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FREE AND CONTROLLED RADICAL POLYMERIZATIONOF POLYMERS FOR THERMORESPONSIVE HYDROGELS

TREBALL DE FI DE GRAU

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Abstract

This work has been realized in '*Makromolekure Chemie',* the polymers research group in the University of Siegen (Germany) headed by Prof. Dr. Ulrich Jonas aided by Mrs. Petra Frank. One of the research line in this group is *Surface-Attached Hydrogel Layer Systems* in which all my projects had been included and some of them has biomedical aims.

The first project was to study a method for the synthesis of an azido functionalized initiator, 4,4 '-azobis-(3-azidopropyl)-(4-cyanopentanamide) by reaction of two products, 4,4' -azobis (4 -cyano pentanoyl chloride) and 3 -amino-1 - azidopropane which were also synthesized in this work . The initiator, which will be examined later, intended to be reacted by free Radical Polymerization with an alkyne terminal group polymer having UCLT properties. The next study will consist of couple this UCST polymer via 'click chemistry' with a polymer LCST as might be the NIPAAm which its chemical behaviour is known for previous studies.

The study of the polymerization of this monomer, NIPAAm, has been developed in this study via ATRP reactions using different solvents, initiator-catalyst systems, reactions conditions and, also, by varying the ratio of monomer to initiator.

This monomer is also used to form a copolymer together with two other monomers: MAA (Methacrylic acid) and BPAAm (N-(4-benzoylphenyl) acrylamide). This NIPAAm-MAA-BPAAm copolymer, with an approximate ratio of (85:5:10) was used as the adhesion polymer into Petri dishes and crosslinked. Subsequently, the same copolymer but with a different relation (94 :5:1) was used and studied as a hydrogel layer, the stability of which was studied against of different solutions such as water, ethanol and two buffer solutions : Tris -HCl pH 7.5 and Borate pH: 10.

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Abbreviations

ATRP	Atom Transfer Radical Polymerization
BPAAm	*N*-(4-benzoylphenyl)acrylamide
CH_2Cl_2	Dichloromethane
CRP	Controlled Radical Polymerization
CuCl	Copper Chloride
DMSO	Dimethyl Sulfoxide
EBIB	Ethyl α-bromoisobutyrate
EBP	Ethyl 2-bromopropionate
HCl	Hydrochloric acid
IR	Infrared
KOH	Potassium hydroxide
LCST	Lower Critical Solution Temperature
MAA	Methacrylic acid
MCP	Methyl 2-chloropropionate
Me_6TREN	Tris[2-(dimethylamino)ethyl]amine
NaN_3	Sodium azide
NIPAAm	*N*-isopropylacrylamide
NMR	Nuclear Magnetic Resonance
RAFT	Reversible Addition-Fragmentation chain Transfer
RP	(Free) Radical Polymerization
UCST	Upper Critical Solution Temperature
TRIS	2-Amino-2-hydroximethyl-propane-1,3-diol

1. Introduction

The work shown in this report has been done in '*Makromolekulare Chemie* (MC)*'* in the '*Universität Siegen'* (NRW, Germany). This research group is headed by Prof. Dr. Ulrich Jonas specialized in organic and polymeric chemistry and aided by the collaboration of Mrs Frank.

MC group works in general in the area Radical Polymerizations (RP)[1] and Controlled/Living Radical Polymerizations (CRP). This last one is nowadays more interesting and it is included in one of the project that I have worked in, especially ATRP which is a type of CRP. A second type of CRP, which is studied in the MC group, is RAFT polymerization.

There are three main lines of research:

1. *Surface-Attached Hydrogel Layer Systems*: Study for the preparation of hydrogels made with polymers or copolymers, which are chemically crosslinked, usually by light-induced reactions. This study is highly attractive for sensors, actuators, adhesives, and coatings and medical applications

2. *Hierarchically Assembled Colloid Structures*: Study the deposition available of colloidal crystals to modify its angle and incident wavelength dependent transmission of optical and the acoustic or elastic waves in crystals called photonic or phononic crystals.

3. *Structural and Viscoelastic Properties of Semifluorinated Alkane Monolayers at the Air/Water Interface*: Investigate the hierarchical self-organization of semifluorinated alkanes at the air/water interface in order to develop a detailed structure-properties relationship for this very particular class of materials.

My project was included in the *Surface-Attached Hydrogel Layer Systems* research. The basic project was to synthesize an initiator containing an azido end group. Then this initiator will be studied the coupling of a polyNIPAAm (poly N-Isopropylacrylamide) and an alkyne group via free Radical Polymerization (RP).

For the synthesis of an azido terminated initiator, it was necessary to prepare two educts in advance. The synthesis routes have been already studied in this project. The first

[1] All the concepts mentioned here are explained detail in the next section (Theorical Background)

educt required is 3-amino-1-azido propane, formed in the reaction between 3-bromopropylamine hydrobromide and sodium azide. The second is the reaction of 4,4'-azobis-(4-cyano pentanoic acid) and phosphorous pentachloride to synthesize 4,4'-azobis-(4-cyano pentanoyl chloride). Once both educts are prepared, they should form the desired initiator, as presented in the scheme below (Fig.1):

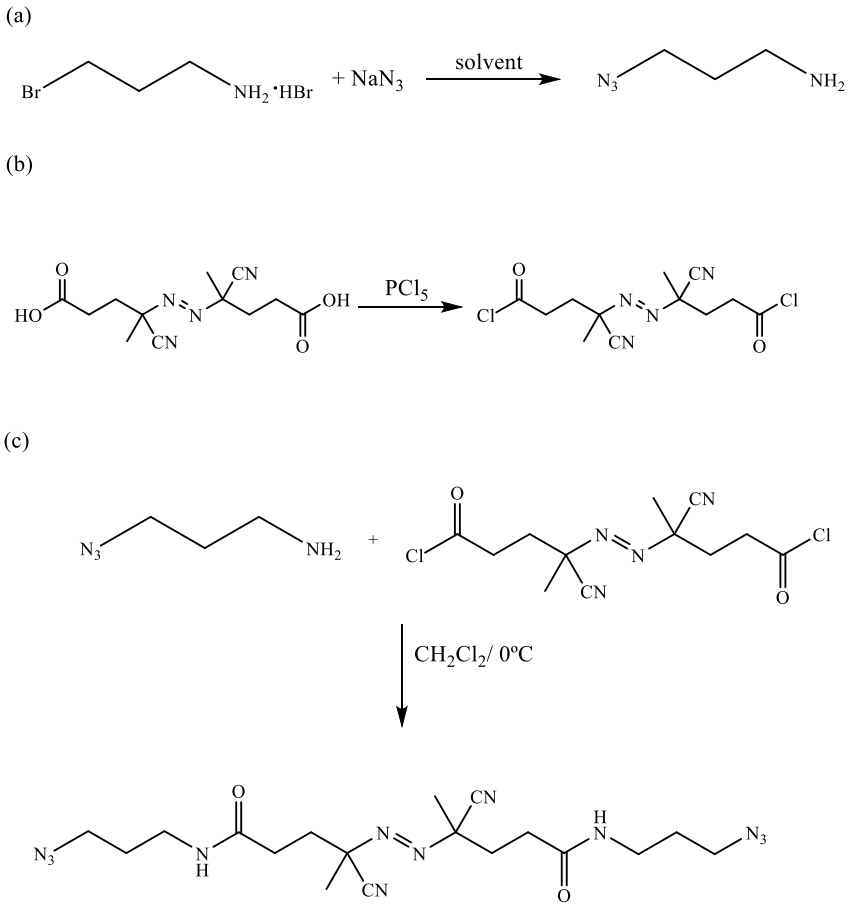


Fig.1: Complete synthesis of azido end group initiator. (a) shows the 3-amino-1-azide propane synthesis while (b) the acyl chloride educt and (c) the synthesis of azido functionalized azo initiator.

This synthesized initiator was studied and then used as initiator in RP to couple an UCST (Upper Critical Solution Temperature) alkyne end group polymer and a LSCT (Lower Critical Solution Temperature) polymer, such as polyNIPAAm, via click chemistry.

Another project was to study the ATRP reaction with NIPAAm as monomer and using Me6TREN as ligand. This polymer was used because its behaviour is known, so different experiments it in different conditions, initiators, catalysts and solvents were used in ATRP reactions, allowing to know exactly how this reaction works and which is the best procedure.

Finally the 3rd and final research project aimed at the study of the stability of polyNIPAAm-copolymer hydrogel layers in different solvents to be used in the study of cell growth substrates.

2. Aims

After making an introduction of the study field in this work is necessary to remark which have been the aims. These are explain below:

- Synthesize an initiator with an azido end group and optimize the reaction.

- Find optimal conditions, solvents, catalyst and initiator for an ATRP with NIPAAm as monomer and Me_6TREN as ligand.

- Study the stability of hydrogel NIPAAm-copolymers in different solutions layers and to test different procedure to prepare these layers.

3. Theorical Background

3.1. Polymers and polymerization

The synthesis of polymers is a reaction of chemical connection of thousands of monomer molecules, forming a macromolecular chain. The synthesized polymers can have different structures e.g. linear, branched or crosslinked. These structures are represented in the Fig.2.

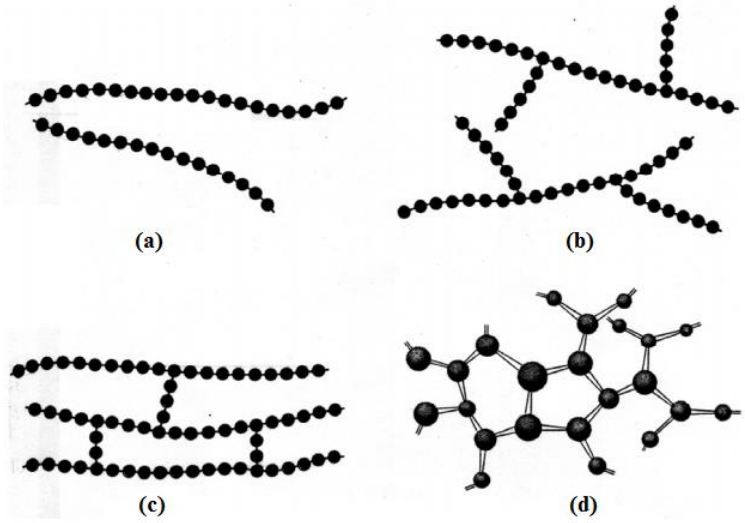


Fig.2: Different polymer structures. Thereby, (a) is linear, (b) is branched and finally, (c) are weakly and (d) highly crosslinked polymers.

Polymers can be synthesized by different mechanisms[1]: *step-growth polymerization*; formed by chemical reaction between at least two functional groups, and *chain polymerization*; which is initiated by an initiator. In this last case, there are different kinds of reaction depending on the type of initiator. The most common type of chain-growth polymerization is the free radical polymerization, which will be explained below.

3.1.1. Free Radical Polymerization

The free radical polymerization is the most versatile type of chain growth. But the general mechanism is similar for all chain polymerizations, which has three or four basic steps [2]: *Initiation*, *propagation*, *termination* and *chain transfer*.

3.1.1.1. Initiation

First of all, the formation of free radicals from the initiator has to happen. In general there are two ways how radicals can be formed: *Transfer of a single-electron* (produces only one single-free radical species) and *homolytic scission* (produces two free radical species).

The homolytic cleavage is mostly done by heating or by photolysis, as it is presented in the next figure (fig.2):

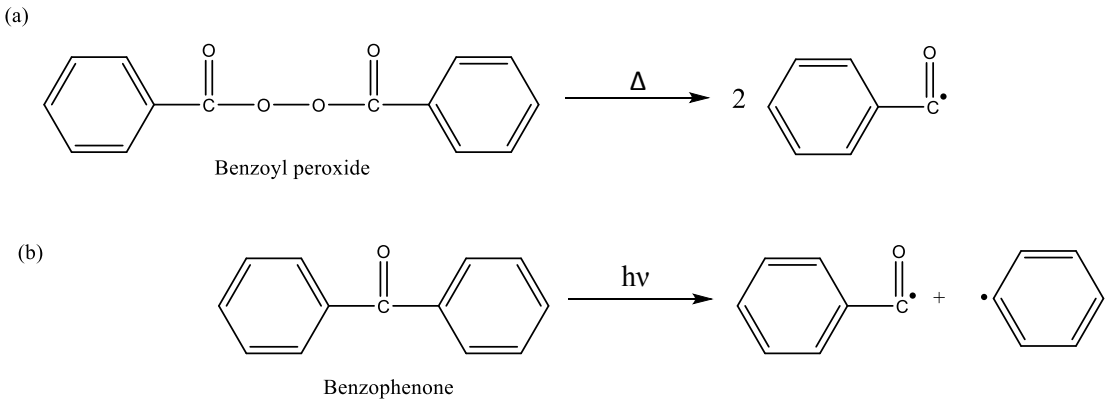


Fig.2: Homolytic scission of initiators. (a) is the homolysis effected by heat of benzoyl peroxide and (b) is the homolytic scission of benzophenone by photolysis

The compounds that contain peroxide (-O-O-) or azo (-N=N-) groups are typically termosensitive initiator for the radical polymerization.

An example of single-electron transfer is shown in the next scheme (Fig.3):

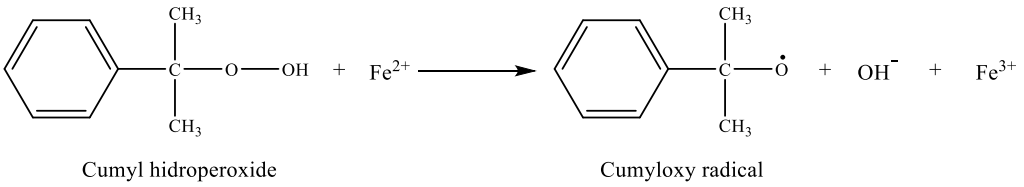


Fig.3: Single-electron transfer radical initiator. Fe^{2+} gives an electron to the OH of cumyl hydroperoxide breaking the peroxide bond (O-O).

Once the radical is formed, it attacks a double bond containing monomer molecule creating an active centre. In principle two different radicals could be formed, but the most stable radical structure will prevail. An example is showed in the Fig. 4, where the initiator R· attacks a styrene monomer forming the starting radical for the propagation.

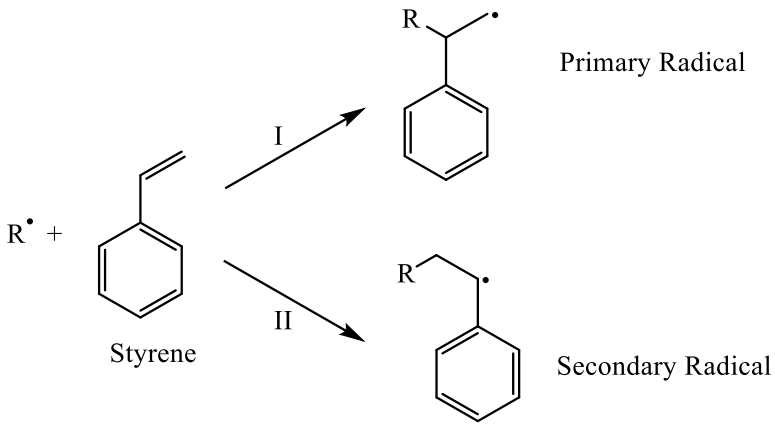


Fig.4: Initiation of styrene polymerization. The initiator (R·) attacks the monomer. The more stable radical will prevail, in this case the secondary radical will be formed.

3.1.1.2. Propagation

In this step the active centre, created in the previous step, continues attacking another monomer and so successively, lengthening the chain, as it is shown in the next scheme (Fig.5):

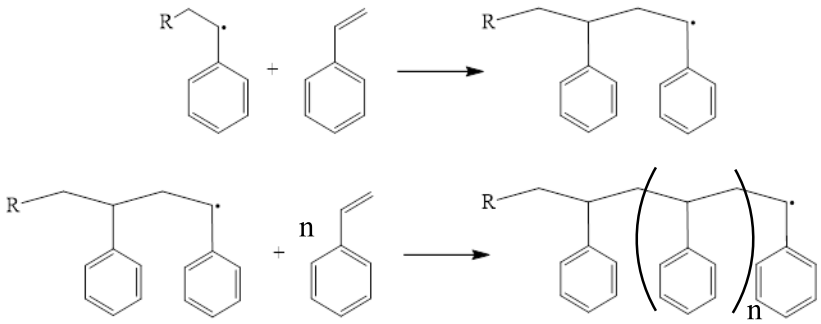
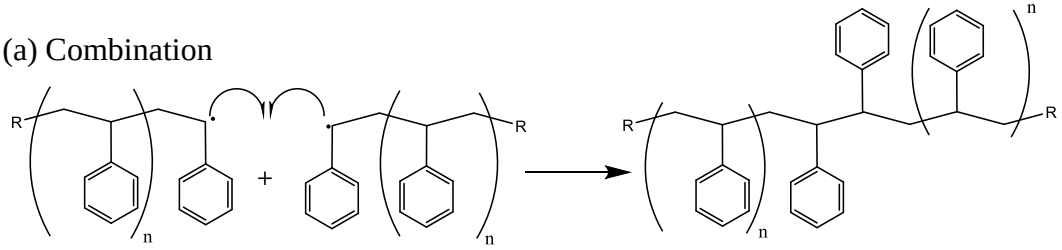


Fig.5: Propagation and chain growing of polystyrene by free radical polymerization (RP).

3.1.1.3. Termination

This step is the last one and there are several possibilities among the termination processes. Two very well studied processes are the ones presented in Fig.6 showing the combination of two polymer radicals and the disproponation with the transfer of a proton radical.

(a) Combination



(b) Disproponation

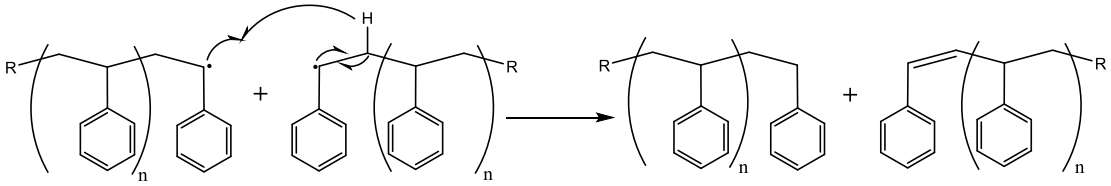


Fig.6: Different kinds of termination in RP. (a) shows the mechanism of combination while (b) disproponation

3.1.1.4. Chain transfer

The transfer of the active centre occurs in most chain-growth polymerization. In this reaction the radical is transferred from the chain end of polymer to another species, which could be e.g. a solvent molecule, a polymer or others. The generic form are presented in the next scheme (Fig.7)

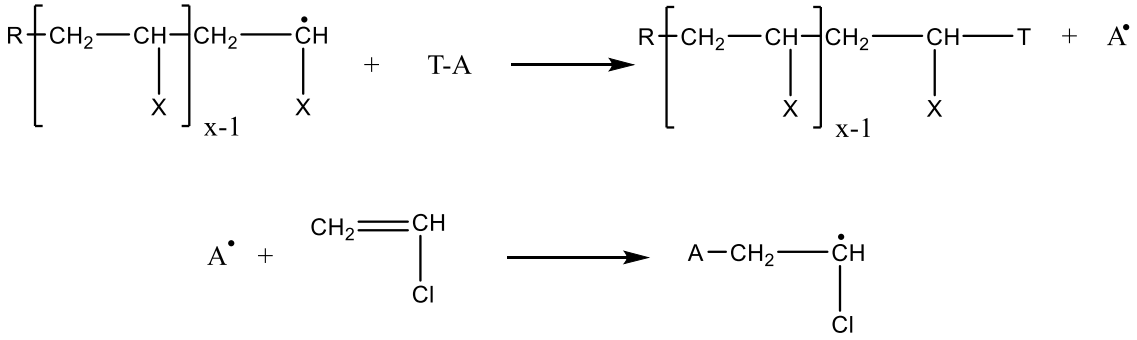


Fig.7: Scheme of the chain transfer reaction

Including special initiators and catalysts systems this mechanisms are fundamental for RAFT and ATRP polymerizations, two types of CRP.

3.2. Controlled Radical Polymerization

Even though Free Radical Polymerization is a widely used reaction, it has some disadvantages. The main limitations are the lack of the of molecular weight distribution (MWD), the difficulty to make well defined block copolymers and the lack of introducing two different functional groups at the opposing chain ends [3]. This control is provided for CRP (Control Radical Polymerization or Living Radical polymerization), which has been studied over the past 60 years in organic and polymer chemistry. In this years, different methods have been developed but the three most important are: *Nitroxide Mediated Polymerization* (NMP), *Reversible Addition Fragmentation chain Transfer* (RAFT) and *Atom Transfer Radical Polymerization* (ATRP)[4], providing almost controlled conditions, obtaining polymers with a narrow mass distribution and partly predictable molar masses. These mechanisms will be briefly explained in the following sections.

Another advantage is, because of using modified initiators or chain transfer agents (CTA) can be incorporated into the polymer which can be used for polymer analogues reactions, e.g. click chemistry of azide and alkyne groups[5].

3.2.1. Nitroxide-Mediated Radical Polymerization (NMP)

NMP was the first developed CRP-method. Salomon *et* al. [6] discovered that nitroxides (R_1R_2N-O·) are persistent radicals which couple rapidly with carbon-centred radicals. Georges[2] showed that propagating radicals can be trapped reversibly and achieved living-like conditions in radical polymerizations. The degrees of distribution were found to be low.

The general mechanism is showed in the next scheme (Fig.8).The equilibrium constant K is defined as k_{diss}/k_{com}, where k_{diss} is the rate coefficient for the dissociation of C-O from alkoxyamine end group presents in the initial polymer while k_{com} is the rate coefficient for combination of the chain radical and the nitroxide radical.

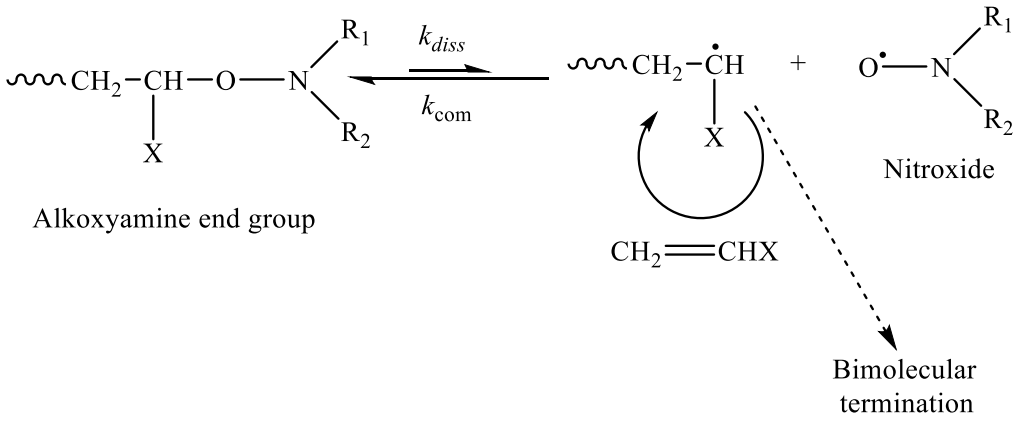


Fig.8: General mechanism of Nitroxide-Mediated Radical Polymerization

3.2.2. Reversible Addition-Fragmentation chain Transfer (RAFT)

RAFT follows an activation/deactivation mechanism, based on a chain transfer process (section 2.1.1.4). In RAFT the initiating radical (R·), which is formed by normal radical initiators e.g. AIBN, reacts with a monomer producing a new radical which then reacts (RM·) with the next monomer[2] (see *Propagation 3.1.1.2*). The Chain Transfer Agent (CTA), normally an active dithioester, is used in excess to ensure the equilibrium to his site. For an easy understanding the mechanism is shown below (Fig.9)

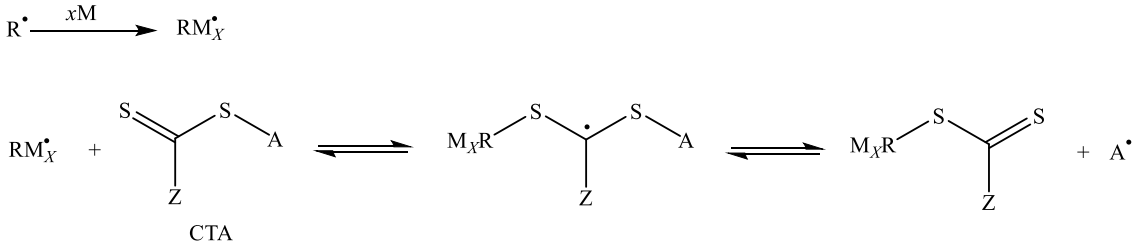


Fig.9 : The chain transfer to the CTA and the propagation mechanism

3.2.3. Atom Transfer Radical Polymerization (ATRP)

ATRP is a transition-metal catalysed radical polymerization, and is the most widely used and effective method of Controlled Radical Polymerization (CRP). The main advantage of ATRP is the ability to control the molecular weight and polymer architecture.

In ATRP, an organic halide in combination with a transition metal is used as catalyst, the most often metal used is Cu(I) but other metals with at least two readily accessible oxidation states separated by one electron are also possible. The catalyst needed in this reaction is a complex of Cu(I)X (X=Br o Cl) with a nitrogen containing ligand. Organic halides RX, mostly alkane bromides or chlorides, are used as initiator, the catalyst-initiator system should have the same halide for a successful polymerization[7].

In ATRP exists an equilibrium between the initiator (R-X) and the free radical (R·) formed by the transition of an electron from transition metal ion which is oxidized and coordinates the nascent halide anion[2]. This radical reacts with the monomer molecule and the process is initiated by propagation. The polymer chain grows by continuous activation-propagation-deactivation cycles whereas the majority chains are in dormant state [8], as it is shown in scheme below (Fig.10). Hence, only very few radicals are available which means in consequence a controlled molecular weight and a narrow weight distribution.

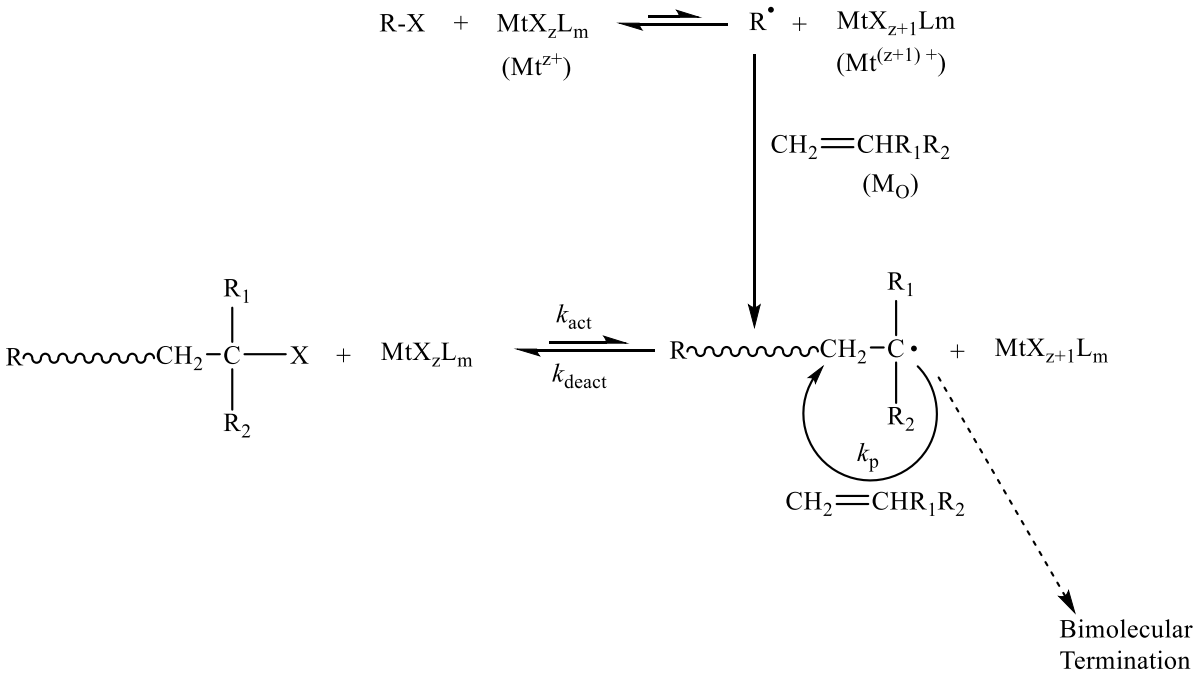


Fig.10: Initiation and mechanism of ATRP. In this scheme, X represents the halogen atom (Cl^- or Br^-), Mt^z the transition metal with its oxidation state, R-X is the initiator and L_n is the ligand.

3.3. "Click" Chemistry

As has been discussed above, the initiator used in CRP is incorporated to the end of polymer chain, which allows polymer analogues reactions e.g. via click chemistry.

This term was introduced by K. Barry Sharpless and his co-workers in the past early nineties. Nowadays, this named "click" reactions are of a great interest for their high specifity, quantitative yields, environmental friendly and highest rate, wide in scope and stereospecific [9].

Click chemistry has had a dramatic and diverse impact in many areas of modern chemistry, highlighting polymer research. Despite not adjusting all the 'click chemistry' requirements, the Huygens cycloaddition 1,3-dipolar azides and alkynes is a great example, as it can be seen in the Fig.11.

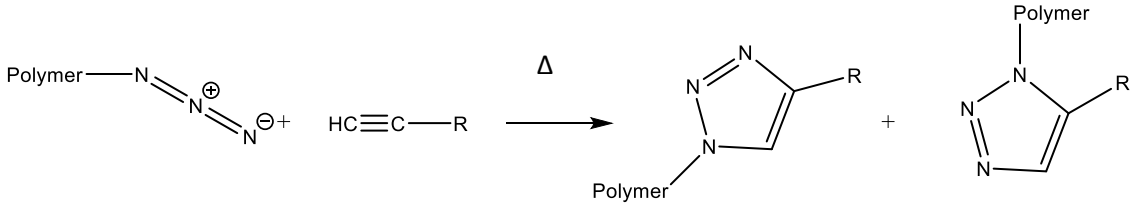


Fig.11: Huygens' cycloaddition. Mixture of regioisomers

This variant of Huygens cycloaddition with copper (I) salt as catalyst, discovered by Sharpless [10], is named CuAAC (Copper-catalyzed Azide-Alkyne Cycloaddition), which fits so well in the definition of "click chemistry". This reaction differs from Huygens reaction that is more selective [11], the reaction can be observed in Fig.12:

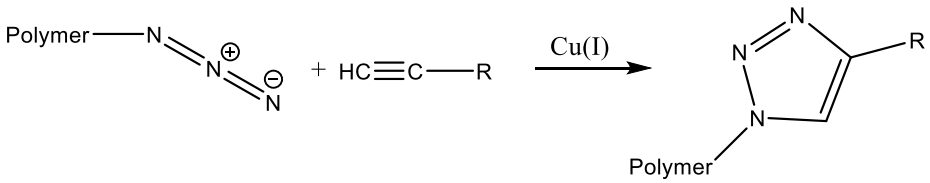


Fig 12: 1,3-dipolar cycloaddition by CuAAC

3.4. Hydrogels

Hydrogels are chemically or physically crosslinked networks of polymers which swell in water and do not dissolve. Usually, hydrogels contain 5-10% of water but in an extreme case i.e superabsorber, the retained water can be 99% [12].

Hydrogels are often biocompatible and therefore interesting materials for cell immobilization or cultivating[13]. Another important medical application is the controlled release of drugs which can diffuse into the pores[12].

In this project the post crosslinkable polymer is achieved by the copolymerization of three monomers (NIPAAm- MAA- BPAAm) crosslink by light-induced. The chemical stability of this gel against several solvents should be studied.

3.5. Smart Polymers

These polymers called *smart polymer* or *stimuli-responsive polymer* are potentially interesting due to strong conformational changes that they can afford when a small physical or chemical stimuli (e.g. temperature, pH, ionic strength) is generated in its environment[14].

Poly N-isopropylacrylamide is known as a thermoresponsive polymer, these polymers have a drastic property change upon a small changes of temperature, this means that the polymer precipitate (cloud point) upon heating or cooling[15].

Polymers which precipitate upon heating are showing a lower critical solution temperature (LCST), other polymers showing precipitation upon cooling have an upper critical solution temperature (UCST). The phase diagram is shown in the Fig.13.

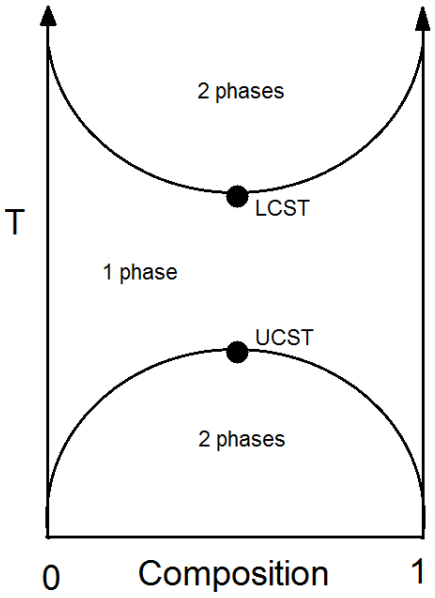


Fig.13: A phase diagram including both an UCST and an LSCT

From the molecular point of view with temperature changes the molecular conformation changes dramatically from a hydrophilic, hydrated polymer coil to hydrophobic collapsed globules[16] as shown in the Fig.14.

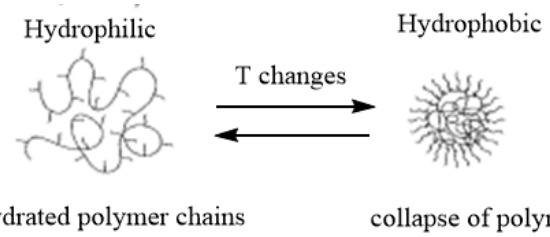


Fig.14: Coil (left) to globule transition (right) depending on the temperature [17](slighty modified).

PolyNIPAAm is a LCST polymer, this temperature is at 32ºC which makes it very attractive for biomedical applications because is close to human body temperature. The amino group can form hydrogen bonding at room temperature, but when this temperature is over LCST these bonds are broken and isopropyl groups are now in the external part of polymer generating the hydrophobic behaviour.

This property is useful for biomedical studies because a tissue or cell can be fixed in polyNIPAAm layer, and then removed without any damage only heating above its LCST.

A long-term project is to synthesize a LCST-UCST block copolymers which only have a narrow temperature window for dissolution. A synthesize rout could be to prepare a LCST-polymer started with an azido terminated initiator like the synthesized 4,4'-azobis-(3-azidopropyl)-(4-cyanopentanamide) and an

UCST-polymer with an alkyne end group following by “clicking” both together. The desired behaviour is schematically show in the Fig.15.

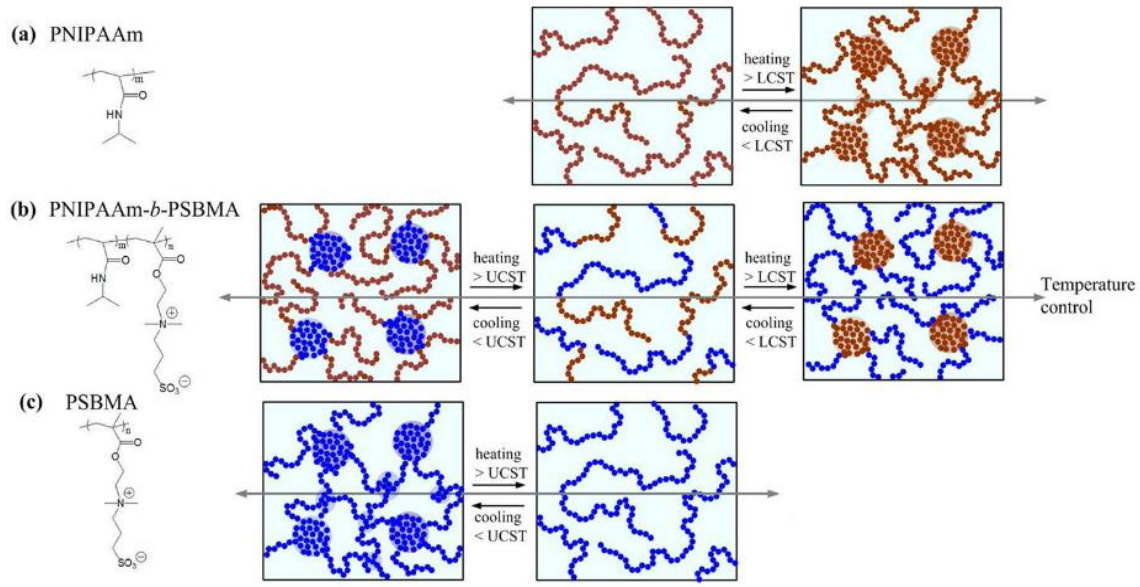


Fig. 15: (a) shows the coil-to-globule transition depending on the temperature of poly(NIPAAm), LCST polymer. On another hand the same transition for the UCST polymer, poly(SBMA), is appeared in picture (c). And in (b) appears the temperature dependence on the LCST-UCST block copolymer. [18]

4. Experimental Part

4.1. Instruments

Infrared (IR) spectrum was made for following the synthesis of 4,4'-azobis-(3-azidopropyl)-(4-cyanopentanamide) showed in (ANNEX 2). IR spectra were recorded on a Bruker Tensor 27 Fourier transform spectrometer. Both solid and liquid samples were measured with Miracle-ATR Diamant from Pike Technologies using blank or relative solvents as background.

For analysis of the molar mass distribution of poly(NIPAAm) a gel permission chromatography was done via a PSS-System (Agilent 1260 series) consisting of a isocratic pump, an UV-detector, a differential refractometer and a column with 103-106 Å pore size (PSS SDV linear 5µ 8×300 mm). Dimethylacetamide containing 0.1 wt.% LiBr was used as eluent with a flow rate of 1.0 ml per min. The concentration of the polymer solution was 0.1-0.5 % and 20 µl were injected per run. Toluene was used as internal standard and PMMA samples for calibration.

For crosslinking an UVP Cl-1000 ultraviolet at 365nm was used, typically with 1 hour irradiation time.

The NMR spectra was recorded with a Bruker Avance 400 spectrometer (1H-NMR, 40MHz), sample was measured at room temperature. The chemical shift δ was specified in ppm. In a mixture of deuterated methanol and deuterium oxide, the solvent peak was used as an internal standard. The spectra was analyzed with software MestreNova.

4.2. Preparation of azido terminated initiator

The synthesis of this compound was described by Basit Yameen *et* al, 2010[19] and had been already studied before in the laboratory but the reaction yield was not very high. Hence, reaction conditions had to be optimized. Different solvents, temperature, reaction times and work-up procedures were chosen.

To achieve this initiator it was necessary to synthesize the two previous educts. Starting from 3-bromopropylamine hydrobromide in the presence of sodium azide, 3-amino-1-azido propane had to be prepared. The second compound 4,4'-azobis-(4-cyano pentanoyl chloride) was synthesized from 4,4'-azobis-(4-cyano pentanoic acid) with phosphorous pentachloride under exclusion of oxygen and moisture. Then these two substances could be coupled in a condensation reaction to the desired product. The reaction path is shown in the scheme (Fig.1) included in section *1. Introduction*. The reactions products were checked in IR spectrum (ANNEX 2)

4.2.1 Chemicals for azido end group initiator synthesis

The commercially available reagents were used as received without any further purification unless specified otherwise. 3-bromopropylamine hydrobromide (98%, Alfa Aesar), sodium azide (99%, Merck. CAUTION extremely toxic), 4,4'-azobis(4-

cyanopentanoic acid) (98%, Fluka Chemika), phosphorous pentachloride (98%, Acros Organics. CAUTION extremely corrosive), sodium chloride, magnesium sulfate (99.5%, Riedel-de Haën) and potassium hydroxide (85%, Roth) were used as received.

The employed solvents were of technical purity unless specified otherwise. Water was obtained from a MilliQ apparatus (18.2MΩ · cm). Diethyl ether (99%, Acros Organics), chloride acid (37%, Fisher Chemical), ethanol (95%, technical grade) were used as received. Dichloromethane (Analytical grade, Fisher Chemicals) and n-Hexane (Technical grade) were stored over 4Å molecules sieves under argon.

4.2.2. Synthesis of 3-amino-1-azido propane

In a 100ml Schlenk flask 3-bromopropylamine hydrobromide (13.7mmoles, 3g) was dissolved in water (30ml) followed by the drop wise addition of NaN_3 (0.5moles, 3g) in 25ml of water under Ar-atmosphere. After completed addition the dropping funnel was replaced with a reflux condenser, and the reaction mixture was stirred at 100ºC overnight.

The reaction progress was followed by TLC using a mixture eluent (13 parts CH_2Cl_2, 7 parts MeOH, 1 part NH_3 aqu.). 2/3 of the water were removed on a rotary evaporator, the mixture was cooled in an ice bath and 100ml diethyl ether were added followed by the addition of powdered KOH until the solution was saturated. The aqueous phase was extracted with diethyl ether five times, 60ml each. The combined organic phase were dried over MgSO4, filtered and the solvent was evaporated.

Yield: 47%

IR [20]: ~3000cm^{-1} (NH_2), 1788cm^{-1} (N_3)

4.2.3. Synthesis of 4,4'-azobis-(4-cyano pentanoyl chloride)

4,4'-azobis(4-cyanopentanoic acid) (14.3mmoles, 4g) was weighted into a 3-necked round bottom flask under Ar-atmosphere, 75ml of dried CH_2Cl_2 were added while stirring. The suspension was cooled down in an ice bath and PCl_5 (0.036moles, 7.43g) was added over 30min.

The reaction mixture was warmed up to room temperature. The volume was reduced to approximately 25ml by distillation. Then, 30ml of dried n-Hexane were added, in order to precipitate the product. To allow complete crystallization, the mixture was cooled in an ice bath. Finally the product was filtrated off under Ar-atmosphere, washed with cold and dried n-Hexane and dried under vacuum.

Yield: 74%

IR: 2360 cm^{-1} (CO_2), 1790cm^{-1} (CO), ~1400cm^{-1} (N=N)

4.2.4. Synthesis of 4,4'-azobis-(3-azidopropyl)-(4-cyanopentanamide)

4,4'-azobis-(4-cyano pentanoyl chloride) (2g, 6.33mmoles) was dissolved in 100ml dry and cold CH_2Cl_2 and transferred into a Schlenk flask under Ar-atmosphere.

The solution was cooled in an ice bath while 3-amino-1-azidepropane (2g, 19.98mmoles) dissolved in 25ml dried CH_2Cl_2 were added drop-wise. The mixture was kept stirring at about 0ºC for 6h. Then the reaction mixture was warmed up at to room temperature covered with aluminium foil. Two phases appeared.

Extract the reaction mixture with 1% HCl until the extract of aqueous phase is neutral. Some NaCl was added to organic phase to ensure that is neutral again, then this phase is filtered and dried with $MgSO_4$ and filtered again. The solvent is removed in a rotary evaporator without heating.

The product obtained was suspended in a small amount of EtOH (10-15ml) followed by drop-wise of cold n-Hexane adding in order to precipitate the product. The supernatant liquid was decanted and the dissolving and precipitating procedure were repeated 5 times to purify the product. Finally the product was dried overnight.

Yield: 66%

IR: 3100-3400 cm^{-1} (NH), 2850-3000 cm^{-1} (CH, CH2), 2095 cm^{-1} (CO) , ~1700cm^{-1} (N_3), 1566-1634 cm^{-1}(NHCO)

4.3. Atom Transfer Radical Polymerization (ATRP) of NIPAAm

4.3.1. Reagents and solvents for ATRP

The commercially available reagents were used as received without any further purification unless specified otherwise. NIPAAm (97%) was recrystallized from toluene/hexane (1:1 v/v). Copper chloride (97%, Merck), Tris[2-(dimethylamino)ethyl]amine (99%, Alfa Aesar), Methyl 2-chloropropionate (98%, Sigma Aldrich), aluminium oxide pH=7 (Fluka AG) were used as received. Tetrahydrofuran (99.5%, Fisher Chemicals) and pentane (Technical grade), the employed solvents, were of technical purity.

4.3.2. Experimental Procedure for ATRP

NIPAAm (2g, 17.1mmoles), CuCl (35mg, 0.35moles) and 2-propanol (4g) were weighted in a Schlenk flask and bubbled with Ar at least 30min. Stock solutions of the Me_6TREN ligand (81.5mg, 0.35moles) and the MCP initiator (43.5mg, 0.35moles) were prepared. Put a septum in all the flasks and excluded the oxygen (freeze, vacuum, defrost and Ar. Three times) out of all the solutions.

The ligand was added with a nitrogen-purged syringe and stirrerred the solution for 20min. Then the initiator was added with another syringe to begin the polymerization and let stirring and heating at 60ºC overnight.

The reaction was stopped when appears polymer in the TLC (n-Hexane: ethyl acetate, 1:2), it is known when a spot remains in the start line unlike monomer, which runs in the TLC. All the solvent was removed in a rotary evaporator followed by the addition of diethyl ether stirring 2h. Separate the ether phase and remove all the solvent.

The reaction mixture was dissolved in THF and precipitated in cold pentane to get the polymer. Filtrated it, after that we had a solid phase (polymer) and liquid pentane phase. The polymer was dried and rotaevaporate the pentane solution.

The polymer was blue coloured by the presence of CuCl which had to be removed. The polymer was dissolved in the minimum amount of 2-propanol and let it stirring overnight. A column with Al_2O_3 pH:7 and sea sand was prepared to eliminate the CuCl, the solution obtained after adding the polymer solution in the column was carried to the rotary evaporator to remove the solvent.

4.4. poly(NIPAAm) hydrogel layers

4.4.1. Reagents and solvents for polyNIPAAm hydrogel layers

The commercially available reagents were used as received without any further purification unless specified otherwise. N-Isopropylcryl amide (NIPAAm,97%) was recrystallized from toluene/hexane (1:1 v/v). AIBN (99%) was recrystallized from methanol. N-(4-benzoylphenyl)acrylamide (BPAAm) was prepared with 4-aminobenzophenone and acryloyl chloride. Methacrylic acid (MAA) was destilled in advance. Ethanol (95%, technical grade) was used as received.

4.4.2. Experimental work for polyNIPAAm hydrogel layers preparation

The petri dishes (Ø: 35mm) were rinsing three times with EtOH HSL. 490µl of 2.5%wt adhesion copolymer solution, NIPAAm- BPAAm- MAA (85:10:5) in EtOH HSL, was added while the petri dishes were shaking and still wet. Then, let them dry in a flat place overnight while shaking, preventing the light focus, and crosslink during 60min at 365nm.

300µl of copolymer NIPAAm- BPAAm- MAA (94:1:5) hydrogel wre added, prepared in advance in the laboratory (ANNEX 3), while petri dishes were shaking. Let them dry in a flat place overnight while shaking, preventing the exposure to UV-Vis radiation, and crosslinked during 60min at 365nm.

Different solutions of copoly(NIPAAm) in EtOH wre prepared: 2%, 1% and 2%+10% SiO_2. 300µl of this solutions were taken with a micropipette and added to the petri dishes while they are shaking. Let dry while shaking in a flat, avoiding the light overnight. Crosslinked at 365nm during 60min. 1ml of different solution were added to study the stability: water, EtOH, Tris-HCl pH: 7.5 and Boronate pH: 10 (ANNEX 4). Let them dry.

5. Results and discussions

5.1. Results of 4,4'-azobis-(3-azidopropyl)-(4-cyanopentanamide)

As it has been explained (Chapter 4.2) this synthesis was done in three steps: the synthesis of 3-amino-1-azidopropane, the synthesis of 4,4'-azobis-(4-cyano pentanoyl chloride) and finally, reacting the products synthesized in these two last synthesis, the synthesis of azido functionalized azo initiator (Fig.1), 4,4'-azobis-(4-cyano pentanoyl chloride).

Acyl chloride and initiator synthesis were successful at the first time, following the procedures are explain in the previous experimental part (section 4.2.3 and 4.2.4) whereas the synthesis of 3-aminoazidopropane was more problematic for the difficult isolation product, and maybe because of the low boiling point of product. Different solvents and conditions were studied, the results obtained are presented in the next table (Table 1).

Table 1: Reaction conditions and results from synthesis of 3-amino-1-azidopropane

Exp. Nr.	Solvent	T (ºC)	Extraction solvent	Notes	Good isolation-Product
ESA002	DMSO (32ml)	80ºC	1L Water (pH≈9-10) + 300mL CH_2Cl_2	-NaN3 is not totally soluble in DMSO, and it stayed attached a little bit in the dropping funnel	NO
ESA005	DMSO (50ml)	80ºC	1L Water (pH≈9-10) + 300mL CH_2Cl_2	-No dropping funnel -It was opened all night→ Not our product	NO
ESA009	Water (25ml)	80ºC	500mL Diethyl ether	After checking a not good isolation, we did: -Make acid the aqueous phase with HCl 0'5M. -Concentrate aqueous phase, remove 2/3 parts of water	NO
ESA010	Water (80ml)	100ºC	1.- CH_2Cl_2 2.-Diethyl ether 3.-Diethyl ether + KOH	1.- Not good isolation of product 2.- Not so many product in the flask after extracting several times. No good isolation 3.-Organic phase of previous extraction was rotaevaporated.	NO

				Aqueous solution was saturated by KOH. Extraction by diethyl ether. No good isolation. (*)	
ESA011	Water (55ml)	100ºC	300mL Diethyl ether + KOH	-Add diethyl ether followed by addition of powdered KOH until aqueous phase is saturated.	YES (47%)

Firstly, DMSO was chosen as solvent but NaN3 was not totally soluble and the process was not successful. For that reason another solvent was chosen, in this case was water, but the isolation of the desired product was also difficult. Different work-up methods were tested, as it is noted in table 1.

The best result was achieved by adding diethyl ether and powdered KOH to the aqueous solution, otherwise the yield was 47%. Hence, further studies are necessary to increase the yield.

5.2. Results of Atom Transfer Radical Polymerization (ATRP)

The target of this series of experiments was to find optimal ATRP-reaction conditions for the polymerization of NIPAAm using different molecular ratios, solvents, catalysts and initiator as it can be observed in the below attached table (Table 2).

All the solvents were nucleophilic and protic as is known from the literature [21], this could be problematic because they could hydrolyze the Cl or Br and the reaction might not be successful.

Another aspect that it has to be considered is that most experiments are needed to check the reproducibility from this method, that is why NIPAAm is used in this reaction because it has been studied in advance and its chemical behaviour is known.

The exclusion of oxygen was absolutely necessary to avoid the oxidation of the copper(I) to copper(II), and the oxygen could capture the radical and inhibits the polymerization.

Some discussion from the results are explained below. Even have a good yield in water (FRP1139-1), the PDI was not good because it was far away from the optimal PDI, which its value is 1.

When shifting the mixture to higher amounts of isopropanol the yield decreases, in pure isopropanol only oligomers were found.

When the solvent was switched to dioxane/water the yield was only 30% with low molecular weight and a broad distribution which might indicate a free radical polymerization instead of the controlled one.

In the next experiments (ESA017 to ESA022), the catalyst-initiator system was changed to copper (I) chloride and MCP. In some of them the yield result was satisfying, but in the case were CuCl was new yields were not good maybe because this was oxidized.

Table 2: Reaction conditions and results in ATRP

Exp. No	Molecular ratio (Mo:CuX:Me6TREN:Initiator)	NiPAAm (g)	T (°C), t (h)	Solvent	CuCl/CuBr	Initiator	Mn / PDI	yield	Tg (°C)
FRP1139-1	200:1:1,25:1	1	25°C, 18h	Water (3ml)	CuBr	EBIB	80.9kDa / 2.28	70 %	140
FRP1139-2	200:1:1,25:1	1	25°C, 18h	Isopropanol (3ml)	CuBr	EBIB	-	-	
FRP1139-3	200:1:1,25:1	1	25°C, 18h	Water / Isopropanol (1:1/3ml)	CuBr	EBIB	62.2kDa / 1.30	93%	136
FRP1142-1	200:1:1,25:1	1	25°C, 18h	Water / Isopropanol (2:8)	CuBr	EBIB	15kDa / 1.25	25%	130
FRP1142-2	200:1:1,25:1	1	25°C, 18h	Isopropanol (3ml)	CuBr	EBIB	830Da / 1.10	1)	53
ESA013-2	200:1:1,25:1	1	25°C, 18h	Dioxan (3ml)	CuBr	EBIB	1.31kDa / 1.14	2)	3) mp 65
ESA014	400:1:1,25:1:20(BPAAm)	1	40°C, 18h	Water / Dioxan (~30:70/6ml)	CuBr	EBIB	2)	2)	2)
ESA015	200:1:1,25:1:20 (BPAAm)	0.5	25°C, 18h	Dioxan / Water (1:1/6ml)	CuBr	EBIB	7.5kDa / 6.41	30%	2)
ESA017	100:2:2:2	2	60°C, 18h	Isopropanol (4g)	CuCl	MCP	7.9kDa / 1.15	55%	121
ESA018	100: 3,2 : 2,5 :2	2	25°C, 18h 60°C, 5h	Isopropanol (4g)	CuCl	MCP	10kDa / 1.12	57%	80
ESA019	200: 2,7 : 2: 2	2	60°C, 18h	Isopropanol (4g)	CuCl (new)	MCP	0.25KDa/ 1.02	4) 5) 7)	2)
ESA020	100: 2 : 2: 2	2	25°C, 48h 60°C, 18h	Isopropanol (4g)	CuCl (new)	MCP	0.29KDa/ 1.43	4) 5) 7)	
ESA022	100 : 2,1 : 2 : 2	1	60°C, 18h	Isopropanol (2g)	CuCl	MCP	5.79kDa/ 1.11	64%	

1) no precipitate in diethyl ether
2) not determined / not measured
3) sample showed melting (mp: melting point) and evaporated
4) no precipitate in pentane
5) not polymerized
6) oxidated when the reaction started
7) no polymers detected, only monomers

5.3. Results of polyNIPAAm hydrogel layers

This study has been researched before, that is why the adhesion copolymer was not developed in this research. The polyNIPAAm layer was synthesized in the laboratory verily this layer is a copolymer of NIPAAm:BPAAm:MAA which the exactly composition (94.86 : 1.74 : 3.4) is measured by a ^{1}H NMR, the spectrum is showed in ANNEX 3.

The main problem for the preparation of these layers lies in the technique. Once the petri dishes are well prepared, the study about the stability of different solutions can be carried out.

The different experiments are explained below, and the two last there are some pictures in the next tables (Table3, Table 4 and Table 5).

1st Experiment

-Add adhesion polymer AAm-EA-BPAAm (85:5:10). 200mg in 6ml water and 4ml ACN. After stirring all the night the solution was still turbid. Hence, a terpolymer NIPAAm : MAA : BPAAm (85: 5: 10) was chosen for the next experiments.

2nd Experiment

-Rinse three times the petri dishes

-Add 490µl of adhesion polymer NIPAAm : MAA : BPAAm (85: 5: 10) 2.5%wt in EtOH HSL. Let dry in a flat place.

-Crosslink during 60min at 365nm

-Prepare pNIPAAm solutions (NIPAAm- BPAAm- MAA) from ESA001:

2% poly(NIPAAm): 100mg in 5ml EtOH abs

1% poly(NIPAAm): 2ml of 2% solution in 2ml EtOH abs

2% poly(NIPAAm) + 10% SiO_2: 100.2mg in 5ml EtOH abs

10% of this previous solution→ 10.02mg of SiO_2 solution (32mg/1ml)

$10.02\text{mg} \times \frac{1ml}{32ml}$ =0.313ml = 313µl of SiO2 solution (PEC062a) to the 2% solution prepared

The silica-nanoparticles were added in order to strengthen the mechanical stability of the hydrogels

-Add poly(NIPAAm) solutions, 300µl, and let dry in a flat surface.

-Add 1ml of different solution to study the stability: water, EtOH, Tris-HCl pH: 7.5 and Borate pH: 10. Let dry.

3rd Experiment:

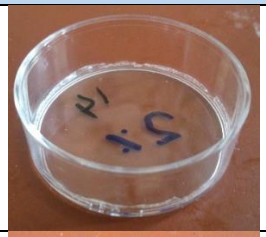
There was an error in second experiment. After adding poly(NIPAAm) hydrogel solution was needed to crosslink!

This experiment was following exactly the same procedure as the 2nd but was crosslinked at 365nm during 60min after adding the poly(NIPAAm) solutions.

Another difference is that we used EtOH HSL instead of EtOH abs for the preparation of poly(NIPAAm) solutions.

The results were visual evaluated and documented by photos as shown in Table 3.

Table 3: Results of stability from the prepared solutions

%pNIPAAm	Num.	Buffer solution	Image
2%	14	Water	
	15	EtOH HSL	
	16	Tris-HCl pH:7.5-8	
	17	Borate pH: 10	
1%	18	Water	

	19	EtOH HSL	
	20	Tris-HCl pH:7.5-8	
	21	Borate pH: 10	
2% + 10%SiO_4	22	Water	
	23	EtOH HSL	
	24	Tris-HCl pH:7.5-8	
	25	Borate pH: 10	

As it can be seen all layers are stable against water, ethanol and borate buffer, because the hydrogel layer stays clear and insoluble. Only Tris-HCl buffer destroys the layer visible at the turbidity.

4th Experiment

We did exactly the same procedure with the same solution than 3rd experiment. After adding the adhesion polymer solution the petri dishes were white, liked turbid.

A new Tris-HCl solution was prepared, this time pH was closer to 7-7.5 measured with pH paper.

In the next table (Table 4) is showed the difference in petri dishes between this two solutions of Tris-HCl (pH: 7-7.5; pH:7.5-8)

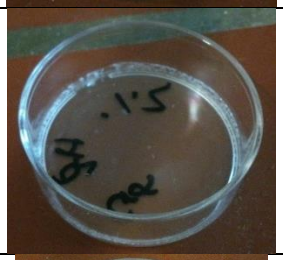
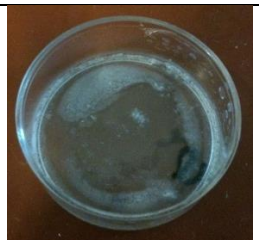
Table 4: Difference between both Tris-HCl solutions depending on pH

% Poly(NIPAAm)	pH:7-7.5	pH:7.5-8
2%		
1%		
2% + 10% SiO_2		

The stability is, in almost all cases, more stable when the pH is 7-7.5 than 7.5-8, could be because at higher pH the COOH undergoes stronger dissociation, they are thus more charged and take up more water to swell stronger, which eventually breaks the bonding to the substrate due to a large swelling stress at the substrate interface.

The others solutions were also studied following the same experimental procedure than in the previous experiment, the results are showed in the next table (Table 5):

Table 5: Different solution stability in petri dishes with polyNIPAAm hydrogel layers

Solvent	2% poly(NIPAAm)	1% poly(NIPAAm)	2% + 10% SiO_2
EtOH HSL			
Water			
Borate pH:10			

Comments:

-Water solvent: The problem was that the notes in the bottom were done with a marker were tried to remove with acetone. Petri dishes are done with polystyrene, and acetone is not a good solvent for them, but anyway, the petri dishes had a good appearance

-Borate pH=10: in this case was not stable maybe because potentially at pH 10 the silane link of the benzophenone crosslinker silane to the silica particles is broken and thus the layer disintegrates

6. Conclusions

In this work had been described a route for the preparation of 4,4'-azobis-(3-azidopropyl)-(4-cyanopentanamide). Two compounds had to be synthesized in advance: 3-amino-1-azido propane and 4,4'-azobis-(4-cyano pentanoyl chloride) . This last one was prepared from the corresponding acid and phosphorous pentachloride under exclusion of moisture and oxygen in good yield (74%).

From 3-bromopropylamine the azide functionalized derivate was synthetized in the presence of an excess of sodium azide, but the isolation from the aqueous phase is not still optimized. Several work-up procedures were tested but the yield was only 47%. The condensation reaction of this two educts to synthesize our azido terminated initiator was successful with a yield of 66%. Even so, the reaction and especially the purification conditions should be optimized.

The polymerization of NIPAAm was studied under ATRP conditions where several solvents, catalyst-initiator systems, reaction conditions and also different ratio monomer to initiator were tested. In most cases polymers could be isolated with molecular weights between 7 and 10kDa (Mn) and narrow weight distribution (~1.1). In conclusion this study was successful.

Finally, the copolymer-NIPAAm hydrogel layers prepared in petri dishes were quite stable in the tested solutions. Only the Tris-HCl buffer seemed to destroy the layer. This buffer solution was tested in the two pH-range of 7.0-7.5 and 7.5-8.0, in the case of more neutral solution the hydrogel layer seemed to be more stable. Also, the preparation technique for this layers has a close relation with the surface and appearance of these hydrogel layers.

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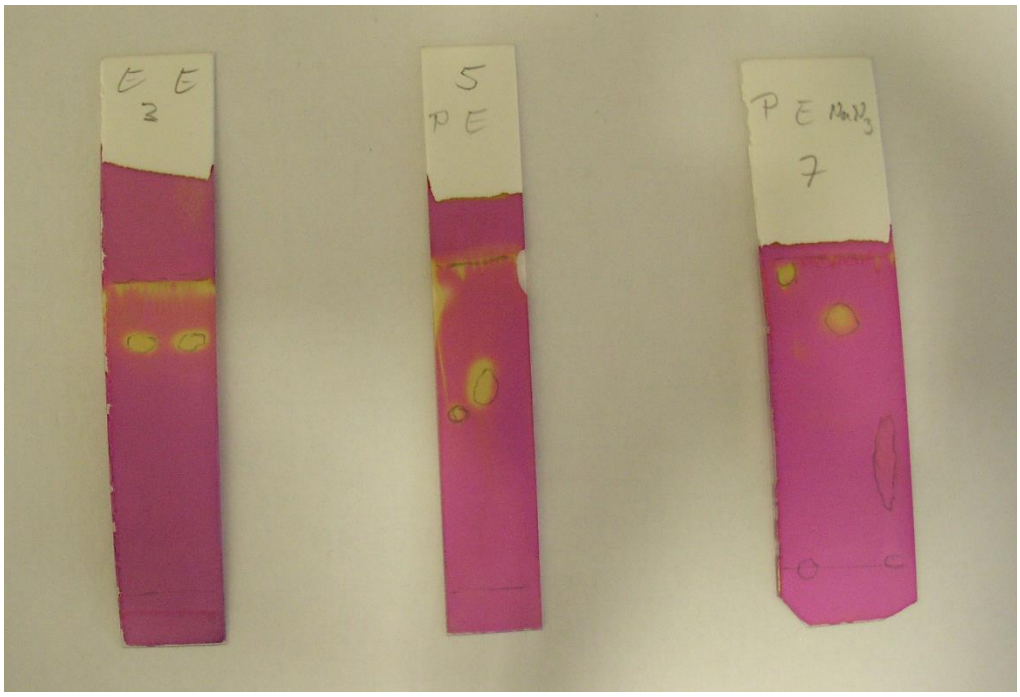
ANNEX 1: TLC of the separation of 3-bromopropyl amino hydrobromide and 3-amino-1-azido-propane

Separation of educt 1-bromo-3amino-propane and product 3-amino-1-azido-propane

Silica-plates, Magic mixture eluent (13 parts CH_2Cl_2, 7 parts MeOH, 1 part NH_3 aqu.)



Legend: E: 1-bromo-3amino-propane

P: 3-amino-1-azido-propane

NaN3

Eluents:

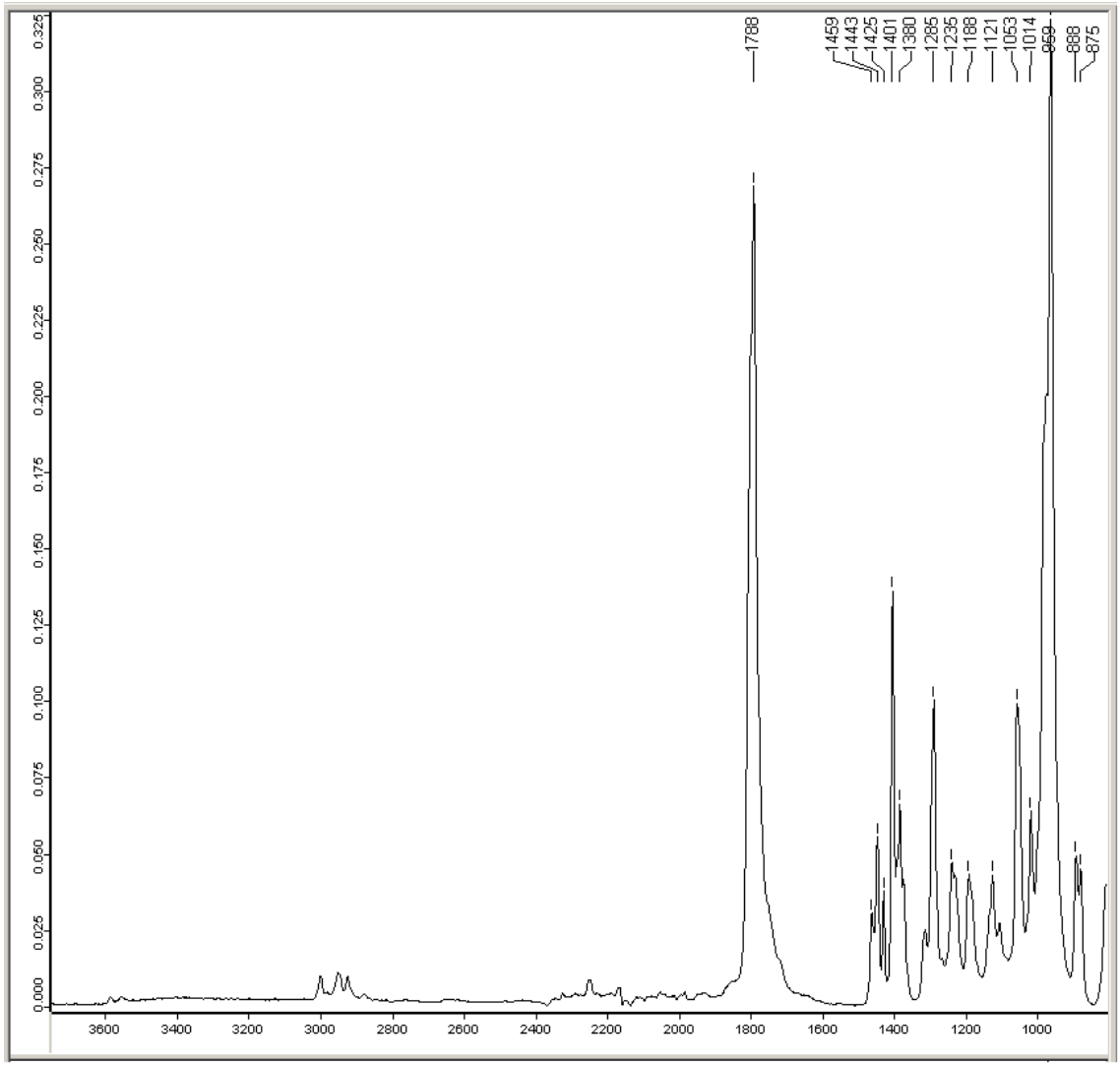
Plate 3: 2,6 ml CH2Cl2, 1,4 ml MeOH, 4 drops NH3 Rf_{Educt}=0,8

Plate 5: 2,6 ml CH2Cl2, 1,2 ml MeOH, 0,1 ml NH3 Rf_{Educt}=0,67; $Rf_{Product}$=0,57

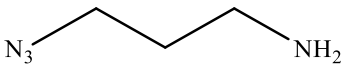
Plate 7: 2,6 ml CH2Cl2, 1,4 ml MeOH, 0,2 ml NH3 (concentrations increased) Rf_{Educt}=0,83; $Rf_{Product}$=0,98; Rf_{NaN3}=0,34

Starting spots of product and sodium azide were visible under UV-light at 254 nm (plate No 7). After separation NaN3 was detected under UV as a broad spot, which is marked, product spot visible, educt with KMnO4 only.

IR 3-amino-1-azido propane



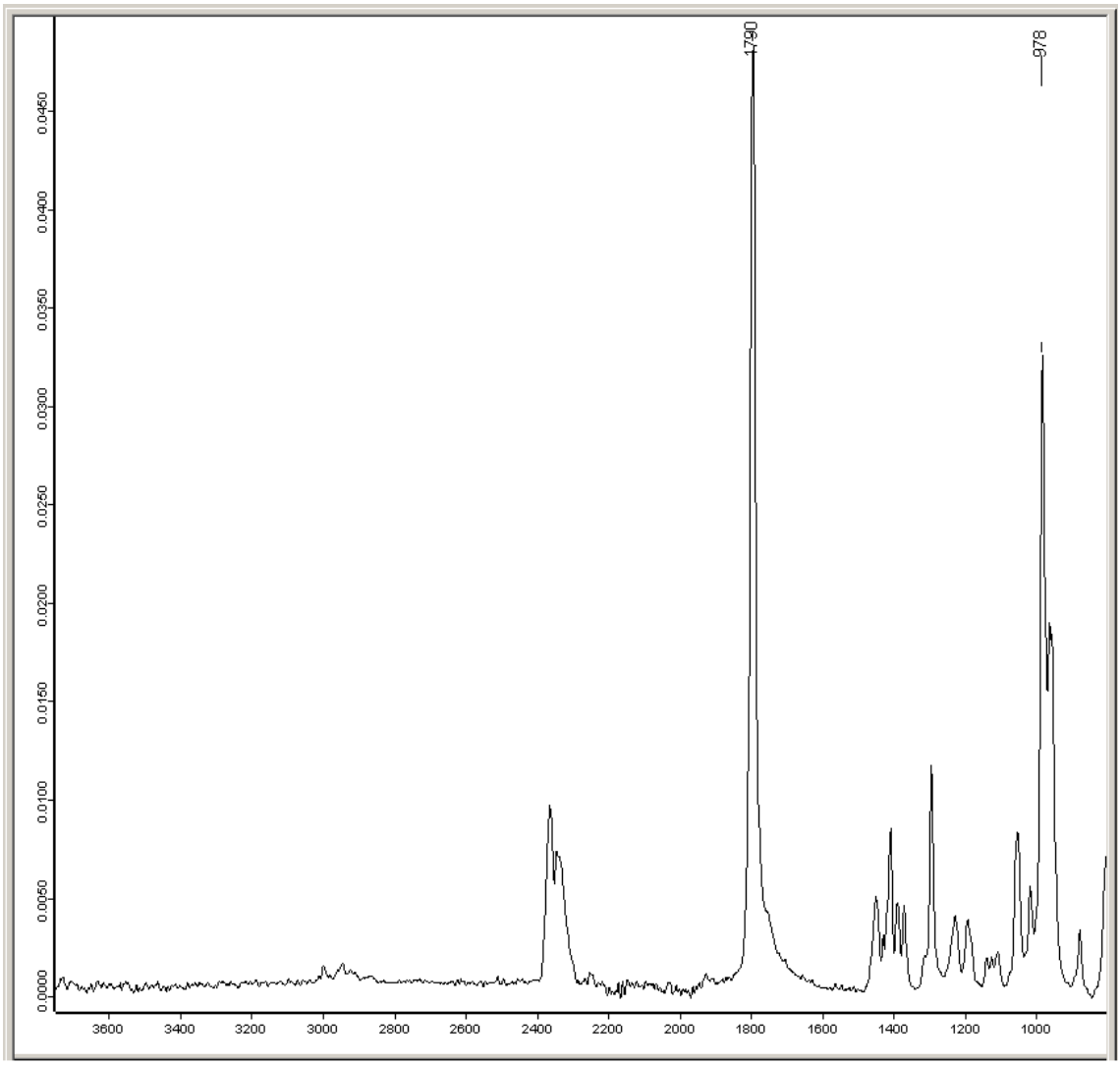
Molecule:



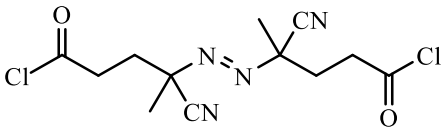
Bands: ~3000cm^{-1} (NH_2)

1788cm^{-1} (N_3)

IR of 4,4'-azobis-(4-cyano pentanoyl chloride)



Molecule:

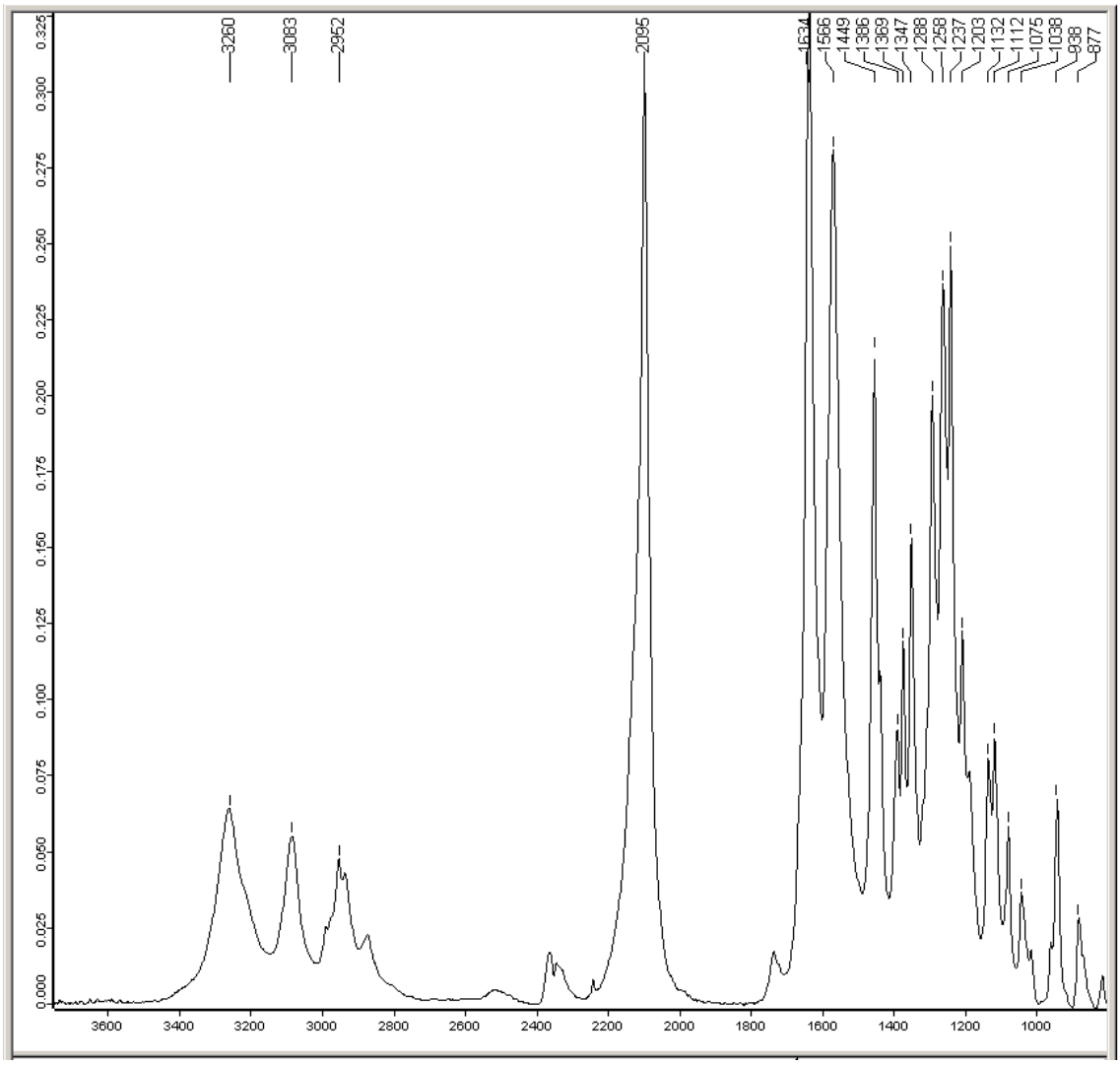


Bands: 2360 cm^{-1} (CO_2)

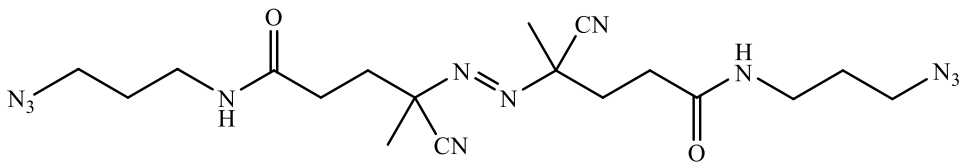
1790cm^{-1} (CO)

~1400cm^{-1} (N=N)

IR of 4,4'-azobis-(3-azidopropyl)-(4-cyanopentanamide)



Molecule:



Bands: 3100-3400 cm^{-1} (NH)

2850-3000 cm^{-1} (CH, CH2)

2095 cm^{-1} (CO)

~1700cm^{-1} (N_3)

1566-1634 cm^{-1}(NHCO)

ANNEX 3: Synthesis of copolymer NIPAAm-MAA-BPAAm (94: 5 :1)

Reagents: NIPAAm was recrystallized from toluene/hexane (1:1 v/v), AIBN from methanol, MAA was distilled from the stabilizer, BPAAm was used as received and dioxane was distilled over calcium hydride. Diethyl ether (99%, Arcros Organics) and tert-butanol.

Instruments: The NMR spectra was recorded with a Bruker Avance 400 spectrometer (1H-NMR, 40MHz), sample was measured at room temperature. The chemical shift δ was specified in ppm. In a mixture of deuterated methanol and deuterium oxide, the solvent peak was used as an internal standard. The spectra was analyzed with software MestreNova.

Experimental Procedure: NIPAAm (1g, 8.84mmols), BPAAm (24.4mg, 0.09mmols) and AIBN (7.22mg, 0.04mmols) were weighted in a Schlenk flask. Then 20ml of dioxane were added, followed by the addition of MAA (40.5mg, 0.47mmols) a reflux condenser under Ar-atmosphere. Cover the flask with aluminium foil and shake at 60ºC overnight. The reaction product was mixed with cold diethyl ether in order to precipitate and then it was filtrated washed with the same solvent. The obtained product was dissolved with tert-butanol followed by filtration. Then the solvent was freeze dried.

Yield: 78%

^{1}H NMR:7.68ppm (H-aromatic, BPAAm), 3.98ppm (-CH, isopropyl NIPAAm),

1.18ppm (CH3, MAA)

Back Bone: 10.47ppm

NIPAAm: (1H) 1ppm

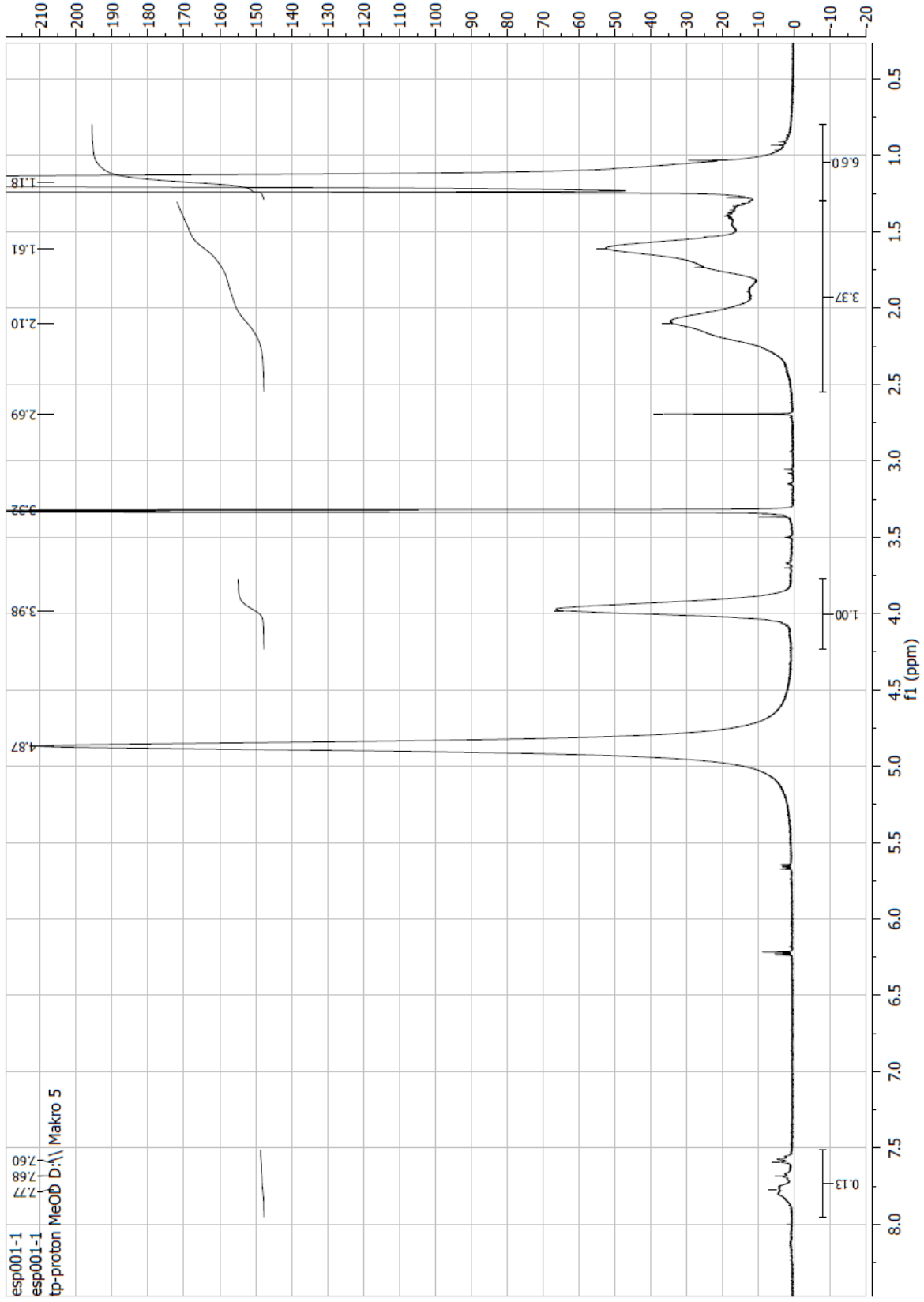
BPAAm: (9H) 0.13ppm

Total: 11.60ppm

NIPAAm: $1H=\frac{1}{11.60}= 0.0862$ $11H= 0.0862 \cdot 11 \cdot 100\% = 94.86\%$

BPAAm: $9H= \frac{0.13}{11.60}= 0.0112$ $14H= \frac{14}{9} \cdot 0.0112 \cdot 100\% = 1.74\%$

MAA= 100% - 94.86%(NIPAAm) – 1.74% (BPAAm) = 3.4%


esp001-1
esp001-1
tp-proton MeOD D:\\ Makro 5
7.77
7.68
7.60
4.87
3.98
3.32
2.69
2.10
1.61
1.18
0.13
1.00
3.37
6.60
f1 (ppm)

ANNEX 4: Solutions to prove the stability in copolymer-NIPAAm layers

Reagents: Water was obtained from a MilliQ apparatus (18.2MΩ · cm). HCl (37%, Fisher Chemical) and NaOH (100%) were used as received. The chemicals 2-Amino-2-hydroximethyl-propane-1,3-diol and sodium borate were used as received.

Preparation of Tris-HCl pH=7.5 : TRIS (6.07g, 50.1mmoles) was dissolved in 30ml of water. The pH until 7.5 was adjusted by a 1M HCl solution.

Preparation of borate pH=10: Prepare a 0.1M NaOH solution. Sodium borate (1.91g, 9.50mmoles) were dissolved in 100ml of water. The solution was prepared with 41ml of 0.1M NaOH solution and 59ml of sodium borate solution.