UNIVERSITY OF PATRAS
INTERDEPARTMENTAL PROGRAM
OF GRADUATE STUDIES ON
POLYMER SCIENCE AND TECHNOLOGY

REVERSIBLE HYDROGELS FROM AMPHIPHILIC
POLYEOLECTROLYTE MODEL MULTIBLOCK
COPOLYMERS

SPECIALISATION THESIS IN SCIENCE AND TECHNOLOGY OF POLYMERS
OF

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Diploma in Chemical Engineering
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PATRA 2009
Acknowledgements

I extend my deepest thanks and appreciation to my advisor, Prof. Constantinos Tsitsilianis, who provided me with my greatest challenges in the Laboratory of Macromolecular Engineering of the Department of Chemical Engineering. I thank him for teaching me how to fulfill my potential and expand my horizons. His valuable insights, knowledge, patience and encouragement, aside from his meticulous guidance through the entire length of the project, have been a strong motivating force. His dedication to science, rational and effective research methodology and acuteness to novelty has been an ideal to exemplify throughout my research work.

I would like to thank my committee, Professors Georgios Bokias and Vlasis Mavrantzas for their support and advice throughout my term. I am grateful to Dr. Spyros Yannopoulos for allowing me to use his facilities at Institute of Chemical Engineering and High Temperature Chemical Processes (ICE-HT), in view of Light Scattering Measurements and to Dr. Christos Tsakiroglou for the Rheology Experiments conducted in his lab. I extend my gratitude to Mary Kollia for her significant help in TEM experiments.

I would like to express my appreciation to Professor Georgios Staikos for his valuable help. I would like to thank my collaborators Prof. Costas Patrickios, Iannis Athanasoulias and Natalia Hadjiantoniou, for their significant contributions to my research efforts.

I also wish to thank all the people who contributed in making my stay at University of Patras a pleasant and rewarding experience.
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In this work, novel amphiphilic polyelectrolytes with multiblock architecture were studied. In particular, a series of model asymmetric ABA, BABAB, ABABABA and BABABABAB (A hydrophobic, B polyelectrolyte) block copolymers were synthesised by group transfer living polymerization in order to control the molecular characteristics of the A and B blocks.

The main objective of the present work was to explore the self-organization ability of the multiblock copolymers to form injectable reversible hydrogels and especially to answer to the question how the block copolymer topology affects the gelation efficiency.

For this purpose, the association behaviour of the multiblock copolymers was compared. The molecular architecture of the comparable copolymers was composed by hydrophilic ionizable 2-(dimethylamino)ethyl methacrylate (DMA, 25 nominal units per block) and the hydrophobic n-butyl methacrylate (BuMA, 5 nominal units per block) bearing three, five, seven and nine blocks.

The great interest that this particular system receives is due to its stimuli responsive character, resulting from the weak polyelectrolyte features of the hydrophilic segments, leading to pH and ionic strength sensitive hydrogels, as promising candidates for drug delivery systems.

In order to define the complex behavior of amphiphilic block copolymers, the first chapter of this thesis is dedicated to associative polymers able to form three-dimensional networks.

Typical gelators usually comprise either amphiphilic graft copolymers, also named hydrophobically modified polymers, or amphiphilic linear ABA block copolymers and ABC terpolymers that can self assemble in water forming transient networks. ABCBA pentablock terpolymers have also been explored as associative polymers yielding physical gels.
Regular copolymers with a multiblock structure, in a selective organic solvent for the internal segments, are able to form a bcc lattice of the spherical domains of the unsolved blocks bridged by the soluble blocks (lattice-type network).

Physical gels are of considerable current attention. However, their structure is usually poorly controlled: the comonomer distribution in the case of copolymer hydrogels is not uniform. Improved structures may broaden hydrogels applications and provide a better understanding of the relationship between their structure and properties.

The novelty of this work is that model asymmetric multiblock copolymers with uniform and precisely known length of the building blocks can predict the importance of topology on the mechanism of association towards the formation of reversible hydrogels.

Further on in this thesis, the properties of these copolymers were studied in aqueous solutions by several techniques such as static and dynamic light scattering, transmission electron microscopy and rheological measurements, in order to explain the aggregation, as well as the network formation mechanism and to explain how the topology of the hydrophobic blocks affects the system. These multiresponsive systems demonstrate a diversity of structural organizations, caused by the BuMA and DMA building blocks.

Rheological results showed that only the heptablock copolymer (ABABABA) with hydrophobic end-blocks formed hydrogels at relatively low copolymer concentrations. The study has been focused, in concentrated solutions of heptablock, on the examination of the effect of pH, temperature and ionic strength onto the hydrogels.

Furthermore, in order to compare the association mechanism of the asymmetrical multiblock copolymer systems and to understand why the pentablock (BABAB) and the nonablock (BABABABAB), bearing only internal hydrophobic segments, do not form a gel at low copolymer concentration, hierarchical study in diluted aqueous solutions has been performed.
The behaviour of these associative polyelectrolytes seems to be governed by the interplay of the hydrophobic attractive interactions of the A blocks and the electrostatic repulsive interactions of the B blocks. Through the global study of these systems it had been revealed that the topology of the A blocks and the degree of ionization of the B blocks influence significantly the association ability of these novel block copolymers.
1.1. Introduction

Water-soluble associative polymers (AP) represent a very important class of polymeric materials with fascinating properties, which have attracted considerable interest in recent years. APs consist of long hydrophilic chains bearing hydrophobic associative groups.

These materials self-organize in aqueous media spontaneously, and exhibit unique rheological properties, that render them very useful rheology modifiers in cosmetics and coatings, suspension stabilizers, drug carriers and injectable hydrogels in pharmaceutical formulations and other water-based applications [1-7].

1.2. Association Leading to Formation of Reversible Hydrogels

The class of Associative Polymers includes charged polymers (ionomers, polyelectrolytes and polyan ampholytes); block copolymers in strongly selective solvents and polymers with hydrogen bonding.

The association between attractive groups leads to the formation of physical bonds. Examples of physical bonds include H-bonding, multiplets in ionomers, hydrophobic interactions in amphiphilic block or graft copolymers, and electrostatic interactions between oppositely charged moieties for polyan ampholytes. The structures formed in solutions of associative polymers depend on polymer concentration, the number of attractive groups per chain and the strength of physical bonds. [2]

Hydrogels belong to a specific class of soft matter, exhibiting particular features and unique properties. In fact they are structured complex bicontinuous
materials constituted of two main components: a three dimensional infinite network (minor component) and the aqueous medium (major component). The network is build in most of the cases from water soluble macromolecular chains (gelators), crosslinked either through permanent covalent bonds, termed chemical (covalent), hydrogels, or through temporary junctions (physical crosslinks), termed physical (reversible) hydrogels.

A reversible hydrogel is fabricated through a hierarchical self-organization process of macromolecular entities of special architecture and functionality which enable the formation of a three dimensional network through non-covalent bonding. Water soluble associative amphiphilic block copolymers are very suitable macromolecular species for the formation of reversible hydrogels since they can be designed at the molecular level through macromolecular engineering.

In the following, the study is focused on the hydrogels based on amphiphilic block copolymers, and especially, on a novel class of hydrogels, created by responsive polymeric materials.

1.3. Association Mechanism of Amphiphilic Block Copolymers

Macromolecules of amphiphilic polymers contain both hydrophobic and hydrophilic groups possessing different affinity to water and polar solvents. In dilute solutions polymers may self associate by forming physical bonds between the attractive groups of the same chain (e.g. unimolecular micelle) or by aggregating into a complex containing several molecules (e.g. multimolecular micelle). At higher polymer concentration a three dimensional transient network is formed, composed from hydrophobic nanodomains (physical crosslinks) interconnected by hydrophilic chains (elastic chains). The creation of a 3D
transient network proceeds through a growth mechanism upon increasing polymer concentration. [2,8-12]

If the hydrophobic blocks are sufficiently long to give association of the hydrophobic blocks, flower like micelles are usually formed with a corona of looping hydrophilic chains. On increasing polymer concentration, some hydrophobic chain ends can disengage from micelles leading to the formation of bridges (loop to bridge transitions) between neighboring micelles, and eventually to the formation of a three dimensional physical transient network in which flower-like micelles act as nodes. [13,14]

Therefore the hydrophobic-hydrophilic balance is a determinant factor for the network formation, which can be changed by varying the content or the length of hydrophobic repeating units, [15] the use of neutral hydrophilic repeating units together with the ionizable ones,[16] and the introduction of external stimuli by varying the solution pH, temperature, and ionic strength. Even so, it is reported that the hydrophobic/hydrophilic balance, even for linear polymer chains, cannot simply be captured by the average polymer chemical composition.

Another factor in the association of amphiphilic block copolymers is the solvent-polymer interaction which determines the conformation of the chains. The conformation of a solvated macromolecule results from several interactions acting within the same macromolecular chain (intramolecular), between different chains (intermolecular) and between the macromolecule and the solvent, i.e.:

- Van der Waals cohesion of uncharged side groups (hydrophobic interactions)
- Electrostatic interactions
- Hydrogen bonds
- Interactions with the solvent (repulsive or attractive)

Hydrophobic interactions can be described as a tendency of hydrophobic groups to reduce their surface of contact with a polar solvent: two hydrophobic groups try to stick together in order to hide their mutual surface of contact. On the other hand, the hydrophilic groups tend to be surrounded by solvent molecules.

Therefore, the hydrophobic–polar interactions give rise to the formation of a
compact structure with mostly hydrophobic core and polar shell, in order to minimise disorder within the aqueous phase. The attractive interaction arises from the hydrophobic effect, the origin of which is the structural adjustment which takes place in water when hydrophobic units are separated from it. [1,12,17].

Amphiphilic polyelectrolytes are polymers with weakly charged groups, i.e. either weak acids or bases, also bearing additional hydrophobic pendant groups. They form an extended structure as a result of mutual repulsion between the charged groups. In the context of amphiphilic polyelectrolytes the most important factor in controlling polymer conformation is the degree of ionization. The association process of amphiphilic block polyelectrolytes is governed by the competition between hydrophobic attraction of the insoluble blocks and intramolecular electrostatic repulsion of the charges in the hydrophilic block. Ways to influence the assembly of charged block are the manipulation of the degree of ionization (e.g. by changing the solution pH), the electrostatic screening of charges by the addition of salts, and changing the solution conditions (e.g. manipulation of the dielectric constant of the medium). [1, 18]

For polyelectrolytes the chain conformation is strongly influenced by the electrostatic interactions. The range of the repulsive Coulomb interaction between the charged molecules is decided by the Debye screening length $\kappa^{-1}$. At room temperature: $\kappa^{-1} = 3.04 \times I^{-1/2}$, where $I$ represents the ionic strength of the solution in mol/L.

Increasing the ionic strength of the solution decreases the repulsive part of the interaction energy between the charged molecules, which would help the aggregation process of amphiphilic polyelectrolytes. At high salt concentrations, the electrostatic interactions are screened and also the effect of added salt produces a faster decay of the inhomogeneity in the charge distribution along the chain.

At finite polymer concentrations some counterions are attracted to polyelectrolyte chains and may condense on them. [1,19] The association of polyions with the counterions maintains the electrical neutrality, but the counterion dimensions can have consequences on the solution properties.
The counterion condensation is due to a fine interplay between the strong electrostatic attraction of counterions to the polymer chain and the loss of translational entropy by counterions due to their localization in the vicinity of polyelectrolytes. In very dilute polyelectrolyte solutions, the entropic penalty for counterion condensation is very high, and almost all counterions leave polyion chains and stay in solution. By increasing the polymer concentration or by decreasing the quality of the solvent, one can induce spontaneous condensation of counterions inside the beads of the necklacelike globule. The reduction of the effective charge of the polyelectrolyte chain by condensing counterions increases the mass of the beads initiating further deficit of counterions that starts the avalanche-like counterion condensation process.

At finite polymer concentrations, this avalanche like condensation results in the phase separation of polyelectrolyte solutions into dilute and concentrated phases. Changes in hydrogen ion equilibrium usually induce the most profound conformational transitions, as the polymer changes from a compact to an extended chain conformation.[1]

**1.4. Associative Polymer Architectures for Network Formation**

The present review focuses on reversible hydrogels which are formed through self-assembling mechanisms of amphiphilic block copolymers by hydrophobic interactions and that are able to respond to an external stimulus like pH and ionic strength.

An important factor for self-organization of these systems towards reversible hydrogels is the architecture/topology of the involved macromolecules and the nature of interactions that are going to be developed in the aqueous medium. Block copolymers are very suitable macromolecular species for the formation of reversible hydrogels since they can be designed at the molecular level through macromolecular engineering. The last decade, an enormous development of synthetic polymerization techniques named controlled/living polymerization
methods offers numerous possibilities in designing of tailor-made block copolymers enabling to self assemble in nanostructured objects of predicted morphology and functionality, like micelles and of course networks.

In Table 1 some macromolecular architectures best suited for creating transient networks are demonstrated.

Table 1. Types of linear and grafted block copolymer architectures able to self-assembly towards a transient network.

<table>
<thead>
<tr>
<th>ABA</th>
<th>A-g-B</th>
<th>ABC</th>
<th>ABCBA</th>
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<tr>
<td><img src="image" alt="ABABA" /></td>
<td><img src="image" alt="ABC" /></td>
<td><img src="image" alt="ABCBA" /></td>
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The common feature of these topologies is that the size of the building blocks, in most of the cases, is highly asymmetric. The short associative blocks mainly are located in the outer part of the macromolecular chain in order to be able to be associated intermolecularly forming physical crosslinks. The nature of these associative blocks may be hydrophobic, charged or moieties capable to form hydrogen bonds which in turn determines the type of interactions among them, i.e. hydrophobic, electrostatic, H-bonding. The long blocks on the other hand are hydrophilic, either ionic or non-ionic, as to prevent precipitation of the polymeric system. The advantage of this macromolecular design is that it gives the possibility to predict the rheological properties of the hydrogel as they strongly depend on the molecular characteristics of the network building macromolecules.

The simplest type of block polyelectrolyte is a diblock copolymer consisting of two covalently bound blocks, A-block hydrophilic (polyelectrolyte) and a hydrophobic B-block. This diblock polyelectrolyte in water takes on a ‘tadpole’-like conformation, because water is a good solvent for the swollen polyelectrolyte block and poor for the
collapsed block B. Several B-blocks prefer to aggregate together into a micelle core through hydrophobic interactions, which is stabilized by the hydrophilic polyelectrolyte-based corona (Figure 1).

\[ \text{Micelle formation of a diblock copolymer in aqueous medium. A: anionic polyelectrolyte. B: hydrophobic block.} \]

Micelle formation occurs as a result of two opposite forces, an attractive force leading to association of the molecules and a repulsive force that prevents unlimited growth of the micelles to a distinct macroscopic phase.[17] The size of diblock polyelectrolyte micelles in aqueous solution results from a balance between the surface tension of the hydrophobic core and the electrostatic repulsion with the charged corona. What makes the block polyelectrolytes unique is the very large driving force and/or the ability to control it by regulating the hydrophilic/hydrophobic balance through a variation of the molecular parameters of the two blocks. This special feature of charged diblock copolymers opens the possibility to design the morphology by varying the ionic strength and/or the pH of the aqueous solution.[1]

Charged polymers release counterions in water which form a more or less diffuse layer around the chain. The structure of this layer determines the effective interaction between neighboring chains. Monovalent ions are relatively easily released and always generate repulsion.
The self-assembly behavior of block copolymers in water is not only determined by the physical properties of the blocks, but also by the topology of the blocks in the linear polymeric molecule.

The degree of stretching or the solubility of one or more blocks can also be varied by changing pH, ionic strength, or temperature, resulting in a stimuli responsive behavior of most block copolymers. The association process of amphiphilic polyelectrolyte block copolymers is governed by the competition between hydrophobic attraction of the insoluble blocks and intramolecular electrostatic repulsion of the charges in the hydrophilic block.

To form a network of micelles, multiblock copolymers with more than one hydrophobic block per chain are needed. ABA triblocks with hydrophobic end blocks can form loops or stay in open configurations. In selective solvents they form flower like micelles and reorganize at higher concentrations into clusters of micelles connected by bridges as depicted in Figure 2. [18]

![Figure 2. From the left to the right: schematic representation of the self-association of telechelic chains in a flower-like micelle above a critical concentration value (left), and of a typical intermolecular aggregation between micelles at high concentration (right). [20].](image)

The influence of the distribution sequence of hydrophilic and hydrophobic monomers on the morphology of the systems can also be exemplified by the study of diblock vs triblock copolymers.

Amphiphilic diblock copolymers bearing a block of hydrophilic monomers and a block of hydrophobic monomers are known to form well-defined spherical micelles with a hydrophobic core and a hydrophilic shell. However, amphiphilic triblock copolymers that carry a hydrophilic block in between two hydrophobic blocks, also named telechelic polymers, form well defined flowerlike micelles, with the hydrophobic core protected by the hydrophilic loops. [21]

The main feature that makes telechelic polyelectrolytes distinct from conventional telechelics (non-ionic hydrophilic part) is that the chain conformation
of the bridging chains of the resulting transient network (Figure 3) depends on its
degree of ionization and can be tuned by external stimuli such as pH and ionic
strength. [2] At a certain pH (depending on the polyelectrolyte nature) and at free
salt conditions the central chain adopts a stretched conformation, which has
fundamental consequences on the association mechanism. The specificity of the
rheological properties, compared to those of neutral telechelic polymer solutions,
is due to intra- and inter-chain Coulombic repulsive and/or attractive interactions,
which control both macromolecular chain rigidity and lead to specific pH-tunable
properties of great potential interest.

![Figure 3. Bridging between hydrophobic block junctions with the formation of a transient network.](image)

In polyelectrolyte physical networks extended bridging is favored due to
stretched conformation of hydrophilic chains.

A cationic PMMA–PDMAEMA–PMMA telechelic polymer had been
investigated in terms of its rheological behavior. Above a critical concentration,
which depends on pH, polymer chain-ends interact via hydrophobic interactions
and form a transient physical network exhibiting peculiar flow behavior. The
viscoelastic behavior was shown to be governed by two pH dependent time
scales: a short time scale controlled by the lifetime of the hydrophobic associative
junctions and a long time scale corresponding to the network relaxation time.
[22,23]

It is known that above a certain concentration the associative telechelic
polyelectrolytes undergo a hierarchical intermolecular association through
hydrophobic attraction of their end-stickers, forming micellar aggregates and a three-dimensional network at elevated concentrations.

Figure 4. Double-logarithmic plot of zero shear viscosity as a function of polymer concentration in aqueous solution at pH 3 and 25°C for (O) MMA$_{32}$-DMAEMA$_{224}$-MMA$_{32}$, (Δ) MMA$_{35}$-DMAEMA$_{180}$-MMA$_{35}$, and (□) MMA$_{16}$-b-DMAEMA$_{252}$-b-MMA$_{16}$. [23]

The critical concentration, $c_{gel}$, above which a physical gel is formed, macroscopically observed by a rapid increase of the solution viscosity, was determined by steady-state shear viscosity measurements. The concentration dependence of the zero-shear viscosity as a function of concentration for the three associative TP is depicted in Figure 4. $c_{gel}$ was determined at the concentration corresponding to the intersection of the straight lines denoting the onset of a transient infinite network formation.

It is obvious that $c_{gel}$ depends on the macromolecular characteristics of the TP. It was found that an increased length of the hydrophilic mid-block (potential bridging chains between the hydrophobic domains) leads to a decrease in critical gel concentration, for a given length of the hydrophobic blocks. For a constant hydrophilic length the critical gel concentration increases with the decrease of the hydrophobic stickers. By AFM imaging (Figure 5), two types of associates were observed at very low concentrations: linear associates of a few macromolecules and starlike “hairy” loose aggregates where the one end of TP is located in an irregular hydrophobic core and the other remains as a dangling end (absence of looping chains). By increasing concentration, finite size clusters (microgels) were
observed with the formation of an infinite transient network at higher concentrations. [23]

Figure 5. AFM topography images of the MMA$_{32}$-b-DMAEMA$_{224}$- b-MMA$_{32}$ deposited on mica at low concentration (10$^{-4}$ wt %) (left image) and of concentration 0.25 wt % at pH 3 (right image). A finite size cluster (microgel) is marked by the yellow cycle.[23]

ABC triblock terpolymers have attracted great interest, in comparison to ABA block copolymers, due to the greater number of different morphologies that have been observed so far in the bulk. Moreover, the introduction of a third block may introduce interesting new functionalities and offers additional parameters to control the copolymer properties, thereby influencing remarkably the self-assembly process. The main motivation for studying ABC triblock terpolymer micelles is related to the presence of a third nanosized compartment in the micellar structure. Depending on the investigated systems, this third compartment can either be soluble or insoluble in the considered solvent, leading to different types of micelles. Since the third micellar compartment is characterized by its own morphology, size, physical and chemical properties, the spectrum of application for ABC block terpolymer micelles is broadly enhanced compared to ABA systems.

A double hydrophilic stimuli-responsive ABC terpolymer, poly(2-vinyl pyridine)-block-poly(acrylic acid)-block-poly(butyl methacrylate) (P2VP-PAA-
PnBMA), had been studied in terms of phase behavior in aqueous media. This multiresponsive molecule had shown a diversity of structural organizations, caused by the combination of the P2VP and PAA building blocks. The transition between different micelles and hydrogels were induced by tuning pH levels in aqueous solutions. At low pH, three-compartment spherical micelles with a positively charged outer corona are formed, which are transformed into a three-dimensional physical network constituted of P2VP and/or PBMA hydrophobic domains interconnected by negatively charged bridging PAA chains upon switching pH.[11],[12]

Linear terpolymers comprising more than three segments that are different in nature, termed ABCBA pentablock terpolymers, have appeared recently, increasing the diversity and functionality towards multifunctional nanostructured polymeric materials.

The novelty of this associative polymer with respect to the classical ABA non-ionic as well as to the telechelic polyelectrolytes is that the bridging chains of the formed reversible hydrogel can exist as positively charged, neutral and/or negatively charged, by adjusting the pH and/or dielectric constant of the medium.

More precisely, multisegmental ABCBA pentablock terpolymer constituted of triblock polyampholyte end-capped at both ends by hydrophobic blocks, (PMMA-PAA-P2VP-PAA-PMMA), had been synthesized by “living” anionic polymerization. The results have shown that in a certain pH range below the isoelectric point, where electrostatic interactions among oppositely charged moieties are present, an intensive thermothickening phenomenon were observed showing that this kind of pentablock terpolymers could be used as multifunctional responsive materials. [24], [25]

Hydrophobically modified polyelectrolytes constituted of PAA grafted by hydrocarbon or fluorocarbon pendant hydrophobes have been prepared and studied in aqueous medium. Above a threshold concentration corresponding to the formation of a reversible network structure, the solutions behave as physical
gels. These amphiphilic graft copolymers A-g-B have been used as associative thickeners, forming physical gels in aqueous formulation.[3]

Since the self-assembly of the copolymer chains is strongly affected by their architecture, it is of interest to examine this effect for multiblock copolymers having more than two inner blocks. For a series of 1,4-butadiene (B)-styrene (S) symmetrical multiblock copolymers, BSB triblock, BSBSB pentablock, and BSBSBSB heptablock copolymers having the same block molecular weights and B/S composition, the rheological behaviour and structure were examined in dibutyl phthalate (DBP) which is an S-selective solvent, i.e., a good solvent for the S blocks and a poor solvent for the B blocks.

It turned out that the behaviour of the pentablock and heptablock copolymers is qualitatively similar to but quantitatively different from the behavior of the triblock copolymer under steady shear flow and after cessation of flow. In particular, the time required for full recovery of the equilibrium elasticity after cessation of flow was significantly different increasing with copolymer molecular weight in the order triblock < pentablock < heptablock.[27]

Thermoplastic hydrogels based on hexablock copolymer (ABABAB) composed of poly(γ-benzyl L-glutamate) (PBLG) as the biodegradable and hydrophobic part and poly(ethylene oxide) (PEO) as the swellable and hydrophilic part were synthesized.[26] Water contents of the copolymers are dependent on the PEO content in the copolymers indicating characteristics of a polymeric hydrogel.

Hydrophobically-modified polyacrylamides (HMPAM) prepared by micellar polymerization technique led to copolymers with a multiblock structure, with variable number and length of the hydrophobic blocks. These systems exhibit in aqueous solution an improved thickening efficiency compared to that of the unmodified polyacrylamide and a pronounced shear thining behavior. The presence of ionic sites on the copolymer backbone can lead to various improvements: 1. a better water-solubility; 2. a stronger thickening efficiency due to the typical coil expansion of polyelectrolytes; and 3. a potential responsiveness to changes in ionic strength and pH. Ion-containing associating copolymers
exhibit a more complicated behaviour than that observed for the corresponding neutral copolymers due to the competition between attractive hydrophobic interactions and repulsive electrostatic interactions. The presence of the ionic sites has two opposite effects on the viscosifying properties: 1. an increase in viscosity due to coil expansion resulting from intramolecular charge-charge repulsions; and 2. a lowering of the degree of association with a concomitant decrease in viscosity arising from intermolecular electrostatic repulsions which hinders the interpenetration of the macromolecular chains.

Owing to their polyelectrolyte character, charged HMPAM are responsive to changes in ionic strength. Upon adding salt, the screening of the charges has also two opposite effects. At the intramolecular level, the chain contraction favours a decrease in viscosity. At the intermolecular level, the macromolecular interpenetration is facilitated and thus promotes enhanced polymolecular associations.

As a consequence, depending on whether which of these effects is dominant, a great variety of behaviours can occur as a function of the molecular characteristics of the samples, polymer concentration and ionic strength. Generally, ionic polyacrylamides require a higher hydrophobe level or the addition of high salt concentrations in order to exhibit intermolecular associations. By an adequate choice of the ionic and hydrophobic group contents, it is possible to obtain a synergistic enhancement of the thickening efficiency. Conversely, for highly charged HMPAM with short hydrophobes, intermolecular associations may be suppressed and the hydrophobically-modified polyelectrolytes exhibit mainly intramolecular associations.

The effect of the hydrophobe content has also been investigated. It was observed that the higher the hydrophobe content, the lower the concentration beyond which the viscosity starts to increase. The optimal hydrophobe content depends on the nature of the hydrophobe group and the ionic units content.

At constant hydrophobe level, the longer the hydrophobic blocks, the greater is the thickening ability. This behavior is an indication that the average
size of the hydrophobic blocks is more effective for the associating properties than their number. [28]

Having in hand these model samples, it became possible to establish molecular structure-macroscopic properties relationships by undertaking a thorough study of the main parameters affecting the rheological behavior of multiblock copolymers.

From these studies it can be concluded that the macromolecular architecture of amphiphilic multiblock copolymers influences the spontaneous self-assembly in specific environmental conditions. Therefore, the macromolecular characteristics play an important role for the design of block copolymers with specific macroscopic properties.

The main objective of the present work was to explore the self-organization ability of the multiblock copolymers to form injectable reversible hydrogels and especially to answer to the question how the block copolymer topology affects the gelation efficiency. Synthetic hydrogels are of considerable current attention.[29] However, their structure is usually poorly controlled: the comonomer distribution in the case of copolymer hydrogels is not uniform. Improved structures may broaden hydrogels applications and provide a better understanding of the relationship between their structure and properties.

The novelty of this work is that model multiblock copolymers with uniform and precisely known length of the building blocks can predict the importance of topology on the mechanism of association towards the formation of reversible hydrogels.
1.5. References

Chapter 2

EXPERIMENTAL TECHNIQUES
2.1. Introduction

Many experimental techniques can be used to characterize the structure and rheological properties of amphiphilic polyelectrolytes. This chapter summarizes the theoretical principles behind the techniques that are utilized during the course of this study. These techniques include static light scattering (SLS), dynamic light scattering (DLS), transmission electron microscopy (TEM) and rheology.

2.2. Light Scattering by Polymeric Solutions\textsuperscript{1-6}

2.2.1. Introduction

Light Scattering occurs when polarizable particles in a sample are placed in the oscillating electric field of a beam of light. The varying field induces oscillating dipoles in the particles which radiate light in all directions. Light Scattering has been used to determine particle size, molecular weight, diffusion coefficients, thermodynamic properties, etc.

2.2.2. Static Light Scattering

In static light scattering the time average value of the scattered intensity is measured as function of the scattering angle. This allows determining the weight average of the molar mass $M_w$, the $z$-average of the squared radius of gyration $<R_g^2>_z$ and the second virial coefficient of the osmotic pressure $A_2$. As mentioned above the oscillating electric field of light induces oscillating dipoles within
molecules and which redistribute light in all directions. The wavelength of the scattered light is identical with the wavelength of the incident beam.

The method is based on Rayleigh theory for the scattering of light by molecules whose linear dimensions are small compared to the wavelength of the light. If the linear dimensions of a molecule are small compared to the wavelength of the light, as for low molecular weight components, then the field is approximately uniform over the dimensions of the molecule. For polymer molecules, different parts experience significantly different fields, oscillate independently and produce light which interferes with itself. By extrapolating the scattered intensity measured at different angles to $\theta = 0^\circ$ eliminates the interference effect, allowing the interpretation of the result in terms of the Rayleigh theory. Thus, the light scattered at the smaller value of $\theta$ remains in phase, while the light scattered at the larger angle significant destructive interference occurs.

Scattered light intensities are measured at several angles for each of several solution concentrations and for the pure solvent at each angle. Subtracting the solvent scattering from the solution value yields the excess intensity scattered by the polymer. For diluted solutions it can be assumed that the contribution of the density fluctuations of solution and solvent are the same. Therefore the scattering of the dissolved substance is given by:

$$R(\theta) = R(\theta)_{\text{solution}} - R(\theta)_{\text{solvent}}$$

Where $R(\theta)$ represents the Rayleigh ratio as a function of scattering angle $\theta$. Scattering experiments are usually reported in terms of the Rayleigh ratio defined by:

$$R(\theta) = \left( \frac{I_\text{s}}{I_\text{i}} \right) \frac{R^2}{I_i} = \frac{I_\text{s}}{I_i} R^2$$

For large particles the dependence of the scattered intensity is expressed by the form factor $P(\theta)$, which describes the large particle size effect.

$$P(\theta) = 1 - \frac{16\pi^2}{3\lambda_s^2} \sin^2 \left( \frac{\theta}{2} \right)$$
An expansion for the inverse structure factor $P(\theta)$ can be obtained by noting that for small values of $x$, $(1-x)^{-1} \sim 1+x$.

$$\lim_{\theta \to 0} \frac{1}{P(\theta)} = 1 + \frac{16\pi^2}{3\lambda_s^2} R_G^2 \sin^2\left(\frac{\theta}{2}\right)$$

Where $<R^2_0>^{0.5}$ is the average root-mean-square radius of gyration, as a primary parameter obtained from light scattering, without shape model assumption.

The Zimm equation for large particles is given by the expansion of $\frac{1}{P(\theta)}$ with a first order Taylor series:

$$\frac{Kc}{\Delta R_\alpha} = \frac{1}{M_w} \left(1 + \frac{16\pi^2}{3\lambda_s^2} \sin^2\left(\frac{\theta}{2}\right) \left(\frac{R^2}{s^2}\right)\right) + 2\lambda c$$

The optical constant is given by $K = \frac{2\pi^2}{\lambda^4} n_0 \left(\frac{dn}{dc}\right)$, where $\lambda$ represents the wavelength in vacuum of the laser, $n_0$ is the refractive index increment of the solvent, $dn/dc$ is the specific refractive index increment of the solution.

The Rayleigh Ratio is proportional to the corrected scattered light intensity, with a proportionality constant determined by calibration against a reference substance (toluene, benzene) with a known Rayleigh Ratio:

$$\Delta R_\theta = \frac{\sin\theta n_0^2}{(I_{ref})_{90^\circ}} \frac{dn}{dc}(R_{ref})_{90^\circ}$$

where $(R_{ref})_{90^0}$ and $(I_{ref})_{90^0}$ are Rayleigh ratio and the intensity at 90$^0$. The ratio $n_0^2/n_{ref}^2$ is the correction of the refractive index.

The constant $K$ is given by: $K = \frac{2\pi^2}{\lambda^4} n_{ref}^2 \left(\frac{dn}{dc}\right)^2 (I_{ref})_{90^\circ}$. The proportionality with $\lambda^4$ is for vertically polarized incident light. Considering that $K_v = \frac{2\pi^2}{\lambda^4} n_{ref}^2 \left(\frac{dn}{dc}\right)^2 (I_{ref})_{90^\circ}$ where $K_v$ represents the experimental optical constant, then:

$$K = K_v \left(\frac{dn}{dc}\right)^2 (I_{ref})_{90^\circ}.$$
Knowing that: \( \Delta I = \sin \theta \Delta I_\theta \), the final relationship is obtained:

\[
\frac{Kc}{\Delta R(\theta)} = \frac{1}{M_w} \left( 1 + \frac{16\pi^2}{3 \lambda^2} \sin^2 \frac{\theta}{2} \left\langle R_g^2 \right\rangle \right) + 2A_2c
\]

This equation is the basis of a graphical technique established by Zimm, for simultaneously extrapolating light scattering data to both zero angle and zero concentration. This is achieved by plotting the data \( \frac{Kc}{\Delta R(\theta)} \) as a function of \( \sin^2(\theta/2) + Kc_2 \), where the arbitrary constant is chosen to give a convenient spacing of the data, with reciprocal concentration units so that \( kc_2 \) to be added to the dimensionless \( \sin^2(\theta/2) \). Two sets of, typically, parallel lines are obtained as depicted in Figure 6. One set consists of angular measurements at each concentration, and one set consists of concentration measurements at each angle. Extrapolating the angular measurements to zero angle for each concentration yields:

\[
\frac{Kc}{\Delta I}_{\theta=0} = \frac{1}{M_w} + 2A_2c.
\]

From the slope the second virial coefficient can be determined.

Extrapolating the concentration measurements to zero concentration for each angle yields:

\[
\frac{Kc}{\Delta R_\theta} = \frac{1}{M_w} \left( 1 + \frac{16\pi^2}{3 \lambda^2} \sin^2 \frac{\theta}{2} \left\langle R_g^2 \right\rangle \right).\]

The slope divided by the intercept gives \( R_G \).

The intercept of both extrapolated lines – the double extrapolation to zero angle and zero concentration – yields \( M_w \) the weight average molecular weight of the polymer solution:

\[
\frac{Kc}{\Delta I}_{c, \theta=0} = \frac{1}{M_w}.
\]
Figure 6. Typical Zimm plot. The experimental data points are at the grid intersection points except along the $\theta = 0$ and $c = 0$ lines.

For the measurement of the scattered light intensity as a function of the scattering angle, lasers are used, due to their high light intensity, low divergence of the beam and high stability with respect to the special position. This allows to measure in the very dilute regime.

Figure 7. Schematic representation of light scattering procedure

As a source of high intensity monochromatic light, the laser source makes it possible to carry out light scattering experiments on samples of greatly reduced volume.

The detection of the scattered intensity is performed by a photomultiplier tube or an Avalanche photodiode. In a goniometer setup (Figure 7) the complete secondary detection optics is rotated around the center of the apparatus. The sample cuvette is placed in a decaline bath which minimizes reflections (refractive index matching to glass) and serves as temperature bath.
2.2.3. Dynamic Light Scattering

According to the semi-classical light scattering theory when light impinges on matter, the electric field of the light induces an oscillating polarization of electrons in the molecules. Hence the molecules provide a secondary source of light and subsequently scatter light. The frequency shifts, the angular distribution, the polarization, and the intensity of the scatter light are determined by the size, shape and molecular interactions in the scattering material. If the particles or molecules are illuminated with a laser, the intensity of the scattered light fluctuates at a rate that is dependent upon the size of the particles as smaller particles are “kicked” further by the solvent molecules and move more rapidly. Analysis of these intensity fluctuations yields the velocity of the Brownian motion and hence the particle size.

Whereas in static light scattering the time average of the scattering intensity is measured in dynamic light scattering the fluctuations of the scattering intensity due to Brownian motion of the particles are correlated by means of an intensity-time autocorrelator.

The second order autocorrelation curve is generated from the intensity trace as follows:

\[
g^{(2)}(\tau) = \frac{\langle I(t) \cdot I(t + \tau) \rangle}{\langle I(t) \rangle^2}
\]

Where \( g^{(2)}(\tau) \) is the autocorrelation function at a particular delay time, \( \tau \), and \( I \) is the intensity. At short time delays, the correlation is high because the particles do not have a chance to move to a great extent from the initial state that they were in. The two signals are thus essentially unchanged when compared after only a very short time interval. As the time delays become longer, the correlation starts to exponentially decay to zero, meaning that after a long time period has elapsed, there is no correlation between the scattered intensity of the initial and final states. This exponential decay is related to the motion of the particles, specifically to the diffusion coefficient.
If the sample is monodisperse then the decay is simply a single exponential. The Siegert equation relates the second order autocorrelation function with the first order autocorrelation function $g^{(1)}(\tau)$ as follows:

$$g^{(2)}(\tau) = 1 + \beta [g^{(1)}(\tau)]^2$$

where the parameter $\beta$ is a correction factor that depends on the geometry and alignment of the laser beam in the light scattering setup.

The correlation function of the electric field $g^{(1)}(\tau)$ is given by:

$$g^{(1)}(\tau) = \exp(-\Gamma \cdot \tau)$$

Where $\Gamma$ is called the decay or relaxation rate and $\tau_r = 1/\Gamma$ is called the decay or relaxation time. For dilute systems where the decay is entirely due to translational, diffusive motion of the center of mass of the scatterer, it is valid: $\Gamma = D_T q^2$, where $D_T$ is the translational diffusion coefficient and $q$ is the magnitude of the scattering wave vector. This magnitude is given by,

$$q = \frac{4\pi n_0}{\lambda_0} \cdot \sin(\theta/2)$$

Where $n_0$ is the refractive index of the liquid, $\lambda_0$ is the wavelength of the laser in vacuum, and $\theta$ is the scattering angle. $q^{-1}$ defines a length scale against which distances between scatters is compared. Depending on the anisotropy and polydispersity of the system, a resulting plot of $\Gamma / q^2$ vs. $q^2$ may or may not show an angular dependence. Spherical particles will show no angular dependence, hence no anisotropy. A plot of $\Gamma / q^2$ vs. $q^2$ will result in a horizontal line. Particles with a shape other than a sphere will show anisotropy and thus an angular dependence when plotting of $\Gamma / q^2$ vs. $q^2$. The intercept will be in any case the $D_0$.

For dilute solutions the diffusion coefficient can be measured as a z-average, and applying the Stokes-Einstein equation the hydrodynamic radius of a sphere can be calculated:

$$R_H = \frac{kT}{6\pi \eta D_0}$$
$K_B$ is Boltzmann’s constant, $T$ is the absolute temperature in Kelvin, $\eta$ is the bulk viscosity of the liquid in which the particle moves, and $R_H$ is the hydrodynamic radius of the assumed sphere.

Hydrodynamic size includes the particle “dry” size and whatever is permanently attached to, and moves with, the surface. In the case of hard mechanical spheres with relatively short chain surfactants added to impart stability, the difference between the “dry” and hydrodynamic diameters is usually not significant. However, when the particle is much smaller and the attached species are much larger, as may be the case with polymer additives, then there may be significant difference between the “dry” and hydrodynamic diameters. Interesting case involves polyelectrolyte systems which may significantly change size as a function of pH.

Dynamic Light Scattering is also known as Photon Correlation Spectroscopy. This technique is one of the most popular methods used to determine the size of particles. Shining a monochromatic light beam, such as a laser, onto a solution with spherical particles in Brownian motion causes a Doppler Shift when the light hits the moving particle, changing the wavelength of the incoming light. This change is related to the size of the particle. When the particles are very small compared with the wavelength of the light, the intensity of the scattered light is uniform in all directions (Rayleigh scattering); for larger particles (above approximately 250nm diameter), the intensity is angle dependent (Mie scattering).

If the light is coherent and monochromatic, as from a laser for example, it is possible to observe time-dependent fluctuations in the scattered intensity using a suitable detector such as a photomultiplier capable of operating in photon counting mode.
The laser passes through a collimator lens and then hits the cell with the solution. The light is scattered and detected by a photomultiplier that transform a variation of intensity into a variation of voltage.

As illustrated in Figure 8, there is another collimating lens before the photomultiplier. The use of both the collimating lenses is essential in this experiment: the first lens allows focusing the beam into the cell, so that the area that we will hit is far enough from the side of the cell. The second lens is used to get an amount of scattered light that is neither too much nor insufficient.

The photomultiplier is positioned at a scattering angle of 90 degrees; indeed for this scattering angle it is possible to neglect the nonlinearity of the line width with the scattering angle. This is because at this angle $\Delta\Gamma/\Gamma < 0.1$. After the photomultiplier, the signal is immediately preamplified and then sent to the computer where the voltage is elaborate.

In Figure 9 it is shown a schematic DLS instrumentation. The time dependence of the intensity fluctuation is most commonly analysed using a digital correlator. Such a device determines the intensity autocorrelation function which can be described as the ensemble average of the product of the signal with a delayed version of itself as a function of the delay time. The "signal" in this case is the number of photons counted in one sampling interval. At short delay times, correlation is high and, over time as particles diffuse, correlation diminishes to zero and the exponential decay of the correlation function is characteristic of the diffusion coefficient of the particles.
The method for analyzing the autocorrelation function can be achieved through an inverse Laplace transform known as CONTIN (regularized non-negatively constrained least squares). The CONTIN analysis is ideal for heterodisperse, polydisperse and multimodal systems.
2.3. Rheological Measurements

2.3.1. Introduction

Rheology is defined as the science that deals with the deformation of materials as a result of an applied stress. It is a powerful tool that is capable of characterizing the properties and microstructure of many polymeric and non-polymeric systems. Rheology provides information that is important in designing and optimizing material properties. The next section is a brief summary of different rheological techniques that have been used in this study.

2.3.2. Rheology of Polymers

Complex fluids exhibit unusual mechanical responses to applied stress or strain due to the geometrical constraints that the phase coexistence imposes. The mechanical response includes transitions between solid-like and fluid-like behavior as well as fluctuations. Their mechanical properties can be attributed to characteristics such as high disorder, caging, and clustering on multiple length scales. The dynamics of the particles in complex fluids are an area of current research. Energy lost due to friction may be a nonlinear function of the velocity and normal forces. The topological inhibition to flow by the crowding of constituent particles is a key element in these systems. The mechanical properties of polymers are highly dependent on temperature and on the time-scale of any deformation; polymers are viscoelastic and exhibit some of the properties of both viscous liquids and elastic solids. Viscoelasticity implies that the material has the characteristics both of a viscous liquid which cannot support a stress without flowing and an elastic solid in which removal of the imposed stress results in complete recovery of the imposed deformation.
Application of stresses for relatively long times may cause some flow and permanent deformation in solid polymers while rapid shearing will induce elastic behavior in some macromolecular liquids. It is also frequently observed that the value of a measured modulus or viscosity is time dependent and reflects the manner in which the measuring experiment was performed. These phenomena are examples of viscoelastic behavior.

Three types of experiments are used in the study of viscoelasticity. These involve creep, stress relaxation, and dynamic techniques. In creep studies a body is subjected to a constant stress and the sample dimensions are monitored as a function of time. When the polymer is first loaded an immediate deformation occurs, followed by progressively slower dimensional changes as the sample creeps towards a limiting shape. Stress relaxation is an alternative procedure. Here an instantaneous, fixed deformation is imposed on a sample, and the stress decay is followed with time. A very useful modification of these two basic techniques involves the use of a periodically varying stress or deformation instead of a constant load or strain. The dynamic responses of the body are measured under such conditions.

### 2.3.3. Steady-State or Simple Shear Flow

Under steady, meaning equilibrium conditions, viscosity data are measured for exactly one shear rate or shear stress. The measurement itself is not influenced by any other external factors. These measurements are therefore performed at constant temperature and shear rate and plotted against time.

In a steady shear experiment, a steady shear stress is applied on the sample and the resultant shear rate is measured. The apparent viscosity is defined as the ratio of the shear stress to the shear and reported as a function of the shear rate (or shear stress):

$$\eta(\dot{\gamma}) = \frac{\tau}{\dot{\gamma}}$$
According to the behavior of $\eta$ as function of $\dot{\gamma}$, the fluid can be classified as Newtonian (constant viscosity), pseudoplastic/shear thinning (viscosity decreases with shear rate), or dilatants/shear thickening (viscosity increases with shear rate) as demonstrated in Figure 10.

![Figure 10: Viscosity as a function of shear rate showing different material response; Newtonian, shear thinning and shear thickening.](image)

In steady measurements the material is deformed by continuous rotation. The cone rotates in one direction at constant or variable shear stress or shear rate. The dynamic viscosity is calculated from the measured velocity or torque and the preset shear stress or strain. The most widely used form of the general viscous constitutive relation for steady shear is the power law model:

$$\tau = m\dot{\gamma}^n \quad \text{or} \quad \eta = m\dot{\gamma}^{n-1}$$

with $m$ representing a function of temperature. Nearly all non-Newtonian materials show shear thinning, $n<1$, but some, particularly concentrated suspensions, show regions of shear thickening, $n>1$. Thickening often signals other complications such as instability, phase separation and lack of reversibility. One of the obvious disadvantages of the power law is that it fails to describe the low shear rate region. Since $n$ is usually less than one, at low shear rate $\eta$ goes to infinity rather than to a constant $\eta_0$ as observed experimentally.

The temperature dependence of viscosity can often be as important as its shear rate dependence.
A relation that is valid over a wide range of temperature is Andrade-Eyring equation:

\[ \eta_0 = Ke^{\frac{E_0}{RT}} \]

This equation was derived from the hypothesis that small molecules move by jumping into unoccupied sites or holes. With polymers, viscosity may be governed by successive jumps of segments of chain.

### 2.3.4. Dynamic or Small Amplitude Oscillatory Shear Flow

The dynamic rheological technique is a useful tool in probing microstructures of materials without disrupting these structures.

A complete description of the viscoelastic properties of a material requires information over very long times. A different experimental approach, called a dynamic experiment, involves stresses and strains that vary periodically. The frequency of this alternation is \( \nu \) cycles/s or \( \omega (= 2\pi \nu) \) rad/s.

Suppose an oscillating strain of frequency \( \omega \) is induced in a sample:

\[ \gamma = \gamma_0 \sin(\omega t) \]

where \( \gamma_0 \) is the maximum amplitude of the strain. In a dynamic experiment, the stress will be directly proportional to the strain if the magnitude of the strain is small enough. Then, if the stress is applied sinusoidally the resulting strain will also vary sinusoidally.

The dynamic experiment is usually carried out using very small strain amplitude and the sample is said to be within the linear viscoelastic (LVE) region. In the LVE region the sample response is independent of the applied strain amplitude and the stress generated due to the sinusoidal shear will again be sinusoidal:

\[ \tau = \tau_0 \sin(\omega t + \delta) \]

Here, \( \delta \) is the phase angle. For elastic solids both stress and strain will be in phase (\( \delta=0^\circ \)) while for Newtonian fluids, the stress and strain will be completely out of phase (\( \delta=90^\circ \)).
Correspondingly, viscoelastic materials exhibit a phase angle between 0 and 90°. Using trigonometric identities, the stress wave can be decomposed into an in-phase and out-of-phase component.

\[ \tau = \tau_0 \cos(\delta) \sin (\omega t) + \tau_0 \sin(\delta) \cos (\omega t) \]

It is convenient to consider the stress as a vector having two components, one in phase with the displacement \(x'\) and one 90° out of phase \(x''\) and to define corresponding in-phase, out-of-phase and the resultant moduli. The sinusoidal motion is illustrated in Figure 11.A. and the vector in Figure 11.B.

The vector moduli in shear are defined by:

\[ G^* = G' + iG'' \]

where \(G^*\) = complex (resultant) modulus, \(G'\) = in-phase or storage modulus, and \(G''\) = out-of-phase or loss modulus.

The in-phase component corresponds to the ability of the material to elastically store energy and the out-of-phase component corresponds to its ability to dissipate energy. Moreover, the shear storage (elastic) modulus \((G')\) and the shear loss (viscous) modulus \((G'')\) is defined as the ratio of the corresponding stress component to the strain amplitude, as follows:

\[ G' = \frac{\tau'}{\gamma_0} = \frac{\tau_0}{\gamma_0} \cos \delta = G^* \cos \delta \]
\[ G'' = \frac{\tau''}{\gamma_0} = \frac{\tau_0}{\gamma_0} \sin \delta = G^* \sin \delta \]
$G'(\omega)$ is associated with the periodic storage in the sinusoidal deformation process, while the loss parameter $G''(\omega)$ on the other hand reflects the non recoverable use of applied mechanical energy to cause flow in the specimen.

$$\tan \delta \text{ (the loss factor or loss tangent)} = \frac{G''}{G'}$$

Tan $\delta$ measures the ratio of the work dissipated as heat to the maximum energy stored in the specimen during one cycle of a periodic deformation. The conversion of applied work to thermal energy in the sample is called damping. It occurs because of flow of macromolecular segments past each other in the sample.

From the magnitudes of the viscous stress to the strain rate, the dynamic viscosity can be defined as: $\eta' = \frac{G''}{\omega}$

An elastic part of the complex viscosity can be also defined: $\eta'' = \frac{G'}{\omega}$

Where the magnitude of complex viscosity is $|\eta'| = \left( \eta'^2 + \eta''^2 \right)^{1/2} = \frac{1}{\omega} |G'|$

The $\eta'(\omega)$ is an energetic dissipation term related to $G''(\omega)$ and has a value approaching that of the steady flow viscosity $\eta$ in very low frequency measurements.

The frequency dependence of $G'$ and $G''$, the dynamic mechanical spectrum (Figure 12), provides the most important information about the microstructure of a material.
Figure 12: Elastic (G') and viscous (G") moduli as function of angular frequency (ω) showing the typical behavior of polymer solutions (melts) and elastic gels.

For example, gels exhibit G’ that is larger than G” with both moduli independent of frequency. Polymer melts show G’ and G” at low frequencies that are dependent on $\omega^2$ and $\omega$, respectively. For viscoelastic materials, the overlap frequency (the frequency at which G’ and G’ curves intersect) gives information about the relaxation time of the system. The plateau modulus, the value of G’ at high frequency, gives information about the strength of the structures formed in the material.

2.3.5. Stress relaxation

Stress relaxation experiments correspond to the situations in which the deformations sketched in Figure 13 are imposed suddenly and held fixed while the resulting stresses are followed with time.

Figure 13. Stress response $\tau$ versus time for a step input in strain $\gamma$. 
When a polymeric liquid is subjected to a step increase in strain, the stress relaxes in an exponential fashion. If a purely viscous liquid is subjected to the same deformation, the stress relaxes instantly to zero as soon as the strain becomes constant. An elastic solid would show no relaxation.

A shear relaxation experiment measures the shear relaxation modulus $G(t)$:

$$G(t) = \frac{\tau(t)}{\gamma_0}$$

where $\gamma_0$ is the constant strain. For small strains, typically $\gamma_0 < 0.5$, all the data shown as a double logarithmic plot fall on the same curve (Figure 14). At short times the relaxation modulus approaches a constant value known as the plateau modulus $G_e$. This linear dependence of stress relaxation on strain is called linear viscoelasticity. Linear viscoelasticity for a particular material can be defined as the region of stress in which strain varies linearly with stress. Concentrated polymeric liquids show a short time relaxation followed by a constant modulus plateau, eventually followed by flow at long times. Molecular weight distribution and long chain branching of the polymer also have a strong effect on the long time relaxation of $G(t)$.

![Figure 14. Stress relaxation data in a log of the time dependent shear modulus is plotted versus log time.](image-url)
For times which are small compared with the relaxation time $\tau$, the elasticity predominates and the modulus is constant. For times which are long compared with $\tau$, the modulus drops to zero, indicating totally viscous behavior.

Experimentally it is difficult to jump the strain $\gamma$ instantaneously to $\gamma_0$. A short rise and stabilization time, typically 0.01-0.1 s is required for current instruments; therefore it is difficult to get data for $t < 0.1$s by stress relaxation methods.

For larger strains, the relaxation modulus is no longer independent of strain:

$$G(t, \gamma) = \frac{\tau(t, \gamma)}{\gamma_0} \text{ for } \gamma > 1$$

This is known as nonlinear viscoelastic behavior. In the linear regime, at small strains or small strain rates, the experimental results can be used to predict results for any volume preserving deformation. For higher strains and strain rates the response is nonlinear. Therefore, there are developed empirical correlations among nonlinear measurements that allow one to predict a useful range of nonlinear behavior.

### 2.3.6. Stress Controlled Rheometer

Shear stress controlled instruments shown in Figure 15 come as torsional or rotational viscometers and high performance capillary rheometers. In a rotational viscometer the test sample is placed between two symmetrical rotating bodies. Mainly plate/plate, plate/cone and coaxial cylinder measuring systems are used.

The force leading to deformation of the sample is defined by the applied torque. Since the test substance exerts a certain resistance to rotation that depends on the viscosity, the measuring probe rotates at a certain speed. The shear rate is calculated from the frequency ($f = \omega/2\pi$) and the geometry of the
measuring system. The viscosity is obtained from the ratio of the shear stress to the shear rate.

![Schematic illustration of a controlled stress rheometer](image)

**Figure 15.** Schematic illustration of a controlled stress rheometer

The temperature is usually controlled by a Peltier element. High cooling and heating rates can be achieved with a Peltier element. The heat generated must be removed via a water bath. Because only the bottom plate is cooled, a temperature gradient builds up in the sample that can be significant depending on the thickness of the sample.

### 2.3.7. Cone and Plate Geometry

The cone and plate (Figure 16) is very useful and simple test geometry due to its homogeneous deformation. It’s very useful for low and high viscosity materials, though the low viscosity is limited by inertia corrections, secondary flow and loss of sample at edges. The attractiveness of the cone and plate geometry lies in the fact that shear rate and shear stress are independent of position and can be easily calculated. The cone-and-plate measuring system has the most advantage of the shear rate across the entire surface is uniform.
Due to the cone angle $\beta$ the ratio of the corresponding radius to the plate gap is constant for every point on the surface of the cone $\tan \beta = \frac{h}{R}$ and for small angles $\beta$ and consequently $\tan \beta$ can always be set equal to $\beta$ (in radians). This means that the shear rate is constant in the cone-plate model across the entire sample: $\dot{\gamma} = \frac{\omega}{\beta}$.

The cone angle must be small to allow the simplification $\tan \beta = \beta$. To prevent wearing of the cone tip and friction arising from contact with the bottom plate, the cone tip is flattened by 30 to 100 µm. The smaller the angle (or gap in a parallel plate system), the higher the maximum shear rate obtainable.

The model has the advantage that a constant shear rate prevails throughout the gap of the cone-plate measuring system but also allows measurement of relatively high shear rates, small sample amounts and easy cleaning. But the cone plate model has one minor disadvantage. Liquids like water are very difficult to handle on the bottom plate because they tend to run off the plate (no raised rim). During the measurement, at the latest, the sample will be expelled from the measuring gap by centripetal forces.
2.4. Transmission Electron Microscopy \(^{12,13}\)

Transmission electron microscopy (TEM) is a microscopy technique whereby a beam of electrons is transmitted through an ultra thin specimen, interacting with the specimen as it passes through. An image is formed from the interaction of the electrons transmitted through the specimen; the image is magnified and focused onto an imaging device, such as a fluorescent screen, on a layer of photographic film.

When electrons are accelerated up to high energy levels (few hundreds keV) and focused on a material, they can scatter or backscatter elastically or inelastically, or produce many interactions, source of different signals such as X-rays, Auger electrons or light (Figure 17). Some of them are used in transmission electron microscopy (TEM).

![Figure 17. Interactions between electrons and material](image)

The scattering processes experienced by electrons during their passage through the specimen determine the kind of information obtained. Elastic scattering involves no energy loss and gives rise to diffraction patterns. Inelastic interactions between primary electrons and sample electrons at heterogeneities such as grain boundaries, dislocations, second phase particles, defects, density variations, etc., cause complex absorption and scattering effects, leading to a
spatial variation in the intensity of the transmitted electrons. In TEM one can switch between imaging the sample and viewing its diffraction pattern by changing the strength of the intermediate lens.

A transmission electron microscope shown in Figure 18 is constituted of: (1) two or three condenser lenses to focus the electron beam on the sample, (2) an objective lens to form the diffraction in the back focal plane and the image of the sample in the image plane, (3) some intermediate lenses to magnify the image or the diffraction pattern on the screen. If the sample is thin (< 200 nm) and constituted of light chemical elements, the image presents a very low contrast when it is focused.

![Ray diagram for a transmission electron microscope in image mode. In diffraction mode, another intermediate lens is inserted to image on the screen the diffraction pattern of the back focal plane.](image)

**Figure 18.** Ray diagram for a transmission electron microscope in image mode. In diffraction mode, another intermediate lens is inserted to image on the screen the diffraction pattern of the back focal plane.

One short coming of TEM is its limited depth resolution. Electron scattering information in a TEM image originates from a three-dimensional sample, but is projected onto a two dimensional detector. Therefore, structure information along the electron beam direction is superimposed at the image plane.
Image contrast is obtained by interaction of the electron beam with the sample. Several contrast effects play a role. In the resulting TEM image denser areas and areas containing heavier elements appear darker due to scattering of the electrons in the sample.

The theoretical resolution of presently used electron microscopes is about hundred thousands times better than that of light microscopes. Additionally, the great advantage the electron microscope offers is about a 1000 fold increase in resolution and a 100 fold increase in depth of field.

There are several disadvantages of electron optics, though. Electrons are high-energy particles, which will easily be affected by any matter they encounter. When they do encounter matter, the interaction results in the emission of all the lower forms of energy, therefore, electrons cannot penetrate a specimen very deeply. In addition, for that reason a microscope has to be kept at a high vacuum. The TEM is ideal for studies in ‘synthetic’ systems, yet the disadvantage exists for biological samples, where the specimen is always dead.
2.5. References

Chapter 3

EXPERIMENTAL PART
3.1. MATERIALS AND METHODS

3.1.1. Introduction

The main objective of the present work was to explore the self-organization ability of the multiblock copolymers to form injectable reversible hydrogels and especially to answer to the question how the block copolymer topology affects the gelation efficiency. Synthetic hydrogels are of considerable current attention.[1] However, their structure is usually poorly controlled: the comonomer distribution in the case of copolymer hydrogels is not uniform. Improved structures may broaden hydrogels applications and provide a better understanding of the relationship between their structure and properties.

The novelty of this work is that model multiblock copolymers with uniform and precisely known length of the building blocks could reveal the importance of topology on the mechanism of association towards the formation of reversible hydrogels.

A series of model asymmetric ABA, BABAB, ABABABA and BABABABAB (A hydrophobic, B polyelectrolyte) block copolymers with controlled molecular characteristics of the A and B blocks were used in this study.

Details of the synthesis are reported elsewhere [2]. The building blocks of the copolymers was, the hydrophilic (ionisable) poly[2-(dimethylamino)ethyl methacrylate] block (DMA, with 25 repeating units) and the hydrophobic poly(n-butyl methacrylate) block (BuMA, with 5 repeating units).
The chemical formulas and the names of the two monomers used are shown in Figure 19:

![Chemical structures and names of the monomers used as building blocks of the copolymers.](image)

Figure 19. Chemical structures and names of the monomers used as building blocks of the copolymers.

Four copolymers of three, five, seven and nine blocks, were prepared with the following macromolecular architecture shown in Figure 20.

![Schematic representation of the structures block copolymers in this study.](image)

Figure 20. Schematic representation of the structures block copolymers in this study. The blue color represents the DMA units, the red color the BuMA units.

### 3.1.2. Polymer Selection Criteria

(2-Dimethylamino)ethyl methacrylate (DMA) has aliphatic tertiary amino groups, and after polymerization PDMA forms typical stimuli-responsive polymers exhibiting a combined temperature and pH sensitivity. PDMA homopolymer is a weak cationic polyelectrolyte with a $pK_a$ at about 7.0 due to protonation of the tertiary amine group. At pH 8 or above, DMA homopolymer has very low or zero charge density, and hydrogen bonding is solely responsible for its water
solubility. Under these conditions, the neutral DMA chains exhibit a temperature-dependent solubility and has a lower critical solution temperature (LCST) in the range from 32 °C to 53 °C depending on the molecular weight, pH, and salt concentration. [3,4] Its thermally responsiveness and LCST, very close to average human body temperature, make it a promising candidate for drug delivery. [5]

A number of recent studies concern block copolymers bearing the ionizable hydrophilic 2-(dimethylamino)ethyl methacrylate (DMA) and/or its diethyl homologue (DEAEMA) as one of the blocks. PDMA-containing block copolymers include amphiphilic block copolymers PDMA-PMMA, [1] doublehydrophilic diblock copolymers P2VP-b-PDMA, [6] and/or PDMA-b-PDEAEMA [4] with interesting responsive micellar properties, PMMA-PDMA-PMMA model chemically cross-linked gels, [7,8] and finally biocompatible macromolecules capable of transferring genetic materials to cells. [9]

At low pH, PDMA is protonated and transformed to a positively charged polyelectrolyte, PDMA is water-soluble over the whole pH range, and it is partially protonated in the range of physiological pH (7.2). Therefore, this homopolymer is a nice candidate for macromolecular engineering of polymers suitable for biomedical applications such as carriers of nucleic acid [3] and/or DNA. [9]

Methacrylate hydrogels have been increasingly studied as matrices for tissue engineering. Hydrogels designed for use as tissue engineering scaffolds may contain pores large enough to accommodate living cells, or they may be designed to dissolve or degrade away, releasing growth factors and creating pores into which living cells may penetrate and proliferate. Poly (n-butyl methacrylate) [PBMA] non-degradable polymer has been used in bone cements because of such advantages as lower exotherm, higher fracture toughness and superior fatigue life as well as lower toxicity of soft tissues and dental pulp. [10] Poly n-butyl methacrylate has also the advantage with respect to PMMA that exhibits lower glass transition temperature at about 20 °C. Poly n-butyl methacrylate is also preferred as a building block for associative polymers because is more hydrophobic due to n-buthyl pendant groups.
3.1.3. Light Scattering Measurements

**Static Light Scattering.** The static light scattering (SLS) experiments were carried out using a thermally regulated ((0.1 °C) BI-200SM goniometer provided by Brookhaven Instruments Corporation equipped with a He-Ne laser (632.8 nm).

**Dynamic Light Scattering.** Autocorrelation functions \( g(q,t) \) were measured with a full multiple digital correlator (ALV-5000/FAST) equipped with 280 channels. The light source was an argon ion laser (Spectra Physics 2020) operating in single mode at 488 nm with a constant output power of about 150 mW. The correlation functions were analyzed by the constrained regularized CONTIN method through CoVA-Jacek Gapinski 2001 software for measurements at 90°.

For the angular dependence measurements the autocorrelation functions had been measured with a BI-9000AT/ Turbocorr digital correlator from a light source of He-Ne laser (632.8 nm). CONTIN analysis was performed through BI-DLSW software.

The apparent diffusion coefficients, \( D_{\text{app}} = \Gamma / q^2 \ (q = (4\pi n/\lambda) \sin(\theta/2), \ n \text{ is the refractive index of the solvent}) \), were determined at the peak of the decay rate distributions and the apparent hydrodynamic radii via the Stokes-Einstein equation:

\[
R_{h,\text{app}} = k_B T / (6\pi \eta D_{\text{app}})
\]

where \( k_B \) is the Boltzmann constant and \( \eta \) is the viscosity of the solvent at temperature \( T \).
3.1.4. Transmission Electron Microscopy

High Resolution Transmission Electron Microscopy (TEM) experiments were carried out on a JEM 2100 microscope operating at 200 kV. Aqueous aggregate solutions were used to prepare the TEM samples. A drop of the dilute solution was placed onto copper grids precoated with carbon and let evaporate at room temperature. TEM images give a direct visualization of the particles which provides direct information on the size, shape, dispersion, structure and morphology of nanosystems.

3.1.5. Rheology

The rheological measurements were carried out using a stress controlled Rheometric Scientific SR 200 rheometer, equipped with a cone and plate geometry (diameter = 20 mm, cone angle = 4°, truncation = 11 µm). After each sample loading, a delay of 5 minutes was applied prior to any measurement, in order to erase the mechanical history. The temperature, fixed at 25 ± 0.1 °C, was controlled by a water bath circulator.

The linear viscoelastic regime was established by oscillatory strain sweeps using different frequencies between 0.628 and 62.8 rad/s. Dynamic oscillatory sweeps were made in linear regime at 25 °C. Steady shear measurements were similarly made at 25 °C.

The temperature ramp and the stress relaxation tests were performed with a stress controlled rheometer (AR-2000ex, TA Instruments) in a cone-plate geometry with a steel cone of radius R = 3.0 cm and angle 2°. Samples were loaded such that the normal stress never exceeded 0.1 N.
In order to probe the temperature-dependent rheological properties, heating of the samples was done with a rate of 1 °C/min at oscillation frequency ($\omega$) of 6.283 rad/s.

The rheometer is equipped with a Peltier control system that allows accurate control ±0.1° of temperature. A solvent trap was used to minimize changes in concentrations due to water evaporation.

### 3.1.6. Sample Preparation

For the static and dynamic light scattering experiments the most concentrated sample solutions were directly prepared to the final desired concentration. For the light scattering measurements a mother solution with a concentration of 0.1 wt % was prepared. The proper amount of polymer was weighed in a screw-capped vial. First, an equivalent amount of HCl (1 N) was added to ionize the tertiary amine groups of the PDMAEMA block, and then triply distilled water (Millipore) was added to the final volume. Samples of lower polymer concentration were obtained by the appropriate dilution of the more concentrated solutions with aqueous HCl solution of a given pH and equilibration at room temperature for several hours. Several concentrations ranging from 0.1 to 0.02 wt % were prepared by dilution. The ionic strength of the solutions was adjusted to 0.1; 0.2; 0.3; 0.5 and 1M NaCl. The samples were left 24 h for equilibration prior the measurements. Finally the solutions were carefully filtered through 0.45 µm Millipore filters.

For the rheological experiments the solutions were directly prepared to the final desired concentration following the same procedure. Vigorous stirring and centrifugations were used for homogenation.
3.2. SYNTHESIS AND CHARACTERIZATION

3.2.1. Synthesis

A series of model asymmetric ABA, BABAB, ABABABA and BABABABAB (A hydrophobic, B polyelectrolyte) block copolymers were synthesized by group transfer living polymerization and sequential monomer addition in order to control the molecular characteristics of the A and B blocks.

Four copolymers of three, five, seven and nine blocks, were prepared with the following macromolecular architecture:

\[ \text{BuMA}_5 \text{-b-DMA}_{25} \text{-b-BuMA}_5 \]
\[ \text{DMA}_{25} \text{-b-BuMA}_5 \text{-b-DMA}_{25} \text{-b-BuMA}_5 \text{-b-DMA}_{25} \]
\[ \text{BuMA}_5 \text{-b-DMA}_{25} \text{-b-BuMA}_5 \text{-b-DMA}_{25} \text{-b-BuMA}_5 \]
\[ \text{DMA}_{25} \text{-b-BuMA}_5 \text{-b-DMA}_{25} \text{-b-BuMA}_5 \text{-b-DMA}_{25} \text{-b-BuMA}_5 \]

Group Transfer Polymerization (GTP) is a controlled living polymerization method suitable for the preparation of block copolymers composed of methacrylic repeating units. It has been applied on an industrial scale (DuPont) for the fabrication of amphiphilic block copolymer dispersing agents for pigmented water based inks. GTP proceeds through an anionic dissociative mechanism in the presence of nucleophilic anions as catalysts. The method demonstrates the fundamental characteristics of a ‘living’ polymerization, namely narrow molecular weight distribution, control of molecular weight derived from the monomer/initiator stoechiometry, and the ability to construct block copolymers. One of the advantages of GTP versus classical anionic polymerization apart from the experimental conditions (i.e. polymerization at room temperature) consists of the ease of block copolymerization by sequential addition of the monomers irrespective of the order of monomer addition.[11,12]
Model amphiphilic block copolymers of the hydrophilic ionizable 2-(dimethylamino)ethyl methacrylate (DMA, 25 nominal units per block) and the hydrophobic n-butyl methacrylate (BuMA, 5 nominal units per block) bearing three, five, seven and nine blocks were synthesized using group transfer polymerization as shown in Figure 21.

1,4 -Bis(methoxytrimethylsiloxy)methylene)cyclohexane was used as the bifunctional initiator.[2]

Figure 21. Synthetic sequential addition procedure and structures of the multiblock copolymers of this study. D and Bu are further abbreviations for DMA and BuMA, respectively.

### 3.2.2. Characterization

Samples of the multiblock copolymers were characterized in terms of their \( M_W \) and composition using GPC and \(^1\)H NMR, respectively. GPC was performed on a Polymer Laboratories system equipped with a Waters 515 isocratic pump, an ERC-7515A Polymer Laboratories refractive index detector and a PL Mixed “D” column. The eluent was THF, pumped at 1 mL min\(^{-1}\). The instrumentation was calibrated using poly(methyl methacrylate) (PMMA) standards supplied by Polymer Laboratories.

The \(^1\)H NMR spectra of the polymer precursors in CDCl\(_3\) were recorded using a 300 MHz Avance Bruker spectrometer equipped with an Ultrashield magnet. The copolymer composition was calculated from the ratio of the peak area due to the two azamethylene protons in DMA at 2.6 ppm to the peak area due to the two oxymethylene protons of BuMA at 3.9 ppm.[2]
Table 2 shows the number-average MWs, Mns and the compositions of the multiblock copolymers. The Mns were close to, but systematically higher than, the theoretically expected MWs calculated from the monomer/initiator ratios. This indicates some initiator deactivation. The molecular weight distributions (MWDs) were narrow with polydispersity indices (PDIs, Mw/Mn) equal to or lower than 1.25 in all cases. This confirms the homogeneity of the lengths of the segments.

Table 2. Molecular weights and compositions of the multiblock copolymers based on DMA and BuMA, synthesized via GTP

<table>
<thead>
<tr>
<th>No.</th>
<th>Polymer formula(^a)</th>
<th>Theoretical MW(^b)</th>
<th>GPC Results</th>
<th>(% mol) BuMA</th>
<th>(^1)H NMR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>M(_n)</td>
<td>M(_w)/M(_n)</td>
<td>Theoretical</td>
</tr>
<tr>
<td>1</td>
<td>D(_{25})</td>
<td>4129</td>
<td>5550</td>
<td>1.15</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Bu(<em>{5})-b-D(</em>{25})-b-Bu(_{5})</td>
<td>5551</td>
<td>7285</td>
<td>1.17</td>
<td>28.6</td>
</tr>
<tr>
<td>3</td>
<td>D(<em>{25})-b-Bu(</em>{5})-b-D(<em>{25})-b-Bu(</em>{5})-b-D(_{25})</td>
<td>13412</td>
<td>19614</td>
<td>1.31</td>
<td>11.8</td>
</tr>
<tr>
<td>4</td>
<td>Bu(<em>{5})-b-D(</em>{25})-b-Bu(<em>{5})-b-D(</em>{25})-b-Bu(<em>{5})-b-D(</em>{25})-b-Bu(_{5})</td>
<td>14834</td>
<td>21515</td>
<td>1.32</td>
<td>21.1</td>
</tr>
<tr>
<td>5</td>
<td>D(<em>{25})-b-Bu(</em>{5})-b-D(<em>{25})-b-Bu(</em>{5})-b-D(<em>{25})-b-Bu(</em>{5})-b-D(<em>{25})-b-Bu(</em>{5})-b-D(<em>{25})-b-Bu(</em>{5})-b-D(_{25})</td>
<td>22695</td>
<td>33157</td>
<td>1.23</td>
<td>13.8</td>
</tr>
</tbody>
</table>

\(^{a}\) D: DMA, Bu: BuMA

\(^{b}\) The contribution from the initiator fragment of 198 g mol\(^{-1}\) was included in the calculation.

Figure 22 presents the GPC traces for each multiblock copolymer and its precursors. The chromatogram of the block copolymers is shifted to shorter elution times (higher MWs) compared to its precursors, as expected. All chromatograms are unimodal.
Figure 22. Gpc traces together with the architecture of the linear multiblock copolymers and the corresponding precursors for each block copolymer: (a) triblock, (b) pentablock, (c) heptablock and (d) nonablock.

However, the broad distribution of the peaks corresponding to the five, seven and nine block copolymers, indicating a higher polydispersity, arising from some termination during the blocking reaction. Thus, the growth of the two end-blocks of the multiblock occurred successfully, without significant termination.

The percentage compositions determined from $^1$H NMR were close to those expected theoretically based on the comonomer feed ratio, confirming full monomer-to-polymer conversion during synthesis. The DMA content in the
copolymers was kept relatively high, at 70 mol% or higher, to secure water-compatibility even when the DMA units were neutral.

3.2.3. Hydrogen Ion Titration

Representative hydrogen ion titration curves of the homopolymer and the corresponding copolymers are shown in Figure 23. The structure of each copolymer is indicated above each plot. The pK\textsubscript{a} can be calculated as the pH in which the DMA blocks are half ionized. The behavior is typical of weak polyelectrolytes, for which the degree of ionization is determined by the solution pH.
Figure 23. pH dependence with the degree of ionization for the multiblock copolymers

The degree of ionization of each sample was calculated as the number of HCl equivalents added divided by the number of DMA unit equivalents (calculated from the polymer dry mass and composition) present in the sample. The acid titration curves were obtained by plotting the calculated degrees of ionization against the measured solution pH. The effective pK of the DMA units of each multiblock copolymer was estimated from its acid titration curve as the pH at 50% ionization are presented in Table 2. The effective pKs of the DMA units in all polymers were determined to be around 7.0, in agreement with previous work.

Table 3. pKs of the DMAEMA monomer repeating units in the multiblock copolymers

<table>
<thead>
<tr>
<th>No.</th>
<th>Polymer formula</th>
<th>pK</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>D_{25}</td>
<td>7.3</td>
</tr>
<tr>
<td>2</td>
<td>Bu_{5}-b-D_{25}-b-Bu_{5}</td>
<td>6.9</td>
</tr>
<tr>
<td>3</td>
<td>D_{25}-b-Bu_{5}-b-D_{25}-b-Bu_{5}-b-D_{25}</td>
<td>7.1</td>
</tr>
<tr>
<td>4</td>
<td>Bu_{5}-b-D_{25}-b-Bu_{5}-b-D_{25}-b-Bu_{5}-b-D_{25}-b-Bu_{5}-b-D_{25}</td>
<td>7</td>
</tr>
<tr>
<td>5</td>
<td>D_{25}-b-Bu_{5}-b-D_{25}-b-Bu_{5}-b-D_{25}-b-Bu_{5}-b-D_{25}-b-Bu_{5}-b-D_{25}</td>
<td>7</td>
</tr>
</tbody>
</table>

The charge density of weak polyelectrolytes is dependent on the solution pH. The dissociation behavior of each functional group along the chain is affected by the dissociation behavior of its nearest neighbors. Intuitively, the apparent basicity of the weak polybase increases with progressive ionization of the polymer because of the difficulty of attaching protons from polyions with increasing charge. Hence, the acid/base equilibrium of the chains is described by a modified version of the Henderson–Haselbalch equation:
The $pK_a(app)$ is an apparent dissociation constant that reflects the overall acid/base equilibrium of the polyelectrolyte chains, and $\alpha$ is the degree of dissociation. The parameter $n$ is related to the extension of the polymer chains, which is related to their charge density.[13]

For low pH, a fully charged system will be obtained due to the ionization of the DMA units which resulted in electrostatic repulsions between the positively charged chains, and in the build-up of an osmotic pressure created by the counterions to the charges. For high pH values the tertiary amine will remain uncharged and the polyelectrolyte will resemble uncharged system.

The stabilization of an intermediate state of alternating protonated and deprotonated groups leads to an intermediate plateau in the titration curve.

\[
pH = pK_a(app) - n \log \left( \frac{1-\alpha}{\alpha} \right)
\]
3.3. References

Chapter 4

ASSOCIATION PHENOMENA IN DILUTE AQUEOUS REGIME
4.1. Introduction

The main objective of the present work was to explore the self-organization ability of the multiblock copolymers to form injectable reversible hydrogels and especially to answer to the question how the block copolymer topology affects the gelation efficiency.

For this purpose polymer aqueous solutions of controlled pH, temperature and ionic strength conditions were prepared and investigated in aqueous solutions by the means of static and dynamic light scattering, (SLS, DLS), Transmission Electron Microscopy and Rheology .

The association ability of these amphiphilic polyelectrolytes was first investigated through rheological measurements. Salt free solutions of 5 wt% polymer concentration in water of pH 6 were prepared for all samples. From optical inspection of the solutions, it was observed that only the heptablock copolymer form a free-standing transparent physical gel. This is also reflected in the viscosity profiles of the solutions depicted in Figure 24. The profiles of all copolymers out of the heptablock show Newtonian behaviour with viscosities lower than 50 mPa.s. In the contrary the heptablock copolymer solution exhibits the behaviour of a structured fluid as can be observed in Figure 24b by its complex response to the applied shear stress.

Figure 24. (a) Viscosity (in mPa.s) as a function of inclination angle (shear rate) for triblock (B₅-D₂₅-B₅), pentablock (D₂₅-B₅-D₂₅-B₅-D₂₅) and nonablock (D₂₅-B₅-D₂₅-B₅-D₂₅-B₅-D₂₅-B₅) copolymer aqueous solutions. (b) Steady state shear viscosity as a function of shear stress for the heptablock (B₅-D₂₅-B₅-D₂₅-B₅-D₂₅-B₅) copolymer aqueous solutions. In all cases the polymer concentration was 5 wt%, pH 6 and 25°C.
The viscosity profile is characterized by several distinct regions. A Newtonian plateau is observed at low stresses followed by a remarkable shear-thickening and an abrupt shear-thinning effect, marked by several orders of magnitude viscosity drop. These results reveal that only the heptablock, bearing hydrophobic groups at both ends, can form a three-dimensional transient network through intermolecular hydrophobic association of the end-blocks. The significant shear-thinning effect, marked by an apparent yield stress ($\tau_Y$), is consistent with the breakage of the infinite 3D structure into finite-size clusters or microgels. This behaviour resembles to that of telechelic polyelectrolytes capable to form injectable hydrogels [1].

**Figure 25.** Digital photographs of the pentablock and the nonablock solutions at 10 wt% (left image) and a free standing gel of heptablock at 4 wt% (right image).

In the digital photographs (Figure 25) it is depicted a free standing gel formed by the heptablock at 4wt%, while for the pentablock and the nonablock no gel formation was observed even at high polymer concentration (i.e. $C_P=10$ wt %). At first sight, the nature of the end-blocks of the multiblock copolymer seems to be the key factor for the network formation.

### 4.2. Study of the Association Mechanism by Light Scattering

In order to better understand the association efficiency of these amphiphilic copolymers and to elucidate the influence of their molecular characteristics on the association mechanism they were examined by Light Scattering.
In the following all the experiments were carried out at pH 6 where the PDMA middle block was protonated about 90% to ensure the polyelectrolyte character of the associative polymer.

Static Light Scattering experiments were performed in order to determine the characteristics of the aggregates, the apparent aggregation number ($N_{agg}$) and the radius of gyration ($R_g$). In Figure 26, the concentration dependence of the inverse scattering intensity extrapolated to zero angle, $(Kc/\Delta I)_{\theta=0}$ (inset of Fig. 26) where $K$ is the optical constant and $I$ the scattering intensity difference between the solution and the solvent, is shown.

**Figure 26.** Inverse scattering intensity extrapolated to zero angle as a function of concentration of A. $B_5$-$D_{25}$-$B_5$-$D_{25}$-$B_5$-$B_5$, B. $D_{25}$-$B_5$-$D_{25}$-$B_5$-$D_{25}$, C. $D_{25}$-$B_5$-$D_{25}$-$B_5$-$D_{25}$-$B_5$-$D_{25}$ in salt free aqueous solutions of pH=6. Inset: angle $\theta$ dependence of inverse scattering intensity for different concentrations.
From the extrapolated value of zero concentration, the apparent molecular weight of the aggregates and therefore the aggregation number \(N_{\text{agg}} = \frac{M_{\text{w,micelle}}}{M_{\text{w,unimer}}}\) can be estimated. The value of \(M_{\text{w(app)}}\) was found \(9.88 \times 10^7\) for the heptablock (Figure 26.A), which gave a mean \(N_{\text{agg}}\) equal to 2880. Additionally, the pentablock, (Figure 26.B), and the nonablock, (Figure 26.C) presents a significantly lower aggregation number.

Finally the apparent radius of gyration of the aggregates was calculated by the slope of the linear part of the q dependence of the inverse scattering intensity extrapolated to zero concentration \((Kc/\Delta I)_{c=0}\), \(R_g\) was found to be 722nm for the heptablock. However, as it can be observed in the inset of Figure 26 the angular dependence of the inverse scattering intensity was not regular, with a slope variation with the concentration, suggesting high polydispersity of the aggregates. Therefore the radius of gyration determined by SLS represents average values. In all cases these values are much higher than the contour length of the PDMAEMA polyelectrolyte blocks (~19nm for the pentablock and the heptablock and ~31 nm for the nonablock), suggesting the formation of nonmicellar aggregates.

The characteristics of the aggregates determined by Static Light Scattering at pH=6, are presented in Table 4.

<table>
<thead>
<tr>
<th>No.</th>
<th>Polymer formula</th>
<th>(M_w)</th>
<th>(N_{\text{agg}})</th>
<th>(R_g/\text{nm})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(D_{25}^-B_5^-D_{25}^-B_5^-D_{25})</td>
<td>(3.3 \times 10^5)</td>
<td>16</td>
<td>138</td>
</tr>
<tr>
<td>2</td>
<td>(B_5^-D_{25}^-B_5^-D_{25}^-B_5^-D_{25}^-B_5)</td>
<td>(9.88 \times 10^7)</td>
<td>2880</td>
<td>722</td>
</tr>
<tr>
<td>3</td>
<td>(D_{25}^-B_5^-D_{25}^-B_5^-D_{25}^-B_5^-D_{25}^-B_5^-D_{25})</td>
<td>(8.6 \times 10^5)</td>
<td>23</td>
<td>241</td>
</tr>
</tbody>
</table>
In order to come to a deeper understanding of this behaviour Dynamic Light Scattering study was performed.

**4.2.1. Pentablock D_{25}\text{-}B_{5}\text{-}D_{25}\text{-}B_{5}\text{-}D_{25}**

In Figure 28 the distribution of relaxation times, obtained by CONTIN analysis of the correlation functions (Figure 27) at a scattering angle of 90° for five different concentrations for the pentablock is depicted. Monomodal distribution of the relaxation times has been observed in all the range of concentration studied.

![Graph](image-url)

**Figure 27.** The representative normalized correlation functions, for concentrations of (a) 0.2 mg/mL; (b) 0.4 mg/mL; (c) 0.6 mg/mL; (d) 0.8 mg/mL and (e) 1 mg/mL aqueous polymeric solution of pentablock D_{25}\text{-}B_{5}\text{-}D_{25}\text{-}B_{5}\text{-}D_{25}, at pH 6 and scattering angle 90°.
A single relaxation mode was appeared attributed to the diffusion of aggregates. The corresponding apparent hydrodynamic radius was $R_H=60\text{nm}$, as calculated using the Stoke-Einstein equation for spherical particles, and
extrapolation to zero concentration as depicted in Figure 29. In all the concentration range studied monomodal distribution functions were obtained.

![Graph showing variation of apparent hydrodynamic radius as a function of concentration for the aqueous solution of the D$_{25}$B$_5$-D$_{25}$B$_5$-D$_{25}$ pentablock, pH=6.]

**Figure 29.** Variation of apparent hydrodynamic radius as a function of concentration for the aqueous solution of the D$_{25}$B$_5$-D$_{25}$B$_5$-D$_{25}$ pentablock, pH=6.

### 4.2.2 Heptablock B$_5$-D$_{25}$-B$_5$-D$_{25}$-B$_5$-D$_{25}$-B$_5$

For the heptablock B$_5$-D$_{25}$-B$_5$-D$_{25}$-B$_5$-D$_{25}$-B$_5$ in the entire range of dilute solutions CONTIN analysis shows two relaxation time distributions as illustrated in Figures 30 and 31.

![Graph showing representative normalized correlation functions for concentrations of (a) 0.2 mg/ml; (b) 0.4 mg/ml; (c) 0.6 mg/ml; (d) 0.8 mg/ml and (e) 1 mg/ml aqueous polymeric solution of heptablock B$_5$-D$_{25}$-B$_5$-D$_{25}$-B$_5$-D$_{25}$-B$_5$, at pH 6 and scattering angle 90°.]

**Figure 30.** The representative normalized correlation functions, for concentrations of (a) 0.2 mg/ml; (b) 0.4 mg/ml; (c) 0.6 mg/ml; (d) 0.8 mg/ml and (e) 1 mg/ml aqueous polymeric solution of heptablock B$_5$-D$_{25}$-B$_5$-D$_{25}$-B$_5$-D$_{25}$-B$_5$, at pH 6 and scattering angle 90°.
Figure 31. The inverse Laplace transformation performed by CONTIN analysis, for concentrations of (a) 0.2 mg/ml; (b) 0.4 mg/ml; (c) 0.6 mg/ml; (d) 0.8 mg/ml and (e) 1mg/ml aqueous polymeric solution of heptablock B\textsubscript{5}-D\textsubscript{25}-B\textsubscript{5}-D\textsubscript{25}-B\textsubscript{5} at pH 6 and scattering angle 90\degree.

At low concentrations (i.e. $c_p=0.2\text{mg/ml}$) two well dissolved relaxation peaks appeared with hydrodynamic sizes of $R_H=84\text{ nm}$ and $R_H=549\text{ nm}$ as calculated using the Stokes-Einstein equation for spherical particles. As the concentration is increased the population of the smaller aggregates decreases at
the expense of bigger ones. The fast relaxation mode should be correlated with small aggregates. The second slow mode relaxation peak corresponds to clusters of aggregates.

Inspection of the angular dependence (Figure 32.a.) of relaxation rates $\Gamma$ ($=1/\tau$) of the fast mode (Figure 32.b.) revealed a $q^2$ dependence at low $q$ which is consistent with the diffusive relaxation process of the aggregates.

The second slow mode relaxation peak demonstrates the onset of bigger aggregate formation, i.e. clusters arising through extending association of dangling sticky ends of the smaller aggregates. In both cases these relaxation modes are of diffusive character (linear at low angles Figure 32.(b) and (c)) corresponding to populations of different hydrodynamic size entities. Similar results have been observed for PMMA-PDMA-PMMA block copolymers revealing two types of associates at dilute acid solutions, which had been attributed to an ‘end-to-end’ association of low number of molecules, and to “hairy” loose aggregates as visualized by AFM. These types of association had been attributed to the stretched conformation of the PDMA, arisen from the repulsive electrostatic interactions, and the relatively short length of PDMA, implying small number of Kuhn segments that prevent looping.[2] It seems that the same association mechanism is valid for the present system.
Figure 32. Representative normalized correlation functions dependence with the scattering angle (a) and characteristic $q^2$ dependence of relaxation rate $\Gamma$ for a concentration $c=1$ mg/ml associated with (b) the first relaxation process and, (c) the second relaxation process of Fig.23.II.e.

The corresponding apparent hydrodynamic sizes of the two populations (assuming translational diffusion of particles) and by extrapolation to zero concentration were determined at 100nm and 518 nm as depicted in Figure 33.

Figure 33. Variation of apparent hydrodynamic radius corresponding to the fast relaxation mode (▲) and to the slow relaxation mode (▲) as a function of concentration for the aqueous solution of the B$_5$-D$_{25}$-B$_5$-D$_{25}$-B$_5$-D$_{25}$-B$_5$ heptablock, pH=6.

The appearance of a secondary population of large aggregates in the heptablock comparing with its precursor (pentablock) it’s ascribed to the additional hydrophobic end-blocks which contribute to further intermolecular association of the hydrophobic BuMA stickers leading to a particle size increase.
DLS was employed to follow the association mechanism of the nonablock DMA$_{25}$-BuMA$_{5}$-DMA$_{25}$-BuMA$_{5}$-DMA$_{25}$-BuMA$_{5}$-DMA$_{25}$ in a range of concentrations from 0.02 to 0.1 wt%. Characteristic plots of the autocorrelation functions and the corresponding inverse Laplace transformation performed by CONTIN are presented in figure 34 and 35.

**Figure 34.** The representative normalized correlation functions, for concentrations of (a) 0.2 mg/ml; (b) 0.4 mg/ml; (c) 0.6 mg/ml; (d) 0.8 mg/ml and (e) 1 mg/ml aqueous polymeric solution of nonablock D$_{25}$-B$_{5}$-D$_{25}$-B$_{5}$-D$_{25}$-B$_{5}$-D$_{25}$, at pH 6 and scattering angle 90°.
Figure 35. The inverse Laplace transformation performed by CONTIN analysis, for concentrations of (a) 0.2 mg/ml; (b) 0.4 mg/ml; (c) 0.6 mg/ml; (d) 0.8 mg/ml and (e) 1 mg/ml aqueous polymeric solution of nonablock D_{25}-B_{5}-D_{25}-B_{5}-D_{25}-B_{5}-D_{25}-B_{5}-D_{25}, at pH 6 and scattering angle 90°.

As depicted in Figure 35 bimodal distribution of the relaxation times corresponding to two decay times of the correlation functions (Figure 34) can be observed at low concentrations (0.2 - 0.6 mg/ml) of the nonablock. As the concentration is increased the population of the bigger aggregates decreases at the expense of smaller ones. At higher concentrations (i.e. c_P = 0.8 and 1 mg/ml) only a single mode relaxation peak is observed.

The inspection of the relaxation rate as a function of scattering wave vector revealed a q^2 dependence at low q which is consistent with the diffusive relaxation process of the micelles. The angular dependence of relaxation rate Γ (=1/t) of the slow relaxation mode for a c_P=0.8 mg/ml is depicted in Figure 36.
Figure 36. $q^2$ dependence of relaxation rate $\Gamma$ for a concentration $c=0.8$ mg/ml associated with the relaxation process of Fig. 35.d.

The slope of the plot yields a translational diffusion coefficient of $D_{\text{app}} = 4.60379 \times 10^{-13}$ m$^2$/s corresponding to a hydrodynamic radius of $R_H = 532$ nm.

The average relaxation time was determined from the peak of the relaxations curves. The apparent hydrodynamic radius was calculated through Stokes-Einstein equation and plotted vs concentration. Extrapolation of the data to zero polymer concentration (essentially equivalent to zero particle concentration) gave the values of the hydrodynamic radius $R_H$ of the aggregates. The apparent $R_H$ corresponding to the fast relaxation mode was found 108 nm comparable with that of the heptablock (100nm), while for the slow mode relaxation peak an $R_H = 739$nm was observed corresponding to big aggregates. (Figure 37)

In salt free conditions polyelectrolytes exhibit expansion at low polymer concentration. This phenomenon (i.e. polyelectrolyte effect) is caused by a decrease in concentration of closely associated counterions surrounding the polyelectrolyte proportional to the decrease in polyelectrolyte concentration.[3]
The slope of the curve of Figure 37, corresponding to the slow diffusion mode, is significant negative revealing strong polyelectrolyte effect for the nonablock. This effect induces significant uncertainties on the determination of the real size of the associates and has to be suppressed by increasing the ionic strength of the medium.

4.3. Influence of Ionic Strength on the Association of Model Multiblock Copolymers

The amphiphilic multiblocks studied also present important dependence on its properties in aqueous solutions, from the effect of external stimulus, such as addition of salt. In solutions containing charged polymers at various concentrations of added salt the dynamics is much more complex. The strength of electrostatic interactions present at low and intermediate salt concentrations increases with decreasing salt content.

The conformation of a flexible polyelectrolyte can be changed by varying the added low molar mass salt. The addition of low molar mass salt results in the screening of the electric charges on the polyion. Short-range electrostatic
interactions are incorporated in the electrostatic persistence length while long range electrostatic interactions are involved in the excluded volume. The number of effective charges on the chain can be smaller than the nominal number of fixed charges on the chain due to counterion condensation.[4,5]

Flexible polyelectrolytes are characterized by a small intrinsic persistence length. In polyelectrolyte theory the total persistence length is the sum of the intrinsic persistence length and the electrostatic persistence length. The intrinsic persistence length represents the stiffness of the uncharged chain. The electrostatic persistence length is due to the electric charges on the chain.

For charged associative polymers, the addition of salt is found to screen electrostatic interactions in the micelle corona to cause the thickness to decrease. Addition of salt also promotes larger micellar aggregation numbers with higher number of charged blocks because of electrostatic screening of repulsions between chains.[6,7]

For Light Scattering measurements with polyelectrolyte macromolecules, it is often convenient to adjust the supporting electrolyte composition to a level such that $k^{-1}$ is comparable to the geometric dimensions of a chain element. In that case, the effects of intramolecular electrostatic interactions on the chain dimensions will be largely suppressed. For flexible polyelectrolytes in the ionic strength range from 0.001 up to 0.5 M the ionic strength dependence of the electrostatic persistence length shows a scaling behaviour close to $k^{-1}$.[8]
Static Light Scattering experiments were performed in order to determine the characteristics of the aggregates in the presence of salt. In Figure 38, the concentration dependence of the inverse scattering intensity extrapolated to zero angle, \((Kc/\Delta I)_{\theta=0}\) (inset of Fig.38) is shown.

![Graph](image_url)

**Figure 38.** Inverse scattering intensity extrapolated to zero angle as a function of concentration of A. B$_5$-D$_{25}$-B$_5$-D$_{25}$-B$_5$-D$_{25}$-B$_5$, B. D$_{25}$-B$_5$-D$_{25}$-B$_5$-D$_{25}$, C. D$_{25}$-B$_5$-D$_{25}$-B$_5$-D$_{25}$-B$_5$-D$_{25}$-B$_5$-D$_{25}$-D$_{25}$ in aqueous solutions of pH=6 and NaCl 0,1M. Inset: angle \(\theta\) dependence of inverse scattering intensity for different concentrations.

From the extrapolated value of zero concentration, the apparent molecular weight and therefore the aggregation number has been estimated. The characteristics of the systems determined by Static Light Scattering of aqueous solutions, at pH=6, salt free and in 0,1M NaCl are presented in Table 5.
Table 5. Characteristics of polymeric aggregates determined by SLS at pH=6 and different ionic strength.

<table>
<thead>
<tr>
<th>No.</th>
<th>Polymer formula</th>
<th>$M_w$</th>
<th>$N_{agg}$ Salt-free</th>
<th>$N_{agg}$ 0.1M NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>D$_{25}$-B$<em>5$-D$</em>{25}$-B$<em>5$-D$</em>{25}$</td>
<td>3.92 x 10$^5$</td>
<td>16</td>
<td>18</td>
</tr>
<tr>
<td>2</td>
<td>B$<em>5$-D$</em>{25}$-B$<em>5$-D$</em>{25}$-B$<em>5$-D$</em>{25}$-B$_5$</td>
<td>2.13 x 10$^5$</td>
<td>2880</td>
<td>6203</td>
</tr>
<tr>
<td>3</td>
<td>D$_{25}$-B$<em>5$-D$</em>{25}$-B$<em>5$-D$</em>{25}$-B$<em>5$-D$</em>{25}$-B$<em>5$-D$</em>{25}$</td>
<td>8.75 x 10$^5$</td>
<td>23</td>
<td>24</td>
</tr>
</tbody>
</table>

The value of $M_{w(app)}$ was found 2.127 x 10$^8$ g/mol with a corresponding aggregation number of 6203 for the heptablock, as depicted in Figure 38.A, which is twice than that observed in the salt free solution, due to the screening of the internal DMA chains that favor the association.

In the case of the pentablock and the nonablock with end chain charged blocks the aggregation number is not influenced by the screening of the charges with values of $N_{agg} = 16$ and $N_{agg} = 24$, respectively.

Moreover the average radius of gyration is decreasing leading to a more compact structure by the reduction of repulsive electrostatic interactions corresponding to 94nm for the pentablock and 196nm for the nonablock. The same association through compactation phenomenon had been observed also for the heptablock leading to a decrease of average gyration radius about 5 times. The fact that the association number can change with the added salt, as in the case of the heptablock, is further evidence that the aggregates are able to exchange chains and to reequilibrate on the timescale of the measurements, therefore a reduction of the solubility of the PDMA corona has consequences on both size and association number as reported for similar systems.[6,9-10]

Dynamic Light Scattering experiments were involved to monitor the association phenomena under the influence of ionic strength, NaCl 0.1M, at pH 6 where the DMA block are protonated, as a function of concentration for the three polymeric systems.
4.3.1. Pentablock $D_{25}$-B$_5$-$D_{25}$-B$_5$-$D_{25}$

Characteristic plots of the autocorrelation function shift with the scattering angle for the pentablock at a concentration of 0.2 mg/mL, at pH 6 and fixed ionic strength (NaCl 0.1M) is shown in Figure 39.

![Graph of autocorrelation function shift](image)

**Figure 39.** The representative normalized correlation functions, for a concentration of 0.2 mg/ml aqueous polymeric solution of pentablock $D_{25}$-B$_5$-$D_{25}$-B$_5$-$D_{25}$, at pH 6 and 0.1M NaCl as a function of scattering angle.

The autocorrelation functions show two decays, revealing polydisperse species with different mobility. Small particles as they diffuse over a distance $q^{-1}$ change their orientation many times. Particles of a size comparable to or larger than $q^{-1}$ essentially preserves their orientation as they travel a distance smaller than their size. [11-13] Measurements of angular dependence are employed in order to verify the diffusive nature of the system dynamics.
Figure 40. Characteristic $q^2$ dependence of relaxation rate $\Gamma$ for a concentration $c=0.2$ mg/ml aqueous polymeric solution of pentablock $D_{25}$-$B_5$-$D_{25}$-$B_5$-$D_{25}$, pH=6, NaCl 0.1M, Extrapolation to zero angle, $D_T=1.49 \times 10^{-11}$ m$^2$/s corresponding to $R_H=16$ nm (A), $D_T=2.47 \times 10^{-12}$ m$^2$/s corresponding to $R_H=93$ nm (B).

The inspection of the relaxation rate as a function of scattering wave vector depicted in Figure 40 revealed a $q^2$ dependence at low $q$ which is consistent with the diffusive relaxation process of single particles. In the limit of low $q$ the angular distribution of the scattered intensity becomes independent of particle shape. At higher values of $q$ the relaxation rate dependence deviates from linearity and its curvature depends on the particular geometry of the studied particles.[11-13]

The apparent diffusion coefficient was determined from the slope of the relaxation rate $\Gamma$ versus the scattering wave vector $q^2$. Consequently, the apparent hydrodynamic radius at infinite dilution has been obtained from the linear best fit of the apparent hydrodynamic radius calculated through Stokes-Einstein equation with the copolymer concentration extrapolated to zero polymer concentration as shown in Figure 41. In dilute block polyelectrolyte solutions with added low molar mass salt the apparent hydrodynamic radius is a linear function of the polyelectrolyte concentration.[3] The apparent hydrodynamic radius extrapolated to zero polyelectrolyte concentration represents the dynamics of an average single polyelectrolyte chain.
Figure 41. Concentration dependence of the apparent hydrodynamic radius for the aqueous solution of the D_{25}-B_{5}-D_{25}-B_{5}-D_{25} pentablock, 0.1M NaCl, pH=6. Extrapolation to zero concentration $R_{H, c=0} = 9$ nm (●), and $R_{H, c=0} = 88$ nm (▲).

The characteristics of the aggregates determined by both SLS and DLS in salt and salt-free conditions are presented in Table 6.

<table>
<thead>
<tr>
<th>Pentablock</th>
<th>$N_{agg}$</th>
<th>$R_H$ (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salt –free pH=6</td>
<td>16</td>
<td>60</td>
</tr>
<tr>
<td>0.1 M NaCl, pH=6</td>
<td>18</td>
<td>9</td>
</tr>
</tbody>
</table>

In the presence of salt the pentablock presents an increased mean aggregation number, with the formation of two populations of different size, which could be ascribed to the presence of unimers and aggregates, respectively. The hydrodynamic radius corresponding to the fast relaxation mode remains relatively constant with the concentration which demonstrates no micelle formation.

In dilute salt-free solutions the intrachain electrostatic interactions dominate over the interchain ones, leading to a stretched conformation. With low salt concentration, the electrostatic interactions are screened. Screening of the
intrachain electrostatic interactions increases chain flexibility by reducing its persistence length leading to chain contraction. As salt is added and screens the electrostatic repulsions, additional unimers are incorporated into the aggregates to increase their size [15] as revealed for the slow relaxation mode corresponding to aggregates. As a result more polymer molecules are required to stabilize the aggregates leading to an increase in hydrodynamic size as observed for other amphiphilic polyelectrolytes.[16,17]

The average size of the aggregates is monotonically decreasing with the concentration due to the predominance of the intermolecular electrostatic interactions of the DMA blocks that cause chain contraction. This decrease of the chain size is due to reduction of the net polymeric charge by condensed counterions which weakens intrachain electrostatic repulsion. [3] This strong polyelectrolyte effect proves the predominance of the electrostatic interactions of the DMA blocks onto the association mechanism of the pentablock, which prevents further growth of the aggregates.

### 4.3.2. Heptablock B₅-D₂₅-B₅-D₂₅-B₅-D₂₅-B₅

Figure 42 shows the autocorrelation functions measured at different scattering angles ranging from 25° to 90° for a given polymer concentration. For low scattering angles the decay rate of the correlation function is usually interpreted as single particle diffusion.[13] As it can be observed in the figure the decay rates become faster as the scattering angle is increased and the correlation functions also deviate from a simple single exponential decay.
Figure 42. The representative normalized correlation functions, for a concentration of 0.2 mg/ml aqueous polymeric solution of heptablock B_{5}-D_{25}-B_{5}-D_{25}-B_{5}, at pH 6 and 0.1M NaCl as a function of scattering angle. The black arrow indicates the increase of the scattering angle.

Figure 43 shows the decay rate distributions corresponding to the correlation functions presented in figure 42 as determined by CONTIN analysis. It is observed that only one peak appears at low angles in the distribution of the decay rates and an additional peak appears at higher scattering angles (i.e. above 55°). The low scattering angle findings is to be identified with the diffusion of molecular centres of mass.[11-13] The faster decay at high angle results from the contribution of internal dynamics of the diffusing particles.
Figure 43. Time relaxation distributions obtained from dynamic light scattering at different scattering angles in aqueous polymeric solution of 0.2 mg/ml heptablock B_5-D_25-B_5-D_25-B_5-D_25-B_5, at pH 6 and 0.1M NaCl.

The typical distribution of relaxation times given in Fig. 43 is dominated by the slow mode. In addition to the slow mode, faster modes are seen at high scattering angles. It should be noted that in Light Scattering experiments, larger weighting factors are given to larger particles (Figure 44 A). Therefore the scattering intensity is proportional to the square of the particle volume or to the sixth power of the particle radius (Figure 44 B).[13] The slow diffusive mode associated with the existence of large aggregates per unit volume is clearly separable from the fast diffusive mode with decreased amplitude.
Figure 44. Representative DLS CONTIN (A) intensity weighted and (B) volume weighted size distribution for the heptablock B$_5$-D$_{25}$-B$_5$-D$_{25}$-B$_5$-D$_{25}$-B$_5$ at a scattering angle of 90°, for a polymer concentration 0.2 mg/ml, in 0.1 M NaCl at pH=6.

In Figure 44 the distribution of relaxation times, for the B$_5$-D$_{25}$-B$_5$-D$_{25}$-B$_5$-D$_{25}$-B$_5$ obtained by CONTIN analysis at a scattering angle of 90° for a concentration of 0.02wt%, at fixed ionic strength (NaCl 0.1M) shows a bimodal distribution. These two relaxation modes can be attributed to small aggregates and clusters of aggregates respectively.

The inspection of $q^2$ dependence on the relaxation rate shown in Figure 45 revealed a diffusive translational character at low values of $q^2$, while at higher scattering wave vectors the contribution from rotational diffusional effects is observed.
Figure 45. Characteristic $q^2$ dependence of relaxation rate $\Gamma$ for different concentrations of aqueous polymeric solution of heptablock $\text{B}_5\text{D}_{25}\text{B}_5\text{D}_{25}\text{B}_5\text{D}_{25}\text{B}_5$, $\text{pH}=6$, $\text{NaCl}$ 0.1M. Solid red lines fit a linear dependence in the low $q$ limit ($q \times R_H < 1$), where $R_H$ is the hydrodynamic size of the diffusing particles.

For low $q$ values only the translational motion of the polymers was analysed since DLS data at relatively large wave vectors cannot be interpreted in terms of translational dynamics.[13] The deviation from linearity of the relaxation rate with the scattering vector could be ascribed to hydrodynamic interactions (i.e. hydrophobic or electrostatic) which affects the dynamics of the aggregates.

The apparent hydrodynamic radius, assuming translational diffusion, was calculated using the Stoke-Einstein equation for spherical particles for the corresponding relaxation modes, and extrapolated to zero concentration as depicted in Figure 46.
Figure 46. Variation of apparent hydrodynamic radius, corresponding to the fast relaxation mode (●) and to the slow relaxation mode (○) depicted in Figure 45 as a function of concentration for the aqueous solution of the B₅-D₂₅-B₅-D₂₅-B₅-D₂₅-B₅ heptablock, 0.1M NaCl, pH=6.

The heptablock containing internal charged blocks has the tendency to form finite sized clusters in the presence of salt. The average size of these clusters is decreasing with the concentration due to predominance of intramolecular interactions that cause chain contraction. The response of the fast mode associated with the small size population is less sensitive to the variation of concentration. The size of the big clusters of aggregates decreases more significant upon increasing polymer concentration, but not as abruptly as in the case of the pentablock. This behavior could be ascribed to a higher hydrophobic content in the heptablock.

Table 7. Characteristics of polymeric aggregates determined by SLS and DLS for the heptablock at pH=6 and different ionic strength.

<table>
<thead>
<tr>
<th>Heptablock</th>
<th>N_{agg}</th>
<th>R_{H} (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salt –free pH=6</td>
<td>2880</td>
<td>100</td>
</tr>
<tr>
<td>0.1 M NaCl, pH=6</td>
<td>6203</td>
<td>85</td>
</tr>
</tbody>
</table>

Table 7 presents the characteristics of the heptablock aggregates for different ionic strength. Two opposite effects on the association of the heptablock upon salt addition can be observed. First, salt addition promotes an increased
aggregation number due to a change in the hydrophobic/hydrophilic balance, as the electrostatic repulsion between the charged groups is screened.

Second, as the ionic strength is increased the electrostatic repulsive forces among neighboring ionic sites are diminished and the polymer contracts [17], leading to a decrease in the hydrodynamic size.

### 4.3.3. Nonablock D$_{25}$-B$_5$-D$_{25}$-B$_5$-D$_{25}$-B$_5$-D$_{25}$-B$_5$-D$_{25}$

Figure 47 shows a representative semilog plot of the normalized correlation function of 0.02 wt% nonablock D$_{25}$-B$_5$-D$_{25}$-B$_5$-D$_{25}$-B$_5$-D$_{25}$-B$_5$-D$_{25}$ at pH 6 and in 0.1 M NaCl at different scattering angles ranging from 25 to 90°. The correlation functions show a deviation from a simple single exponential decay by increasing scattering angle, therefore the solution is characterized by a single relaxation process at low angles, whereas the higher angles by two relaxation modes.

![Figure 47](image_url)

**Figure 47.** The representative normalized correlation functions, for a concentration of 0.2 mg/ml aqueous polymeric solution of nonablock D$_{25}$-B$_5$-D$_{25}$-B$_5$-D$_{25}$-B$_5$-D$_{25}$ at pH 6 and 0.1M NaCl as a function of scattering angle. The black arrow indicates the increase of the scattering angle.

We first analyzed the $q^2$ dependence of the relaxation rate for the two relaxation modes in the limit of small scattering vector (i.e. $q \times R_H < 1$), where the decay rate constant is proportional to the translational diffusion coefficient.[11-13]
These results are shown in Figure 48.

**Figure 48.** Characteristic $q^2$ dependence of relaxation rate $\Gamma$ for a concentration of 0.2 mg/mL aqueous polymeric solution of nonablock $D_{25}$-$B_5$-$D_{25}$-$B_5$-$D_{25}$-$B_5$-$D_{25}$-$B_5$-$D_{25}$, pH=6, NaCl 0.1M corresponding to A: the fast decay of the correlation function and B: the slow decay of the correlation function.

The decay rate of the fast mode was almost proportional to squared scattering vector at low angles, as expected of uniformly sized particles; see Figure 48 A. Sometimes, small deviations from linearity become apparent at high values of the scattering vector.

In most DLS studies on polyelectrolyte solutions, the slow relaxation rate shows a $q$-dependence stronger than that expected for simple diffusion; this phenomenon is generally attributed to the dynamics of large multichain domains, clusters, or temporal aggregates.[16]

The apparent hydrodynamic radius was calculated through Stokes-Einstein equation and plotted vs concentration and the extrapolation of the data to zero particle concentration gave the values of the hydrodynamic radius $R_H$ (Figure 49). The apparent $R_H$ corresponding to the fast relaxation mode was found 14nm, while for the slow mode relaxation peak is observed an $R_H=199$nm corresponding to big aggregates.
The abrupt decrease of hydrodynamic radii is due to the contribution of the end chain hydrophilic segments which results in a more compact structure at high ionic strength. In 0.1M NaCl the polymer backbone shrinks due to the shielding of the electrostatic charges and the disruption of the hydrophobic interaction. The behavior of the nonablock seems to resemble to that of polyelectrolytes in salt solution.

The dynamic light scattering results demonstrated a sharp shrinking of the hydrodynamic sizes in the presence of salt for the nonablock, with a slight increased aggregation number as revealed from the data in Table 8.

Table 8. Characteristics of polymeric aggregates determined by SLS and DLS for the nonablock at pH=6 and different ionic strength.

<table>
<thead>
<tr>
<th>Nonablock</th>
<th>$N_{agg}$</th>
<th>$R_H$(nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salt –free pH=6</td>
<td>23</td>
<td>108</td>
</tr>
<tr>
<td>0.1 M NaCl, pH=6</td>
<td>24</td>
<td>14</td>
</tr>
</tbody>
</table>
The size variation for the nonablock indicates that under conditions at which the electrostatic interactions between the ionic DMA groups along the polymer chains are diminished (high ionic strength) leads to a shrinking of the dimensions of the aggregates.

However, the hydrodynamic size corresponding to the slow relaxation modes in both systems of pentablock and nonablock decreases sharply with the concentration proving a predominance of the electrostatic interactions of the charged DMA blocks. The addition of salt is expected to screen the electrostatic interactions in the charged corona leading to more compact structures.[3-6] The decrease of the size with the concentration at constant salt content should result in a shrinking of the corona formed by the DMA blocks. This behavior has been observed for the two relaxation modes of the nonablock. This trend contrasts with the pentablock \( D_{25-B_5-D_{25-B_5-D_{25}}-D_{25}} \) behavior where the slow mode is independent of the concentration and the difference is most certainly caused by the additional charged end-blocks.

The study of the influence of salt concentration on the association mechanism of the asymmetrical multiblocks supports the conclusion that in the case of the heptablock the association is dominated mainly by intermolecular associations between the hydrophobic stickers. Contrary, for the nonablock and the pentablock, the additional hydrophilic end-chain segments prevent the extending association of the internal hydrophobic blocks.

Since CONTIN analysis reports weight-intensity distributions with strongly exaggerated proportion of the large particles, the volume weighted diameter distribution was preferred in order to have a better view on the aggregation of the studied systems.

As depicted in Figure 50 the representative volume weighted diameter distributions measured for each multiblock copolymer at a \( 45^0 \) scattering angle shows the self-assembly dictated by the topology of the BuMA stickers. The digital photographs in Figure 50 left confirm a higher scattering intensity for the heptablock relatively to the pentablock and the nonablock.
Figure 50. (Left) Digital photographs of the scattering intensity for the three samples and (right) representative DLS CONTIN volume weighted size distribution for the pentablock D_{25}-B_{5}-D_{25}-B_{5}-D_{25} (red line), the heptablock B_{5}-D_{25}-B_{5}-D_{25}-B_{5}-D_{25}-B_{5} (blue line) and the nonablock D_{25}-B_{5}-D_{25}-B_{5}-D_{25}-B_{5}-D_{25}-B_{5}-D_{25} (green line) at a scattering angle of 45°, for a polymer concentration 1 mg/mL, in 0.1 M NaCl at pH=6.

The influence of salt content on the association mechanism has been investigated in order to reduce the polyelectrolyte effect on the association mechanism. With the addition of salt, the range of the electrostatics becomes screened, quantified by the Debye screening length, and the structural features in the polyelectrolyte solution closely resemble neutral polymer solutions [4,7,18,19]. The role of this Debye screening on the association behaviour determined by the inverse of the scattering intensity as a function of 0.1, 0.2, 0.3, 0.5 and 1.0 M NaCl is shown in Figure 51, for a fixed polymer concentration of 0.1 wt%.
**Figure 51**: Inverse scattering intensity extrapolated to zero angle as a function of concentration of (A) B$_5$-D$_{25}$-B$_5$-D$_{25}$-B$_5$ and (B) D$_{25}$-B$_5$-D$_{25}$-B$_5$-D$_{25}$-B$_5$-D$_{25}$ in acidic aqueous solutions at a concentration of 1 mg/mL, pH=6 for different salt concentrations.

**Figure 52**: Dependence of ionic strength on the aggregation number for polymer solutions of B$_5$-D$_{25}$-B$_5$-D$_{25}$-B$_5$ (■) and D$_{25}$-B$_5$-D$_{25}$-B$_5$-D$_{25}$-B$_5$-D$_{25}$ (●) at a concentration of 1 mg/mL, pH=6.

From the extrapolated zero angle $Kc/\Delta I$ values (Figure 51.A.), an average apparent aggregation number was calculated to be $N_{agg} = 3000$ slightly dependent on the salt concentrations as shown in Figure 52.

Increasing the ionic strength of the solution decreases the repulsive part of the interaction energy between the charged molecules, which would help the aggregation process. [6] In Figure 51. B extrapolation to zero angle gave an apparent $M_w=7.95\times10^5$ g/mol corresponding to an apparent aggregation number $N_{agg}$ of 21 for the highest ionic strength 1M. The very low aggregation numbers of the nonablock can be attributed to the high molar fraction of hydrophilic units of approximately 86 wt% and to the positioning of the end chain hydrophilic DMA which prevents extended associations.
4.4. Association Mechanism Multiblock Copolymers by Transmission Electron Microscopy

In order to generate deeper insight into the structure of the aggregates, TEM was used to study the morphologies of polymeric particles. However, the TEM image as a two-dimensional object is usually hard to distinguish between hydrophobic and hydrophilic domains, unless the natural contrast between them is strong enough. The observations were performed for the dried particles without staining.

High Resolution Transmission Electron Microscopy (HRTEM) is a powerful technique for the visualisation of the morphology of macromolecular self-assemblies from dilute solutions.[17] In this work we have performed TEM experiments on dry heptablock BuMA$_5$-DMA$_{25}$-BuMA$_5$-DMA$_{25}$-BuMA$_5$ and the nonablock DMA$_{25}$-BuMA$_5$-DMA$_{25}$-BuMA$_5$-DMA$_{25}$-BuMA$_5$-DMA$_{25}$-BuMA$_5$ from solutions at different ionic strength.

The aggregates could be directly observed by TEM, and their dimensions were measured readily from the digital micrographs. It was found that the investigated multiblock copolymers yielded multiple morphologies in aqueous solution.

Figure 53 displays TEM images obtained from a concentration of heptablock 0.01wt% in salt free at pH=5. The polymeric aggregates appear as large clusters that tend to percolate. Most of the chains are in extended
conformation forming bundles of averagely 4nm in width consistent with strong intramolecular electrostatic repulsions in PDMA protonated blocks.
The PBuMA blocks appear as associated dark beads as expected from their hydrophobic nature. The formed aggregates are irregular finite sized clusters in accordance with the results observed by DLS, where the bridging chains adopt a stretched conformation. This type of association can be attributed to the repulsive electrostatic interactions along the PDMA chains and its relatively short length that implies small persistence length which prevents looping in accordance with other findings.[2]
In Figure 54 the morphology of the nonablock associates observed by TEM is depicted. The nonablock copolymer with additional charged external blocks forms in dilute solution and low ionic strength finite sized aggregates. The observed structures could be attributed mainly to the polyelectrolyte character of the protonanted PDMA blocks which prevent the extended association of the internal BuMA stickers. Due to the repulsive electrostatic interactions and the position of the charged blocks at the end of the chain the clusters are not further growing towards a three-dimensional network.

Figure 54. TEM images (A,B) of 0.01 wt % nonablock D_{25}-B_{5}-D_{25}-B_{5}-D_{25}-B_{5}-D_{25}-B_{5}-D_{25} in aqueous solution at pH=5. Scale bar lengths are indicated.
DLS results had shown that the aggregates formed by the nonablock had a bimodal distribution, confirmed by the TEM results in Fig. 54A, where it is clearly seen that the morphology presents two types of associates: small unimers and larger compounds. Picture 54.B revealed that the aggregates showed spherical shape. The diameter of the latter was less than 200 nm, which was much lower than that observed by dynamic light scattering in solution. Possible reasons for the difference results between TEM and DLS are as follows. First, the aggregates overlapped and shranked during the process of water evaporation. Second, the hydophobic core of the aggregates has a higher contrast as it can be observed by TEM as the chain density in the corona was probably too low to make visible contrast to the background in Figure 54A.

In order to suppress the electrostatic repulsive interactions that develop due to the charged DMA blocks the ionic strength of the medium has been increased.

Figure 55 shows TEM micrographs demonstrating the morphologies obtained from the multiblock copolymers at initial concentration of 0.02 wt % in NaCl 0,1M.

The TEM image depicted in figure 55 A for the heptablock reveals both spherical and nonspherical objects corresponding to highly aggregated clusters with sizes ranging from 50 to 200 nm. The heptablock forms large irregular shaped structures, which are probably formed by aggregation of several smaller objects. The addition of salt to the solution facilitates the aggregation of heptablock molecules with a growth of the size of the aggregates, contrary to typical polyelectrolyte behaviour, where the chain dimensions are reduced by charge screening. These results are in agreement with what was observed in the DLS experiments and confirm the conclusion that the association mechanism is predominated by the hydrophobic interactions of the BuMA stickers.
Figure 55. TEM image of 0.02 wt % B$_5$-D$_{25}$-B$_5$-D$_{25}$-B$_5$-D$_{25}$-B$_5$ and block copolymer in aqueous solution, NaCl 0.1 M and pH=6 together with magnified view of the aggregates. Scale bar lengths are indicated.
The nonablock with hydrophilic end blocks showed a dispersed morphology (Figure 56). As revealed from DLS measurements the aggregates formed by the nonablock in salt conditions are corresponding to two different populations of small aggregates and respectively to larger clusters with a more compact average hydrodynamic size than in salt-free condition.

Figure 56. TEM image of 0.02 wt % D_{25}-B_{25}-D_{25}-B_{25}-D_{25}-B_{25}-D_{25} block copolymer in aqueous solution, NaCl 0.1 M and pH=6 together with magnified view of the aggregates. Scale bar lengths are indicated.

The HRTEM imaging is qualitatively in good agreement with the DLS results taking into account the dry state, overlapping and adsorption phenomena in the TEM experiments, therefore expecting for the size and shape of the aggregates to be different from those in dilute solution.
4.5. Conclusions

Static and Dynamic Light Scattering were used to determine the structural characteristics of multiblock copolymers D\textsubscript{25}-B\textsubscript{5}-D\textsubscript{25}-B\textsubscript{5}-D\textsubscript{25}, B\textsubscript{5}-D\textsubscript{25}-B\textsubscript{5}-D\textsubscript{25}-B\textsubscript{5}-D\textsubscript{25}, D\textsubscript{25}-B\textsubscript{5}-D\textsubscript{25}-B\textsubscript{5}-D\textsubscript{25}-B\textsubscript{5}-D\textsubscript{25} in dilute aqueous solution and as function of ionic strength. In both salt and salt-free regime, it has been determined that the heptablock has a high association number comparing with the pentablock and the nonablock with charged end-blocks.

The hierarchical study of the heptablock in diluted aqueous solutions proved the formation of small aggregates and clusters of aggregates arising through extending association of the smaller entities. Additionally, the nonablock presents a significantly decreased aggregation number, with extended dimensions, due to the electrostatic repulsive interactions along the DMA chains that favours stretching.

The dynamic light scattering results demonstrated a decrease of the clusters size with the concentration, and a shrinking of the hydrodynamic size with the addition of salt. The DLS study had revealed strong polyelectrolyte effect for the nonablock and the pentablock with charged end blocks. This effect has been suppressed by increasing the ionic strength of the medium.

The addition of salt to the solution facilitates the aggregation of heptablock molecules with a growth of the size of the aggregates, due to a strengthening of the hydrophobic interactions. The study of the influence of salt concentration on the association mechanism of the two asymmetrical multiblocks (the heptablock and the nonablock) supported the conclusion that in the case of the heptablock the association is dominated mainly by intermolecular associations between the hydrophobic stickers. Contrary, for the nonablock, the additional hydrophilic end-chain segments prevent the extending association of the internal hydrophobic blocks.

The aggregate morphologies were studied by transmission electron microscopy (TEM) at different ionic strength.
Evidence from both scattering measurements and transmission electron microscopy (TEM) measurements suggested that the system formed various morphologies dictated by the topology of the hydrophobic stickers. The heptablock with hydrophobic end-blocks associates into a percolated structure with cylindrical shaped hydrophilic domains (bundles) which shrinks in the presence of salt.

The different associative behaviour that the asymmetric multiblock copolymers revealed by Light Scattering and TEM measurements showed a trend upon the nature of the end blocks.

In the case of the heptablock, the telechelic end blocks favour extended association in water with the formation of a physical transient network above a threshold concentration. The pentablock and the nonablock with hydrophilic polyelectrolyte end-blocks had shown low aggregation numbers corresponding to finite size aggregates, as well as no evidence of aggregates growth towards a three-dimensional network.
4.6. References

Chapter 5

ASSOCIATION IN SEMIDILUTE AQUEOUS REGIME
5.1. Rheological Measurements as a Function of Polymer Concentration

Rheological measurements showed that only the heptablock copolymer (ABABABABA) bearing hydrophobic end-blocks formed hydrogels at relatively low copolymer concentrations as depicted in Figure 24. The gelation efficiency of amphiphilic multiblock copolymers based on the same building blocks for another series with constant molecular weight and hydrophobic molar ratio, but variant size of the hydrophilic and hydrophobic segments was investigated by our group. It was found that the low length of the triblock chain in the present case prevents the formation of a three-dimensional transient network while at higher molecular weight (\(\sim 10^4\) g/mol) a fragile physical gel was obtained at about 5 wt%. Moreover, the heptablock copolymer described here presented a hydrophilic/hydrophobic ratio and block lengths that enabled the greatest thickening efficiency.

Therefore, the investigation was focused in the rheological properties of the heptablock copolymer solutions at pH 6 at which central PDMAEMA blocks are protonated.

5.1.1. Steady Shear

Figure 57 shows characteristic flow curves of the heptablock at different polymer concentrations. The system, at 0,1 and 0,25 wt % almost exhibits the classic behavior of a Newtonian liquid (curves not shown). At 1 wt% the behavior of a viscoelastic fluid was observed, which was characterized by a low shear Newtonian plateau followed by a smooth shear thinning effect at higher shear rates. As the polymer concentration increased above 3,5 wt %, a drastic change in the flow behavior was observed. The zero shear viscosity determined at the low shear plateau exceeded \(10^4\) Pa s which is 2 orders of magnitude higher than that at 2 wt% polymer and 6 orders of magnitude higher than that of the medium,
revealing a remarkable strengthening of the polymeric structure of the complex fluid. This behavior is also marked by a vivid shear thinning effect characterized by an apparent yield stress of about 90 Pa, suggesting a disruption of the physical network into smaller aggregates.

![Figure 57. Viscosity profile for different concentrations of a polymer solution B₅-D₂₅-B₅-D₂₅-B₅-D₂₅-B₅ block copolymer aqueous solution at pH 6 and 25°C.](image)

At even higher polymer concentrations (4-6 wt%), a decrease in the zero shear viscosity was observed. The drop in viscosity upon increasing concentration may be ascribed to electrostatic screening effects due to counterion condensation along the elastic polyelectrolyte segments, leading to a decrease in the number density of the elastically active chains.

At elevated concentrations a Newtonian plateau is observed at low stresses followed by a smooth shear-thickening and an abrupt shear-thinning effect, marked by several orders of magnitude viscosity drop.

The configuration of the heptablock chains is influenced by two antagonistic factors: the hydrophobic attractive interactions between BuMA segments that favor chain folding and the electrostatic repulsive interactions between the adjacent positive charges along the DMAEMA chains that favor stretching. Therefore, the association phenomena proceed through both electrostatic and hydrophobic interactions.[1]
The hydrophobic stickers, which are at fixed positions, can associate to form reversible aggregates, either can be free. Furthermore, it is assumed that the free sticker ("dangling" ends) can find many sites to attach to, besides the one it was just recently attached with, leading to a shear thickening followed by a shear thinning beyond a critical shear stress. At the intermediate shear stress corresponding to the shear thickening effect, the shear flow forces both intramolecular associated and “dangling” ends to form new elastically active intermolecular bridges which harden the transient network. When the structure is submitted to an increasing stress, there is only a competition between association and dissociation of efficient associative junctions from the network, therefore at high shear stress, the gradually fragmentation of the network leads to a decrease of the viscosity. The shear-thinning region corresponds to a gradual decrease of viscosity level, usually attributed to an apparent yield stress. The increase in the yield stress with the concentration, contrary to the evolution of viscosity, could be ascribed to strengthening of the network as system becomes denser and the intermolecular distance between the stickers decreases favoring association and the disruption of the physical network is more difficult.

5.1.2. Oscillatory shear

Figure 58 shows the variations of the storage (G’) and loss (G’”) moduli as a function of the angular frequency for different concentrations of heptablock in water at pH 6. The results observed indicate that the frequency dependence of the shear moduli corresponds to a gel like behavior since for all the concentrations depicted and in the whole frequency range studied, G’ is higher that G’”. It should be noted that the oscillatory measurements had been performed in the linear viscoelastic regime.
Figure 58. Dynamic moduli $G'$ (up triangle), $G''$ (square) as a function of frequency for concentrations of 1 wt% ($\uparrow$), 2 wt% ($\uparrow$), 3.5 wt% ($\uparrow$), 4 wt% ($\uparrow$), 5 wt% ($\uparrow$) and 6 wt% ($\uparrow$) for heptablock BuMA$_{5-25}$-DMAEMA$_{25}$-BuMA$_{5-25}$-DMAEMA$_{25}$-BuMA$_{5}$ aqueous solutions, pH=6, at 25 °C.

At low polymer concentration (1-2 wt %) the sample behaves as fragile gels since it is frequency dependent. It is apparent that with increasing frequency, the structural changes due to the disruption of the polymer network are manifested.

By increasing concentration, the storage modulus increases significantly at 3.5 and 4 wt%. Moreover the storage modulus is more than 1 order of magnitude higher than the loss modulus $G''$, for 4 wt %, and both moduli are almost frequency independent (at low frequency values) exhibiting elastic behaviour typical for strong physical gels.

Interestingly, at even more elevated concentrations, corresponding to 5 and 6 wt% the elastic modulus $G'$ is decreasing and the $G'$-$G''$ difference is narrower with increasing concentration.
5.1.3. Low-shear Viscosity Behavior of the Heptablock

The concentration dependence of the maximum viscosity observed in the stress dependence plots (Figure 57) together with digital photos of some solutions is presented in Figure 59.

![Figure 59. Double logarithmic plot of the maximum shear viscosity, \( \eta \), as a function of polymer concentration of heptablock B5-D25-B5-D25-B5-D25-B5 in aqueous solution of pH 6 and 25°C. The digital photos in the right show solutions of different polymer concentrations in wt%.](image)

Several concentration regimes can be identified according to the viscosity versus concentration plot. At low concentrations and in the dilute regime, polymer solutions exhibit Newtonian response and the viscosity values are comparable to that of the medium. Above a critical gel concentration named \( c_{gel} \) the viscosity increases dramatically following a scaling law \( \eta \sim C^{7.5} \) as has been observed for the associative ABA telechelic polyelectrolytes [2-4]. The \( c_{gel} \), denoted the onset of the 3D network formation, was determined at about 0.6 wt\%, which is remarkably low regarding other APs with comparable molecular weight. As can be observed from the digital photos, a free-standing gel is attained above \( c=3.5 \) wt\%. While even above \( c=2 \) wt\% the solutions resist strongly to flow.

Importantly, the present system exhibits an unusual behavior at elevated concentrations with respect to the ABA type telechelic polyelectrolytes [2-4]. Above \( c=3.5 \) wt\% the viscosity increases even more abruptly with a power exponent of 13.8, revealing the formation of a very stiff gel. In order to better
understand this behavior we should distinguish two kinds of stickers. The internal stickers \((-D_{25} - B_{5} - D_{25} - B_{5} - D_{25})\) bearing at both ends charged moieties which develop repulsions that prevent association (named “open” stickers) and the end stickers \((B_{5} - D_{25} - B_{5} - D_{25} - B_{5} - D_{25} - B_{5})\) which are highly associative (“closed” stickers) as in the heptablock telechelic, as schematically depicted in Figure 60. This is corroborating with the fact that the nonablock copolymer cannot form a network as all the stickers are internal. The concentration effect mentioned above (increase of the power exponent) could be attributed to the strengthening of the network by transformation of “open” stickers to “closed” stickers as the system becomes denser and the intermolecular distance between the internal hydrophobic parts decreases favoring association.

<table>
<thead>
<tr>
<th>“open” stickers</th>
<th>“closed” stickers</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="“open” stickers" /></td>
<td><img src="image2.png" alt="“closed” stickers" /></td>
</tr>
</tbody>
</table>

**Figure 60.** Schematic representation of internal or “open” stickers and of end or “closed” stickers of BuMA building blocks that influence the association of the heptablock.

Further increase of concentration results to a viscosity decrease, which may be ascribed to electrostatic screening effects due to counter ion condensation along the elastic polyelectrolyte segments. In this case, the number density of the elastically active chains decreases.
5.2. Effect of pH on the Rheological Behavior

5.2.1. Steady Shear

The study of the rheological properties of the heptablock hydrogels by varying the pH of the medium was also performed. In Figure 61(a) the low shear viscosity has been plotted as a function of pH for a 4 wt% polymer concentration. As can be observed, the viscosity passes through two maxima prior to decrease sharply at high values of pH. Above pH 8 a GEL-SOL transition was observed due to the strong deprotonation of the tertiary amine pendant groups of the PDMA blocks which result to very low ionization degree (Figure 61.(b)).

Since the ionization degree is a monotonous decreasing function of pH, this cannot justify alone the complex behavior of the system. As has been discussed for the ABA telechelic polyelectrolytes [5-9] the behavior of these associative systems is governed by the interplay of the attractive interactions of the hydrophobic blocks and the electrostatic repulsive interactions of the polyelectrolyte blocks. The electrostatic interactions evidently depend on the
degree of ionization and the ionic strength of the medium (see next section). As has been reported for the PMMA-PDMA-PMMA, constituted from similar in nature blocks, the viscosity passes through a maximum, in the vicinity of pH 4 where the degree of ionization of the PDMA chains is about 90% [10]. The second viscosity maximum, in the present case, could be ascribed to further contribution of the additional hydrophobic interactions of the internal stickers which are mainly “open”. Upon further decreasing the charge density of the PDMA chains above pH 6, the intermolecular attractive interactions of the internal hydrophobic domains predominate and the “open” stickers become “close”, increasing therefore the number of elastic chains resulting to strengthening of the 3D network and in turn increasing the viscosity of the system. This could interpret the fact that the viscosity at pH 6.5 (second maximum) is higher than that at pH 4 which does not occur in the PMMA-PDMA-PMMA triblock copolymers bearing only external stickers.

![Figure 62. Viscosity profile of a 4 wt% polymer solution B5-D25-B5-D25-B5-D25-B5 block copolymer aqueous solution at pH 4.5 (▲), 5.5 (▲) and 6.5 (▲) and 25°C.](image)

Figure 62 shows the flow curves of the heptablock B5-D25-B5-D25-B5-D25-B5, which exhibit smooth shear-thickening at low-shear followed by a shear thinning behavior at higher shear stress. At pH 5.5 which corresponds to a minimum in low shear viscosity, the flow curve presents two drastic shear-thinning regions. This kind of strong nonlinear behavior, corresponding to
discontinuities in the viscosity versus shear stress curve, has been observed with other network forming associative polymeric systems.[11,12] The apparent yield stress, that is the stress at which viscosity departs from the viscosity maximum, has also a maximum value about 90 Pa, at pH 6,5 as shown in Figure 62. On the other hand at pH 5,5 this apparent yield stress takes its minimum value (~ 30 Pa). The above results demonstrate that the network is strengthening at pH 6,5 due to additional physical crosslinking.

5.2.2. Oscillatory shear

Dynamic strain sweeps were conducted first, at different values of pH and a frequency of 1Hz, in order to establish the linear viscoelastic regime. In Figure 63, representative plots of the storage ($G'$) and loss ($G''$) modulus are depicted as a function of strain, $\gamma$, at 1 Hz, for a 4 wt % polymer for pH 5, 6 and 6,5, together with the variation of the elastic modulus with deformation for a wider range of pH.

![Figure 63](image-url)  
Figure 63. Storage modulus $G'$ (up triangle) and loss modulus $G''$ (square) as a function of strain $\gamma$ at 1 Hz of a 4%wt BuMA$_5$-DMAEMA$_{25}$-BuMA$_5$-DMAEMA$_{25}$-BuMA$_5$-DMAEMA$_{25}$-BuMA$_5$ polymer solution, at 25 °C.

The linear viscoelastic regime (LVR) is defined as the regime below the critical value $\gamma_{LVR}$ where $G'$ remains invariant with respect to strain. The critical strain $\gamma_{LVR}$ value is about 5% at pH=6,5. This low $\gamma_{LVR}$ value classifies this gel to the category of the so-called “weak” physical gels that, in fact, are structured
fluids behaving as liquids at relatively large deformations.[13] However, the magnitude of the elastic modulus in the LVR is high (more than $10^5$ Pa for $c_p = 4$ wt % at pH=6.5), which is rather unexpected for a weak gel. Therefore, this behaviour could be ascribed to a “fragile” strong type of gel.

It should be noted that in the range of pH 6 to 6.5 and at pH 4.5 corresponding to maxima in the viscosity versus pH plots, present no strain hardening phenomenon. This should be attributed to an increased number of elastic chains upon decreasing charge density of the polyelectrolyte PDMA chains above pH 6. In this case, chain rearrangement by shearing cannot any more occur, and this leads to fast gel softening.

Accordingly, frequency sweep experiments were performed in the linear viscoelastic regime.

![Figure 64. Dynamic moduli $G'$, $G''$ as a function of frequency at 1% strain at pH=4.5 (●, ○) and at pH=6.5 (■, □) of a 4%wt BuMA$_5$-DMAEMA$_{25}$-BuMA$_5$-DMAEMA$_{25}$-BuMA$_5$-DMAEMA$_{25}$-BuMA$_5$ polymer solution, at 25 °C.](image)

These measurements have shown that at 4 wt% polymer concentration, the system behaves already as a strong physical gel, since the storage modulus, $G'$, is higher than the loss modulus, $G''$ in the entire frequency range investigated (Figure 64) and the $G'$–$G''$ intersection is not visible as it has been shifted to very low frequencies. Moreover, the storage modulus is higher at pH 6.5 due to a higher number of elastic chains.
5.3. Temperature effect on the Rheological Behavior

5.3.1. Temperature Ramp Test

To evaluate the effect of charge density of the PDMA blocks on the rheological behavior of the aqueous solutions of the linear associative multiblock copolymer, the Temperature Ramp Test was performed. This test allowed monitoring the evolution of the rheological properties during heating/cooling cycles for the two solutions corresponding to maxima in the viscosity versus pH profile. The assays were carried out within a temperature interval between 10 and 50°C, establishing a ramp of 1°C/min between the 2 consecutive values and allowing a reasonable margin of 10 min for this variation in temperature to take place, at constant rate of 0.1 s⁻¹. The rheometer was equipped with a Peltier control system that allowed accurate control (±0.1°C) of temperature between 10°C-50°C.

The viscosity of the two samples is plotted as a function of temperature (Figure 65) showing significantly different behavior for the two systems.

![Figure 65](image_url)

Figure 65 . A. Viscosity as a function of temperature for 4 wt % BuMA₂₅-DMAEMA₂₅-BuMA₂₅-DMAEMA₂₅-BuMA₂₅-DMAEMA₂₅-BuMA₅ copolymer solution at pH = 4.5 (○, heating and ■, cooling) and pH = 6.5 (□, heating and ■, cooling) at 1°C/min and 0.1 s⁻¹ shear rate. B. Viscosity as a function of time for the heptablock 4 wt% at 10°C and 0.1 s⁻¹ shear rate for pH 4.5 (●) and 6.5 (■).
The differences on the molecular characteristics imposed by a different charge density of the PDMA chains impose important differences on their temperature dependent rheological behavior. The second viscosity maximum, in the plot of low shear viscosity versus pH (Figure 61) has been ascribed to the further contribution of the additional hydrophobic interactions of the internal stickers which are mainly “open” which become “close”, upon further decreasing the degree of ionization above pH 6, with a strengthening of the network corresponding to an increase viscosity value.

Therefore, in Figure 65 B it is demonstrated that the viscosity drop at the start-up of heating is a time dependent effect, since the decrease is observed at 10°C for about 500s corresponding to an increase in temperature until ~ 18°C. Since the initial viscosities of the samples tested by temperature ramp were not reproducible with those found in the time dependent viscosity, the observed variation could be due to sample time and shear history dependence on the rheological properties. It is observed that the viscosity shows a maximum at the start-up and then gradually decreases to a constant level. This overshoot depends on both the instrument and the sample, since the latter needs time to reach the constant conditions.

It can be also seen from Figure 65.A that the shear viscosities decrease first remarkably with increasing temperature up to a certain value (subtracting the time effect), then it remains constant for a large range of temperature.

It clearly appears that the network structure at higher temperature is no longer affected since the viscosity reaches a plateau. The fact that viscosity is nearly invariant with temperature should be ascribed to the stability of the structure over the temperature range examined. Interestingly, no change in viscosity of the copolymer had been observed even at 50°C, since though the cloud point for PDMA blocks in water has been shifted well above 40°C [14,15], probably due to the prevalence of the hydrophobic stickers to the transient network.

However, in both cases the viscosity undergoes a hysteresis effect over a complete cycle of temperature change. This leads to a partial recovery of their
values at the end of the heating/cooling sweep test. The recovery process of the system upon cooling also differs. The polymer solution at pH 4.5 exhibits an almost complete recovery in the range of 28 to 50°C (i.e. the viscosity plateau region) followed by a linear increase with cooling, attaining the initial viscosity value (plateau in Figure 65 B). Contrary the copolymer solution with a lower charge density of the PDMA chains (i.e. pH=6.5), corresponding to the highest viscosity value in Figure 47, shows a complex recovery process. During cooling, two distinct ramifications of the heating and cooling curves (viscosity hysteresis) are observed, with retardation onto attaining the previous structure.

5.3.2. Oscillatory Temperature Sweep

The properties of the gels upon heating were probed via oscillatory measurements using oscillation frequency of 1Hz in the linear regime and heating the gels at 1 °C/min with a fixed strain amplitude of 4% for the sample at pH=6.5 and 1% for the sample at pH=4.5, to obtain a sufficient response in all temperature ranges. The results are shown in Figure 66. Moreover, these are within the area of linear viscoelasticity for the whole temperature ramp. The storage modulus is greater than the loss modulus in all cases, which indicates the stable and more elastic component of the samples within this temperature range.
The behavior of $B_5-D_{25}-B_5-D_{25}-B_5-D_{25}-B_5$ is similar for both systems, the increasing temperature increases the molecular motions and leads to faster exchange rate of the transient junctions. On the other hand, even though the presence of the stickers should create tighter associations, the ionized DMA blocks increase the exchange due to the thermal motion in the studied range leading to a decrease of moduli with heating with thermothinning behaviour at the startup of heating.

### 5.4. Effect of ionic strength on the Rheological Behavior

#### 5.4.1. Steady Shear

Finally the influence of ionic strength on the properties of the heptablock hydrogel was carried out at pH 6 by adding NaCl in 4 wt% polymer solutions. In Figure 67, digital photos of solutions of different salt content are demonstrated. At 0.1N salt, a free-standing gel, retaining its transparency, is formed whereas at
higher ionic strength phase separation occurs. Obviously when salt is added to the system, the electrostatic interactions are screened provoking shrinkage of the 3D network which leads to network breakage and eventually to phase separation as clearly can be seen in the 1N salt solution (Figure 67 left).

![Image](88x438 to 234x631)

**Figure 67.** Shear viscosity as a function of shear stress for heptablock copolymer aqueous solutions of 4 wt%, at pH=6 (∆) and, pH=6, NaCl 0.1M (∆) at 25°C. The digital photos show solutions of different salt concentration.

The presence of salt has significant consequences on the rheological properties of the hydrogel as revealed by comparing the viscosity profiles at free salt and 0.1N NaCl conditions. The viscosity decreases about two orders of magnitude and the apparent yield strength one order of magnitude upon adding 0.1N salt (Figure 67) which again should be ascribed to the network structure alteration due to electrostatic screening.

### 5.4.2. Oscillatory shear

In figure 68, representative plots of storage and loss modulus (G', G''), are shown as a function of deformation at 1Hz for a 4 wt% solution of heptablock at pH 6 and different ionic strength.
Figure 68. Storage modulus $G'$ (solid symbol) and loss modulus $G''$ (open symbol) as a function of strain $\gamma$ at 1 Hz of a 4%wt BuMA$_5$-DMAEMA$_{25}$-BuMA$_5$-DMAEMA$_{25}$-BuMA$_5$ polymer solution, at 25°C at pH 6 aqueous solution (▲) and 0.1 M NaCl (▲△).

The addition of salt leads to two significant effects. First the elastic modulus plateau in the linear viscoelastic regime is reduced and the critical $\gamma_{LVR}$ value is higher in 0.1M NaCl. The above behaviour can be explained by the screening of the electrostatic interactions in the presence of electrolyte, leading to a more flexible conformation of the elastic chains. This can facilitate the formation of intramolecular hydrophobic associations at the expense of intermolecular junctions. Therefore the transient gel formed in the presence of salt is weaker than in pure water which will result in a decrease of the elastic modulus. The low value of $\gamma_{LVR}$ in pure water should be attributed to the more stretched conformation of the polyelectrolyte (PDMA) chains that connect the hydrophobic stickers. In this case, chain elongation by shearing cannot be adsorbed, and this leads to faster gel softening.

To confirm the existence of a strong physical network for the heptablock in salt solution, oscillatory shear experiments were performed within the linear viscoelastic regime, and compared with the relaxation spectra for the salt free solution. Figure 69 shows the variations of the storage ($G'$) and loss ($G''$) moduli as a function of the angular frequency ($\omega$) for BuMA$_5$-DMAEMA$_{25}$-BuMA$_5$-DMAEMA$_{25}$-BuMA$_5$ in water and in 0.1M NaCl solution. At 4
wt % concentration and pH=6 the values of both \(G'(\omega)\) and \(G''(\omega)\) are smaller in salt solution than in pure water.

![Graph](image)

**Figure 69.** Dynamic moduli \(G', G''\) as a function of frequency at 1% strain of the heptablock copolymer 4 wt % in water (\(G'\) and \(G''\)) and in 0.1M NaCl solution (\(G'\) and \(G''\)), at pH=6 and 25°C

The results obtained for the heptablock in salt solution indicate that the frequency dependence of the complex shear modulus corresponds to a gellike behavior, as observed for the same sample in pure water. In the whole frequency range studied, \(G'\) is higher than \(G''\). The crossover frequency cannot be observed because of the limited range of experimentally accessible frequencies. The terminal zone cannot be explored since the network relaxation time related to the detachment of a hydrophobe from a junction is very high. Stress relaxation experiment for the determination of the relaxation times is presented below.
5.4.3. Stress Relaxation

The 4 wt% polymer solutions at pH 6 salt free and 0.1N NaCl conditions were submitted to stress relaxation measurements in the linear (strain = 4%) viscoelastic regime. Measurements of the stress relaxation function are appropriate for viscoellastic fluids with relaxation times larger than 10 s. Stress relaxation functions are plotted in Figure 70. There is a clear difference in relaxation behaviour between the two systems:

For the heptablock solution in salt free conditions, the stress relaxation function is well described by a stretched exponential function of the first part of the curve in Figure 56,

\[ G(t, \gamma) = G_0(\gamma) \exp \left[-\left(\frac{t}{\tau(\gamma)}\right)^\alpha\right] \]  

where \( G_0 \) denotes the elastic modulus extrapolated as \( t \to 0 \), \( \tau \) is the relaxation time and \( \alpha \) the stretched exponent.

It was obtained the instantaneous elastic modulus \( G_0 \sim 258 \) Pa, the exponent \( \alpha \sim 0.7 \), and the characteristic time \( t \sim 11 \) 000 s. For the second relaxation mode, corresponding to an elastic modulus of 64 Pa, the decay is not visible in the time scale of the experiment since it had been shifted towards very high values of the relaxation time.

The copolymer in 0.1 NaCl conditions had given a relaxation function \( G(t) \) that exhibits a two-step relaxation pattern, proving the existence of a fast and a slow relaxation mode.

Experimental data are properly fitted by eq (2), a sum of stretched exponential functions that describes the fast initial decay and the long time relaxation.

\[ G(t, \gamma) = G_{0,1}(\gamma) \exp \left[-\left(\frac{t}{\tau_1(\gamma)}\right)^{\alpha_1}\right] + G_{0,2}(\gamma) \exp \left[-\left(\frac{t}{\tau_2(\gamma)}\right)^{\alpha_2}\right] \]  

where \( G_0 \) is the instantaneous modulus and \( \tau \) a characteristic relaxation time; the indices 1 and 2 referring to the relaxation modes. The exponent \( 0 < \alpha < 1 \) is the
stretched exponent that quantifies the departure from the mono-exponential function; it measures the broadness of the time relaxation distribution, the smaller $\alpha$ values corresponding to the broader distributions. Table 9 presents the rheological parameters corresponding to the best fit of experimental results using equations 1 and 2.

The curves through the data demonstrate rather good fits to eq 1 and 2 with small deviations at long $t$.

**Figure 70.** Stress relaxation modulus of a 4 wt% polymer solution at pH=6 (○) and in NaCl 0,1 M conditions at pH=6 (○), for a strain amplitude of $\gamma=4\%$. The continuous lines through data represent the best-fitting calculation curves obtained from equation 2 (black line).

The calculated values of $G_0$, $\tau$, and $\alpha$ are listed in Table 9. The $G_0$ values decreases by salt addition, and their magnitudes are in good agreement with the results of oscillatory measurements.

**Table 9.** Rheological parameters $G_{0,1}$, $G_{0,2}$, $\tau_1$, $\tau_2$ and $\alpha_1$, $\alpha_2$ obtained from the fit of stress relaxation modulus of a 4 wt% polymer solution using equations (1) and (2).

<table>
<thead>
<tr>
<th>Sample</th>
<th>$G_{0,1}$ / Pa</th>
<th>$G_{0,2}$ / Pa</th>
<th>$\tau_1$ / s</th>
<th>$\tau_2$ / s</th>
<th>$\alpha_1$</th>
<th>$\alpha_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>7B 4wt% pH=6</td>
<td>258</td>
<td>64</td>
<td>11000</td>
<td>-</td>
<td>0,7±0,1</td>
<td></td>
</tr>
<tr>
<td>7B 4wt% NaCl 0,1M pH=6</td>
<td>2,24</td>
<td>0,7</td>
<td>80</td>
<td>5300</td>
<td>0,6</td>
<td>0,8±0,1</td>
</tr>
</tbody>
</table>
A two step relaxation mechanism was also observed for the PMMA-PDMA-PMMA, constituted from similar in nature blocks in the linear and nonlinear regime and in salt free conditions.[10] The major differences in the relaxation response of between these systems can be attributed to the different topology of the additional blocks and to the different nature (ionic/screened charges) of the bridging hydrophilic chains.

From a molecular point of view, the fast relaxation mechanism may be attributed to polymer chains that relax following a process controlled by the disengagement of the hydrophobic blocks from associative junctions. This effect is due to the difficulty for a given hydrophobic end group, which disengage from an associative junction, to find a new available partner since the hydrophilic polyelectrolyte “spacers” had been shrinked.[16], [17]

The slow relaxation mechanism may be attributed to polymer chains which are elastically active in the network and cannot relax on short times because of electrostatic interactions with neighboring chains. These interactions are either electrostatic repulsions between positively charged PDMA blocks or dipole attractions arising from counterion condensation. Such electrostatic interactions act as “electrostatic entanglements”, which tend to slow down the chain relaxation.[10] The decrease of the relaxation time for the salt solution comparing with salt-free solution can be ascribed to the screening of these “electrostatic entanglements” which now allows faster relaxation of the elastic chains.

Thus, the presence of two distinct relaxation time scales for is attributed to an inhomogeneous network structure composed of two polymer chain populations: highly stretched chains whose relaxation is governed by the effective lifetime of an associative junction (fast relaxation), and weakly stretched chains whose relaxation is hindered by electrostatic interchain interactions or counterion condensation (slow relaxation).

The above behavior can be explained by taking into account the effect of salt on the size of the macromolecular chains in solution. In the presence of electrolyte, the chains have a more flexible conformation due to the screening of the electrostatic repulsions developed between charges of the same sign. This
can facilitate the formation of intramolecular hydrophobic association at the expense of intermolecular junctions. Once the chain dimensions are reduced by charge screening the transient network formed in the presence of salt is weaker, which will result in a decrease of both the viscosity as depicted in figure 70 and the instantaneous modulus (table 9). However, at such a high polymer concentration, degree of chain overlap and electrostatic entanglements prevent the network from disruption.
5.5. Conclusions

Rheological measurements showed that only the heptablock copolymer (ABABABABA) bearing hydrophobic end-blocks formed hydrogels at relatively low copolymer concentrations. Therefore, the investigation was focused in the rheological properties of the heptablock copolymer solutions.

The onset of network formation has been studied at pH 6 ensuring polyelectrolyte character of the PDMA blocks. The critical gel concentration was determined at about 0.6 wt%, remarkably low regarding other associative polymers with comparable molecular weight. Moreover, at higher polymer concentrations (3-4 wt%) the heptablock exhibits an unusual behaviour with respect to simple telechelic polyelectrolytes with an increase in the power exponent in the viscosity vs concentration dependence, revealing the formation of a very stiff gel. The behaviour of the system in this concentration regime has been ascribed to a contribution of the internal PBuMA stickers to the strengthening of the network, as the system becomes denser and the intermolecular distance between the internal hydrophobic blocks decrease, favouring association. Further increase in concentration resulted in a viscosity decrease due to counterion condensation along the elastic chains that have an electrostatic screening effect.

Moreover, polymers containing ionic sites can be potentially responsible for changes in the ionic strength and pH. The study of the rheological properties of the heptablock hydrogels by varying the pH of the medium was also performed. In the case of charged associating polymers, a more complicated rheological behavior is observed comparing with that of the corresponding neutral polymers because of the competition between attractive (hydrophobic) and repulsive (electrostatic interactions). Thus, the presence of the charged units may have two opposite effects on the thickening properties: an increase in the viscosity due to coil expansion and a decrease due to a lowering of the hydrophobic association degree arising from the charge repulsion, hindering the interpenetration and overlapping of the macromolecules. For the present system
it was found that the viscosity passes through to maxima in the vicinity of pH 4.5 and pH 6.5 prior to sharply decrease at high values of pH where the tertiary amine pendant groups of the PDMA blocks are strongly deprotonated. The first viscosity maximum had been reported for telechelic polyelectrolytes type ABA constituted by similar in nature blocks. The second maximum in viscosity has been attributed to a predominance of intermolecular attractive interactions of the internal hydrophobic stickers upon charge density decrease, leading to a transition of the “open” stickers to “closed”, contributing to an increased number of elastic chains and a strengthening of the network.

The temperature dependence on the samples corresponding to the maxima values of viscosity vs pH had demonstrated a gradual decrease in viscosity at the start-up of heating (from 10°C to about 20°C) followed by a viscosity plateau for a large temperature range (from ~20°C until 50°C). It was also proved that the drop of viscosity is not temperature induced, but a time dependent effect. These results had revealed that the network structure is stable over the temperature range examined.

Finally the influence of the ionic strength on the properties of the heptablock hydrogels was performed. It was found that the addition of salt leads to several significant effects. First, the Newtonian viscosity and the critical yield stress is decreased due to network weakening by electrostatic screening. This had been also proved by a decrease in the elastic modulus in the linear viscoelastic regime as revealed by oscillatory measurements. Importantly, the critical $\gamma_{LVR}$ value in salt free is slightly lowered due to the stretched conformation of the polyelectrolyte that refrain further elongation.

Stress relaxation tests had demonstrated the existence of two relaxation modes both in salt free and salt conditions. The presence of two distinct relaxation time scales for is attributed to an inhomogeneous network structure composed of two polymer chain populations: highly stretched chains whose relaxation is governed by the effective lifetime of an associative junction (fast relaxation), and weakly stretched chains whose relaxation is hindered by electrostatic interchain interactions or counterion condensation (slow relaxation).
Due to the shrinkage of the polyelectrolyte elastic chains, the hydrophobic blocks that is disengaged from an associative junction encounter difficulties onto finding a new available partner for further association. Therefore the two relaxation modes are shifted toward lower values in salt solution.
5.6. References

In this work, novel amphiphilic polyelectrolytes with multi-block architecture were explored with respect to their ability to form hydrogel. In particular, a series of model asymmetric ABA, BABAB, ABABABA and BABABABAB (A hydrophobic, B polyelectrolyte) block copolymers were investigated as possible gelators in aqueous media. Rheological results showed that only the heptablock copolymer (ABABABA) with hydrophobic blocks at both ends formed hydrogels at relatively low copolymer concentrations. This result reveals the importance of the macromolecular topology of the hydrophobic and hydrophilic blocks. The behaviour of the system and especially its ability to self-organized in a 3D infinite reversible network (physical hydrogel) is governed by the interplay of the hydrophobic attractive interactions of the A blocks and the electrostatic repulsive interactions of the polyelectrolyte B blocks.

Light Scattering and TEM experiments were used to elucidate the influence of the topology of the building blocks onto the association efficiency of the systems in low concentration. The dilute solution studies in both salt and salt-free conditions, had shown that the heptablock with hydrophobic end blocks, forms extended aggregated structures of extremely high association number. Contrary, the pentablock and the nonablock bearing only internal stickers forms finite size aggregated of significantly lower aggregation numbers, due to the polyelectrolyte blocks that prevent the extended association through hydrophobic interactions.

Due to the fact that the hydrophilic block is a weak polyelectrolyte, the electrostatic interactions are strongly sensitive to pH and ionic strength. This can explain the various phenomena observed for the heptablock copolymer hydrogel, regarding its concentration, pH and ionic strength dependence of the rheological properties.
Regarding the concentrated solutions:

The critical gel concentration was determined at about 0.6 wt\%, which is remarkably low regarding other associative polymers with comparable molecular weight.

Importantly, the present system exhibits an unusual behavior at elevated concentrations with respect to the ABA type telechelic polyelectrolytes, with an increase of the power exponent. This behavior has been attributed to the strengthening of the network by the transformation of “open” internal stickers, bearing at both ends charged moieties which develop repulsions that prevent association, to “closed” highly associative stickers as the system becomes denser and the intermolecular distance between the internal hydrophobic parts decreases favoring association. This was supported by counterion condensation which weaken the electrostatic repulsions. Further increase of concentration resulted to a viscosity decrease, which has been ascribed to electrostatic screening effects due to counter ion condensation along the elastic polyelectrolyte segments leading to a decrease in the number density of the elastically active chains.

From practical point of you, the heptablock copolymer exhibits high gelation efficiency forming a free-standing hydrogel above 3.5 wt\% polymer concentration, characterized by viscosities more than six orders of magnitude higher than that of the medium.

The pH dependence on the rheological properties has shown a peculiar behavior comparing with telechelic polyelectrolytes constituted from similar in nature blocks. In the SOL-GEL-SOL transition the viscosity passes through two maxima prior to decrease sharply at high values of pH. The second viscosity maximum was attributed to further contribution of the additional hydrophobic interactions of the internal stickers upon further decrease in the charge density of the PDMA resulting to strengthening of the 3D network and in turn increasing the viscosity of the system.

The temperature effect on the rheological properties of the heptablock hydrogel was negligible. The fact that viscosity is nearly invariant with
temperature, even at $50^\circ$C, is due to the stability of the structure over the temperature range examined.

The presence of salt has significant consequences on the rheological properties of the hydrogel. At 0.1N salt, a free-standing gel, retaining its transparency, is formed whereas at higher ionic strength phase separation occurs. The viscosity decreases about two orders of magnitude upon adding 0.1N salt due to the network structure alteration provoked by electrostatic screening.

Stress relaxation tests had shown a two step relaxation mechanism for both salt and salt-free hydrogels, with an increase of the relaxation time for the salt-free solution. Thus, the presence of two distinct relaxation time scales for is attributed to an inhomogeneous network. The two slow relaxation times in salt-free hydrogel have been shifted towards very high values proving that the system behaves as a very stiff gel.

Summarizing the results from the study of these different associative multiblock copolymers we could conclude that the differences in the topology of the building blocks play a key role in the type of self-organization of these systems.

These kind of polymeric materials may found applications as thickeners in cosmetics, coatings and other water-born formulations, and as injectable hydrogels in pharmaceutical applications since its gelation ability is preserved in physiological pH and ionic strength.