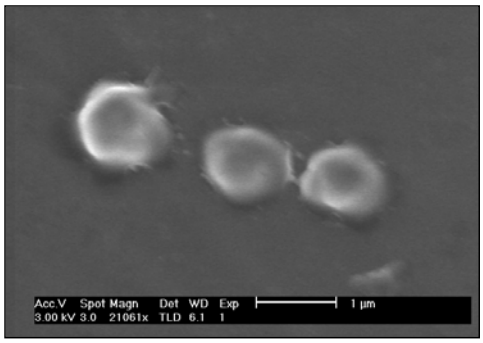
Figure 2. *Histogram of polyNIPAAM (m3) particles established by measuring the diameter of 80 particles from TEM micrographs*

According to Wu et al. (Wu et al. 1994) the initially formed small particles (precursor particles) are colloidally unstable and the nucleation of colloidal stable particles will be from the coagulate association of precursor particles. Therefore, the presence of small particles with an average diameter of 80 nm (Figure 3 and Figure 4), which probably correspond to remaining "precursor particles", indicate the possible necessity for longer polymerization time (Theodore 1985). It is apparent from these micrographs that new particles are formed throughout the reaction, in agreement with previous observations on particle formation and growth (Killgoar et al. 1977; Theodore 1985). In some cases aggregation of the particles or bridging of particles, could be also observed. Both phenomena are probably a combination of particle growth through polymerization of residual monomer (Killgoar et al. 1977).



(a)





(b) (c)

Figure 3. *Morphological characteristics of polyNIPAAM (m4) particles by* (a) *TEM and* (b-c) *low-voltage SEM*

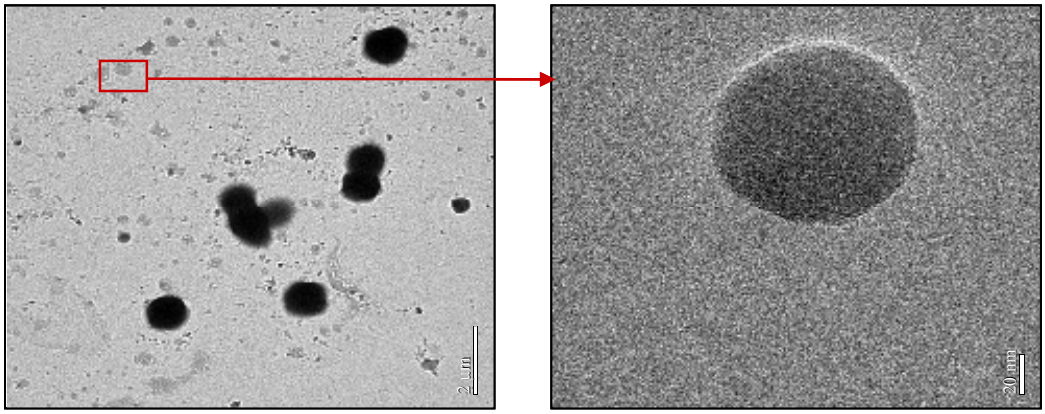
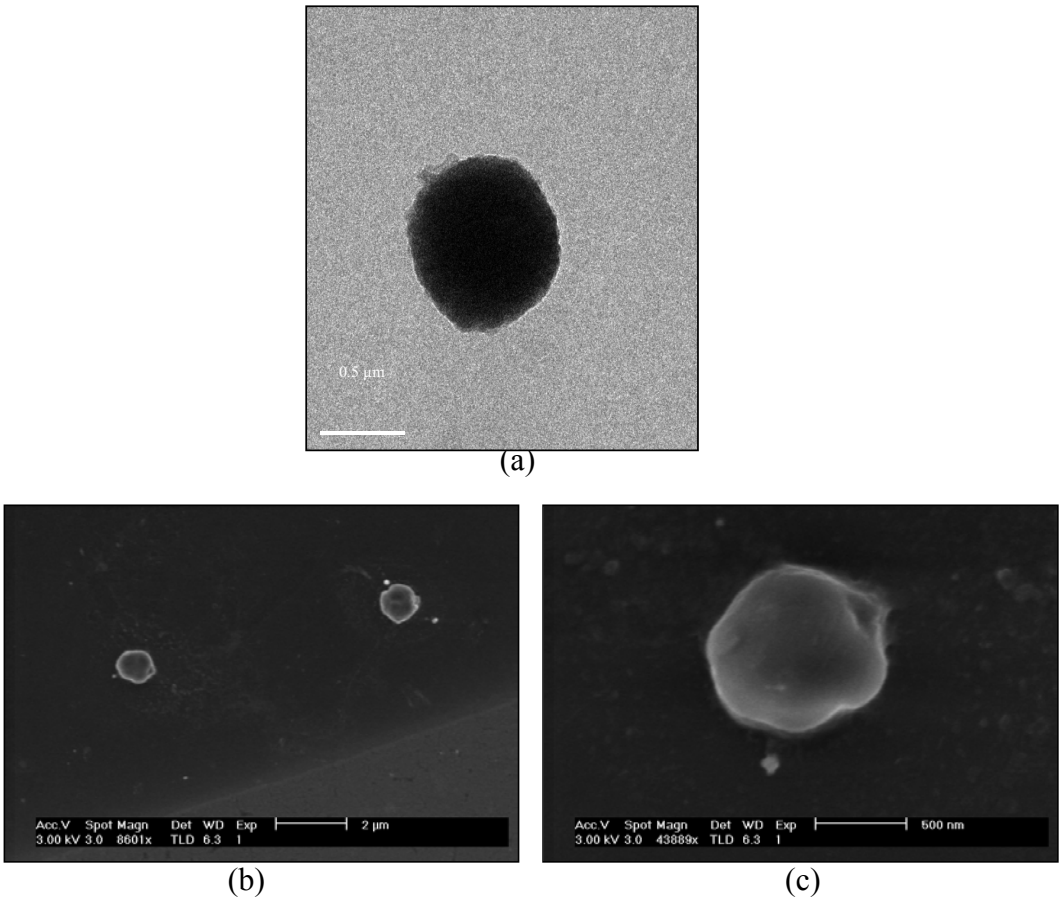


Figure 4. *TEM image of polyNIPAAM (m4) microgels solution with small particles indicating possible continuation of nucleation process*



(a)

(b)

(c)

Figure 5. *Morphological characteristics of polyDEAAM (md1) particles by* (a) *TEM and* (b-c) *low-voltage SEM*

Figure 5 presents the polyDEAAM microgels prepared at 400 rpm via a Surfactant-Free Emulsion Polymerization route under the same experimental conditions as the ones for the preparation of polyNIPAAM microgels. The method results in spherical particles having a regular size and shape and an average diameter around 866 nm.

Figure 6 shows a gradual increase in the density of the polymer from the outer edge to the center of a particle taking the morphology of a "core-shell structure". Even if spherical particles do not usually show this behavior in the TEM similar observation has been done by Pelton et al. for polyNIPAAM particles (Pelton et al. 1986). The smaller average diameter observed in the case of polyDEAAM md3 microgels, even when a smaller stirring rate was applied during the polymerization process compared to polyDEAAM md4 microgels, together with the presence of small particles "attached" on the surface (shell) of the gel, which are probably colloidal unstable "precursor particles", indicate possible continuation of nucleation process (Theodore 1985; Wu et al. 1994).

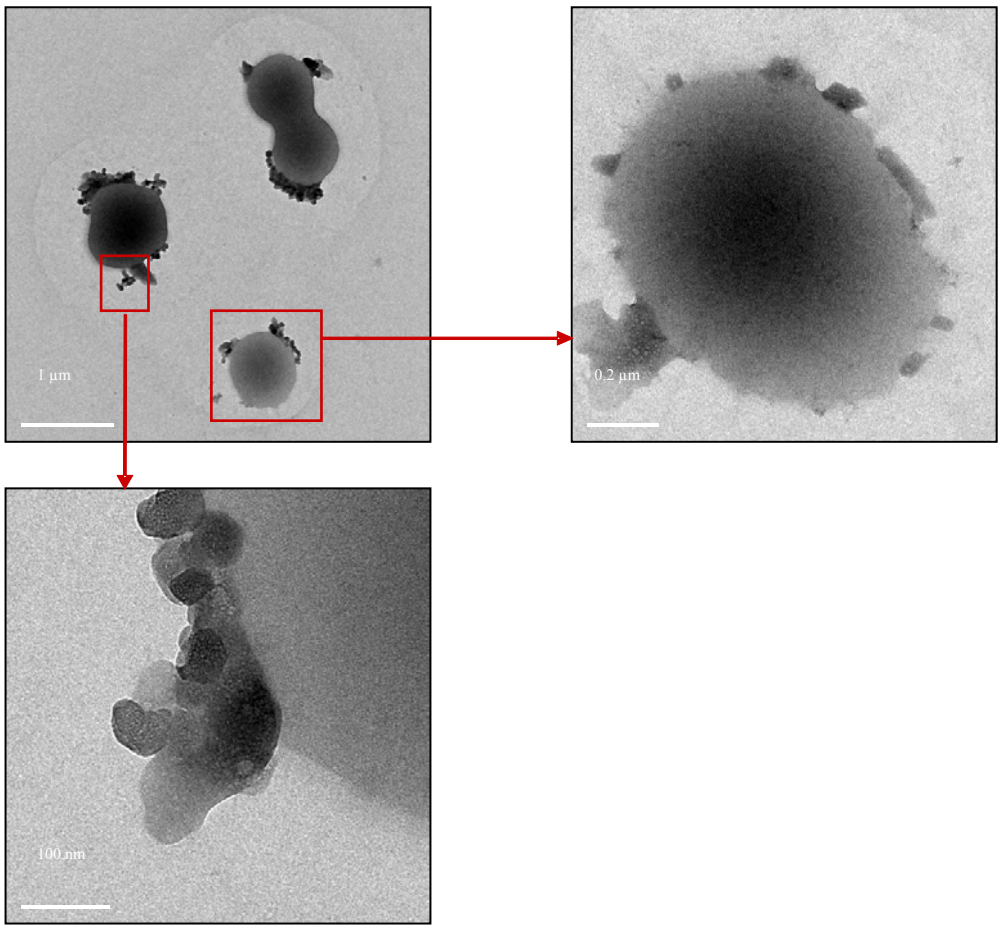
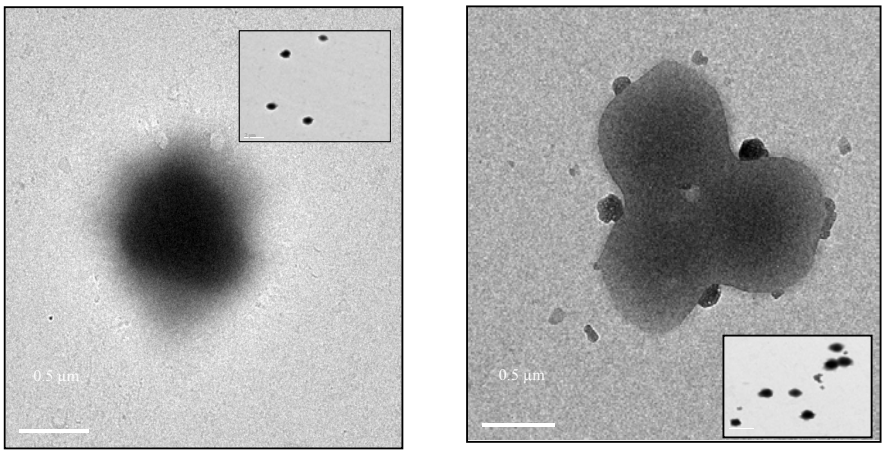


Figure 6. *Morphological characteristics of polyDEAAM (md3) particles by TEM*

The paradoxical results obtained in the case of polyDEAAM md3 compared with the other polyDEAAM microgels, are probably also due to an undesirable termination caused by the presence of oxygen.

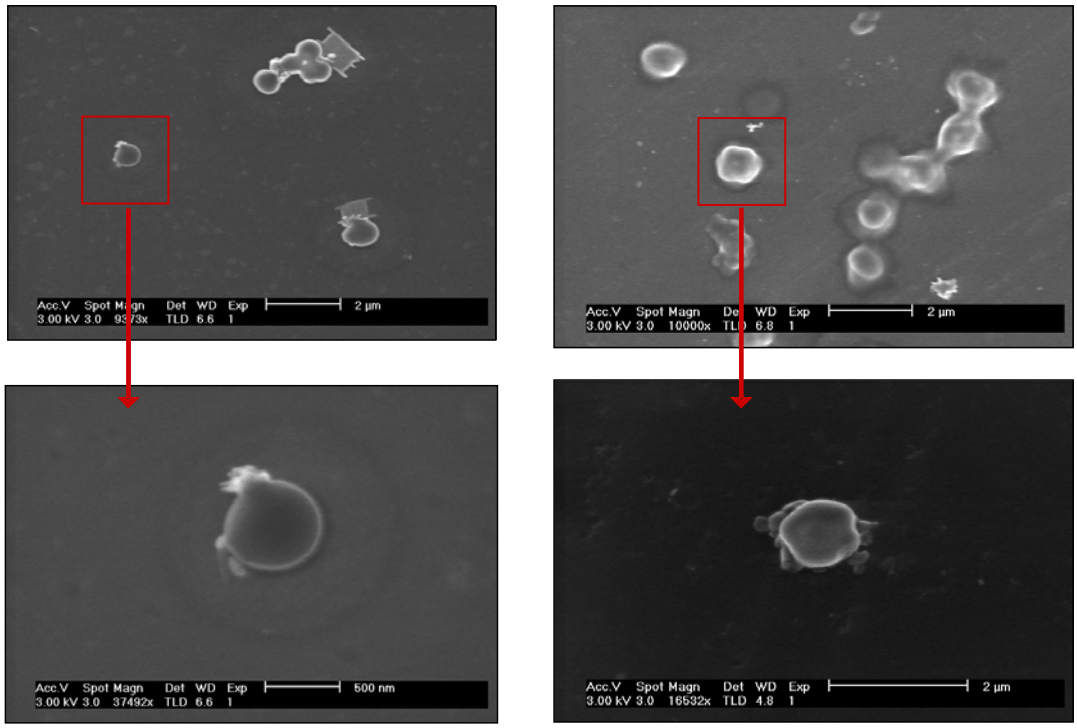
Figure 7 presents polyDEAAM md4 particles produced at a stirring rate of 700 rpm. Particles showed regular spherical shape with an average size around 875 nm. Some isolated cases with aggregated particles and few colloidally ustable "precursor particles" have been observed indicating once more a possible continuation of the nucleation progress.



(a) (b)

Figure 7. *Morphological characteristics of polyDEAAM (md4) particles by TEM*

Figure 8 shows the corresponding SEM images of polyDEAAM md3 and md4. Again, images with more stable colloidal particles having a spherical morphology correspond to the md4 microgels, while some unstable colloidal "precursor particles" are observed in the images of the md3 microgels.



(a) (b)

Figure 8. *Morphological characteristics of polyDEAAM* (a) *md3 and* (b) *md4 particles by low-voltage SEM*

1.4 Summary

Surfactant-Free Emulsion Polymerization could be applied for the synthesis of polyDEAAM microgels. Varying the stirring rate during the polymerization process, an effect on the final particle size could be observed; the average particle diameter decreases with increasing stirring rate. The effect seems to be more pronounced for polyNIPAAM microgels compared to polyDEAAM microgels. Also, increasing the stirring rate the need for longer polymerization time is observed. This observation derives from the presence of small particles, possibly colloidal unstable "precursor particles", in the microgel solution, indicating the continuation of nucleation process. Oxygen can undesirably terminate the polymerization process playing an important role to the final morphology of the microgels.

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Perspectives

The recent technology of scaling down materials to micro- or even to nano- size is becoming a powerful key for a variety of scientific and technological applications in the field of micro- or nano-technology. Adapting this new generation trend and taking into account the necessity of finding a good candidate, which could be used as drug delivery system, the last Part of this investigation was dedicated to the synthesis and characterization of such micro-materials (microgels). Microgels have advantages over the other synthetic materials, which are among the others, their small size and fast kinetic response.

Our investigation presents a novel stimuli-responsive microgel, consisting of *N,N'*-diethylacrylamide and this work is to our knowledge the first reported preparation of polyDEAAM microgel system.

The fact that certain parallels could be already drawn between the behaviour of linear and macroscopic networks (hydrogels) under certain conditions of the surrounding environment could be used as a guideline for the investigation of the external conditions influencing the structure and properties of microgels. Furthermore, optimisation of the synthesis conditions and investigation of the factors affecting the final size and morphology of the microgels, such as surfactant, initiator, crosslinking density, monomer concentration, monomer composition, polymerization time is suggested. Additional characterization of the physical and chemical properties of microgels by a variety of experimental techniques, such as light scattering and calorimetry techniques is also necessary. Swelling ratio measurements of microgels would provide useful information about their response kinetics to environmental stimuli.

In conclusion, further investigation and optimisation of the preliminary results obtained for microgels, in correlation with the results obtained for polymers and hydrogels of the present investigation, could be used to gain more insight into the field of drug delivery.

Abbreviations and Symbols

I. Abbreviations

CST	critical solution temperature (°C)
LCST	lower critical solution temperature (°C)
nDEP-barrier	negative dielectrophoresis barrier
SR	swelling ratio
UCST	upper critical solution temperature (°C)
WR	water retention
WU	water uptake

List of chemicals

AIBN	2,2-azoisobutyronitrile
APS	ammonium persulfate
BIS	*N,N*-methylenebisacrylamide
DEAAM	*N,N*-diethylacrylamide
DMAAM	*N,N*-dimethylacrylamide
DMF	dimethylformamide
EAAM	*N*-ethylacrylamide
EMAAM	*N*-ethyl,*N*-methylacrylamide
KPS	potassium persulfate
MPA	3-mercaptopropionic acid
NIPAAM	*N*-isopropylacrylamide
PBS	phosphate buffer saline
PLAAM	*N*-pyrrolidinoacrylamide
TEMED	*N,N,N',N'*-tetramethylenediamine
THF	tetrahydrofuran

List of apparatus

^{1}H NMR	proton Nuclear Magnetic Resonance
HSRC	High Speed Refrigerated Centrifuge
MALDI-TOF MS	Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

SEM	Scanning Electron Microscopy
TEM	Transmission Electron Microscopy

II. Symbols

PART I-PART II

- ***General Introduction***

Flory – Huggins theory

k	Boltzmann's constant (= 1.3807 x 10^{-23} J K^{-1})
n_1	number of moles of solvent
n_2	number of moles of polymer
N	Avogadro's number (= 6.022 x 10^{23} mol^{-1})
r	ratio of the molar volume of the polymer to that of the solvent
R	gas constant (= 8.3144 J mol^{-1} K^{-1})
T	temperature (K or °C)
V	volume of the swollen gel (m^3)
V_0	volume of the unswollen polymer (m^3)
V_1	molar volume of the solvent (m^3 mol^{-1})
x_1, x_2	number of segments in species

Greek symbols

$\alpha_s = \alpha_x = \alpha_y = \alpha_z$	factors related with an alternation of the network dimensions
Δ	chemical potential of the penetrating solvent (J mol^{-1})
$\Delta G_{elastic}$	Gibbs free energy due to deformation of the network chains (J)
ΔG_m	Gibbs free energy of mixing (J)
ΔH_m	enthalpy of mixing (J)
ΔS_m	entropy of mixing (J K^{-1})
μ_1	chemical potential of the solvent in the solution (J mol^{-1})
μ_1^0	chemical potential of the solvent in the pure liquid (J mol^{-1})
ν_e	effective number of chains in the network
Π	osmotic pressure (Pa)

Π_{elas}	osmotic pressure due to deformation of the network chains to a more elongated state (Pa)
Π_{mix}	osmotic pressure due to polymer-solvent mixing (Pa)
ρ	effective crosslink density (mol m^{-3})
φ_1	volume fractions of solvent (m^3)
φ_2	volume fractions of polymer (m^3)
χ	polymer-solvent interaction parameter

PART I

- ***Chapter 1***

I	initiator
K'	weighting parameter
k_d	rate constant for the initiator dissociation
k_i	rate constant for the initiation step
k_p	rate constant for propagation
k_t	rate constant for termination
M	monomer
M_i	mass of a given unimolecular oligomer species in a given sample (g)
M_n	number-average molar mass (g mol^{-1})
M_w	mass-average molar mass (g mol^{-1})
N_i	number of the molecules of that weight in the preparation
PD	polydispersity
P_n	degree of polymerization
r	temperature-zone distance (m)
R	radical
V_{rms}	root-mean-square value of the AC voltage (V)
XY	telogen

Greek symbol

μ_1, μ_2	molar fraction of a given monomer (1,2: comonomers) (mol)

- ***Chapter 2***

Jones-Dole equation

A	viscosity A coefficient (L mol^{-1})
B	viscosity B coefficient (L mol^{-1})
c	ion concentration (mol L^{-1})
n	viscosity (Pa s or N m^{-2} s)
n_0	viscosity of water (Pa s or N m^{-2} s)

Setschenow equation

C_s	salt concentration (mol L^{-1})
K_s or k_s	salting out coefficient for a given salt (L mol^{-1})
S_i	solubility of the polymer in a salt solution (mol L^{-1})
S_i^0	solubility of the polymer in pure water (mol L^{-1})

PART II

Hydrogels Swelling Mechanism

C_0	equilibrium concentration of a solvent in a polymer (mol/L)
D	diffusion coefficient for the solvent in the polymer (m^2 s^{-1})
D_c	collective diffusion coefficient (m^2 s^{-1})
k_0	Case-II relaxation constant
l	gel thickness (m)
$M_{s\infty}$	total amount of water sorbed by the gel at the equilibrium state (m^3)
M_{st}	total amount of water sorbed by the gel at time t (m^3)
n	number of particles
R	radius of equilibrium swelling for a spherical gel (m)
t	time (min)

Greek symbols

α	radius for a cylindrical and spherical polymer and the half-thickness for a slab polymer (m)
ΔR(t)	radius changes at time t (m)

ΔR_0	total radius change (m)
π	mathematical constant, 3.14
τ	network relaxation time (s)

Solute diffusion

D_{sw}	drug diffusion coefficient in the swollen phase ($m^2\ s^{-1}$)
De	Debora number
K	constant related to the characteristics of the gel
M_∞	swelling ratio at equilibrium
M_t	swelling ratio as a function of time
n'	exponent describing the Fickian or anomalous swelling mechanism
S_w	swelling interface number
v	velocity of the penetrating swelling front ($m\ s^{-1}$)

Greek symbols

$\delta_{(t)}$		time-dependent thickness of the swollen phase (m)
$\theta_D = L^2/D_s$	L	sample thickness (m)
	D_s	solvent diffusion coefficient ($m^2\ s^{-1}$)
λ_m		mean relaxation time of the hydrogel / solvent system (min)

- ***Chapter 1***

W x C	W	weight of the combined monomers per 100 mL of water (g)
	C	mass of crosslinker expressed as a percentage of the total amount of monomer plus crosslinker
W_d		dried weight of the gel (g)
W_s		weight of water in the swollen gel after reaching the equilibrium in water (g)
W_t		weight of the gel at regular time intervals (g)
W_w		weight of the wet sample (g)

Greek symbols

χ_H	enthalpic polymer-solvent interaction parameter
χ_S	entropic polymer-solvent interaction parameter

- ***Chapter 3***

R_n	viscosity radius (nm)

Free-volume theory

B	proportional constant
D_m	diffusion coefficient within the gel ($m^2\ s^{-1}$)
D_w	diffusion coefficient in water ($m^2\ s^{-1}$)
H	water content within the gel (m^3)
q	cross-sectional area of the solute (m^2)
V_w	free volume of water (m^3)

Greek symbol

ψ(q)	probability of pores having larger cross-sectional area than q

- ***Chapter 4***

M*in*	amount of insulin initially presented in the gel at time 0 (mol L^{-1})
M*t*	amount of insulin released (mol L^{-1})

APPENDIX

Table 1. *Molar Mass of monomers*

Monomer	Molar Mass (g/mol)
N,N-diethylacrylamide	127.2
N,N-dimethylacrylamide	99.2
N-ethyl,*N*-methylacrylamide	113.2
N-isopropylacrylamide	113.2
N-pyrrolidinoacrylamide	128.2

PART I

Chapter 1.

- **MALDI-TOF-MS**

Sample preparation / Experimental conditions: MALDI-TOF mass spectrometric analyses were carried out on a PerSeptive Biosystems Voyager-DE STR instrument equipped with a 2 meter ion flight tube and delayed extraction system. A fresh 10mg/ml solution of 3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid) in solvent A (30 % CH_3CN containing 0.1 % trifluoroacetic acid) was used as matrix. The sample was dissolved in solvent A to make 5 mg/ml solutions. These were then diluted at 1:10 with the matrix solution and 1 μl of the mixture was loaded on a gold target plate and allowed to dry. Spectra were obtained in linear mode using a 337 nm radiation from a nitrogen laser with 20 kV accelerating voltage and 256 scans were averaged. External calibration of the mass scale was performed with horse myoglobin under the same conditions (1 μl of a 1 mg/ml solution in the matrix were loaded on the target).

- **Phosphate Buffer Saline (PBS)**

For the preparation of 10 mM PBS solution, pH 7.4, the following quantities are used:

0.2 g KH_2PO_4 } phosphate buffer 10 mM
1.15 g Na_2HPO_4 }

8 g NaCl, 0.2 g KCl

We add 1L of H_2O and we adjust the pH with HCl to 7.4.

PART II

Chapter 1.

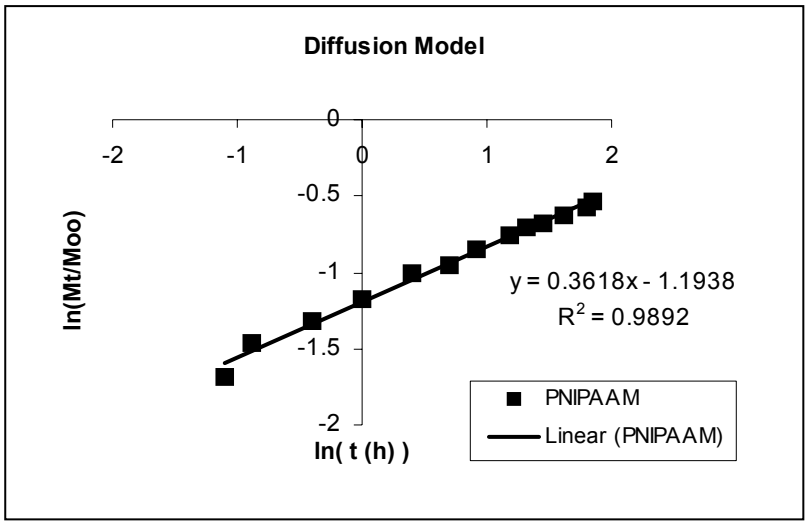

Figure 1. *Diffusion model of polyNIPAAM (10x4) gels*

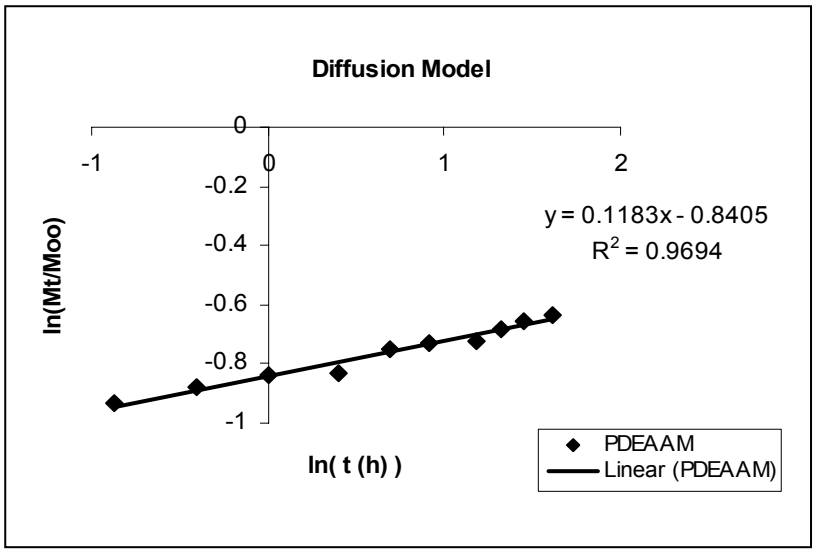

Figure 2. *Diffusion model of polyDEAAM (10x4) gels*

Chapter 4.

Table 2. *Characteristics of Proteins* (Brissova 1996)

Protein	M_w	R_n (nm)
Thyroglobulin	670,000	8,60
β-Galactozidase	518,000	6,86
Apoferitin	443,000	6,06
Gatalase	232,000	5,23
Glucose oxidase	186,000	5,20
γ-Globulin	158,000	5,23
Alcohol dehydrogenase	150,000	4,55
Albumin (dimmer)	132,000	4,76
Alkaline phosphatase	86,000	3,30
Transferin	77,000	3,92
Albumin	66,000	3,62
Ovalalbumin	44,000	2,83
β-Lactoglobuline	35,000	2,70
Hemoglobin (dimer)	32,000	2,40
Carbonic anhydrase	29,000	2,01
Chymotrypsinogen	25,700	2,50
Ovomucoid	25,000	2,75
Myoglobin	17,000	1,91
α-Lactalbumin	15,500	2,02
Lysozyme	14,000	1,85
Ribonuclease	13,700	1,75
Cytochrome c	11,700	1,63
Aprotinin	6,700	1,50
Insulin	5,700	1,34

Table 3. *Characteristics of Dextran Solutes* (Brissova 1996)

Dextran	M_w	R_n (nm)
Dextran 670	676,000	20,5
Dextran 410	403,000	15,8
Dextran 270	262,000	12,8
Dextran 150	143,000	9,4
Dextran 80	79,800	7,0
Dextran 50	50,000	5,6
Dextran 25	22,700	3,8
Dextran 12	11,700	2,7
Dextran 5	5,700	1,9
Dextran 1	1,200	0,9

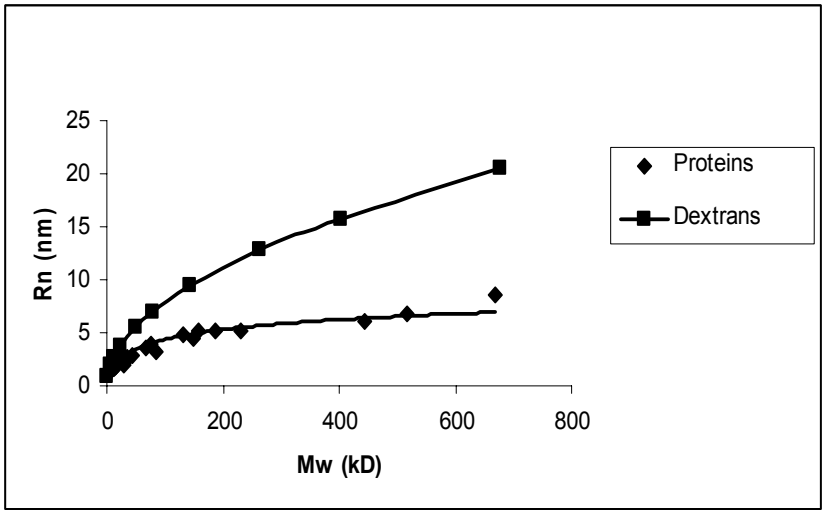

Figure 3. *Dependence of viscosity radius* (R_n) *on solute molecular weight*

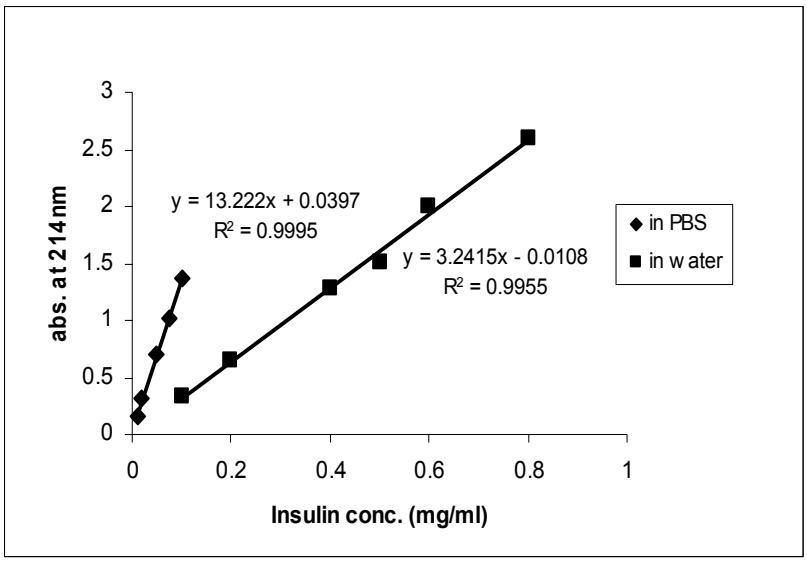

Figure 4. *Insulin calibration curve at 214 nm*

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PART III

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- Scheme 2. Applications of microgels

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PUBLICATIONS

Co-nonsolvency effects in the thermoprecipitation of oligomeric polyacrylamides from hydro-organic solutions, Marilia Panayiotou, Frederic Garret-Flaudy, Ruth Freitag. (2004) *Polymer* (45), 3055-3061

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CONFERENCES AND WORKSHOPS

Oral Presentation

- "Synthesis and Characterization of Thermoresponsive Hydrogels", M. Panayiotou , R. Freitag, **MACRO 2004** (World Polymer congress: 40th International Symposium on Macromolecules), 4-9 July 2004, Paris
- "Synthesis and Characterization of Thermoresponsive Polymers", M.Panayiotou, R.Freitag, **EUPOC 2002** (Polymeric Gels: Interesting Synthetic and Natural Soft Materials), 2-7 June 2002, Gargnano (Brescia), Italy

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- "Synthesis and Characterization of Thermoresponsive Polymers", M.Panayiotou, R. Freitag, **Polymerisation and Polymers**: Concepts, Methods, Mechanisms, Catalysts and Applications, 1-6 September 2002, Champery, Switzerland
- "Synthesis and Characterization of Thermoresponsive Polymers", M.Panayiotou, R. Freitag, **Molecular Biotechnology**: Chemical Engineering and bioengineering of Processes, 23-28 September 2001, Champery, Switzerland

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