



Departamento de
Química Orgánica
Universidad Zaragoza

 Facultad de Ciencias
Universidad Zaragoza

Block copolymers for stimuli responsive nanometric systems

Final Master Project (TFM)
Master in Nanostructured Materials
for Nanotechnology Applications

Liquid Crystal and Polymer Group
University of Zaragoza
2015/2016

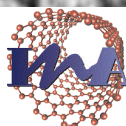
Miriam Abad Andrés

Tutored by: Dr. Luis T. Oriol Langa
Dra. Milagros Piñol Lacambra

 **icma**
Instituto de Ciencia
de Materiales de Aragón



CSIC
CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS



Instituto Universitario de Investigación
en Nanociencia de Aragón
Universidad Zaragoza

ABSTRACT: “BLOCK COPOLYMERS FOR STIMULI RESPONSIVE NANOMETRIC SYSTEMS”

Amphiphilic block copolymers are able to self-assemble and form polymeric micelles able to encapsulate hydrophobic molecules, or vesicles able to encapsulate both hydrophobic and hydrophilic molecules. By using stimuli responsive polymers, the encapsulated molecules can be controlled release. Light is one of the most investigated stimuli because it can be remotely and locally applied. Photoresponsive polymers are composed by moieties that react or isomerize when they are irradiated with light of a particular wavelength. Azobenzene is one of these functional molecules whose light activity is due to its reversible photoisomerization *trans-cis-trans*. Azobenzene can be covalently bound to polymeric chains, which is the most frequent situation, or can be bound using the principles of Supramolecular Chemistry, which constitutes a far less explored approach. The aims of this project were:

The synthesis and characterization of amphiphilic supramolecular block copolymers containing units of 2,6-diacylaminopiridine (DAP, analog to adenine) capable of binding, via hydrogen bonds, azobenzenes having thymine terminal groups, including the

- study of association constants in supramolecular amphiphilic block copolymers.
- study of aggregation of amphiphilic supramolecular block copolymers in aqueous solutions by microfluidics to check a fast and reproducible methodology of preparing polymeric nanoparticles.

According to these objectives, amphiphilic block copolymers containing DAP units and poly (ethylene glycol) were first synthesized by controlled RAFT polymerization. Azobenzenes having thymine terminal groups are linked through a triple hydrogen bond to the DAP units to give amphiphilic supramolecular block copolymers. All copolymers were characterized by spectroscopic techniques.

Association constant (K_a) for H-bond complex was calculated by $^1\text{H-NMR}$ titrations. It has a lower value (4 M^{-1}) in THF than in chloroform (42 M^{-1}) thus H-bonds are weakened due to a solvation effect. Reproducible polymeric aggregates from both block copolymers and supramolecular block copolymers were obtained by microfluidics in THF/water systems. In chloroform/water systems no reproducible results were obtained, so further studies are required.

ABBREVIATIONS LIST

$^1\text{H-NMR}$	Hydrogen nuclear magnetic resonance
AIBN	2,2'-Azo-bis-isobutyronitrile
BC	Block copolymer
CDCl_3	Deuterated Chloroform
CHCl_3	Chloroform
CTA	Chain transfer agent
DAP	Monomer with the 2,6-diacylaminopyridine unit
DCM	Dichloromethane
DLS	Dynamic light scattering
DMF	<i>N,N</i> -dimethylformamide
FTIR	Fourier transform infrared spectroscopy
GPC	Gel permeation chromatography
HPLC	High performance liquid chromatography
KBr	Potassium Bromide
M_n	Number average molecular mass
PEG	Polyethyleneglycol
PTFE	Polytetrafluoroethylene (Teflon)
RAFT	Reversible Addition-Fragmentation Transfer
tAZO	Thymine derivative that contains 4-isobutyloxyazobenzene group
TEM	Transmission electron microscopy
THF	Tetrahydrofuran
THF-d^6	Deuterated Tetrahydrofuran
UV	Ultraviolet
UV-vis	Ultraviolet-visible

Index

1.- INTRODUCTION	1
1.1.- Block copolymers	1
1.2.- Amphiphilic BCs. Self-assembly	3
1.3.- Preparation of polymeric nanoparticles	5
1.4.- Microfluidics	6
1.5.- Polymeric self-assemblies from photoresponsive amphiphilic BCs: Antecedents in the CLIP group.....	7
2.- OBJECTIVES	9
3.- RESULTS AND DISCUSSION	11
3.1.- Synthesis and characterization of amphiphilic BCs	11
3.2.- Synthesis and characterization of supramolecular amphiphilic BCs	15
3.3.- Study of association constants in supramolecular amphiphilic BCs.....	17
3.4.- Preparation and characterization of polymeric nanoparticles by microfluidics	19
3.5.- Study of the light response of supramolecular polymeric self-assemblies	25
4.- EXPERIMENTAL SECTION	27
4.1.- Copolymers PEG ₂₀₀₀ - <i>b</i> -PDAP and PEG ₁₀₀₀₀ - <i>b</i> -PDAP synthesis and characterization	27
4.2.- Supramolecular BCs synthesis.	28
4.3.- Study of association constants in supramolecular amphiphilic BCs.....	29
4.4.- Preparation of polymeric nanoparticles by microfluidics	29
5.- CONCLUSIONS	30
APPENDIX	
A.- FTIR and ¹ H-RMN spectra	A-1
B.- Gel permeation chromatography (GPC)	A-4
C.- Hydrogen bond study	A-6
D.- Preparation of polymeric nanoparticles by microfluidics	A-12
E.- Instruments and techniques	A-16

INTRODUCTION

1.1. Block copolymers

A polymer is a molecule of high relative molecular mass, whose structure comprises the multiple repetition of units known as repeating units derived from molecules of low relative molecular mass or monomers. Polymer synthesis from their monomers is called polymerization.

Polymers can be classified according to their origin (natural, synthetic or semisynthetic polymers) or to their structure (linear, crosslinked, branched, hyperbranched or dendritic polymers). Polymers also can be classified distinguishing between homopolymers and copolymers. Homopolymers are polymers that have only one constitutional repeating unit. Copolymers are polymers that have two or more different constitutional repeating units. Depending on how the repeating units are distributed along the polymeric chain, there are several types of copolymers (Figure 1.1): e.g. alternating, statistical, block, graft or gradient, in the case of copolymers having two different repeating units.

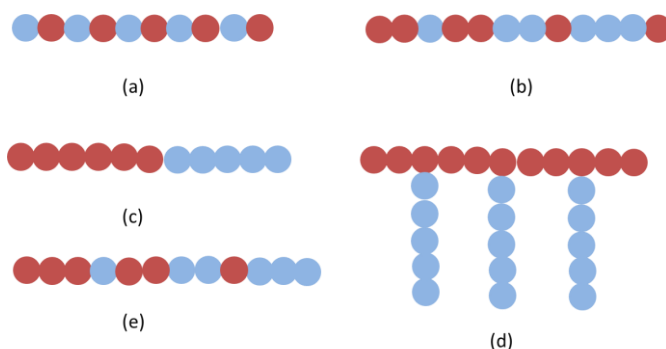


Fig.1.1. Types of copolymers: a) alternating, b) statistical, c) block, d) graft and e) gradient.

A block copolymer (BC) is a polymer having two or more repeating units that are joined together forming blocks (Figure 1.1.c). It also can be defined as two or more homopolymers that are covalently linked. If blocks are not similar, they tend to segregate into different domains, but as they are chemically connected, extension of these domains is limited. So, they try to reach the lower interfacial energy, and consequently a minimization of the area at the interface, avoiding unfavorable contacts between blocks. As a consequence, BCs present self-organized nanoscale morphologies. This fact, together with their chemical and physical properties and the convenient size and shape variability of microdomains by changing their molecular weights and

compositions, make them attractive for nanotechnological applications.¹ Equilibrium morphologies, both in bulk and in solution, for diblock copolymers are shown in Figure 1.2. These three dimensional structures have feature sizes from 5 to 200 nm.²

These morphologies are determined by three main parameters or variables: the overall degree of polymerization (N), the relative volume fraction of each block (f_A, f_B, \dots, f_i) and the block-block interaction parameter(s) (χ), whose value is larger when the blocks are highly incompatible.³ So, it is possible to design a BC depending on the morphology that is wanted or required.

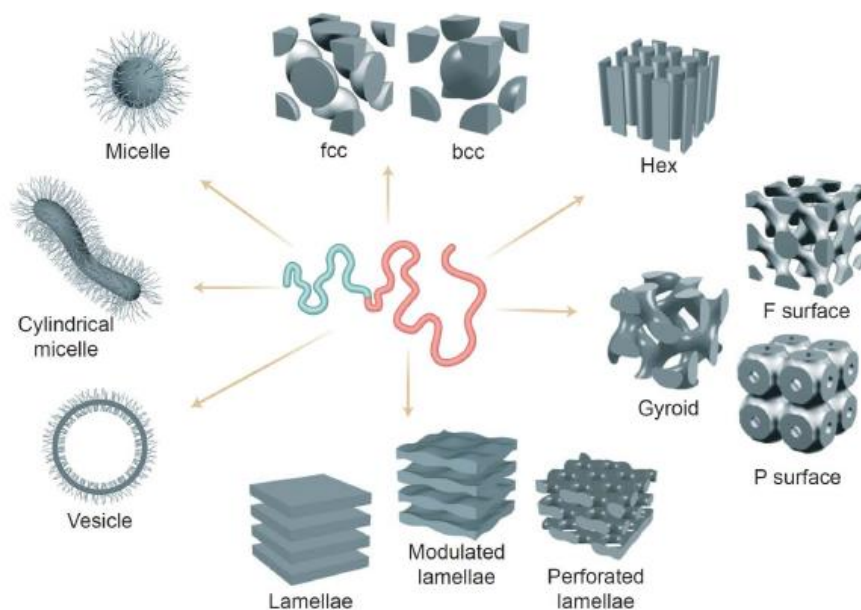


Fig.1.2. BCs self-organization both in bulk and in solution: spheres, hexagonally close-packed cylinders, gyroid networks, lamellae, vesicles and micelles. (Ref. 2)

Regarding to the synthesis of BCs, polymerization methods are different than the conventional ones because accurate control of the macromolecular structure is necessary. One option is to employ anionic polymerization although it requires severe reaction conditions (low temperature, high-purity solvents, inert atmosphere...) and direct use of hydrophilic monomers is restricted. Controlled/living free-radical polymerization methods, which were developed in the recent years of the last century, are a better option. Indeed, the use of these methods has broadened the number of BCs that can be prepared with a high structural control. The key factor of these methods is

¹ C. Park, J. Yoon, E.L. Thomas, *Polymer*, **2003**, 44, 6725-6760.

² D.G. Bucknall, H.L. Anderson, *Science*, **2003**, 302, 1904-1905.

³ C.M. Bates, M.J. Maher, D.W. Janes, C.J. Ellison, C.G. Willson, *Macromolecules*, **2014**, 47, 2-12.

that the termination step is suppressed (actually it is highly minimized) by keeping a low radical concentration.⁴ For this purpose, radicals are masked as a compound with a labile bond. Controlled/living free-radical polymerization methods include Stable Free Radical Polymerization (SFRP) or Nitroxide Mediated Polymerization (NMP), Atom Transfer Radical Polymerization (ATRP) and Reversible Addition-Fragmentation Transfer (RAFT) polymerization.

1.2. Amphiphilic BCs. Self-assembly

A BC formed by a hydrophobic and a hydrophilic block is known as amphiphilic block copolymer. It can segregate and self-assemble in selective solvents, because it tends to avoid unfavorable interactions with the surrounding environment and, at the same time, to maximize the favorable ones. Consequently, when an amphiphilic BC is dispersed into water, the hydrophobic block tends to interact with itself to minimize contact with water, because it is unfavorable from an enthalpic point of view, while the hydrophilic block is solvated by water molecules. Thus, BCs are able to self-assemble in water, forming organized structures that have at least one dimension at the nanoscale.

These nanostructures are mainly micelles and vesicles. Polymeric micelles, which can mainly be spherical (generally called polymeric nanoparticles) or cylindrical, have a hydrophobic internal core and a hydrophilic outer shell. Hydrophobic molecules (e.g. drugs) can be encapsulated, and carried, into these micelles, so higher concentrations than their water solubility can be reached in aqueous media. Polymeric vesicles (also having a spherical morphology) present an inside hollow cavity delimited by a spherical double-wall layer or membrane. Vesicles are particularly interesting because of their hydrophilic inside and their hydrophobic membrane allows encapsulating both hydrophilic (in the hollow cavity) and hydrophobic (embedded inside the membrane) molecules, as it is shown in Figure 1.3.

Therefore, polymeric micelles and vesicles can be employed as nanocarriers for drug delivery. Apart from stabilizing and carrying cargo molecules, drug delivery using these polymeric nanocarriers can be controlled by the response to specific stimuli, such as pH, temperature or light. In this case, both efficiency and specificity of dosage will be

⁴ C.Grande, F. Zuluaga, *Rev. Iberoam. Polim.*, **2010**, 11, 339-359.

improved. For this purpose, BCs that form these structures should include in their structure functional groups or units able to induce changes as response to external stimuli.

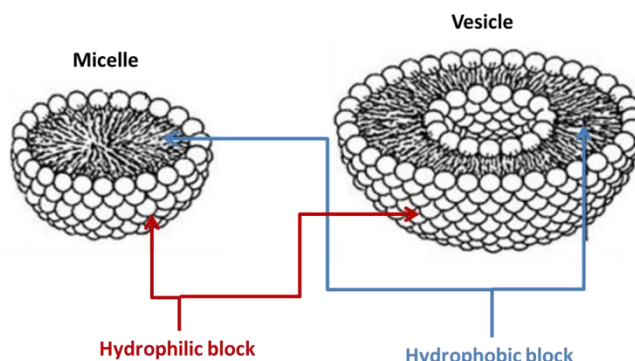


Fig.1.3. Schematic representation of self-assembling of BCs resulting in micelles and vesicles.

1.2.1. Light responsive amphiphilic BCs

One of the most employed stimuli is the light, because it can be very precisely controlled in time and space. In these polymers, photoresponse is associated with functional groups that are able to isomerize or react when they are irradiated with light of a particular wavelength. One of these groups is azobenzene, composed by two aromatic rings linked by an azo bond (N=N), that presents two isomers: *trans* (E) and *cis* (Z).

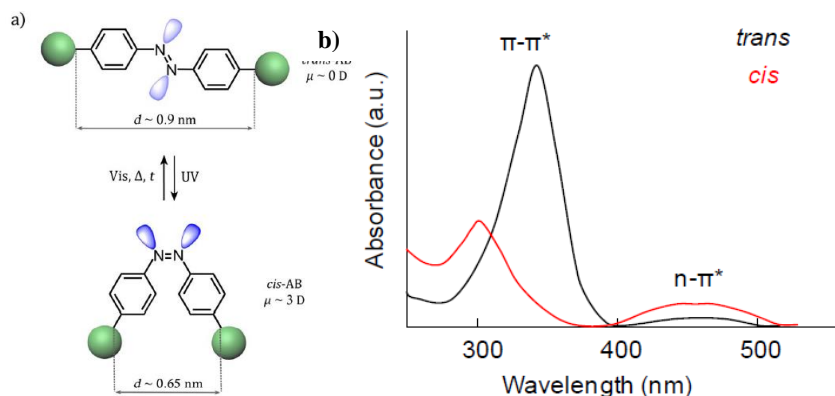


Fig.1.4. a) Azobenzene isomerization (Ref. 5) b) General UV spectra of two azobenzene isomers (Ref. 6).

Azobenzene UV-vis spectrum shows two characteristic bands: one of higher intensity in UV region corresponding to a $\pi\text{-}\pi^*$ transition, and another in the visible region corresponding to an $n\text{-}\pi^*$ transition (Figure 1.4.b). The spectral maxima depend on substituents. *Trans* isomer is thermodynamically stable, but when it is irradiated with UV light, turns on *cis* isomer. Changes on the geometric shape and polarity occur in the

photoisomerization, since *cis* isomer loses the planarity of *trans* isomer and is more polar, so it is more hydrophilic than *trans* isomer. This photoisomerization is a reversible process and it is possible to return to *trans* isomer either by using visible light or thermally (Figure 1.4.a).^{5,6}

1.3. Preparation of polymeric nanoparticles

Polymeric nanoparticles (or in general polymeric micelles and vesicles), either derived from BCs or other type of polymeric compounds, can be synthesized either during the polymerization or once formed the polymers by subsequent processing of the polymeric material. Different methods are available and the choice of a specific method depends on the type of polymeric system, the specific nanoparticle application, the requested size and particle distribution and the use of additives or surfactants, amongst others.⁷

One of the methods that employs preformed polymers is nanoprecipitation, a one-step, simple method, that is performed by adding an organic solution that contains the polymer into an aqueous solution in a drop-wise manner under constant stirring. The organic solvent has to be miscible with water and easy to remove by evaporation. In this way nanoparticles are instantaneously formed during the polymer solution diffusion to aqueous phase. Particle size is influenced by the rate of polymer addition and the stirring speed.^{7,8} Another version of nanoprecipitation is co-solvent methodology. It is based on the slow addition of water (or aqueous solution) into a polymer organic solution, being the organic solvent miscible with water. It is a good strategy for preparing polymeric nanoparticles.

Other simple method is dialysis, which is based on introducing into a dialysis tube a polymer dissolved in an organic solvent, and placing this dialysis tube in another solvent that is not miscible in the polymer one, and where the polymer is not soluble. Nanoparticles are formed due to the progressive aggregation of the polymer when the solvent inside leaves dialysis tube due to solvent outside displacement. Dialysis tube must have a proper weight cut-off to avoid nanoparticles release.⁷

⁵ R. Klajn, *Pure Appl. Chem.*, **2010**, 82, 2247-2279.

⁶ E. Blasco, *Tesis Doctoral*, Facultad de Ciencias, Universidad de Zaragoza, Julio **2013**.

⁷ J. P. Rao, K.E. Geckeler, *Prog. Polym. Sci.*, **2011**, 36, 887-913.

⁸ Y. Zhang, H.F. Chan, K.W. Leong, *Adv. Drug Deliv. Rev.*, **2013**, 65, 104-120.

Emulsification-based methods are nanoparticle formation methods in two steps. In the first one, an organic solution which contains the polymer dissolved is vigorously agitated or sonicated in the aqueous phase to form emulsified droplets. The particle size and distribution depends on the emulsified system used, and they can be more controlled by using surfactants. Then, the nanoparticle formation is completed when the organic phase is removed. Organic solvent removal can be carried out by different methods depending on the kind of solvent: by simple or under reduced pressure evaporation, by diffusion (dialysis) or by adding a salt (salting-out effect).^{7,8}

There are other less common approaches to synthesize polymeric nanoparticles such as layer by layer method (based on the electrostatic interaction between oppositely polyelectrolites),⁸ employing supercritical fluids as solvents (rapid expansion of supercritical solution),⁷ and even by top-down methods such as particle replication in non-wetting template (PRINT) and step-flash imprint lithography (S-FIL).⁸

1.4. Microfluidics

During the last years, a novel method for preparing polymeric nanoparticles has emerged using microreactors and microfluidics. A microreactor is a reactor whose principal dimensions are comprised between tens to hundreds of micrometers, so it comprises small fluid volumes (from 10^{-9} to 10^{-18} L).⁹ Because of the small channel dimensions, the surface/volume ratio is large and the diffusion lengths are short, so mass and heat transport processes are fast and predictable. Therefore, microreactors are suitable to have a better control in nanoparticle preparation because heating and mixing are more uniform.¹⁰

In addition to the high and predictable heat and mass transfer rates, working in microreactors offers several advantages. One of the most important advantages is the high reproducibility achieved, which is very important when the process is scaled up. In other methodologies, results may be different for different batches, or even in different regions of the same batch, because parameters such as temperature or concentrations are variable. In a microreactor these parameters are better controlled, so high

⁹ J.I. Park, A. Saffari, S. Kumar, A. Günther, E. Kumacheva, *Annu. Rev. Mater. Res.*, **2010**, 40, 415-443.

¹⁰ L. Gomez, M. Arruebo, V. Sebastian, L. Gutierrez, J. Santamaría, *J. Mater. Chem.*, **2012**, 22, 21420-21425.

reproducibility is achieved, and also particle dimensions and shape can be tuned by modifying these parameters, and hence corresponding size-dependent properties. For instance, decreasing flow sometimes implies decreasing in particle size.⁹ In addition, microfluidic processes can be easily scaled up by increasing the number of microreactors.

However this technique is not recommended when precipitate at microscale appears or when compound tend to nucleate on the microreactor's walls or to be stuck there. In case that this happened, the microreactor would be clogged (maybe irreversibly), so a previous reactions study is required.

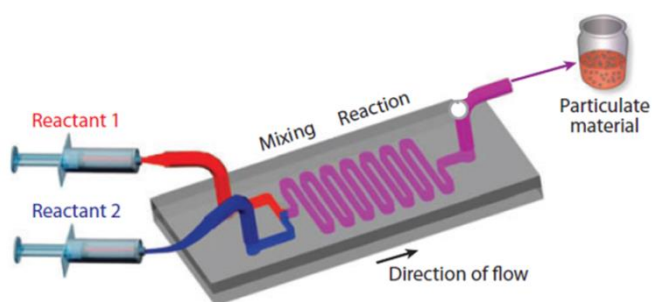


Fig. 1.5. Scheme of synthesis of particulate material in a flow-based microfluidic reactor.

(Adapted from ref. 9)

In Figure 1.5 a scheme of a preparation of nanoparticles in a flow-based microfluidic reactor is shown, where two streams that contain the reactants are injected in two separate inlets, and they enter to the microreactor through opposite sides, so a good mixture is accomplished before the nanoparticles formation takes place (even emulsification is achieved without employing sonication). The flow goes through the microreactor and the product is collected at the end. These microreactors can be made of steel, glass or even polymers (but these last ones cannot be employed with several solvents).

1.5. Polymeric self-assemblies from photoresponsive amphiphilic BCs: Antecedents in the CLIP group

Different photoresponsive polymeric self-assemblies from amphiphilic BCs have been prepared and studied in the Liquid Crystals and Polymers (CLIP) group of the University of Zaragoza, due to their potential as drug delivery systems stimulated by

light. The first system described by this group was based on amphiphilic linear-dendritic block copolymers functionalized with 4-azobenzene units.^{11, 12} However, the synthesis of these covalent BCs is highly time-demanding and with a limited versatility as any structural modification on the final macromolecule involves the previous synthesis of the appropriate monomers or precursors.

More recently, supramolecular BCs have been prepared from BCs containing 4-vinylpyridine,^{13, 14} or 2,6-diacylaminopyridine¹⁵ units able to link functional units by molecular recognition through hydrogen bonding. In the last case, azobenzene derivatives having a thymine terminal group have been linked by triple hydrogen bonds to 2,6-diacylaminopyridine units (analogs of adenine) giving rise to light responsive BCs in an easy manner. Several structural modifications have been explored in these supramolecular polymers in order to decrease interactions between azobenzene moieties and facilitate their isomerization. The modifications include the supramolecular modification of the polymer with mixtures of hydrocarbon and azobenzene thymine derivatives,¹⁶ or the copolymerization of a hydrophilic oligoethylene glycol monomer with the 2,6-diacylaminopyridine one to form the hydrophobic block.¹⁷ In this previous work, nanoobjects were prepared using co-solvent method,¹¹⁻¹⁸ by slowly adding water to the organic solution until aggregation occurs, or using an ultrasonic dispersion methodology.¹⁸ The co-solvent method usually affords very good results but the methodology requires a long time of preparation and no complete reproducible results were obtained with the supramolecular BCs modified with azobenzene moieties. In order to check and optimize other different methods to achieve more reproducible results this Final Master Project was planned.

¹¹ J. del Barrio, L. Oriol, C. Sánchez, J.L. Serrano, A. Di Cicco, P. Keller, M.H. Li, *J. Am. Chem. Soc.*, **2010**, 132, 3762-3769.

¹² E. Blasco, J. del Barrio, C. Sánchez-Somolinos, M. Piñol, L. Oriol, *Polym. Chem.*, **2013**, 4, 2246-2254.

¹³ J. del Barrio, E. Blasco, L. Oriol, R. Alcalá, C. Sánchez-Somolinos, *J. Polym. Sci. Part A: Polym. Chem.*, **2013**, 51, 1716-1725.

¹⁴ J. del Barrio, E. Blasco, C. Toprakcioglu, A. Koutsoubas, O.A. Scherman, L. Oriol, C. Sánchez-Somolinos, *Macromolecules*, **2014**, 47, 897-906.

¹⁵ A. Concellón, E. Blasco, M. Piñol, L. Oriol, I. Díez, C. Berges, C. Sánchez-Somolinos, R. Alcalá, *J. Polym. Sci. Part A: Polym. Chem.*, **2014**, 52, 3173-3184.

¹⁶ L. Labarta, *Final Bachelor Project*, Faculty of Sciences, University of Zaragoza, June **2014**.

¹⁷ M. Abad, *Final Bachelor Project*, Faculty of Sciences, University of Zaragoza, June **2015**.

¹⁸ R.T. Buratto, *Final Master Project*, Faculty of Sciences, University of Zaragoza, September **2015**.

2. OBJECTIVES

The aim of this Final Master Project is the synthesis and characterization of amphiphilic BCs, supramolecular light-responsive BCs derived from them and the study of their aggregation process in water by employing microfluidics. According to the previous work, these amphiphilic BCs contain a hydrophilic block composed by polyethylene glycol (PEG), and a hydrophobic block that contains 2,6-diacylaminopyridine moieties (DAP). These moieties are able to bind thymine derivatives containing functional units by forming multiple hydrogen bonds. In this project, functional units will be azobenzenes. In this way, supramolecular light-responsive amphiphilic BCs are formed. The preparation of polymeric nanoparticles by microfluidics is studied both in the starting amphiphilic BCs and in the functional supramolecular ones.

According to what has been said above, the plan and the specific tasks proposed for this project have been the following ones:

Task 1. Synthesis and characterization of amphiphilic BCs: The first task of this project is the synthesis of two amphiphilic BCs, named as PEG₁₀₀₀₀-*b*-PDAP and as PEG₂₀₀₀-*b*-PDAP whose general chemical structure is shown in Figure 2.1. Both have a hydrophilic block of PEG, which has a molar mass around 10000 in the case of PEG₁₀₀₀₀-*b*-PDAP, and around 2000 in the case of PEG₂₀₀₀-*b*-PDAP. The hydrophobic block (PDAP) is a polymethacrylate with pendant DAP units. The BCs will be approached by a RAFT

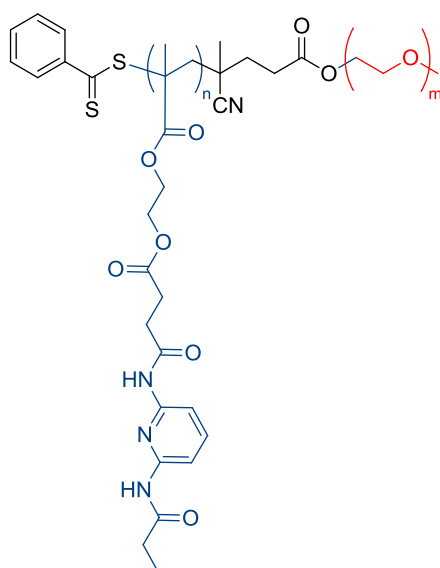


Fig.2.1. General polymeric structure of PEG_x-*b*-PDAP copolymers (x=2000 (m=45) and x=10000 (m=227)).

Task 2. Synthesis and characterization of supramolecular amphiphilic BCs: In order to reach the supramolecular side chain polymers, the starting amphiphilic BCs will be functionalized with 4-isobutyloxyazobenzene units with a thymine terminal group (tAZO). The chemical structure of the supramolecular polymers is shown in Figure 2.2. Thymine units are able to form three hydrogen bonds with the 2,6-diacylaminopyridine units. The supramolecular copolymers are named as PEG_x-*b*-PDAP·tAZO, and their structure is shown in Figure 2.2.

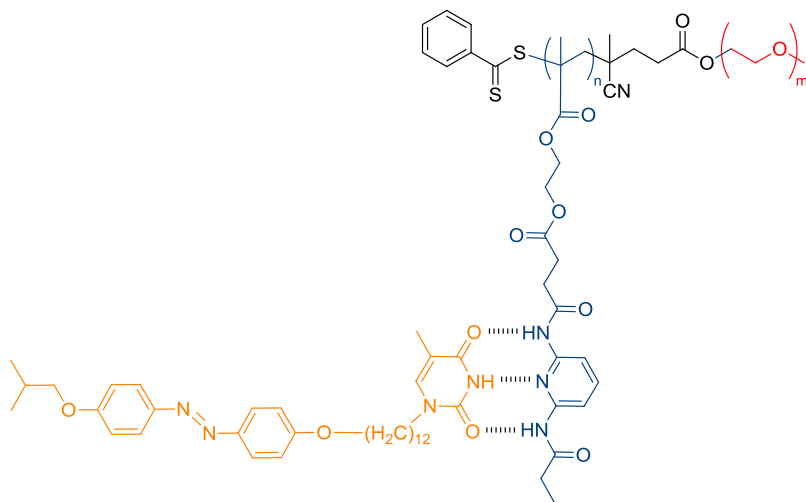


Fig.2.2. Chemical structure of the final supramolecular copolymers. Broken lines represent the H-bonding between a repetitive PDAP units from the block copolymer and the tAZO units.

Task 3. Study of association constants in supramolecular amphiphilic block copolymers: H-bond formation between the thymine and the 2,6-diacetylaminopyridine groups will be verified by ¹H-NMR in two different solvents (THF and chloroform) as well as the binding affinity of the H-bond complex will be quantified using ¹H-NMR titration experiments to calculate the association constant (K_a).

Task 4. Preparation and characterization of polymeric nanoparticles by microfluidics: The main target of this project is the preparation of polymeric micelles or alternatively polymeric vesicles, by employing microfluidics. Self-assembly of both PEG_x-*b*-PDAP and PEG_x-*b*-PDAP·tAZO in water will be investigated by using different experimental conditions. The study of the polymeric aggregates will be carried out by dynamic light scattering (DLS) and by transmission electron microscopy (TEM).

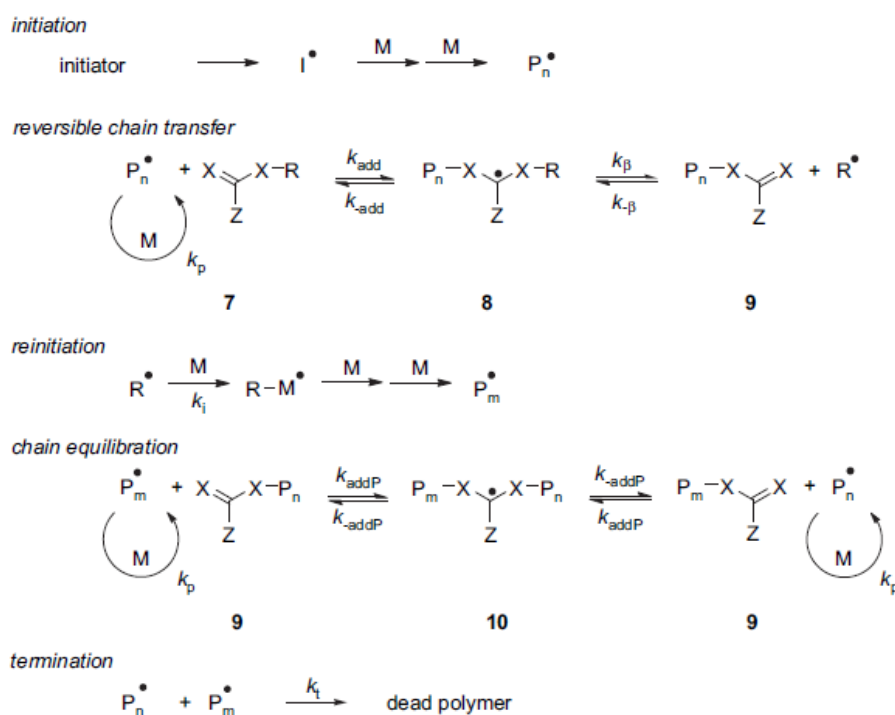
Task 5. Study of the light response of supramolecular polymeric self-assemblies: Changes on the morphology of the self-assemblies under exposure to UV light will be investigated by UV-vis spectroscopy and TEM.

3. RESULTS AND DISCUSSION

3.1. Synthesis and characterization of amphiphilic BCs

Copolymer synthesis was carried out by using RAFT polymerization, a controlled radical polymerization first described by Rizzardo, Moad and Thang's group.^{19, 20}

The RAFT polymerization uses an initiator (usually AIBN or an organic peroxide) combined with a dithioester, a xanthate or a dithiocarbamate that acts as a chain transfer agent (CTA). The CTA is chosen according to the monomer to be polymerized, and determines the control over both molecular mass and polydispersity. In order to achieve this purpose, the key role in the RAFT polymerization mechanism is played by a sequence of addition-fragmentation equilibria as it is shown in Scheme 3.1.



Scheme 3.1. RAFT polymerization mechanism (Ref. 20)

Both initiation and termination steps take place as in a traditional free-radical polymerization. In the first polymerization steps, a propagating radical (P_n^\bullet) is formed. This propagating radical reacts with CTA and then X-R bond is broken. Thus, a polymer linked to the CTA by one of its ends is obtained. In this polymer, the reactive end is deactivated but it can be fragmented again, so this polymer is known as “latent

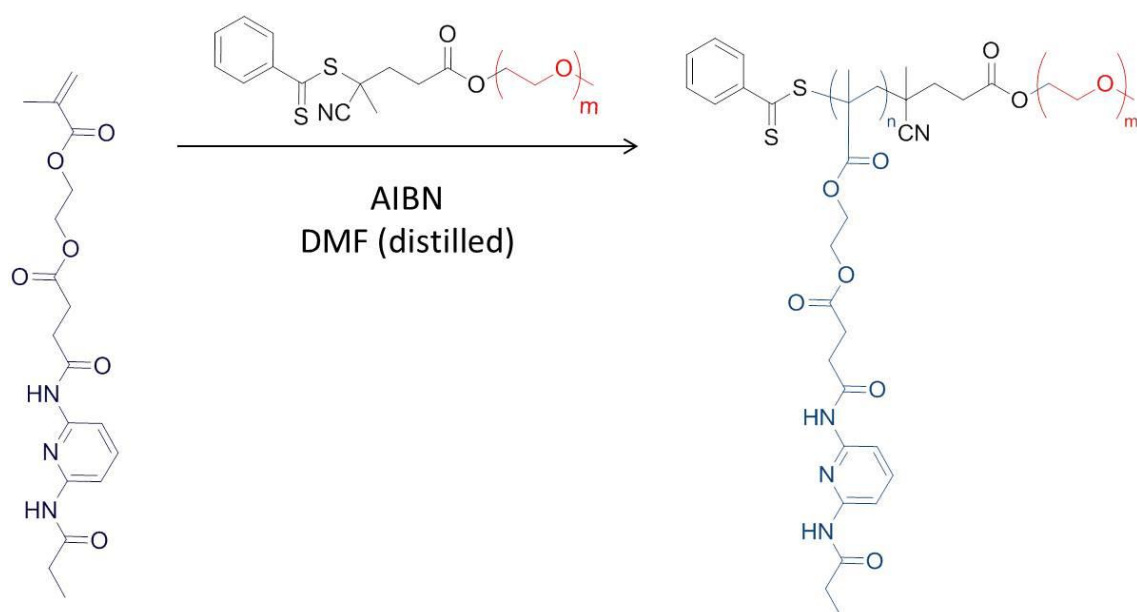
¹⁹ J. Chiefari, Y.K.B. Chong, F. Ercole, J. Krstina, J. Jeffery, T.P.T. Le, R.T.A. Mayadunne, G.F. Meijs, C.L. Moad, G. Moad, E. Rizzardo, S.H. Thang, *Macromolecules*, **1998**, 31, 5559-5562.

²⁰ G. Moad, E. Rizzardo, S.H. Thang, *Polymer*, **2008**, 49, 1079-1131.

polymer”. At the same time, a new radical ($R\bullet$) is formed which continues reacting with the monomer to form a new propagation radical ($P_m\bullet$). The quick equilibrium between the two propagating radicals and the “latent polymer” provides the same growth probability to all polymeric chains, which results in a low polydispersity of the polymers. As it happens in all free-radical polymerizations, termination step cannot be completely suppressed. However, in RAFT polymerization, when polymerization is finished, many polymeric chains keep CTA as terminal group and can be isolated as stable materials.

These polymers with a terminal CTA can be employed as transfer agents in subsequent polymerization process with other monomers in order to obtain block copolymers. In this case, they are called macro-CTA.

Two target PEG_{2000} -*b*-PDAP and PEG_{10000} -*b*-PDAP copolymers were obtained by RAFT polymerization as it is shown in Scheme 3.2 using a polyethylene glycol (PEG) macro-CTA (commercially available).



Scheme 3.2. RAFT polymerization reaction to obtain PEG_{2000} -*b*-PDAP and PEG_{10000} -*b*-PDAP.

The macro-CTA is a dithiobenzoate that is recommended for the polymerization of methacrylates.²¹ For PEG_{2000} -*b*-PDAP, PEG with a molar mass around 2000 was used and for PEG_{10000} -*b*-PDAP, PEG of molar mass around 10000. AIBN (2,2'-azo-bis-

²¹ SigmaAldrich® commercializes these macro-CTAs, so it provides information to costumers in the following webpage:
<http://www.sigmaaldrich.com/materials-science/polymer-science/raft-polymerization.html#classes>

isobutyronitrile) is employed as a radical thermal initiator, and freshly distilled DMF (*N,N*-dimethylformamide) as solvent.

Theoretical molar mass of each copolymer can be estimated using equation 3.1, in which $[M_{DAP}]$ is DAP monomer initial concentration, $[macro-CTA]$ is macro-CTA initial concentration, MW_{DAP} is DAP monomer molar mass, and $MW_{macro-CTA}$ is macro-CTA molar mass.

$$M_n(Theoretical) = \left(\frac{[M_{DAP}]}{[macro-CTA]} \times MW_{DAP} \times conversion \right) + MW_{macro-CTA}$$

Equation 3.1.

In this project, several polymer batches have been synthesized and employed. PEG₂₀₀₀-*b*-PDAP, which was previously synthesized in CLIP group laboratories, was again prepared in this project. PEG₁₀₀₀₀-*b*-PDAP was also previously synthesized in CLIP group laboratories, but two new samples were prepared in the laboratory having different polymerization degree in the hydrophobic block. Summarizing, in this project five polymer batches were employed: two of PEG₂₀₀₀-*b*-PDAP, one synthesized within the framework of this project, and three of PEG₁₀₀₀₀-*b*-PDAP, two prepared within the framework of this project. Their synthesis and characterization is explained below.

RAFT polymerizations were carried out under inert atmosphere, making previously three argon-vacuum cycles in order to remove oxygen, because it is a radical inhibitor. Afterwards, the flask was introduced into a preheated bath at 80°C. At this temperature, AIBN decomposes homolytically. If the reaction does not start at a fixed temperature, radical concentration will not have a constant value so the mechanism equilibrium will be disrupted. After 5 hours, the reaction was quenched introducing the flask into liquid nitrogen. Reaction time was fixed according to previous work. After polymerization, the polymers were precipitated into cold diethyl ether and they were isolated by centrifugation. They were purified by re-precipitation in cold diethyl ether.

Polymers were characterized by FTIR and ¹H-NMR. FTIR spectra confirmed the presence of the functional groups expected for these BCs. It also was confirmed by ¹H-NMR spectra (see appendix section A).

In addition, the average degree of polymerization of the hydrophobic block (*n*) can be calculated by ¹H-NMR from the integrations of selected peaks taking into account that

the degree of polymerization of the PEG block (m) is known (commercially available but also verified by mass spectrometry). Thus, taking relative integrations of PEG block (protons labeled b and c that appear around 3.6 ppm, see Figure 3.1) as reference (considering a degree of polymerization (m) equal to 45 for PEG₂₀₀₀- b -PDAP and equal to 227 for PEG₁₀₀₀₀- b -PDAP) and the ones of the peaks corresponding to DAP repeating units (protons labeled f and g that appear around 4.2 ppm, see Figure 3.1), the average number of DAP repeating units (n) was calculated. Once known m and n , the total average molecular mass of polymers can be easily calculated. Obtained results, collected in Table 3.1, show that both batches of PEG₂₀₀₀- b -PDAP are very similar (their behavior and properties were identical) but in the case of PEG₁₀₀₀₀- b -PDAP, batch C is different from the other batches ($n=31$ DAP units for C and 18 for A and B). Along this report, the batch is indicated together with the nomenclature.

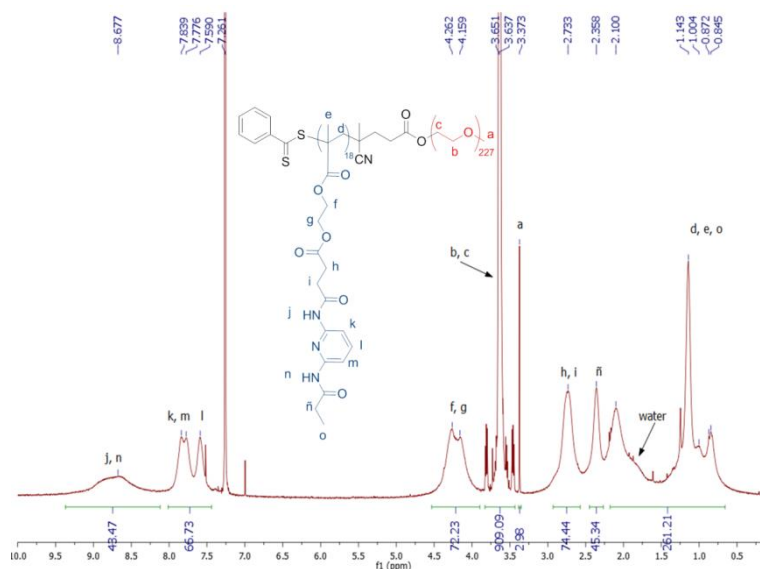


Fig. 3.1. PEG₁₀₀₀₀- b -PDAP-(B) ¹H-NMR spectrum (CDCl₃, 400 MHz) δ (ppm)

Gel permeation chromatography (GPC) measurements, employing a light scattering detector and poly (methyl methacrylate) standards, were carried out using THF solutions (columns are available in this solvents) to study the relative molecular mass and dispersity of the prepared BCs (see appendix section B). First, it should be remarked that no peaks corresponding to macro-CTA or DAP monomers were detected when chromatograms under the same experimental conditions were compared, which evidences the block copolymer formation. However, it was observed that elution time increased when hydrophobic block increased (it is higher for BCs than for macro-CTA), in an opposite way to the expected in GPC, because molecules that have a bigger hydrodynamic volume should elute before. This unexpected behavior can be due to

interactions between the hydrophobic block of the BCs and the material of the chromatographic columns, giving rise not only this behavior but also to the appearance of other peaks. This phenomenology has been previously observed in polymers that interact with chromatographic columns.²²

As a consequence of this behavior, the average molecular masses obtained by GPC are masses relatives to the standards and they clearly differ from the theoretically estimated ones, as it is shown in Table 3.1. An accurate value of polydispersity could not be calculated.

Table 3.1. BCs average molecular mass.

Polymer	Theoretical M _n (g/mol)	¹ H-RMN M _n (g/mol)	DAP units number (n)	GPC M _n (g/mol)
PEG ₂₀₀₀ - <i>b</i> -PDAP-(A) ^b	---	6150	11	---
PEG ₂₀₀₀ - <i>b</i> -PDAP-(B)	5750	5400	9	14285
PEG ₁₀₀₀₀ - <i>b</i> -PDAP-(A) ^b	----	16795	18	---
PEG ₁₀₀₀₀ - <i>b</i> -PDAP-(B)	29273	16795	18	11205
PEG ₁₀₀₀₀ - <i>b</i> -PDAP-(C)	28869	21700	31	7645

b: polymers previously synthesized in CLIP group.

3.2. Synthesis and characterization of supramolecular amphiphilic BCs

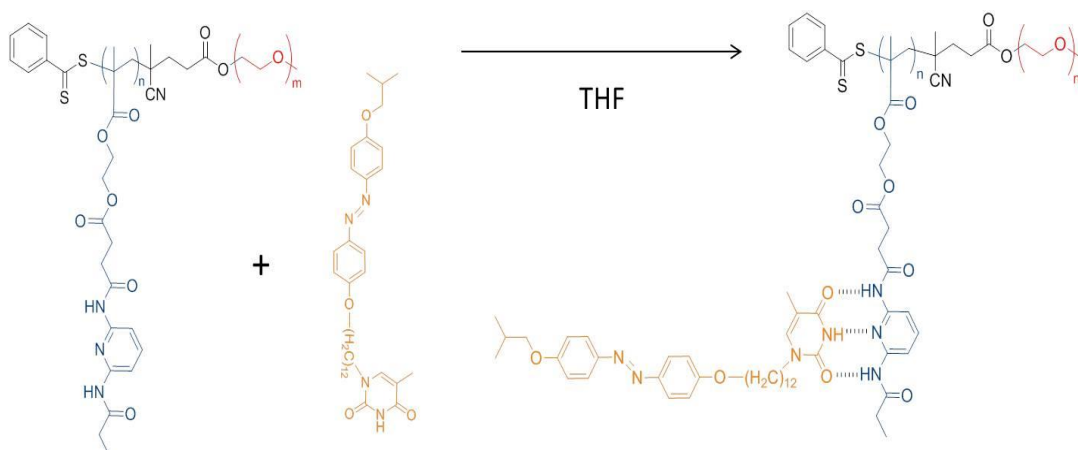
Supramolecular BCs were prepared using the amphiphilic BCs and a thymine derivative that contains the chromophore group 4-isobutyloxyazobenzene (tAZO) (see Scheme 3.3) and previously synthesized in the CLIP group laboratories.

These supramolecular BCs were prepared weighting the corresponding amounts of BC and tAZO, assuming that all DAP units will be linked to thymine (1:1 stoichiometry). Both components were dissolved in THF that was gradually evaporated (under mechanic stirring) to, finally obtain the supramolecular BCs.

Table 3.2. Hydrophilic/hydrophobic weight percentages for BCs and supramolecular BCs.

Polymer	BCs	Supramolecular BCs
PEG ₂₀₀₀ - <i>b</i> -PDAP-(A)	33% : 67%	16% : 84%
PEG ₂₀₀₀ - <i>b</i> -PDAP-(B)	37% : 63%	19% : 81%
PEG ₁₀₀₀₀ - <i>b</i> -PDAP-(A and B)	60% : 40%	37% : 63%
PEG ₁₀₀₀₀ - <i>b</i> -PDAP-(C)	46% : 54%	25% : 75%

²² A. Concellón, *Final Bachelor Project*, Faculty of Sciences, University of Zaragoza, June 2012



Scheme 3.3. Supramolecular BCs formation.

These supramolecular copolymers were characterized by FTIR and $^1\text{H-NMR}$, in order to verify hydrogen bonding.

FTIR spectra were registered using KBr pellets and are collected in appendix C.1 section. An example is shown in Figure 3.2. Stretching N-H band wavenumber ($3300\text{--}3500\text{ cm}^{-1}$) slightly decreases in supramolecular copolymer compared with initial copolymer and with the thymine derivative, due to hydrogen bonding between thymine and 2,6-diacylaminopyridine units weaken N-H bonds.

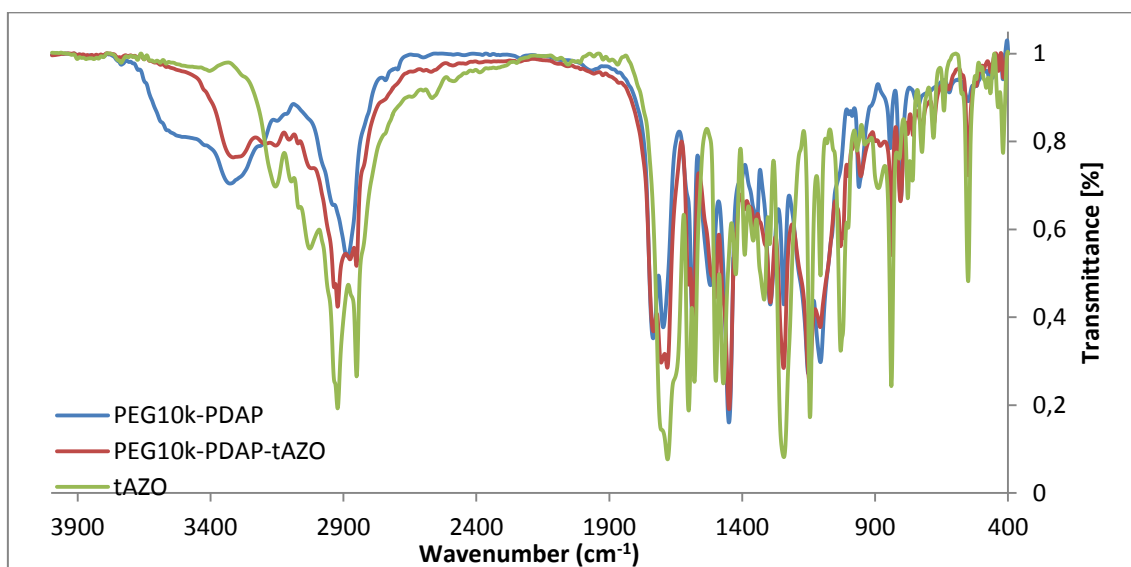


Fig. 3.2. $\text{PEG}_{10000}\text{-}b\text{-PDAP-(A)-tAZO}$ and their starting materials ($\text{PEG}_{10000}\text{-}b\text{-PDAP-(A)}$ and tAZO) FTIR spectrum.

However, the molecular recognition and H-bond formation is better verified by $^1\text{H-NMR}$, because when supramolecular assembly takes place, protons involved are deshielded, so their chemical shifts increase compared with the corresponding peaks in

the starting materials. An example of this is behavior (see Figure 3.3, the rest are in appendix C.2 section) displays the region from 8 to 10 ppm at which H involved in the triple hydrogen bonding appears. It can be seen that protons coded H_a of the amide group of DAP and proton coded H_b of the thymine ring are shifted to higher chemical shifts in the supramolecular BC spectra (green line) with respect to the starting components.

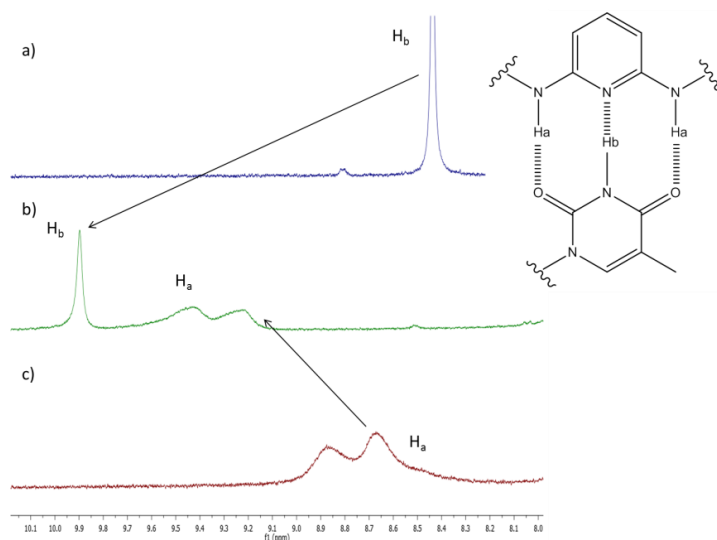


Fig.3.3. ¹H-NMR spectra (CDCl₃, 400 MHz) [10.2-8.0 ppm] of: tAZO (a, blue), PEG₁₀₀₀₀-*b*-PDAP-(A)·tAZO (b, green) and PEG₁₀₀₀₀-*b*-PDAP-(A) (c, red).

3.3. Study of association constants in supramolecular amphiphilic BCs

Once verify the formation of hydrogen bonding, binding affinity of the H-bond complexes was studied by ¹H-NMR titration experiments. Two different solvents were chosen: deuterated tetrahydrofuran and chloroform. This study was performed with the collaboration of Dra. P. Romero.

3.3.1. H-bond study in THF-d⁶

For this study, PEG₁₀₀₀₀-*b*-PDAP-(A) was employed. Previous to the ¹H-NMR titration, H-bond formation in THF-d⁶ was verified. The corresponding ¹H-NMR spectrum (see Figure 3.4.) shows that H-bond complex is weaker in THF than in chloroform, due to lower displacements of the involved signals. This can be explained because of the THF polarity and solvation weaken the H-bond assembly.

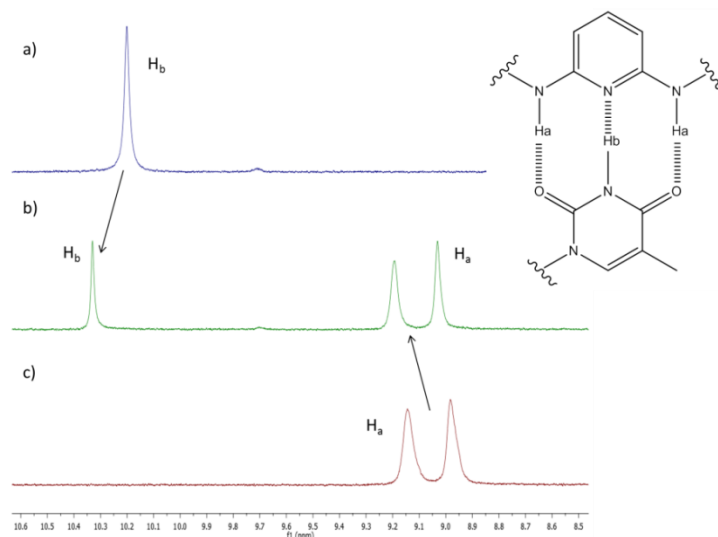


Fig.3.4. $^1\text{H-NMR}$ spectra (THF-d^6 , 400 MHz) [10.6-8.5 ppm] of: tAZO (blue), $\text{PEG}_{10000}\text{-}b\text{-PDAP-(A)\cdot tAZO}$ (green) and $\text{PEG}_{10000}\text{-}b\text{-PDAP-(A)}$ (red).

$^1\text{H-NMR}$ titration was carried out by recording $^1\text{H-NMR}$ spectra of different samples having the same amount of $\text{PEG}_{10000}\text{-}b\text{-PDAP-(A)}$ and variable amounts of tAZO (see appendix C.3.1 section). After that, difference between chemical shifts versus tAZO concentration was plot, and by fitting to 1:1 binding isotherm, the association constant was calculated applying Benesi-Hildebran method²³ (Figure 3.5). Thus, it was observed that K_a is approximately 5 M^{-1} . So the binding affinity of the H-bond complex is weak in tetrahydrofuran, according to the literature.²³

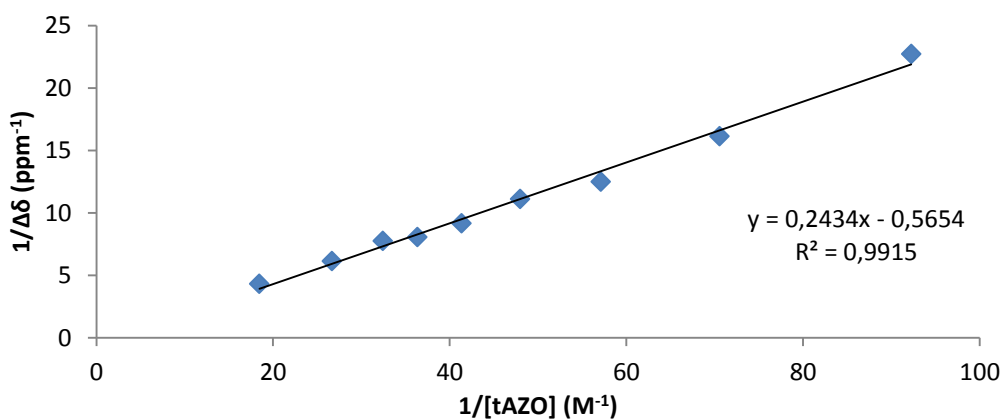


Fig.3.5. Benesi-Hildebrand plot using DAP protons (H_a).

3.3.2. H-bond study in chloroform.

For this study, $\text{PEG}_{10000}\text{-}b\text{-PDAP-(B)}$ was employed. The same methodology than in the case of tetrahydrofurane was followed (see appendix C.3.2 section), but using CDCl_3 .

²³ L. Fielding; *Tetrahedron*, **2000**, 56, 6151-6170

Chemical shift difference versus tAZO concentration was plot, and the association constant was calculated applying Benesi-Hildebran method²³ (Figure 3.6). In this case, it was observed that K_a is approximately 42 M^{-1} . This value is one order of magnitude larger than the one obtained in tetrahydrofuran, so it proves that the binding affinity of the H-bond complex is stronger in chloroform than in tetrahydrofuran.

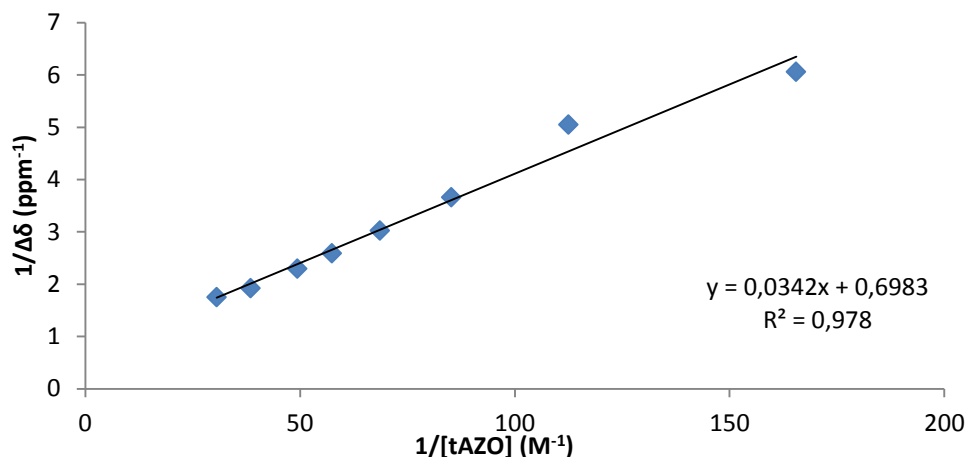


Fig.3.6. Benesi-Hildebrand plot using DAP protons (H_a).

3.4. Preparation and characterization of polymeric nanoparticles by microfluidics.

As was mentioned above, the preparation of polymeric nanoparticles was accomplished by microfluidics to check the feasibility of this methodology to these materials. This part of the project has been made with the collaboration of Dr. V. Sebastián and Dr. M. Arruebo.

Polymeric aggregates have been prepared mixing instantaneously an organic solution that contains the polymer and Milli-Q water, using a commercial slit interdigital microstructured mixer. Both organic solution and water are fed into the micromixer using two syringe pumps. A diagram of the synthetic process is shown in Figure 3.8. The micromixer, which has a volume of $8 \mu\text{L}$, is designed for dividing the feeds into 15 channels of 40 microns that merge at the outlet, achieving instant mixing. Flow values can be modified to obtain different total flow values and different values of the phase ratio. These parameters are modified to optimize the methodology for each polymer, depending on the viscosity and the miscibility of the solvent. After the aggregates preparation, the samples have been characterized by using two techniques: dynamic

light scattering (DLS), to know their hydrodynamic diameter, and transmission electron microscopy (TEM), to know the morphology of the polymeric aggregates.

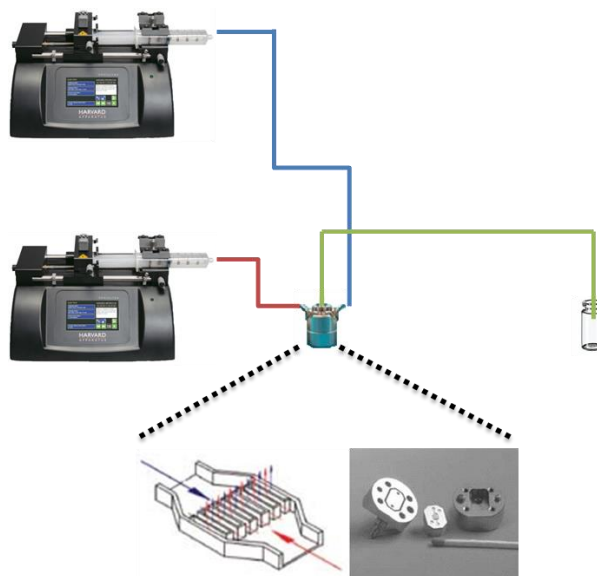


Fig.3.8. Diagram of the experimental set-up for the continuous preparation of polymeric aggregates. Left bottom inset: scheme of the flow arrangements inside the micromixer (reagents come from both sides of central slit from where the mixed products come out). Right bottom inset: image of the micromixer opened.

In this project, two different organic solvents have been used: THF and chloroform. In the case of THF, all the samples were dialyzed after being prepared in order to remove THF. In the case of chloroform, the solvent was removed by three different methodologies: by sonication, by using an orbital shaker and by gradual evaporation.

3.4.1. THF/water systems

As this methodology had not been employed for this kind of BCs before, the first step was to optimize flow values and phase ratio. For this purpose, PEG₁₀₀₀₀-*b*-PDAP-(A) was employed under four different total flow values: 0.4, 2, 10 and 50 mL/min. For each value, two phase ratio values were tried: 60% and 80% of water. The best results in phase mixture were obtained for 10 and 50 mL/min. Consequently, these flow values were then employed using additional phase ratio values with both BCs (PEG₁₀₀₀₀-*b*-PDAP-(A and B) and PEG₂₀₀₀-*b*-PDAP-(A): 60%, 70%, 80%, 90% and 95% of water. The results obtained at 10 mL/min are not very different than the obtained at 50 mL/min (see appendix section D.1). So, the optimal flow chosen was 10 mL/min because the amount of material employed is lower than the employed working at 50 mL/min, and the pumps work better at lower flows (50 mL/min was very close to pump limit speed).

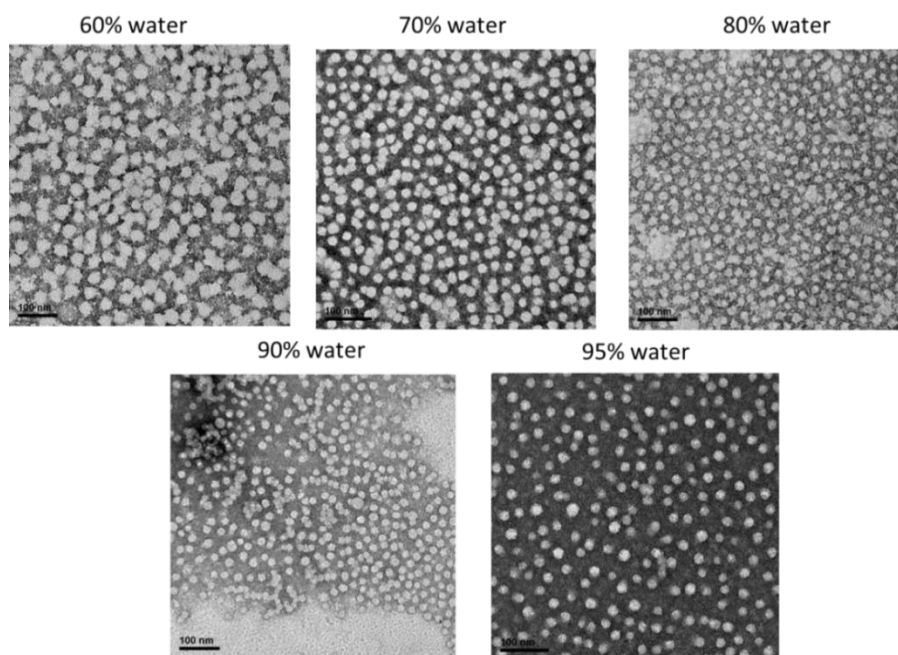


Fig.3.9. TEM images of polymeric nanoparticles obtained by microfluidics using THF/water in different proportions at 10mL/min for PEG₁₀₀₀₀-*b*-PDAP-(A and B).

Working under these conditions, homogeneous spherical micelles were obtained for PEG₁₀₀₀₀-*b*-PDAP-(A and B) (Figure 3.9). Regarding to their diameters, they gradually decrease from approx. 35 to 15 nm when the percentage of water increases from 60 to 95% according to the average values detected by TEM. The hydrodynamic diameter was calculated by DLS and a decrease from 62 to 31 nm was observed that puts in evidence the influence of the experimental conditions on the properties of the polymeric nanoparticles.

This influence on the morphology was more evident in the case of PEG₂₀₀₀-*b*-PDAP. For 90% and 95% of water, spherical micelles were also obtained, as for PEG₁₀₀₀₀-*b*-PDAP-(A and B), with diameters between 20-30 nm. However, for 60%, 70% and 80% of water, vesicles were obtained, although some spherical micelles were also observed. Vesicles had not homogeneous diameters and they were comprised between approx. 80-150 nm (Figure 3.10). DLS results were in accordance with these TEM observations.

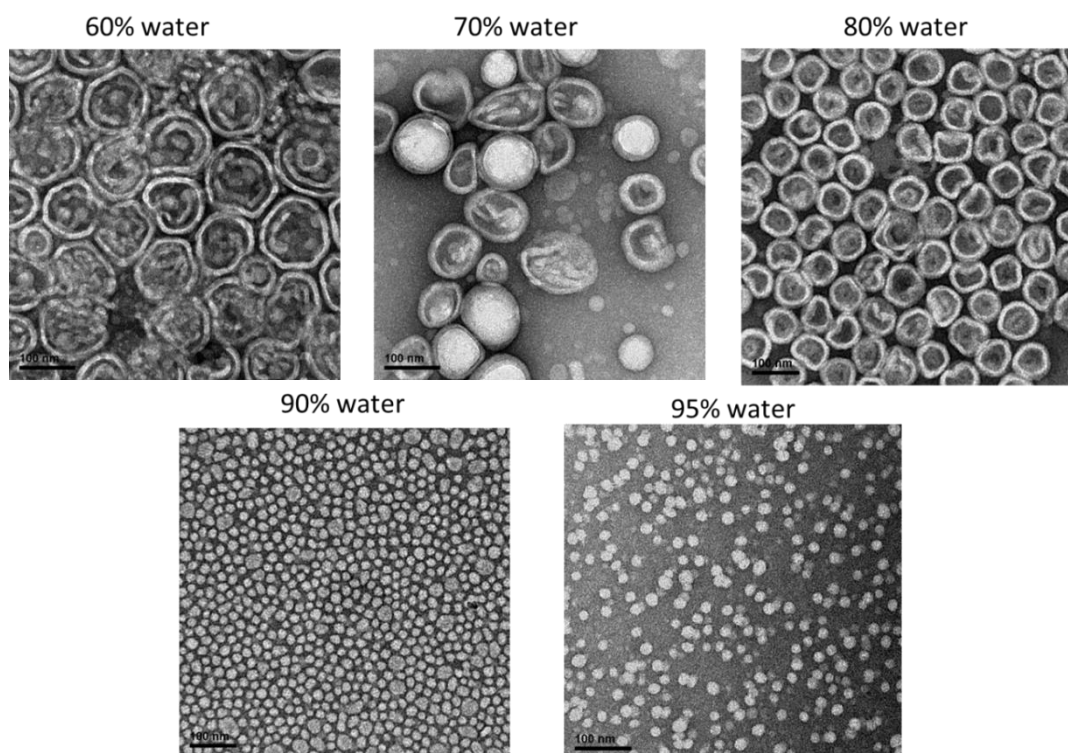


Fig.3.10. TEM images of polymeric aggregates obtained by microfluidics using THF/water in different proportions at 10mL/min for PEG₂₀₀₀-*b*-PDAP-(A).

A similar study was carried out using the supramolecular BCs PEG₁₀₀₀₀-*b*-PDAP-(A)·tAZO and PEG₂₀₀₀-*b*-PDAP-(A)·tAZO. The flow value was fixed at 10 mL/min and different percentages of water were studied: 60%, 70%, 80%, 90%, 95% of water. However, stable polymeric nanoparticles were only obtained for percentages of 90% and 95% of water in both copolymers. For the rest of percentages precipitation was observed a few seconds after sample collecting in all cases. Therefore, new trials were made for the same water percentages but employing a flow value of 50 mL/min, in order to facilitate a better mixing. However stable aggregates were again obtained only for 90% and 95% of water being the results of both flows very similar (see appendix section D.1). In the case of PEG₁₀₀₀₀-*b*-PDAP-(A)·tAZO, homogenous spherical micelles with diameters around 25-30 nm were observed (Figure 3.11). These sizes are bigger than the ones from PEG₁₀₀₀₀-*b*-PDAP-(A and B) samples (20-15 nm), which evidences that azobenzenes were incorporated to the polymeric micelles thus increasing the final diameter of the nanoparticles. Similar micelles were observed for PEG₂₀₀₀-*b*-PDAP-(A)·tAZO, but in this case, bigger polymeric aggregates with irregular shape and size around 120-200 nm were also observed (Figure 3.12), which seems to be related

with a higher tendency to collapse the nanoparticles. All the samples prepared and their characterization data are shown in Table D.1, in appendix section D.

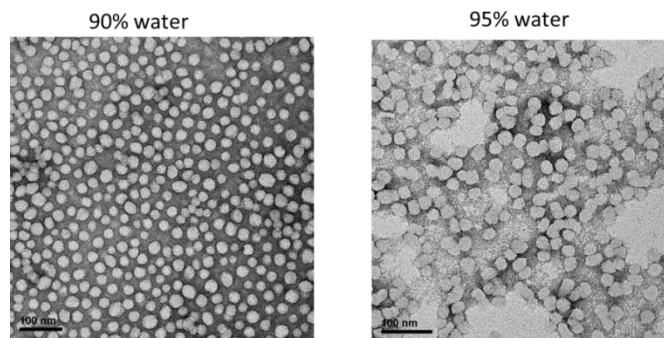


Fig.3.11. TEM images of polymeric nanoparticles obtained by microfluidics using THF/water in different proportions at 10mL/min for PEG₁₀₀₀₀-*b*-PDAP-(A)·tAZO.

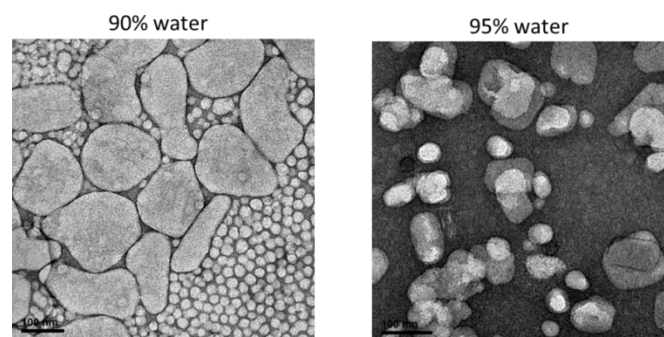


Fig.3.12. TEM images of polymeric aggregates obtained by microfluidics using THF/water in different proportions at 10mL/min for PEG₂₀₀₀-*b*-PDAP-(A)·tAZO.

3.4.2. Chloroform/water systems

In the study of H-bonding by ¹H-NMR it was observed a stronger interaction in chloroform than in THF. This solvent was also employed in the preparation of polymeric nanoparticles in an attempt to improve the results obtained for supramolecular copolymers with the system THF/water. Chloroform is not miscible with water, so high flows are required to obtain a good emulsion. Consequently, only 50 mL/min flow was employed in this case (the maximum flow allowed by the pump). In a first experiment without polymer (only with the solvents) it was observed that using this flow without polymer no emulsion was achieved. However, when PEG₁₀₀₀₀-*b*-PDAP-(B) is introduced, the BC is as a surfactant that stabilizes an emulsion for percentages of 90% and 95% of water in solvent mixture.

As it is said above, three different methods for removing chloroform from the samples were tried: by sonication, by using an orbital shaker and by gradual evaporation. It was

proved that the morphologies obtained depend on the removing method used, and the better results were obtained after sonicating (see appendix section E) the samples during 15 s with an amplitude value of 40% (Figure 3.13). In this way, polymeric nanoparticles with a diameter between 20-40 nm were obtained.

Thus, these conditions were applied to PEG₁₀₀₀₀-*b*-PDAP-(C) (having a different length of the hydrophobic block), PEG₂₀₀₀-*b*-PDAP-(B), PEG₁₀₀₀₀-*b*-PDAP-(B)·tAZO and PEG₂₀₀₀-*b*-PDAP-(B)·tAZO, but no emulsion was achieved for these materials, and in some cases a precipitate appears in the sample after phase separation. Even so, these samples were characterized, and although amorphous matter dominates, in the case of PEG₁₀₀₀₀-*b*-PDAP-(C) and PEG₁₀₀₀₀-*b*-PDAP-(B)·tAZO some spherical and cylindrical micelles appear (Figure 3.14). Consequently, these conditions are not appropriate for preparing polymeric aggregates from these supramolecular BCs. However, it is relevant the influence of the balance hydrophobic/hydrophilic in the preparation of polymeric nanoparticles, as deduced from the results of the two different copolymers PEG₁₀₀₀₀-*b*-PDAP having a different length of the hydrophobic block.

All the samples synthesized and their characterization data are shown in Table D.2, in appendix section D.

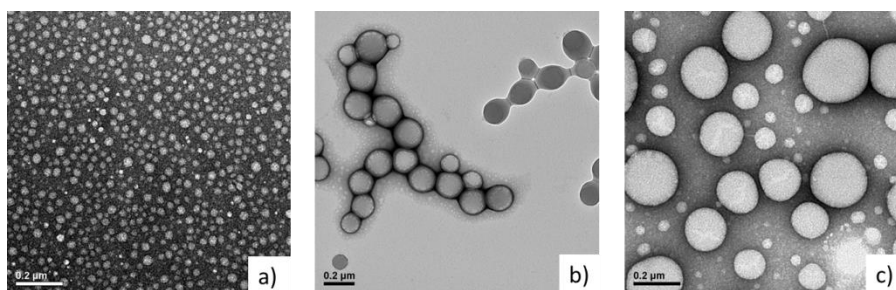


Fig.3.13. TEM images of polymeric aggregates obtained by microfluidics using CHCl₃/water at 50mL/min for PEG₁₀₀₀₀-*b*-PDAP-(B), employing different CHCl₃ removing methods: a) by sonication, b) by using an orbital shaker and c) by gradual evaporation.

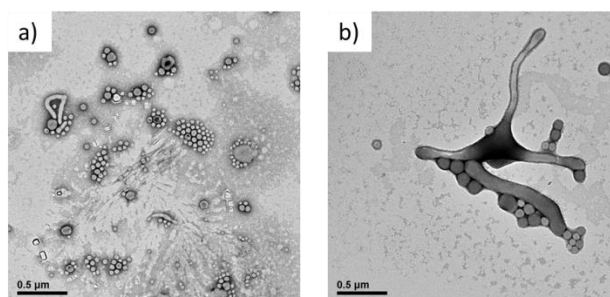


Fig.3.14. TEM images of polymeric aggregates obtained by microfluidics using CHCl₃/water at 50mL/min and sonicating for: a) PEG₁₀₀₀₀-*b*-PDAP-(C) and b) PEG₁₀₀₀₀-*b*-PDAP-(B)·tAZO.

3.5. Study of the light response of supramolecular polymeric self-assemblies

The light response of supramolecular polymeric nanoparticles (PEG₁₀₀₀₀-*b*-PDAP-(A)·tAZO and PEG₂₀₀₀-*b*-PDAP-(A)·tAZO synthesized at 10 mL/min and 90% of water) was studied. For this purpose, samples were exposed to UV light inside a quartz short path length cuvette at a 10 cm distance. After each minute of exposure, a UV-vis spectrum is recorded to follow the isomerization *trans* to *cis*. In the case of PEG₁₀₀₀₀-*b*-PDAP-(A)·tAZO, a complete isomerization was accomplished after 5 min and after 35 min in the case of PEG₂₀₀₀-*b*-PDAP-(A)·tAZO. A displacement of the maximum of absorbance and an increase of the n- π^* band are observed in Figures 3.15 and 3.17 upon increasing the time of radiation. In both cases, 30 minutes once finished the irradiation, the return to *trans* isomer is detected and after 24 hours it is completely recovered. However, by TEM no size or shape changes were observed after irradiation (Figures 3.16 and 3.18). This behavior may be due to the sample preparation (there is a delay between irradiation and the complete preparation of the sample). In the case of PEG₂₀₀₀-*b*-PDAP-(A)·tAZO sample, some shape changes are observed in the bigger aggregates after radiation (they become smaller and more spherical).

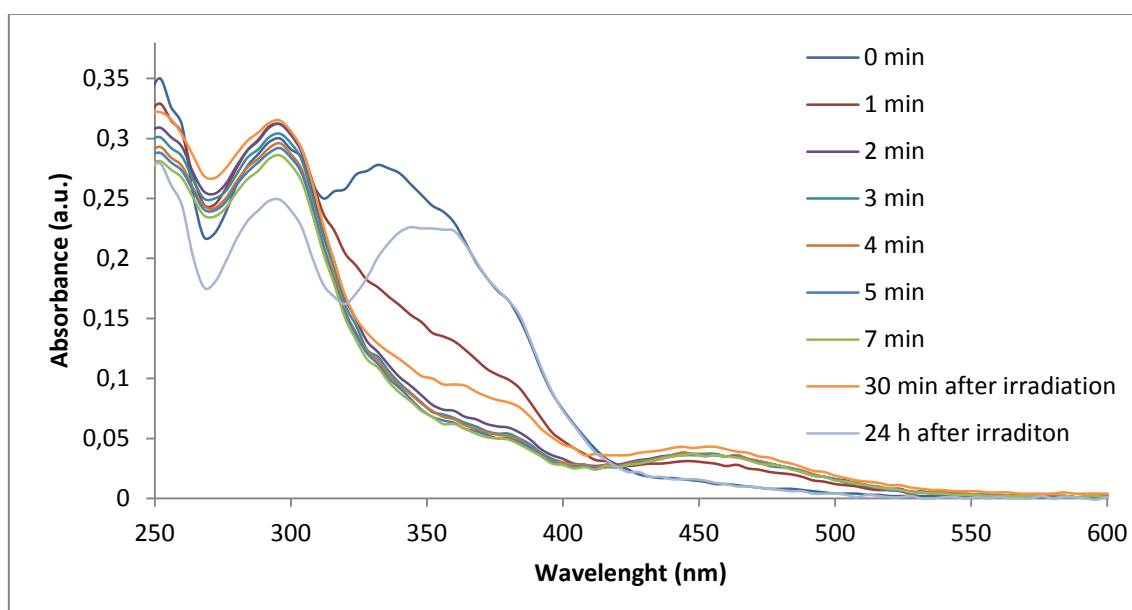


Fig.3.15. UV-vis spectra of PEG₁₀₀₀₀-*b*-PDAP-(A)·tAZO nanoparticles upon UV light radiation.

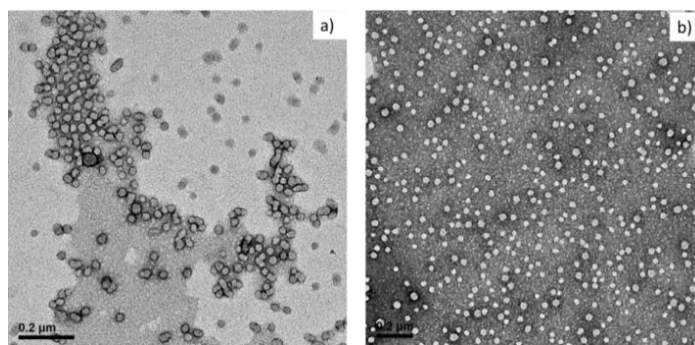


Fig.3.16. TEM images of PEG₁₀₀₀₀-*b*-PDAP-(A)·tAZO nanoparticles: a) before and b) after UV light exposure.

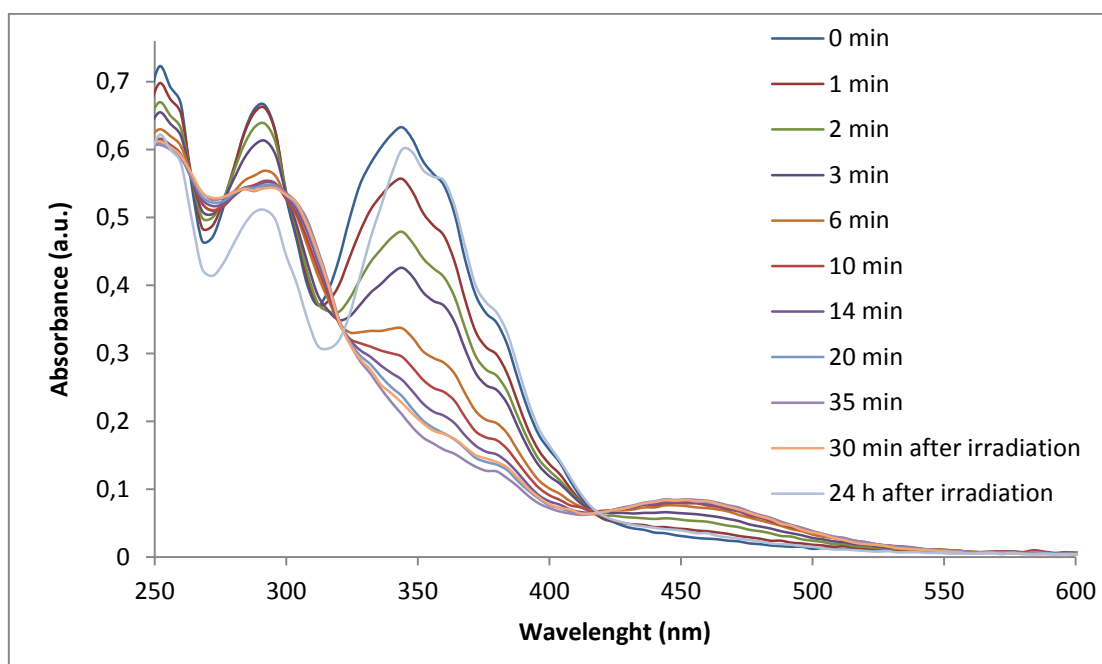


Fig.3.17. UV-vis spectra of PEG₂₀₀₀-*b*-PDAP-(A)·tAZO aggregates upon UV light radiation.

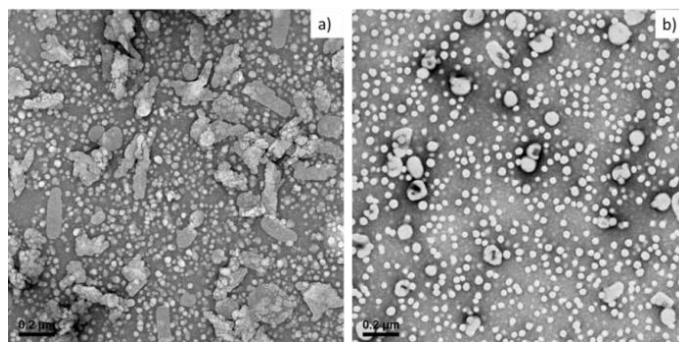


Fig.3.18. TEM images of PEG₁₀₀₀₀-*b*-PDAP-(A)·tAZO aggregates: a) before and b) after UV light exposure.

4. EXPERIMENTAL SECTION

Techniques and instruments that have been employed in this project are described in Section E, in Appendix.

4.1. Copolymers PEG₂₀₀₀-*b*-PDAP and PEG₁₀₀₀₀-*b*-PDAP synthesis and characterization

The DAP monomer, the macromolecular transfer agent (CTA-PEG₂₀₀₀ for PEG₂₀₀₀-*b*-PDAP or CTA-PEG₁₀₀₀₀ for PEG₁₀₀₀₀-*b*-PDAP) and AIBN are added into a Schlenk flask in the amounts that are shown in Table 4.1, 1.5 mL of dry DMF (distilled previously) are also added. Three cycles of Ar/vacuum are carried out and then, the Schlenk flask is placed in a preheated bath at 80 °C. The reaction is maintained under stirring at 80°C for 5 hours. Afterwards, the Schlenk flask is introduced into liquid nitrogen (quenching) and the crude is precipitated into 100 mL (approximately) of cold diethyl ether. For the PEG₁₀₀₀₀-*b*-PDAP, the precipitate is filtered under vacuum and then washed with cold diethyl ether. For the PEG₂₀₀₀-*b*-PDAP, the precipitate is centrifuged and dried under vacuum. In order to purify the compounds and remove residual monomer and other impurities that might remain the copolymer is dissolved in DCM and reprecipitated in cold diethyl ether.

Table 4.1. Reagent amounts employed in copolymer synthesis.

Polymer	DAP (mg)	Macro-CTA (mg)	AIBN (mg)	Yield
PEG ₂₀₀₀ - <i>b</i> -PDAP-(B)	300.1	160.3	2.2	58%
PEG ₁₀₀₀₀ - <i>b</i> -PDAP-(B)	269.9	142.6	3.0	50%
PEG ₁₀₀₀₀ - <i>b</i> -PDAP-(C)	282.9	150.1	3.2	72%

Characterization data:

PEG₂₀₀₀-*b*-PDAP (B)

IR (KBr), ν (cm⁻¹): 3323 (N-H), 1736, 1697 (C=O), 1586, 1518, 1449 (Ar), 1150, 1112 (C-O).

¹H-NMR (CDCl₃, 400 MHz), δ (ppm): 9.12-8.25, 7.98-7.53, 4.52-3.97, 3.81-3.49, 3.37, 2.87-2.57, 2.45-2.27, 2.16-0.71.

PEG₁₀₀₀₀-b-PDAP (B)

IR (KBr), ν (cm⁻¹): 3326 (N-H), 1735, 1697 (C=O), 1586, 1519, 1449 (Ar), 1151, 1105 (C-O).

¹H-NMR (CDCl₃, 400 MHz), δ (ppm): 9.39-8.13, 8.03-7.44, 4.53-3.92, 3.84-3.42, 3.37, 2.96-2.56, 2.49-2.25, 2.16-0.64.

PEG₁₀₀₀₀-b-PDAP (C)

IR (KBr), ν (cm⁻¹): 3324 (N-H), 1734, 1695 (C=O), 1586, 1519, 1449 (Ar), 1151, 1107 (C-O).

¹H-NMR (CDCl₃, 400 MHz), δ (ppm): 9.10-8.22, 7.95-7.44, 4.50-3.94, 3.85-3.43, 3.37, 2.96-2.53, 2.45-2.24, 2.15-0.62.

4.2. Supramolecular BCs synthesis

The corresponding amounts of BC and tAZO (see Table 4.2) are weighted in a vial using a precision balance (accuracy 0.1 mg). They were dissolved in the minimal volume of HPLC-grade THF. The solution is filtered using a 0.2 micron PTFE filter, and then it is maintained at room temperature under constant mechanical stirring several days for a gradual solvent evaporation. Afterwards, the supramolecular block copolymers are finally dried under vacuum.

Table 4.2. Reagent amounts employed in supramolecular BCs synthesis.

Supramolecular Block Copolymer	Block Copolymer (mg)	timAZO (mg)
PEG₂₀₀₀-b-PDAP-(A)·tAZO	60.0	60.4
PEG₂₀₀₀-b-PDAP-(B)·tAZO	40.0	37.5
PEG₁₀₀₀₀-b-PDAP-(A)·tAZO	60.0	36.2
PEG₁₀₀₀₀-b-PDAP-(B)·tAZO	40.0	24.1

4.3. Study of association constants in supramolecular amphiphilic BCs

Samples prepared are described in Tables C.3.1 and C.3.2 of appendix Section D.

4.4. Preparation of polymeric nanoparticles by microfluidics

The nanoparticles in water have been prepared using both starting BCs and supramolecular BCs. Two different organic solvents have been employed, HPLC-grade THF and HPLC-grade CHCl_3 . The general method consists in dissolving a certain amount of the polymer in the organic solvent, reaching a concentration of 5 mg/mL. This solution is filtered through a 0.2 micron PTFE filter, and then it is loaded up in a syringe. Another syringe is loaded up with Milli-Q water. Both syringes are placed on the pumps, and connected to the micromixer. After fixed the flow, both pumps are turned on at the same time, and after waiting several seconds to micromixer's homogenization, sample is collected in a vial.

If the organic solvent is THF, the samples are transferred to a dialysis membrane (pore size 1000 Da) to dialyze against Milli-Q water to eliminate the organic solvent. Water needs to be constantly changed every 24 h during 4 days. If it is CHCl_3 , it is removed by three different methodologies: by sonication, by using an orbital shaker and by leaving it evaporate gradually.

5. CONCLUSIONS

As a summary of this work, it can be said that:

- Using RAFT polymerization is possible to obtain amphiphilic BCs with a controlled structure. The BCs are composed of PEG (hydrophilic) and a polymethacrylate containing units of 2,6-diacylaminopyridine, PDAP (hydrophobic).
- By molecular recognition between thymine derivatives, which contain azobenzene groups, and 2,6-diacylaminopyridine units, light responsive supramolecular amphiphilic BCs were synthesized. The presence of hydrogen bond was demonstrated by $^1\text{H-NMR}$ and FTIR.
- $^1\text{H-NMR}$ titrations were performed to study the binding affinity of the H-bond complex in two different solvents. Association constant (K_a) has a very low value (4 M^{-1}) in THF, thus H-bonds are weak in this solvent due to a solvation effect. In the case of chloroform, K_a is higher (42 M^{-1}), so H-bonds are stronger than in THF.
- Microfluidics was employed for polymeric aggregates preparation in two different systems: THF/water and CHCl_3 /water. In the first one, reproducible and homogenous micelles (15-30 nm) were obtained for $\text{PEG}_{10000}\text{-}b\text{-PDAP}$ and, depending on the water ratio, vesicles or micelles were obtained for $\text{PEG}_{2000}\text{-}b\text{-PDAP}$. Polymeric nanoparticles were also obtained for supramolecular BCs. In the case of CHCl_3 /water system, a good emulsion was not achieved, and consequently no reproducible results were obtained.
- Supramolecular BC nanoparticles were irradiated to study their behavior in the presence of UV light. Although azobenzene isomerization was observed, no size or shape significant changes were observed after irradiation. Nevertheless, the response to light can be employed for the release of encapsulated molecules.

The main conclusion of this Final Master Project is that microfluidic is a suitable technique to prepare polymeric nanoparticles in an easy and reproducible way from amphiphilic BCs. This methodology provides many advantages in comparison with co-solvent methodology previously studied as it has a better reproducibility and a large amount of polymeric nanoparticles can be prepared in a faster way. In the case of supramolecular BCs, this methodology is not of direct application and further studies are required to optimize the conditions of the process.